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Antibióticos de consumo humano: adsorción-desorción en suelos y materiales residuales

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ANTIBIÓTICOS DE CONSUMO HUMANO: ADSORCIÓN-DESORCIÓN EN SUELOS Y MATERIALES RESIDUALES

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ABSTRACT

The discovery of antibiotics represented a major milestone in modern medicine as they contributed to significant reductions in mortality and morbidity rates. However, the intensive and, in many cases, indiscriminate use of these compounds has led to a significant increase in their presence in different environmental compartments. Once administered, antibiotics can be excreted partially unmetabolized and reach the environment through wastewater, sewage sludge, slurry or manure, constituting a risk for the environment when spread on soils. Once they reach the soil, they can be absorbed by plants or leach to water bodies, leading to the entry of these contaminants into the food chain, and potentially impacting both environmental and human health. In addition, if these antibiotics persist for long periods of time in the environment they can lead to the development of antibiotic-resistant bacteria, a growing global concern.

Among the most used antibiotics in human medicine are amoxicillin (AMX) and cefuroxime (CFX), both belonging to the β-lactam group, and azithromycin (AZM), which is part of the macrolide group. These drugs are poorly absorbed in the human gastrointestinal tract, and a very high percentage (up to 90%) is excreted as parent compound through faeces and urine entering wastewaters and reaching wastewater treatment plants (WWTPs) where conventional treatment processes only partially remove them; as a result, these antibiotics persist in treated effluents and accumulate in sewage sludge. These sludges are widely used as fertilizers for agricultural soils, creating a direct pathway for antibiotic contamination of terrestrial ecosystems. Once in the soil, antibiotics undergo a series of physicochemical and biological processes, such as adsorption, desorption, degradation or transformation, determined by both compound-specific properties and soil characteristics. Adsorption/desorption dynamics are particularly critical as they influence antibiotic mobility, bioavailability, potential transfer to water bodies and crops and, consequently, the entry into the food chain, determining their persistence and ecological impact within agroecosystems.

The general objective of this thesis is to study the adsorption and desorption capacity of amoxicillin, cefuroxime and azithromycin by different soils from Galicia. In addition, the potential for the retention of these antibiotics of several locally abundant residues and by-products from the forestry and food industries (ash, pine bark and needles, eucalyptus leaves and mussel shell), will also be studied. These materials will be assessed as potential low-cost bioasorbents for mitigating antibiotic mobility in soil systems and for their use in the decontamination of water containing these antibiotics.

An initial phase of this study involved assessing the presence of various antibiotics commonly used in human medicine within different environmental matrices, including wastewater treatment plant sludges, sludges processed by waste management companies, soils previously amended with these sludges, and vegetation grown on those soils. Specifically, 50 WWTP sludges from different treatment plants in Galicia were analysed (22 sampled in spring and 28 sampled in autumn). Additionally, 13 processed sludge samples obtained from the waste management companies as well as 59 soils, from different areas of Galicia, that had been previously amended with these sludges, were analysed. Vegetation grown on these soils was also collected (27 corn samples and 32 vineyard samples).

For the adsorption/desorption experiments, 23 soils with different physicochemical properties were selected. Batch experiments were performed, in which increasing concentrations of the three previously mentioned antibiotics (cefuroxime, amoxicillin and azithromycin) were added to a fixed amount of soil. These experiments were also carried out using the different bioadsorbents, to determine their capacity to adsorb the three

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antibiotics and to consider the possibility of adding them to soils in order to increase the adsorption of these compounds, or even to use them in water decontamination processes. The experiments with soils and bioadsorbents were conducted under three different systems: a single-compound system (each antibiotic individually), a binary system (amoxicillin combined with ciprofloxacin, another antibiotic widely used in human medicine), and a ternary system (AMX, CFX, and AZM applied simultaneously). In addition, bioadsorbents were incorporated into the soils to evaluate their effect on the soil's adsorption capacity.

Regarding the occurrence of antibiotics, 68% of the sludge samples collected in spring showed the presence of antibiotics, while this percentage decreased up to 32% in the sludges collected in autumn. Processed sludges generally exhibited a lower frequency and concentration of antibiotic residues compared to untreated sludges, suggesting that current treatment methods applied by waste managers are effective to some extent. Specifically, during spring antibiotics were detected in 5 out of 6 samples, compared to only 4 out of 7 in the autumn sampling. Regarding soils, antibiotics were detected in 12% of the samples, but only one antibiotic per sample was found and at lower concentrations. Notably, no antibiotic residues were detected in any of the vegetation samples analysed.

In the single-compound systems, AZM exhibited the highest affinity for the different soils, achieving adsorption rates close to 100% in most cases. This was followed by CFX, while AMX showed the lowest adsorption. Desorption results further confirmed that AZM was the most strongly retained compound, with no detectable desorption across all samples. In contrast, AMX displayed the highest desorption values, reaching up to 17%, whereas CFX exhibited minimal desorption, not exceeding 1% in any case. Soil pH was found to influence adsorption behaviour. Specifically, CFX and AZM showed increased adsorption in soils with higher pH, while AMX adsorption was favoured in soils with lower pH values.

When all three antibiotics (AMX, CFX, and AZM) were simultaneously applied to the soils (ternary system), AZM again demonstrated the highest adsorption rates in most cases. However, both AZM and CFX showed reduced adsorption compared to the single-compound system, particularly at higher concentrations. In contrast, AMX presented more variable results, with evidence of a synergistic effect in some cases. Notably, AMX desorption increased in the presence of CFX and AZM, suggesting that AMX primarily occupied lower-energy adsorption sites.

In the binary system involving AMX and ciprofloxacin (CIP), AMX also showed enhanced adsorption in the presence of CIP. Moreover, desorption was lower in the binary system, particularly at lower concentrations, indicating more stable binding.

Adsorption data for all three antibiotics were fitted to the Freundlich, Langmuir, and linear isotherm models. The best fit was achieved with the Freundlich model, with $R^2>0.9$ in most cases. In the simple systems, the values of the Freundlich KF parameter were similar for CFX and AZM, oscillating between 3.234 and 478.866 $L^n\ \mu mol^{1-n}\ kg^{-1}$, respectively, while for AMX the values were lower, with a maximum of 150.34 $L^n\ \mu mol^{1-n}\ kg^{-1}$. In the ternary system, KF values (ranging from 0.529 to 12.543 $L^n\ \mu mol^{1-n}\ kg^{-1}$) were generally lower than in the single-compound systems, with the greatest reduction observed for CFX. Regarding the binary system AMX+CIP the KF parameter ranged from 1.12 to 129.796 $L^n\ \mu mol^{1-n}\ kg^{-1}$. The Freundlich n parameter ranged from 0.41 to 1.655 for CFX, from 0.306 to 1.33 for AMX, and from 0.128 to 1.327 for AZM. The values of the n parameter obtained in the simple systems were similar to those obtained in the ternary system (ranging between 0.795 and 1.544) and in the binary system AMX+CIP (0.658-1.79). The Langmuir model yielded large fitting errors, rendering it unsuitable, and none of the antibiotics fitted well to the linear model under single-

compound conditions. However, in the ternary system, several soils demonstrated a good fit with the linear model.

Regarding bioadsorbents, in the simple system alkaline bioadsorbents, ash and mussel shell, were the most effective for CFX retention. Wood ash exhibited nearly 100% adsorption and no desorption, making it the most effective material as bioadsorbent for this antibiotic. Mussel shell also showed high adsorption capacity for CFX and null desorption, although its adsorption percentages were slightly lower than those of ash, falling within the range of 71.19% to 85.02%. For AMX, eucalyptus leaves, pine needles and wood ash showed the high adsorption percentages, higher than 76% in all cases, while desorption was null in all cases, indicating irreversible adsorption. Finally, in the case of AZM, ash again displayed the highest retention capacity, with adsorption exceeding 80% at concentrations above 50 μmol L^{-1} and no desorption detected.

In the ternary system, ash remained highly effective, achieving 100% adsorption in many cases, particularly at higher concentrations. Interestingly, compared to the single-compound system, higher adsorption percentages were observed for ash in the ternary system, indicating a possible synergistic effect. In contrast, pine bark (except for AMX) and mussel shell exhibited more reduced adsorption in the ternary system, suggesting competitive interactions among the antibiotics for the adsorption sites.

The incorporation of bioadsorbents (ash, mussel shell, and pine bark) into soils enhanced adsorption in most cases. For CFX, the greatest improvements were observed in soils amended with ash and mussel shell, often reaching complete (100%) adsorption, while unamended soils did not exceed 80% adsorption in most cases. For AMX, the best results were obtained with the addition of pine bark, with increases of up to 40% in certain cases. In the case of AZM, the incorporation of any of the three materials into the soils increased adsorption up to 100% in most soils, while adsorption in unamended soils was below 80% at the highest concentration applied.

The main factors influencing antibiotic adsorption in both soils and bioadsorbents included pH, organic matter content, and the presence of non-crystalline minerals. These properties were found to play a central role in the retention mechanisms of AMX, CFX, and AZM in the studied systems.

Keywords: Antibiotics, adsorption-desorption, bioadsorbents, emerging pollutants, soils.

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RESUMEN

El descubrimiento de los antibióticos supuso un logro fundamental en la medicina moderna al contribuir a una notable reducción de las tasas de mortalidad y morbilidad. Sin embargo, su uso intensivo y, en muchos casos, indiscriminado ha provocado un incremento significativo de su presencia en distintos compartimentos ambientales. Una vez administrados, los antibióticos pueden ser excretados parcialmente sin metabolizar y alcanzar el medio ambiente a través de aguas residuales, lodos de depuradora, purines o estiércoles, lo que supone un riesgo para el medio ambiente cuando se aplican a los suelos. Una vez en el suelo, pueden ser absorbidos por las plantas o llegar a masas de agua, lo que facilita su entrada en la cadena trófica, pudiendo afectar tanto a la salud ecológica como humana. Además, si estos antibióticos persisten durante largos periodos en el medio, pueden favorecer la aparición de bacterias resistentes, una preocupación creciente a nivel mundial.

Entre los antibióticos más utilizados en medicina humana se encuentran la amoxicilina (AMX) y la cefuroxima (CFX), del grupo de los β-lactámicos, y la azitromicina (AZM), perteneciente al grupo de los macrólidos. Estos fármacos se absorben de forma limitada en el tracto gastrointestinal humano, y un porcentaje muy elevado (hasta el 90%) se excreta como compuesto original a través de heces y orina, accediendo a las aguas residuales. Estos compuestos llegan a las estaciones depuradoras (EDAR), donde los tratamientos convencionales sólo los eliminan parcialmente y, como resultado, persisten en los efluentes tratados acumulándose en los lodos. Estos lodos se utilizan frecuentemente como fertilizantes en suelos agrícolas, generando una vía directa de contaminación por antibióticos en entornos terrestres. Una vez en el suelo, los antibióticos experimentan procesos fisicoquímicos y biológicos, como adsorción, desorción, degradación o transformación, determinados tanto por las propiedades del compuesto como por las características del suelo. Las dinámicas de adsorción/desorción son especialmente relevantes, ya que influyen en su movilidad, biodisponibilidad, posible transferencia a cuerpos de agua o cultivos y, en consecuencia, en su entrada en la cadena trófica, determinando su persistencia e impacto ecológico en los agroecosistemas.

El objetivo general de esta tesis es estudiar la capacidad de adsorción y desorción de amoxicilina, cefuroxima y azitromicina por parte de distintos suelos de Galicia. Asimismo, se evaluará el potencial de varios residuos y subproductos locales de las industrias forestal y alimentaria (ceniza, corteza y acículas de pino, hojas de eucalipto y concha de mejillón) para retener estos antibióticos. Estos materiales se estudiarán como posibles bioadsorbentes de bajo coste para mitigar la movilidad de antibióticos en suelos o para la descontaminación de aguas que los contengan.

En una fase inicial del estudio se evaluó la presencia de diversos antibióticos de uso humano en distintos compartimentos ambientales: lodos de depuradora, lodos tratados por gestores autorizados, suelos enmendados previamente con estos lodos y vegetación cultivada sobre dichos suelos. En total se analizaron 50 muestras de lodos EDAR (22 recolectadas en primavera y 28 en otoño), 13 muestras de lodos tratado y 59 suelos de distintas zonas de Galicia previamente enmendados con esos lodos. La vegetación que creció en estos suelos (27 de maíz y 32 de viñedo) también fue analizada.

Para los ensayos de adsorción/desorción se seleccionaron 23 suelos con propiedades fisicoquímicas variables. Se realizaron experimentos de tipo batch, en los que se añadieron concentraciones crecientes de los tres antibióticos mencionados a una cantidad determinada de suelo. Estos ensayos también se realizaron con los bioadsorbentes, para evaluar su capacidad de retener los antibióticos y considerar su uso como enmiendas para aumentar la adsorción en suelos o como materiales para eliminar antibióticos en aguas.

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Los ensayos se realizaron en tres sistemas: uno simple (cada antibiótico por separado), uno binario (AMX con ciprofloxacino, otro antibiótico de uso humano) y uno ternario (AMX, CFX y AZM simultáneamente). Además, se incorporaron bioadsorbentes a los suelos para evaluar su efecto en la capacidad de adsorción de estos.

En cuanto a la presencia de antibióticos, el 68% de los lodos recogidos en primavera contenían antibióticos, mientras que en otoño este porcentaje descendió al 32%. Los lodos tratados presentaron, en general, menores concentraciones y frecuencia de antibióticos que los lodos sin tratar, lo que sugiere cierta eficacia de los tratamientos actuales. En primavera se detectaron antibióticos en 5 de las 6 muestras tratadas, y en otoño en 4 de 7. En suelos, los antibióticos se detectaron en el 12% de las muestras, pero solo se encontró un antibiótico por muestra y a bajas concentraciones. En cuanto a las muestras vegetales analizadas, no se encontró presencia de antibióticos en ninguna de ellas.

En los sistemas simples, la AZM mostró la mayor afinidad por los suelos, alcanzando tasas de adsorción cercanas al 100% en la mayoría de los casos. Le siguió la CFX, mientras que la AMX fue la que menos se adsorbió. La desorción confirmó que la AZM fue el compuesto que se adsorbió más fuertemente, sin desorción detectable. La AMX mostró el mayor porcentaje de desorción (hasta el 17%), mientras que en el caso de la CFX la desorción fue mínima (<1%). El pH del suelo influyó en el comportamiento de la adsorción: CFX y AZM aumentaron su adsorción en suelos de pH más alto, mientras que la AMX se adsorbió mejor en suelos más ácidos.

En el sistema ternario, la AZM mantuvo la mayor adsorción, pero tanto AZM como CFX vieron reducida su retención respecto al sistema simple, especialmente a concentraciones altas. En cambio, AMX mostró resultados más variables, con indicios de efecto sinérgico en algunos casos. La desorción de AMX aumentó en presencia de los otros antibióticos, lo que sugiere su adsorción preferente en sitios de menor energía.

En el sistema binario AMX+CIP, la AMX también mejoró su adsorción y redujo su desorción, sobre todo a concentraciones bajas, indicando una unión más estable.

Los datos de adsorción se ajustaron a los modelos de isoterma de Freundlich, Langmuir y lineal. El mejor ajuste se obtuvo con el modelo de Freundlich ($R^2 > 0,9$). En sistemas simples, los valores del parámetro KF fueron similares para la CFX y AZM, oscilando entre 3,234 y 478,866 $L^n\ \mu mol^{1-n}\ kg^{-1}$, y más bajos para AMX, con un máximo de 150,34 $L^n\ \mu mol^{1-n}\ kg^{-1}$. En el sistema ternario los valores de KF fueron generalmente más bajos, especialmente para la CFX. En el sistema binario AMX+CIP, KF varió entre 1,12 y 129,796 $L^n\ \mu mol^{1-n}\ kg^{-1}$. Los valores del parámetro n oscilaron entre 0,41 y 1,655 para la CFX, entre 0,306 y 1,33 para la AMX y entre 0,128 y 1,327 para la AZM. Estos valores fueron similares a los observados en sistemas ternario y binario. El modelo de Langmuir presentó errores elevados, y el modelo lineal no fue adecuado en sistemas simples, aunque sí mostró buen ajuste en algunos suelos en el sistema ternario.

En cuanto a los bioadsorbentes, en el sistema simple, los materiales alcalinos como la ceniza y la concha de mejillón fueron los más efectivos para retener CFX. La ceniza logró casi un 100% de adsorción y nula desorción, seguida de la concha de mejillón, que presentó unos porcentajes de adsorción entre 71,19% y 85,02%. En el caso de la AMX, las hojas de eucalipto, las acículas de pino y la ceniza superaron el 76% de adsorción, sin desorción detectable. Finalmente, para AZM la ceniza mostró de nuevo la mayor capacidad de retención con porcentajes de adsorción superiores al 80% a concentraciones añadidas por encima de 50 $\mu mol\ L^{-1}$.

En el sistema ternario, la ceniza siguió siendo altamente efectiva, obteniendo porcentajes de adsorción en torno al 100% en la mayoría de los casos y especialmente a altas concentraciones añadidas. Curiosamente, los porcentajes fueron incluso más altos que en el sistema simple, lo que sugiere un efecto sinérgico. Por el contrario, la corteza

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de pino y la concha de mejillón redujeron su eficacia, posiblemente por competencia entre antibióticos.

La incorporación de bioadsorbentes en suelos mejoró la adsorción en la mayoría de los casos. Para la CFX, los mayores aumentos se lograron con la ceniza y la concha de mejillón, llegando en ocasiones al 100% de adsorción, mientras que en los suelos sin enmendar los porcentajes de adsorción no alcanzaron el 80% en la mayoría de los casos. Para la AMX, la adición de corteza de pino incrementó la adsorción hasta un 40% en ciertos casos, y en el caso de la AZM, cualquiera de los tres materiales aumentó la adsorción al 100% en la mayoría de los suelos, mientras que en los suelos sin enmendar los porcentajes de adsorción fueron menores del 80% en la mayoría de los casos.

Los principales factores que influyeron en la adsorción fueron el pH, el contenido de materia orgánica y la presencia de minerales no cristalinos, propiedades que jugaron un papel clave en los mecanismos de retención de los antibióticos estudiados.

Palabras clave: Antibióticos, adsorción-desorción, bioadsorbentes, contaminantes emergentes, suelos

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RESUMO

O descubrimento dos antibióticos supuxo un avance destacado e fundamental na medicina moderna ao contribuír a unha notable redución das taxas de mortalidade e morbilidade. Porén, o seu uso intensivo e, en moitos casos, indiscriminado, provocou un incremento significativo da súa presenza en distintos compartimentos ambientais. Unha vez administrados, os antibióticos poden ser excretados parcialmente sen metabolizar e chegar ao medio ambiente a través de augas residuais, lodos de depuradora, xurros ou esterco, o que supón un risco ambiental cando se aplican aos solos. Unha vez no solo, poden ser absorbidos polas plantas ou alcanzar masas de auga, facilitando así a súa entrada na cadea trófica, podendo afectar tanto á saúde ecolóxica como á humana. Ademais, se estes compostos persisten durante longos períodos no medio, poden favorecer a aparición de bacterias resistentes, unha preocupación crecente a nivel mundial.

Entre os antibióticos máis empregados en medicina humana atópanse a amoxicilina (AMX) e a cefuroxima (CFX), do grupo dos β-lactámicos, e a azitromicina (AZM), pertencente ao grupo dos macrólidos. Estes fármacos absorbense de forma limitada no tracto gastrointestinal humano, e unha porcentaxe moi elevada (ata o 90 %) é excretada como composto orixinal a través das feces e ouriños, accedendo ás augas residuais. Estes compostos chegan ás estacións depuradoras de augas residuais (EDAR), onde os tratamentos convencionais só logran eliminalos parcialmente. Como resultado, persisten nos efluentes tratados e acumúlanse nos lodos. Estes lodos úsanse frecuentemente como fertilizantes en solos agrícolas, xerando unha vía directa de contaminación por antibióticos nos ecosistemas terrestres. Unha vez no solo, os antibióticos experimentan procesos físico-químicos e biolóxicos, como adsorción, desorción, degradación ou transformación, determinados tanto polas propiedades do composto como polas características do solo. As dinámicas de adsorción/desorción son especialmente relevantes, pois inflúen na súa mobilidade, biodispoñibilidade, posible transferencia a masas de auga ou cultivos e, en consecuencia, na súa entrada na cadea trófica, determinando a súa persistencia e impacto ecolóxico nos agroecosistemas.

O obxectivo xeral desta tese é estudar a capacidade de adsorción e desorción da amoxicilina, cefuroxima e azitromicina por parte de distintos solos de Galicia. Así mesmo, avaliarase o potencial de varios residuos e subprodutos locais das industrias forestal e alimentaria (cinza, cortiza e agullas de piñeiro, follas de eucalipto e cuncha de mexillón) para reter estes antibióticos. Estes materiais estudiaranse como posibles bioadsorbentes de baixo custo para mitigar a mobilidade dos antibióticos nos solos ou para a descontaminación de augas que os conteñan.

Nunha fase inicial do estudo avaliouse a presenza de diversos antibióticos de uso humano en distintas matrices ambientais: lodos de depuradora, lodos tratados por xestores autorizados, solos emendados previamente con estes lodos e vexetación cultivada sobre ditos solos. En total analizáronse 50 mostras de lodos EDAR (22 recollidas na primavera e 28 no outono), 13 mostras de lodos tratados, 59 solos de distintas zonas de Galicia que foron previamente enmendados por estes lodos. A vexetación que medrou nestes solos tamén foi analizadas (27 mostras de millo e 32 de viñedo).

Para os ensaios de adsorción/desorción seleccionáronse 23 solos con propiedades físico-químicas variables. Realizáronse experimentos tipo batch, nos que se engadiron concentracións crecentes dos tres antibióticos mencionados a unha cantidade determinada de solo. Estes ensaios realizáronse tamén cos bioadsorbentes, para avaliar a súa capacidade de reter os antibióticos e considerar o seu uso como emendas para aumentar a adsorción en solos ou como materiais para eliminar antibióticos en augas. Os ensaios realizáronse en tres sistemas: un simple (cada antibiótico por separado), un binario (AMX

con ciprofloxacino, outro antibiótico de uso humano) e un ternario (AMX, CFX e AZM simultaneamente). Ademais, incorporáronse bioadsorbentes aos solos para avaliar o seu efecto na capacidade de adsorción destes.

En canto á presenza de antibióticos, no 68 % dos lodos recollidos na primavera detectáronse antibióticos, mentres que no outono esta porcentaxe descendeu ao 32 %. Os lodos tratados presentaron, en xeral, menores concentracións e frecuencia de antibióticos que os lodos sen tratar, o que suxire certa eficacia dos tratamentos actuais. Na primavera detectáronse antibióticos en 5 das 6 mostras tratadas, e no outono en 4 de 7. Nos solos, os antibióticos detectáronse no 12 % das mostras, pero só un antibiótico por mostra e a baixas concentracións. Non se detectaron residuos en ningunha das mostras vexetais analizadas.

Nos sistemas simples, a AZM amosou a maior afinidade polos solos, alcanzando taxas de adsorción próximas ao 100 % na maioría dos casos, seguida da CFX, mentres que a AMX foi a que menos se adsorbeu. A desorción confirmou que a AZM foi o composto máis retido, presentando nula desorción, mentres que a AMX amosou a maior desorción (ata o 17 %) e a CFX a máis baixa (<1 %). O pH do solo influíu no comportamento de adsorción: CFX e AZM aumentaron a súa adsorción en solos de pH máis alto, mentres que a AMX adsorbeuse mellor en solos máis ácidos.

No sistema ternario, a AZM mantivo a maior adsorción, pero tanto a AZM como a CFX viron reducida a súa retención respecto ao sistema simple, especialmente a altas concentracións. Pola contra, a AMX amosou resultados máis variables, con indicios de efecto sinérxico nalgúns casos. A desorción de AMX aumentou en presenza dos outros antibióticos, o que suxire a súa adsorción preferente en sitios de menor enerxía.

No sistema binario AMX+CIP, a AMX tamén mellorou a súa adsorción e reduciu a súa desorción, sobre todo a concentracións baixas, indicando unha unión máis estable.

Os datos de adsorción axustáronse aos modelos de isotermas de Freundlich, Langmuir e lineal. O mellor axuste obtívose co modelo de Freundlich ($R^2 > 0,9$). En sistemas simples, os valores do parámetro KF foron similares para CFX e AZM, oscilando entre 3,234 e 478,866 $L^n\ \mu mol^{1-n}\ kg^{-1}$, e máis baixos para AMX, alcanzando un máximo de 150,34 $L^n\ \mu mol^{1-n}\ kg^{-1}$. No sistema ternario os valores de KF foron xeralmente máis baixos, especialmente para a CFX. No sistema binario AMX+CIP, KF variou entre 1,12 e 129,796 $L^n\ \mu mol^{1-n}\ kg^{-1}$. Os valores do parámetro n oscilaron entre 0,41 e 1,655 para a CFX, entre 0,306 e 1,33 para a AMX e entre 0,128 e 1,327 para a AZM. Estes valores foron similares aos observados nos sistemas ternario e binario. O modelo de Langmuir presentou erros elevados, e o modelo lineal non foi axeitado en sistemas simples, aínda que si amosou bo axuste nalgúns solos no sistema ternario.

En canto aos bioadsorbentes, no sistema sinxelo, os materiais alcalinos como a cinza e a cuncha de mexillón foron os máis efectivos para reter a CFX. A cinza logrou case un 100 % de adsorción e nula desorción, seguida da cuncha de mexillón (71,19 %–85,02 %). No caso da AMX, as follas de eucalipto, as agullas de piñeiro e a cinza superaron o 76 % de adsorción, sen desorción detectable. Para a AZM, a cinza amosou de novo a maior capacidade de retención, sendo superior ao 80% a concentracións engadidas maiores de 50 $\mu mol\ L^{-1}$.

No sistema ternario, a cinza seguiu sendo altamente efectiva (ata o 100 % de adsorción). Curiosamente, as porcentaxes foron incluso máis altas que no sistema simple, o que suxire un efecto sinérxico. Pola contra, a cortiza de piñeiro e a cuncha de mexillón reduciron a súa eficacia, posiblemente pola competencia entre antibióticos.

A incorporación de bioadsorbentes en solos mellorou a adsorción na maioría dos casos. Para a CFX, os maiores aumentos lográronse con cinza e cuncha de mexillón, chegando nalgúns casos ao 100 % de adsorción, mentras que nos solos sin emendar as

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porcentaxes de adsorción foron menores ao 80% na maioría dos casos. Para a AMX, a adición de cortiza de piñeiro incrementou a adsorción ata un 40 % en certos solos. Finalmente, en canto a AZM, calquera dos tres materiais aumentou a adsorción ao 100 % na maioría dos solos, mentres que nos solos sen emendar esta porcentaxe non superou o 80% ao engadir a máxima concentración de antibiótico.

Os principais factores que influíron na adsorción foron o pH, o contido de materia orgánica e a presenza de minerais non cristalinos, propiedades que xogaron un papel clave nos mecanismos de retención dos antibióticos estudados.

Palabras chave: Antibióticos, adsorción-desorción, bioadsorbentes, contaminantes emerxentes, solos

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1.INTRODUCCIÓN

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1. INTRODUCCIÓN

El término “antibióticos” se refiere a compuestos químicos que tienen la capacidad de inhibir el crecimiento de otros microorganismos y destruir sus células (Konstantopoulou, 2016). Aunque generalmente se refiere a los antibacterianos, los compuestos antibióticos se diferencian como antibacterianos, antifúngicos y antivirales, dependiendo del grupo de microorganismos a los que antagonizan (Brooks et al., 2004; Russell, 2004). Todos los antibióticos son selectivamente tóxicos para los organismos objetivo, es decir, bacterias, hongos, helmintos, parásitos y rickettsias, sin tener un efecto tóxico sobre el huésped (Lalchhandama, 2021; Graham, 2023).

Los antibióticos pueden producirse de forma natural a través del metabolismo secundario de microorganismos vivos o pueden ser sintetizados de manera artificial o semisintética por la industria (Kümmerer, 2009; Du and Liu, 2012; Kuppusamy et al., 2018). Hasta ahora, se han aislado al menos 4000 antibióticos de cultivos microbianos y se han desarrollado 30000 de manera semisintética, aunque solo 100 de ellos se emplean en la práctica diaria, tanto en medicina humana y veterinaria, como en agricultura y química de los alimentos (Partalidou, 2016).

Desde su descubrimiento los antibióticos contribuyeron a una fuerte reducción en las tasas de mortalidad y morbilidad. Sin embargo, su uso masivo durante los últimos años ocasionó que residuos de estos compuestos estén presentes en diferentes compartimentos medioambientales, siendo considerados actualmente contaminantes emergentes, ya que pueden ocasionar importantes riesgos para la salud humana y ecológica, tales como la proliferación de bacterias resistentes a los antibióticos, problemas de ecotoxicidad o su entrada en la cadena alimentaria, generando graves riesgos para la salud.

De hecho, la aparición de antibióticos y otros productos farmacéuticos en el medio ambiente se convirtió en una preocupación pública cada vez mayor, ya que desde principios del siglo XXI hay cada vez más estudios que revelan la presencia de una amplia gama de productos farmacéuticos persistentes en suelos y agua (Kim et al., 2007). Un ejemplo de esto es un estudio realizado en España por García-Galán et al. (2013), en el que detectaron 13 sulfonamidas diferentes en suelos y lodos de depuradora de plantas EDAR situadas a lo largo del río Ebro (Cataluña). Asimismo, Conde-Cid et al. (2018) estudiaron la presencia de antibióticos de uso veterinario en purines, suelos de Galicia (de pradera y de maíz) enmendados con purines y en los cultivos de esos suelos. Estos autores encontraron que un 42% de los purines, 17% de los suelos, 72% de las muestras vegetales de pradera y maíz y un 33% de los granos de trigo tenía algún tipo de antibiótico (hasta 5 en la misma muestra en el caso de los purines). En la misma línea, An et al. (2015) detectaron presencia de diferentes antibióticos en suelos de China en un rango de concentración de 0,29 a 1590,16 $\mu g\ kg^{-1}$, correspondiendo la concentración más alta a la clortetraciclina. También en China, Pan et al. (2019) estudiaron la presencia de 10 antibióticos en 530 suelos, obteniendo una frecuencia de detección por debajo del 0,8% para 4 sulfonamidas y 2 macrólidos, mientras que en las tetraciclinas la frecuencia era del 20,9%. Además de los purines, otra vía de entrada de los antibióticos en el suelo es a través de las aguas residuales después de su uso terapéutico en medicina humana, debido a que son pobremente absorbidos en los intestinos y una importante proporción son excretados en heces y orina (hasta un 90%) (Sarmah et al., 2006). Concretamente, a través de estas aguas residuales llegan a las Estaciones Depuradoras de Aguas Residuales (EDAR) donde los procesos utilizados habitualmente no eliminan totalmente estos compuestos y además se generan unos lodos que

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pueden acumular estos antibióticos y que son ampliamente utilizados como fertilizantes en suelos agrícolas.

Los estudios existentes hasta ahora indican que una vez que los antibióticos llegan al suelo pueden sufrir varios procesos fisicoquímicos y biológicos en el sistema suelo-agua como puede ser: lixiviación, degradación o adsorción-desorción por los componentes del suelo. Estos procesos condicionan su movilidad y su-transporte, con lo cual también la probabilidad de que alcance las aguas superficiales, subsuperficiales o subterráneas, así como su disponibilidad para las plantas y microorganismos. La adsorción-desorción es uno de los procesos más importantes que permiten explicar el destino de los antibióticos y su comportamiento en el medio ambiente. Esto indica que la interacción de los antibióticos con el suelo juega un papel importante en su presencia/ausencia en aguas y en su entrada a la cadena trófica.

1.1 HISTORIA DE LOS ANTIBIÓTICOS

El descubrimiento y desarrollo de antibióticos supusieron un importante hito en la historia de la medicina. El uso de microbios productores de antibióticos para combatir enfermedades tiene una historia milenaria. En lugares como Serbia, China, Grecia y Egipto, hace 2000 años ya se utilizaban cataplasmas de pan mohoso como tratamiento para heridas abiertas (Hutchings et al., 2019). Hacia 1859, Louis Pasteur sentó las bases de la "teoría microbiana de la enfermedad", fundamental para el desarrollo posterior de la terapéutica antibiótica, que consiguió que se evolucionara de un enfoque centrado en el órgano afectado hacia una estrategia dirigida al agente etiológico de la infección, el cual podía involucrar múltiples órganos y sistemas durante su desarrollo. En 1871, Joseph Lister descubrió los efectos inhibidores del *Penicillium glaucum* sobre el crecimiento bacteriano (Durand et al., 2019). Años después, en 1897, Ernest Duchesne descubrió la inhibición de *Escherichia coli* por *P.glaucum*, 30 años antes que Flemming. En 1909, Paul Ehrlich introdujo el fármaco a base de arsénico "Salvarsán", que actuaba contra *Treponema pallidum,* la bacteria responsable de la enfermedad de la sífilis (Kourkouta et al., 2018). Una vez que Ehrlich y sus colaboradores anunciaron los efectos clínicos del tratamiento de la sífilis a través del Salvarsan (en 1910), se produjo una enorme cantidad de solicitudes del fármaco, y la Fábrica Química Hochst comenzó a fabricar el compuesto a gran escala (Gensini et al., 2007). A pesar del tedioso procedimiento de inyección y los efectos secundarios, este fármaco, fue un gran éxito y, junto con un Neosalvarsan más soluble y menos tóxico, alcanzó el estatus del fármaco más frecuentemente prescrito hasta su reemplazo por la penicilina en la década de 1940 (Mahoney et al., 1943). En 1928 Alexander Fleming, bacteriólogo del St Mary's Hospital de Londres, descubrió, por accidente, la penicilina: Se trata de un antibiótico β-lactámico producido por el hongo *Penicillium notatum*, que tiene un efecto bactericida al inhibir la biosíntesis de la pared celular bacteriana, lo que permite combatir patógenos infecciosos como *Staphylacoccis aureus*. Un año más tarde, Flemming publicó el descubrimiento de la penicilina en el British Journal of Experimental Pathology, sin embargo, la comunidad científica recibió su trabajo con poco entusiasmo inicial, no siendo hasta 1940 cuándo dos científicos, Howard Florey y Ernst Chain, se interesaron por la penicilina (Tan y Tatsumura, 2015). Mientras, en 1932, fue descubierta la primera sulfonamida, Prontosil, por el equipo de Bayer; Gerhard Domagk, uno de los químicos de esta compañía, demostró su eficacia contra las principales infecciones bacterianas.

Debido al estallido de la Segunda Guerra Mundial, se puso en marcha el proyecto colaborativo angloamericano de la penicilina, cuyo objetivo era producir cantidades suficientes del nuevo antibiótico para las necesidades de las fuerzas aliadas que luchaban y morían a causa de infecciones en los campos de batalla de Europa, África y Asia (Nicolaou and Rigol, 2017).

Gracias al impacto trascendental de la penicilina, en 1945 Flemming, Chain y Florey ganaron el premio Nobel de Medicina y Fisiología. En la figura 1 se muestra un esquema de la evolución cronológica del descubrimiento de los antibióticos.

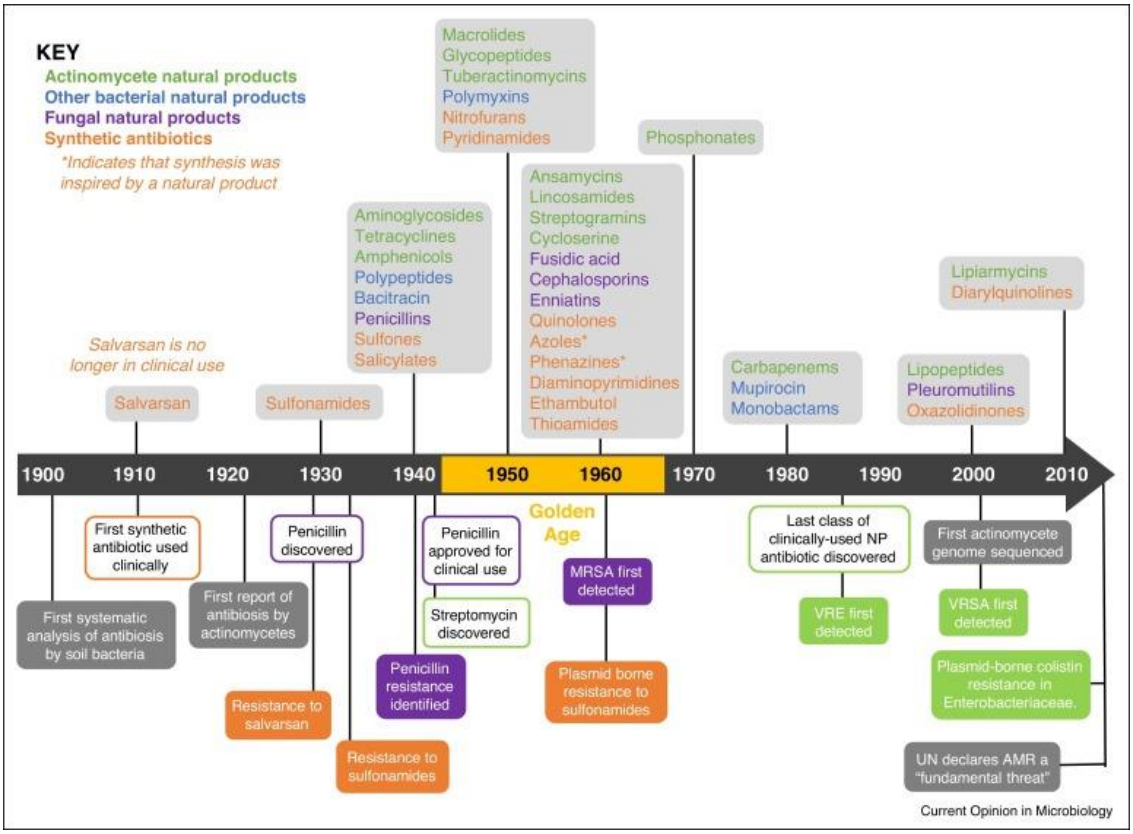

Figura 1. Evolución cronológica del descubrimiento de diferentes antibióticos (Hutchings et al., 2019)

Ocho nuevos ingredientes activos antibacterianos han sido aprobados desde 2017 y sesenta nuevos productos están en proceso de desarrollo desde 2020, incluyendo diez fármacos biológicos (OMS, 2019). El descubrimiento de estos antimicrobianos proporcionó una terapia sencilla y eficaz para las infecciones bacterianas, y desde entonces, los antibióticos han influido enormemente en la salud y la longevidad de los humanos (Dyary et al., 2022).

1.2 CLASES DE ANTIBIÓTICOS

Actualmente se utilizan cientos de antibióticos diferentes en todo el mundo, tanto en humanos como en animales. Para evaluar mejor el destino de los antibióticos en el ecosistema, es esencial conocer su correcta caracterización y una comprensión adecuada de las propiedades fisicoquímicas, ya que, estos datos son imprescindibles para determinar la posibilidad de que un compuesto se concentre en el compartimento ambiental terrestre, acuático o atmosférico (Ozumchelouei et al., 2020). Los antibióticos se pueden clasificar por su estructura química, el espectro bacteriano (estrecho y amplio), la vía de administración (tópica, oral e inyectable), modo de acción u organismo de actividad inhibidora. Sin embargo, la clasificación en base a su estructura química es la más adecuada desde el punto de vista medioambiental, ya que su comportamiento en el medio ambiente va a depender fuertemente de sus propiedades químicas y físicas, las cuales van a determinar su eficacia, toxicidad y posibles efectos secundarios (Kümmerer, 2009; Ozumchelouei et al., 2020).

Entre las clases más comunes de antibióticos, según sus estructuras moleculares o químicas, se encuentran los β-lactámicos, las tetraciclinas, los macrólidos, las quinolonas, las sulfonamidas, los aminoglucósidos, las lincosamidas y la trimetoprima (Grenni et al, 2018; Ozumchelouei et al, 2020). En la tabla 1 se muestra una clasificación de los antibióticos basada en su estructura química y su uso principal. La clase principal son los antibióticos β-lactámicos, que a su vez se pueden clasificar en penicilinas, cefalosporinas, carbapenémicos y aztreonam (Haddad et al., 2024). En esta tesis se van a utilizar dos antibióticos pertenecientes al grupo de los β-lactámicos (amoxicilina y cefuroxima) y uno del grupo de los macrólidos (azitromicina).

Los antibióticos β-lactámicos son considerados de amplio espectro y se caracterizan por contener un anillo β-lactámico en su estructura molecular (Holten, 2000). Estos compuestos son susceptibles a la hidrólisis a pH y temperatura ambiente (Mitchell et al., 2014), y son además sensibles a la degradación química, enzimática y biológica (Yidong et al., 2017). Por otro lado, los macrólidos son compuestos orgánicos que se caracterizan por la presencia de un gran anillo de lactona macrocíclica, al que pueden estar unidos uno o más desoxiazúcares, como la cladinosa y la desosamina. Estos compuestos son sustancias de naturaleza lipofílica, con carácter ácido débil y baja solubilidad en agua (Ozumchelouei et al., 2019).

Tabla 1. Principales clases de antibióticos, con ejemplos de compuestos de cada clase y su principal uso (Grenni et al, 2018; Ozumchelouei et al, 2020).

Clase	Ejemplos	Uso principal
Aminoglucósidos	Amikacina	Veterinario
	Gentamicina	Humano,Veterinario
	Neomicina	Humano,Veterinario
β-Lactámicos	Amoxicilina	Veterinario
	Cloxacilina	Veterinario
	Cefuroxima	Humano
Lincosamidas	Clindamicina	Humano
	Lincomicina	Veterinario
Macrólidos	Azitromicina	Humano
	Claritromicina	Humano
	Eritromicina	Humano, Veterinario
Quinolonas y Fluoroquinolonas	Ciprofloxacina	Humano
	Enrofloxacina	Veterinario
	Ofloxacina	Humano
Sulfonamidas	Sulfacloropiridazina	Humano, Veterinario
	Sulfadiazina	Veterinario
	Sulfametazina	Veterinario
Tetraciclinas	Clortetraciclina	Veterinario
	Oxitetraciclina	Humano, Veterinario, Plantas
	Tetraciclina	Humano, Veterinario
Trimetoprima	Trimetoprima	Humano, Veterinario

1.2.1. Amoxicilina, cefuroxima y azitromicina

La amoxicilina (AMX) es un antibiótico semisintético de amplio espectro, del grupo de las penicilinas y perteneciente a la subclase de los β-lactámicos. Este antibacteriano actúa contra un amplio espectro de bacterias, tanto Gram-positivas como Gram-negativas, por ello se emplea a menudo como fármaco de primera elección para el tratamiento de infecciones de diferente

gravedad, tanto en medicina humana como en veterinaria. Entre las infecciones que se tratan con estes fármacos están las del tracto respiratorio superior, como la faringitis estreptocócica y la bronquitis, infecciones pulmonares, de la piel, del oído y trastornos óseos (Yazidi et al., 2020). Entre el 80-90% de este antibiótico es excretado debido a su pobre absorción por los organismos vivos (Kidak y Doğan, 2018), y, debido a su escasa degradación, puede aparecer en los efluentes de las depuradoras en concentraciones de hasta 120 ng L^{-1} (Andreozzi et al., 2005). En un estudio realizado en la India, se encontró amoxicilina junto con sus productos de degradación en los efluentes de las plantas de tratamiento de aguas residuales en concentraciones de 62 ng L^{-1} (Mutiyar y Mittal, 2014).

En cuanto al comportamiento de la amoxicilina en el suelo, está muy condicionado por el pH del medio, pues este afecta a la ionización del fármaco y a la carga superficial de los coloides del suelo. En general, los antibióticos son compuestos orgánicos polares iónicos, con muchos grupos funcionales iónicos y varias constantes de disociación de ácido (pKa), pudiendo comportarse como cationes, aniones o zwitteriones en función de las condiciones ácido-base. La amoxicilina tiene propiedades anfotéricas debido a la existencia en su estructura de tres grupos funcionales: -NH_2, -COOH e -OH (Anastopoulos et al., 2020). La carga de la molécula de amoxicilina cambia gradualmente en función del pH, debido a los pKa de estos grupos funcionales. El carboxilo tiene un pKa1= 2,68, el grupo amina tiene un pKa2= 7,49 y el hidroxilo fenólico tiene un pKa3= 9,63 (Homsirikamol et al., 2016). A un pH<pKa1, la carga positiva del grupo amino de la amoxicilina determina la carga de la molécula. A valores de pH entre pKa1 y pKa2 la molécula se comporta como un zwitterion con grupos carboxilo desprotonados y grupos amino protonados; por otro lado, a pH entre pKa2 y pKa3 se desprotonan los grupos carboxilo y predominan los grupos amina. Por último, a pH>pKa3 se favorece la desprotonación de los grupos fenólicos y la molécula de la amoxicilina tiene básicamente una doble carga negativa (Pezoti et al., 2016). En la figura 2 se muestra el gráfico de la especiación de la amoxicilina en función del pH.

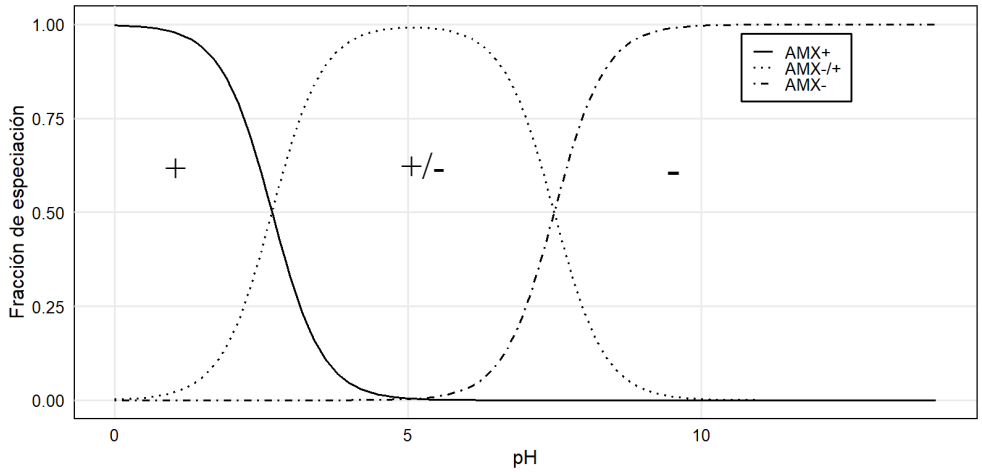

Figura 2. Especiación de la amoxicilina en función del pH

La cefuroxima (CFX) es un antibiótico del grupo de los β-lactámicos, dentro de las cefalosporinas de segunda generación, que se usa principalmente en medicina humana. Exhibe una actividad de amplio espectro contra organismos Grampositivos y Gramnegativos, lo que hace que el fármaco sea eficaz para tratar una amplia gama de infecciones bacterianas, como la faringitis estreptocócica hemolítica β del grupo A, amigdalitis o amigdalofaringitis y otitis

media aguda. En un estudio sobre la presencia de antibióticos en aguas residuales tratadas en Grecia, se encontró que la cefuroxima era la que presentaba las concentraciones más altas, en torno a 6,6 µg L^{-1} (Iatrou et al., 2014).

Este antibiótico presenta dos valores de pKa, 3,15 y 10,97 (Ribeiro y Schmidt, 2017), lo que indica que a pH superior de 10,97 está en forma aniónica, mientras que por debajo de 3,97 está como catión, y entre ambos valores de pH está como zwitterion. En la figura 3 se muestra el gráfico de la especiación de la cefuroxima en función del pH.

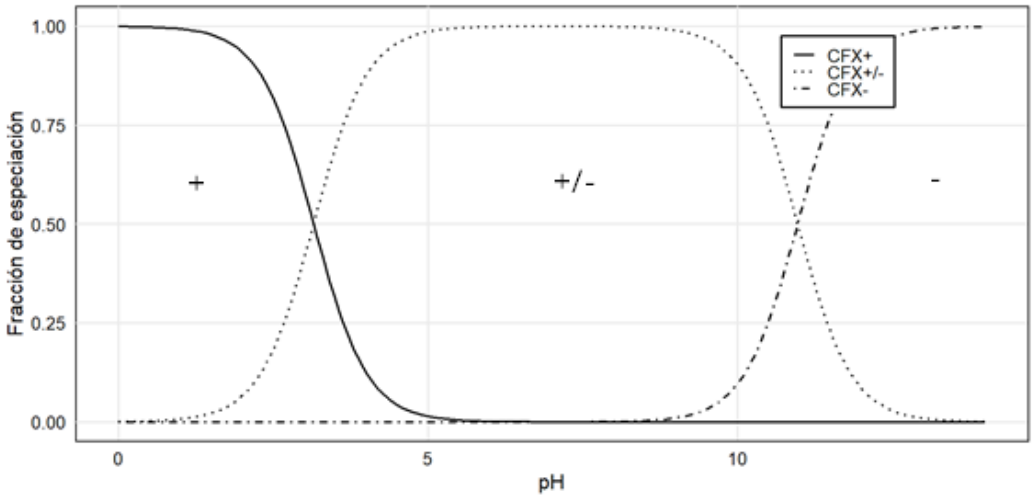

Figura 3. Especiación de la cefuroxima en función del pH

La azitromicina (AZM) es un antibiótico semisintético perteneciente al grupo de los macrólidos de segunda generación y de amplio espectro, pues actúa contra varias bacterias Gram-positivas y Gram-negativas (Vermillion Maier y Tjeerdema, 2018). Es muy utilizado en medicina veterinaria y humana para tratar infecciones de oído medio, faringitis estreptocócica, pneumonía, diarrea del viajero y algunas otras infecciones intestinales.

Aproximadamente el 75% de la azitromicina es excretada a través de heces y orina (Sidhu et al., 2019a). Debido a su baja absorción, los antibióticos tipo macrólidos (azitromicina, claritromicina y eritromicina) se detectan con mucha frecuencia en aguas residuales, con concentraciones medias de 1 µg L^{-1}, mostrando una alta persistencia (Karthikeyan y Meyer, 2006). Aunque para los macrólidos generalmente solo se cita un valor de pKa, en el caso de la azitromicina algunos autores citan dos valores, pKa1 de 8,74 y pKa2 de 9,45, por lo que en la mayoría de los suelos se comportan como un catión (Sidhu et al., 2019b). Por el contrario, otros autores solo señalan un valor de pKa de 7,25 (Babić et al., 2007) o de 8,96 (Zrnčić et al., 2015). En la figura 4 se muestra el gráfico de la especiación de la azitromicina en función del pH.

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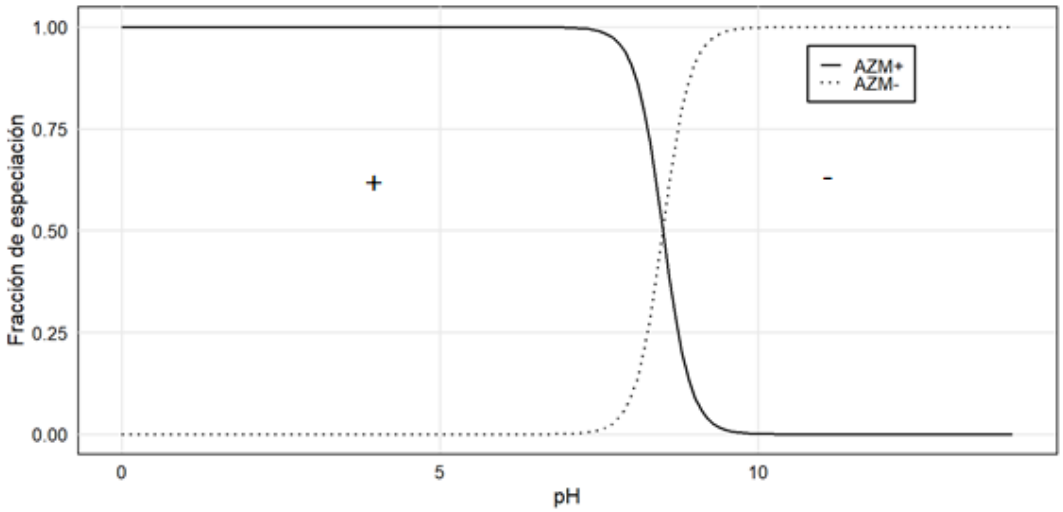
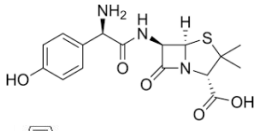
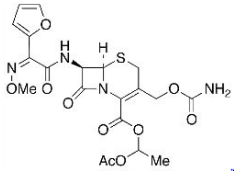
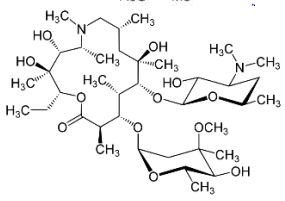


Figura 4. Especiación de azitromicina en función del pH

En la tabla 2 se muestra un resumen de las propiedades fisicoquímicas de los tres antibióticos usados en este trabajo (amoxicilina, cefuroxima y azitromicina).

Tabla 2. Propiedades químicas y físicas de la amoxicilina, cefuroxima y azitromicina. MM: masa molecular; WS: solubilidad en agua.

Antibiótico	Estructura química	pKa	Polaridad (LogKow)	MM (g mol^{-1})	WS (mg ml^{-1})
Amoxicilina		pKa1=2,68[a] pKa2=7,49 pKa3=9,63	0,87[d]	365,41	3,4 [d]
Cefuroxima		pKa1=3,15[b] pKa2=10,97	0,4[e]	424,39	0,065[g]
Azitromicina		pKa1=8,74[c] pKa2=9,45	4,02[f]	749	0,08[h]

[a] Homsirikamol et al. (2016)
[b] Ribeiro y Schmidt, (2017)
[c] Sidhu et al. (2019b)
[d] Palacio et al. (2021)
[e] Mrestani et al. (2004)
[f] McFarland et al. (1997)
[g] Arora et al. (2010)
[h] Adeli, (2017)

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1.3 CONSUMO GENERAL DE ANTIBIÓTICOS

Los antibióticos son ampliamente utilizados en el mundo para el tratamiento de enfermedades infecciosas, tanto en humanos como en animales, desde hace más de 60 años. La cantidad de antibióticos que se consume en todo el mundo no se conoce con precisión debido a que sólo unos pocos países cuentan con estadísticas nacionales del uso, cantidades y patrones de antibiótico; sin embargo, hay estudios que estiman que el consumo global de antibióticos oscila entre 100.000 y 200.000 toneladas anuales (Albero et al., 2018). A pesar de esta falta de datos exhaustivos, investigaciones como la de Klein et al. (2024) que estudiaron el consumo de antibióticos humanos entre 2016 y 2023, con datos de 67 países, evidenciaron un aumento del consumo del 16,3 %, pasando de 29,5 a 34,3 mil millones de dosis diarias definidas (DDD).

En la tabla 3 se muestra el consumo de antibióticos en humanos (sector hospitalario y comunitario combinados) entre los años 2019 y 2023. En el caso de la UE/EEE el consumo medio de antibacterianos en humanos para uso sistémico fue de 19,9 DDD por cada 1000 habitantes por día en 2023, con importantes oscilaciones entre países, desde 9,6 DDD en Holanda a 28,5 DDD en Grecia. España se sitúa en la parte alta del rango (24.3 DDD) con un cuarto puesto (tabla 3), por detrás de Rumanía, Grecia y Bulgaria. A pesar de ello, en 2023 España fue el primer país con mayor reducción de consumo de antibióticos veterinarios (69%) y el tercero en salud humana a nivel europeo (17%) (AEMPS, 2023).

Al analizar por separado el consumo de antibióticos en el ámbito hospitalario y en el comunitario (ECDC, 2023) se obtuvo que, en el sector hospitalario, el consumo medio ponderado por población de la UE/EEE de antibacterianos para uso sistémico fue de 1,6 DDD por cada 1000 habitantes por día en 2023, siendo el subgrupo de antibióticos más consumido el de las penicilinas (34%), seguidas de las cefalosporinas y otras betalactámicas (28%). A continuación, se sitúan las quinolonas (9%), macrólidos, lincosamidas y estreptograminas (8%), sulfonamidas y trimetoprima (4%), representando el resto de los grupos conjuntamente un bajo porcentaje (12%.) Cuando se analiza el sector comunitario este consumo fue de 18,3 DDD por cada 10000 habitantes por día (ECDC, 2023), siendo, al igual que en el sector hospitalario, las penicilinas el subgrupo de antibióticos más consumido (47%). Sin embargo, el patrón de consumo de los siguientes subgrupos varió respecto al sector hospitalario, ocupando el segundo lugar los macrólidos, lincosamidas y estreptograminas (17%), seguidos de las cefalosporinas y otras betalactámicas (12%), las tetraciclinas (9%), las quinolonas (7%), las sulfonamidas y trimetoprima (3%), mientras que el resto de los grupos suponen conjuntamente un porcentaje bajo (5.5%) (ECDC, 2023).

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Tabla 3. Consumo total (sectores comunitario y hospitalario combinados) de antibacterianos en humanos para uso sistémico (grupo ATC J01) en países de la UE/EEE, 2019-2023 (expresado en DDD por 1 000 habitantes por día) (ECDC, 2023)

Country	2019	2020	2021	2022	2023	Trend 2019–2023		Progress towards 2030 target*			
								Change (%) 2019-2023	Recommended reduction (%) 2019-2030	2023	Target 2030
Austria	11.6	8.8	8.8	10.5	11.3			-3%	-3%	11.3	11.2
Belgium	21.4	16.7	17.4	20.5	20.6			-3%	-18%	20.6	17.5
Bulgaria	20.7	22.7	24.4	25.7	26.3		↑	+27%	-18%	26.3	17.0
Croatia	18.8	15.7	18.2	20.2	21.2			+13%	-9%	21.2	17.1
Cyprus	30.1	28.9	25.0	33.5			N/A	N/A	-27%	N/A	22.0
Czechia	16.9	13.4	13.7	17.1	18.1			+7%	-9%	18.1	15.4
Denmark	15.3	14.3	14.4	15.2	16.2			+6%	-9%	16.2	13.9
Estonia	11.8	10.5	10.1	12.4	12.7			+8%	-3%	12.7	11.4
Finland	14.7	11.9	11.3	12.5	12.9			-12%	-9%	12.9	13.4
France	25.1	20.3	21.5	24.3	24.1			-4%	-27%	24.1	18.3
Germany					13.3		N/A	N/A	-9%	13.3	11.5
Greece[a]	34.1	28.1	23.5	32.9	28.5		N/A	-16%	-27%	28.5	24.9
Hungary	14.4	11.2	11.9	14.4	14.2			-2%	-9%	14.2	13.1
Iceland	19.3	16.5	16.8	18.6	18.5			- 4%	N/A	18.5	N/A
Ireland	22.8	18.6	17.8	23.1	22.4			-2%	-27%	22.4	16.6
Italy	21.7	18.4	17.5	21.9	23.1			+6%	-18%	23.1	17.8
Latvia	13.9	11.9	11.6	14.9	14.9			+7%	-9%	14.9	12.6
Lithuania	16.3	14.2	14.1	18.5	18.7			+15%	-9%	18.7	14.7
Luxembourg[a]	21.1	16.1	15.9	19.1	20.2		N/A	-4%	-18%	20.2	17.3
Malta	20.7	16.6	15.8	24.0	22.9			+11%	-18%	22.9	17.0
Netherlands	9.5	8.5	8.3	9.1	9.6			+1%	-3%	9.6	9.2
Norway	14.9	13.9	14.0	15.3	15.5			+4%	N/A	15.5	N/A
Poland	23.6	18.5	20.2	23.6	23.2			-2%	-27%	23.2	17.2
Portugal	19.3	15.2	15.3	18.8	19.7			+2%	-9%	19.7	17.6
Romania	25.8	25.2	25.7	27.6	27.4			+6%	-27%	27.4	18.8
Slovakia	19.3	14.4	16.0	20.8	20.1			+4%	-9%	20.1	17.6
Slovenia	13.0	10.2	10.2	12.4	13.4			+3%	-9%	13.4	11.8
Spain	24.9	19.7	20.0	23.2	24.1			-3%	-27%	24.1	18.2
Sweden	11.8	10.3	10.1	11.2			N/A	N/A	-3%	N/A	11.4
EU/EEA**	**19.8**	**16.4**	**16.4**	**19.3**	**19.9**			**+1%**	**N/A**	**19.9**	**N/A**
EU***	**19.9**	**16.4**	**16.4**	**19.4**	**20.0**			**+1%**	**-20%**	**20.0**	**15.9**

En la figura 5 se muestra la evolución del consumo de antibióticos en atención primaria en España y en Galicia desde el año 2014 hasta el 2023. Durante este período de tiempo el consumo de antimicrobianos en atención primaria en Galicia siempre fue superior a la media española, aunque ambos tuvieron una tendencia descendente hasta el año 2020, en el que se produjo una caída abrupta motivada por la pandemia. Sin embargo, en los años 2022 y 2023 el consumo volvió a casi niveles de 2019 y con un incremento más pronunciado en Galicia (SERGAS, 2024).

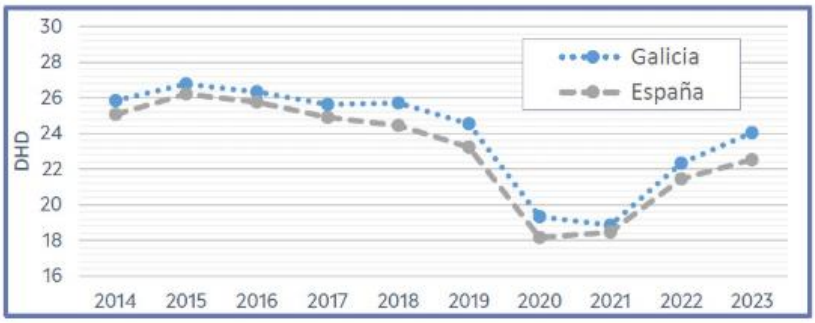

Figura 5. Evolución del consumo de antibióticos en dosis diarias definidas (DDD) desde el año 2014 hasta el 2023 en el ámbito comunitario en Galicia y España (SERGAS, 2024).

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En la figura 6 se muestra el porcentaje de consumo de antibióticos por grupos terapéuticos en atención primaria en la comunidad de Galicia, siendo más de la mitad de los antibióticos consumidos pertenecientes al grupo de las penicilinas. Dentro de este grupo, el más consumido fue la amoxicilina-clavulánico con un 29,2% de las DHD (dosis diarias definidas por cada mil habitantes y día), seguido por la amoxicilina (24,8%), cefalosporinas (13,8%), los macrólidos (12,3%), las fluoroquinolonas (8,8%) y las tetraciclinas (7,2%) (Figura 6) (SERGAS, 2024).

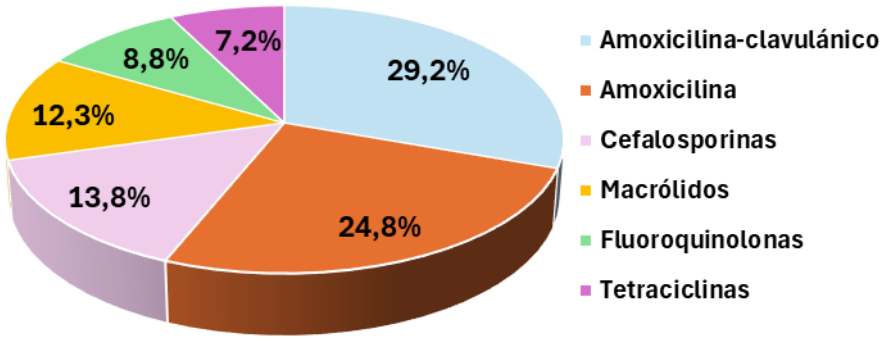

Figura 6. Porcentaje del consumo de antibióticos por grupos terapéuticos en el sector comunitario en Galicia en el año 2023 (Elaboración propia con datos del SERGAS, 2024).

1.4 PRESENCIA DE ANTIBIÓTICOS EN EL MEDIO AMBIENTE

Los antibióticos utilizados en la terapia humana y animal llegan al medio ambiente a través de la orina y las excretas, por lo que optimizar y/o controlar el uso de estos fármacos es clave para reducir la contaminación ambiental. De hecho, como consecuencia del alto consumo de estos fármacos, comentado anteriormente, en los últimos años es frecuente detectarlos en distintos compartimentos ambientales. Cada vez son más los estudios que revelan la presencia y los efectos de estos compuestos en suelos, agua y cultivos (Conde-Cid et al., 2018; Santás-Miguel et al., 2020; Beltrán et al., 2021). Así, Hernández et al. (2019) detectaron la presencia de ocho antibióticos diferentes en aguas residuales tratadas, correspondiendo las concentraciones más altas a dos quinolonas (ciprofloxacino y norfloxacino, con concentraciones de 0,89 y 0,75 µg L^{-1} respectivamente), y a tres macrólidos (azitromicina y claritromicina, con concentraciones de 0,4 µg L^{-1}, y eritromicina con concentraciones de 0,003 µg L^{-1}). Un estudio realizado en muchas muestras de aguas residuales urbanas en Alemania mostró concentraciones de CFX de hasta 6196 ng/L y 1957 ng/L en muestras de influente y efluente, respectivamente (Rossman et al., 2014). Watkinson et al. (2009) detectaron cefalexina en efluentes hospitalarios y afluentes de aguas residuales en el sudeste de Queensland (Australia), en concentraciones de 64 µg L^{-1}. En plantas de tratamiento EDAR de diferentes regiones de Lituania se detectaron tres antibióticos del grupo de los macrólidos, azitromicina, claritromicina y eritromicina, en concentraciones máximas de 593,8 ng L^{-1}, 4113,9 ng L^{-1} e 147,5 ng L^{-1}, respectivamente (Baranauskaite-Fedorova y Dvarioniene, 2023). La azitromicina también fue detectada en aguas residuales tratadas recicladas para uso agrícola (Panthi et al., 2019) en concentraciones de 213 µg kg^{-1} y en biosólidos municipales digeridos anaeróbicamente (Sabourin et al.; 2012).

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Por su parte, Borquaye et al. (2019) encontraron amoxicilina en concentraciones de hasta 76,62 ng g^{-1} en suelos. Este antibiótico es altamente móvil en suelos, por lo que podría transportarse rápidamente a aguas subterráneas y superficiales (Litskas et al., 2018). De hecho, en aguas superficiales muestreadas en China se encontró amoxicilina en concentraciones medias de 1 ng L^{-1} (Li et al., 2020). Un estudio realizado por Walters et al. (2010) en mezclas de biosólidos con suelos obtuvieron que la azitromicina tiene una vida media de 408 a 3466 días. También hay estudios que encuentran diferentes antibióticos en plantas cultivadas con concentraciones de hasta 487 μg kg^{-1} (Wu et al., 2015), mientras que Zhang et al. (2017) encontraron en plantas de *Brassica chinensis* L. tetraciclina, cefalexina y sulfametoxazol en rangos de concentración de 6,9 a11,8 μg kg^{-1}, 26,4 a 48,1 μg kg^{-1} y 18,1 a 35,3 μg kg^{-1}, respectivamente. Estos hallazgos evidencian que los antibióticos presentes en aguas residuales tratadas o enmiendas orgánicas como los biosólidos pueden pasar al suelo y ser absorbidos por las plantas, lo que representa un riesgo potencial de transferencia a la cadena alimentaria

1.4.1 Incorporación de los antibióticos al suelo

Entre el 20% y el 97% de cualquier dosis de la mayoría de los antibióticos administrados a humanos y animales se excreta como substancias activas (Jelic et al., 2015; Quaik et al., 2020). Los antibióticos de uso veterinario se incorporan al suelo a través de la utilización de purines y estiércoles como fertilizantes, o directamente a través de excrementos y orinas de los animales que están pastando, si bien esta vía es menos importante (Sarmah et al., 2006).

En el caso de los antibióticos utilizados en medicina humana, la absorción incompleta de antibióticos por parte de los humanos conlleva el vertido de grandes cantidades de estos fármacos en las plantas de tratamiento de aguas residuales municipales (EDAR) (Phoon et al., 2020), lo que, junto con su ineficacia a la hora de eliminar los antibióticos (Foroughi et al., 2022), es la principal causa de este problema de contaminación.

La eliminación de antibióticos en EDAR mediante tratamientos convencionales está limitada al 50-80%, dependiendo de las propiedades fisicoquímicas de los antibióticos (Abegglen et al., 2009; Massé et al., 2014; Moles et al., 2020). Estudios realizados en hospitales que cuentan con procesos de tratamiento de agua en sus instalaciones, demostraron que las concentraciones de ciertos antibióticos (en mg L^{-1}), incluidas penicilinas, tetraciclinas, macrólidos y fluoroquinolonas eran similares tanto en el influente como en el efluente de la planta depuradora (Pena et al., 2010; Al-Maadheed et al., 2019). Algunos autores indican que, a pesar de los avances en la tecnología de tratamiento, las plantas depuradoras más comunes siguen sin ser lo suficientemente eficaces para eliminar estos contaminantes emergentes (Ahmed et al., 2015; Wang y Wang 2016; Gogoi et al., 2018). Concretamente, en España se han realizado algunos estudios sobre la eficiencia en la eliminación de antibióticos mediante distintos sistemas de tratamiento concluyendo que la digestión anaerobia era la técnica más efectiva (Carballa et al., 2007). Los procesos aeróbicos suelen emplearse para tratar efluentes con baja carga contaminante, con una demanda química de oxígeno (DQO) biodegradable menor a 1000 mg L^{-1}, mientras que los procesos anaeróbicos se utilizan principalmente para efluentes con alta contaminación (DQO biodegradable superior a 4000 mg L^{-1}) (Chan et al., 2009). La digestión anaeróbica es un proceso biológico en el que los microorganismos anaeróbicos descomponen la materia orgánica en ausencia de oxígeno para producir biogás. Este tipo de digestión juega un papel importante en los procesos de tratamiento de aguas residuales, reduciendo la materia sólida orgánica durante el tratamiento de efluentes (Zhang et al., 2019).

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En Galicia, las técnicas de tratamiento utilizadas en las líneas de lodos de las EDAR de algunas de las principales poblaciones son diferentes entre sí (tabla 4). En algunas ciudades, los lodos simplemente se centrifugan (Viveiro), en otras se les añaden espesantes (Vilagarcía de Arousa y Verín), en algunas pasan por un proceso de tamizado, espesadores y filtros prensa (Santiago de Compostela), y en otras cuentan con procesos de digestión anaerobia (Ourense, A Coruña y Lugo).

Tabla 4: Tratamientos utilizados en las principales plantas de lodos EDAR de Galicia (fuente propia)

Vigo	Tamizado, espesamiento, hidrólisis térmica, digestión, deshidratación, secado térmico de los lodos y tratamiento de retornos para eliminación del exceso de amonio
Ourense	Espesador, digestión anaerobia y centrífugas
Vilagarcía de Arousa	Tambor de espesado y centrífuga
Verín	Espesador y centrífuga
O Barco de Valdeorras	Espesador y filtro banda
A Coruña	Digestión anaerobia, centrífuga y secado térmico
Lugo	Espesador, digestión anaerobia, centrífugas y secado térmico
Santiago de Compostela	Tamiz de lodos, espesadores y filtros prensa
Viveiro	Almacén de lodos y centrífuga
Monforte de Lemos	Espesador, digestión aerobia y filtro banda

Los antibióticos que no se eliminan en las EDAR se acumulan frecuentemente en los lodos que se generan en la actividad de estas estaciones (Wang y Wang, 2016 ; Yang et al., 2018 ; Gogoi et al., 2018). Estos lodos suelen utilizarse como enmiendas en suelos agrícolas y en zonas forestales con actividad pastoril. En Europa, más del 50% de los lodos de depuradora producidos se utilizan en agricultura (Kelessidis y Stasinakis, 2012; Malmborg y Magnér, 2015; Ezzariai et al., 2018; Buta et al., 2021). El interés por el uso de lodos de depuradora como fertilizante se debe a sus características como el elevado contenido de nutrientes, las propiedades óptimas para restaurar suelos agrícolas sobreexplotados y la capacidad de mejorar el contenido de materia orgánica y la retención de agua en suelos arenosos o erosionados (Clarke y Smith, 2011 ; Inglezakis et al., 2014 ; Seleiman et al., 2020). Esta práctica demostró ser una de las principales fuentes de antibióticos de consumo humano liberados al medio ambiente, junto con la utilización de las aguas residuales en el riego (Michael et al. 2013; Rivera-Utrilla et al. 2013), aunque en algunos lugares. La persistencia de antibióticos en estos lodos puede provocar la contaminación del suelo y otros compartimentos ambientales, como las aguas superficiales y subterráneas, por procesos de lixiviación o escorrentía, o incluso afectar a la salud humana al entrar en la cadena alimentaria a través de vegetales cultivados en suelos contaminados (Pan y Chu, 2017; Zhan et al., 2022; Gong et al., 2023). Las tasas de disipación de los antibióticos, expresadas como vidas medias, pueden variar de 18,6 a 21,3 días para las sulfonamidas y de hasta 1-2 meses para las tetraciclinas, mientras que, para la enrofloxacina, pueden ser de aproximadamente 7-8 meses (Yang et al., 2018). En algunos países es obligatorio someter a los lodos a tratamientos para eliminar contaminantes antes de su

aplicación al suelo. En España, el Real Decreto 1310/1990, de 29 de octubre indica que solo podrán ser utilizados en la actividad agraria los lodos tratados, es decir, previamente depurados por una vía biológica, química o térmica, mediante almacenamiento a largo plazo o por cualquier otro procedimiento apropiado, de manera que se reduzca de forma significativa su poder de fermentación y los inconvenientes sanitarios de su utilización. Con carácter general, los lodos se tratan en la propia depuradora, aunque en algunos casos son tratados fuera de las depuradoras en instalaciones específicas de tratamiento de residuos (MITECO, s.f.). Concretamente, en Galicia estos lodos EDAR tienen que ser tratados por gestores de residuos antes de ser incorporados al suelo, pero, como indicamos anteriormente, estos tratamientos no garantizan la completa eliminación de antibióticos.

1.4.2. Problemas ambientales asociados a los antibióticos: resistencia bacteriana

Los problemas ambientales y de salud pública relacionados con la presencia de antibióticos en suelos y aguas han sido señalados por diferentes autores (Duan et al., 2020; Lyu et al., 2020). Por ello, en los últimos años el estudio de este tipo de contaminantes emergentes ha provocado un gran interés, debido a que pueden generar problemas de toxicidad, ingresar a la cadena alimentaria a través de los cultivos o llegar a cuerpos de agua, generando riesgos para la salud pública (Kumar et al., 2019; Núñez-Delgado et al., 2019).

La aparición y propagación de infecciones causadas por bacterias resistentes a los antibióticos es una de las amenazas más graves que enfrenta la salud pública y uno de los desafíos más importantes para la medicina (Aslam et al., 2021). La resistencia a los antibióticos es un fenómeno natural en el que las bacterias desarrollan mecanismos que les permiten sobrevivir en presencia de estos compuestos. Dichos mecanismos se clasifican en dos categorías principales: resistencia fenotípica, caracterizada por modificaciones fisiológicas transitorias y no heredables, y resistencia adquirida, en la que la resistencia a los antibióticos se obtiene genéticamente (Olivares et al., 2013). Esta resistencia surge debido a la exposición de las comunidades y poblaciones bacterianas a los agentes antimicrobianos. Estas poblaciones abarcan el microbioma humano y animal, que contiene más de 500 especies de bacterias (Lykov and Volodkin, 2021). El uso excesivo e inadecuado de los antibióticos, entre otros factores, ha provocado el desarrollo y la rápida expansión de estas bacterias resistentes (Davies y Davies, 2010), pudiendo volverse ineficaces estos antimicrobianos y siendo más difíciles de tratar las infecciones, lo que aumenta el riesgo para el medio ambiente y la salud humana (Carvalho y Santos, 2016; O'Flynn et al., 2021; Zheng et al., 2021; Wu et al., 2022). Además, este problema se vio agravado con la COVID-19 que derivó en un aumento adicional del consumo de antibióticos (Race et al., 2020; Rawson et al., 2020; Núñez-Delgado et al., 2022), afectando también a los niveles generales de resistencia a los antimicrobianos (Hsu, 2020). Varios estudios han informado que concentraciones bajas de antibióticos (por debajo de las concentraciones mínimas inhibitorias) contribuyen al enriquecimiento de la resistencia (Gudda et al., 2020). También hay diversos trabajos que concluyen que la fertilización con enmiendas orgánicas incrementa las bacterias y genes resistentes a antibióticos (Pikkemaat et al., 2016; Kuppusamy et al., 2018), detectándose con frecuencia resistencia a tetraciclinas y sulfonamidas en suelos (Kuppusamy et al., 2018). Estas bacterias y genes resistentes pueden persistir en el medio y movilizarse entre distintos compartimentos medioambientales. La principal vía de transferencia de estos genes a los humanos es a través de la cadena alimentaria, constituyendo un riesgo para la salud pública debido a la aparición de infecciones que no responden a tratamientos antibióticos y pueden resultar letales (Kuppusamy et al., 2018). Ante el creciente aumento de cepas resistentes a múltiples fármacos a nivel mundial, la Organización Mundial de la Salud

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(OMS) reconoció en 2014 este fenómeno como una grave amenaza para la salud global, por lo que se implementaron medidas legislativas para limitar o eliminar el uso de antibióticos, incluso en forma de aditivos alimentarios para el ganado, pero también como agentes antibacterianos en la metafilaxis y el tratamiento, ámbito regulado por el Reglamento (UE) 2019/6 (Unión Europea, 2019).

Las estrategias actuales de gestión y control no están funcionando y se prevé que el uso de antibióticos aumente un 200% para 2030 (Klein et al., 2018). Aunque muchos países han establecido planes nacionales de vigilancia para monitorear y regular el uso de antibióticos, es fundamental una respuesta global unificada para enfrentar eficazmente el creciente problema de la resistencia a estos fármacos (Chow et al., 2021). Se estima que para el año 2050 las infecciones resistentes a los antibióticos causarán hasta 100 millones de muertes anuales en todo el mundo (O'Neill, 2016). Solo en la Unión Europea, se estima que las infecciones resistentes a los antibióticos causan más de 30.000 muertes al año, siendo Italia y Grecia los países que tienen el mayor número de casos (Cassini et al., 2019). A pesar de ello, en Europa no existe una legislación que regule la concentración de antibióticos liberados al medio ambiente desde las EDAR (Carvalho y Santos, 2016; Marutescu et al., 2023). La Directiva 2008/105/CE incluye una lista con 33 sustancias o grupos de sustancias prioritarios y sus respectivos estándares de calidad ambiental. En 2015, se añadieron tres antibióticos del grupo de los macrólidos a esta lista (azitromicina, claritromicina y eritromicina) (Barbosa et al., 2016).

Los lodos de depuradora, así como los purines y estiércoles, constituyen un reservorio clave de antibióticos, como se indicó previamente, pero también de bacterias resistentes (ARB) y genes de resistencia a los antibióticos (ARGs). Diferentes estudios evidenciaron que la aplicación prolongada de estos residuos incrementa la abundancia de (ARGs) en el suelo (Chen et al., 2016; Cheng et al., 2016; Wang et al, 2020). El compostaje y la digestión anaerobia o aerobia se han propuesto como métodos eficaces para reducir las concentraciones de estos genes en estiércol y lodos. No obstante, mientras que algunos ARGs disminuyen durante estos procesos, otros aumentan, lo que resalta la necesidad de investigar más a fondo los factores y mecanismos que influyen en sus tasas de degradación (Wang et al., 2020).

1.5 DINÁMICA Y DESTINO DE LOS ANTIBIÓTICOS EN EL SUELO

El suelo juega un papel muy importante en la sustentabilidad de los ecosistemas naturales, por lo que la conservación de este como recurso natural, evitando su degradación y contaminación, constituye actualmente uno de los grandes retos medioambientales. El suelo suele ser el principal sumidero de contaminantes, pero también puede convertirse en una fuente de emisiones cuando se supera su capacidad de retención, dando lugar a suelos contaminados (Pan et al., 2019). Entre los contaminantes están los productos farmacéuticos, productos de cuidado personal, pesticidas, productos biológicos, etc. (Cukierman et al., 2019), muchos de los cuales entran dentro de los denominados "contaminantes emergentes", como es el caso de los productos farmacéuticos.

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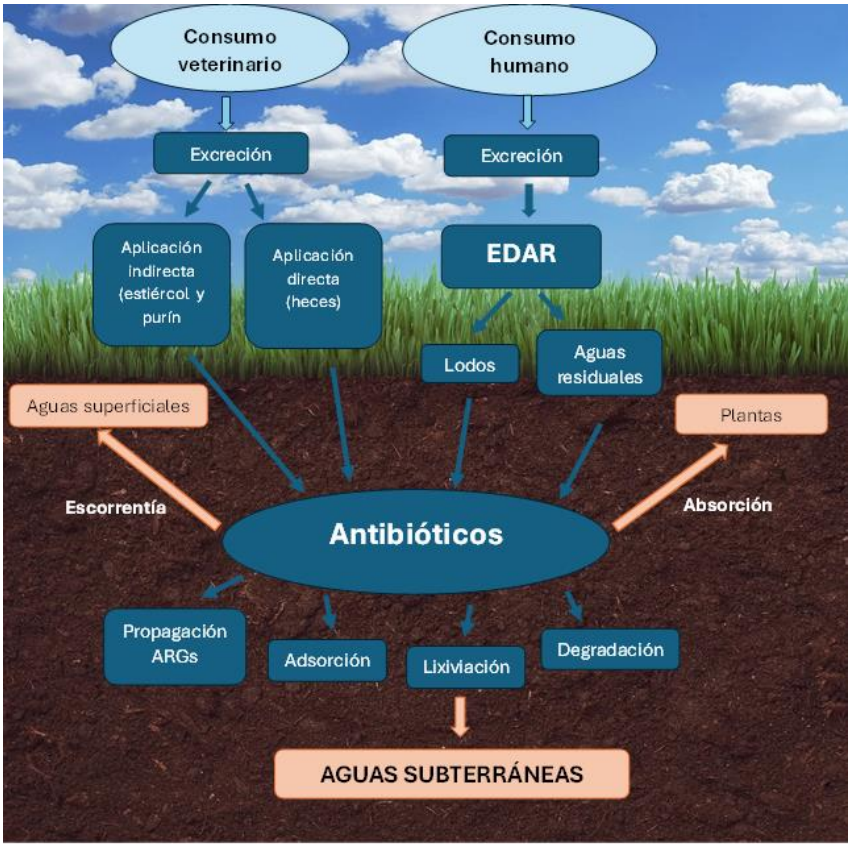

Figura 7. Principales vías de entrada y salidas de los antibióticos en el suelo (fuente propia)

En las últimas décadas se realizaron numerosos estudios en todo el mundo para conocer los niveles de diferentes tipos de antibióticos en enmiendas orgánicas, suelos y cultivos (Sarmah et al., 2006; Martínez- Carballo et al., 2007; Blackwell et al., 2007; Kemper et al., 2008; Zhao et al., 2010; Li, 2014; Conde-Cid et al., 2018), así como en aguas superficiales y subterráneas (Milić et al., 2013; Li, 2014; Carvalho y santos, 2016). Las concentraciones detectadas en los diferentes compartimentos ambientales varían dependiendo de la especie animal, de la naturaleza del antibiótico y la región de muestreo, pudiendo oscilar entre µg kg^{-1} hasta cientos de mg kg^{-1}. La presencia de antibióticos en el suelo puede provocar efectos nocivos sobre organismos edáficos, la aparición de resistencias bacterianas y contaminación de masas de agua subterráneas o superficiales, tal y como se comentó previamente. Esto podría suponer la entrada de estos antimicrobianos a la cadena alimentaria, tanto al consumir productos animales y vegetales que previamente absorbieron antibióticos presentes en los suelos, como a través del agua de bebida (si el antibiótico alcanza los recursos hídricos subterráneos). Por ello, es necesario obtener información sobre los niveles de estos fármacos, y conocer la dinámica de dichos contaminantes en el medio edáfico.

En la Fig. 7 se recogen las principales vías de entrada de los antibióticos en el suelo y los procesos que pueden seguir una vez incorporados. La dinámica de los antibióticos en el suelo depende de las propiedades del mimo (pH, contenido de materia orgánica, cantidad y tipo de arcilla, componentes no cristalinos, capacidad de intercambio catiónico), de las características del antibiótico (estructura molecular, tamaño, forma, solubilidad e hidrofobicidad) y de las condiciones climáticas (radiación solar incidente, temperatura) (Carvalho y Santos, 2016).

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Una vez que se incorporan al suelo, los antibióticos pueden sufrir dos tipos principales de procesos (Pikkemaat et al., 2016): 1) procesos de transformación o degradación, tales como hidrólisis, fotodegradación, biodegradación, oxidación y reducción; 2) procesos de transferencia, entre los que están la adsorción-desorción, lixiviación, escorrentía, difusión, volatilización y toma por las plantas. Todo este segundo grupo de procesos, sobre todo la adsorción-desorción, son los que controlarán la movilidad y el transporte de los antibióticos en el medio y su paso potencial a otros compartimentos (Pikkemaat et al., 2016).

1.5.1. Degradación

La degradación de antibióticos en el suelo consiste en la descomposición de compuestos de alto peso molecular en otros de menor peso e impacto, o en la eliminación total de los antibióticos a través de diversos mecanismos (Nkoh et al., 2024). La degradación constituye uno de los procesos más importantes en la eliminación de los antibióticos del medio ambiente y puede realizarse por mecanismos bióticos (biodegradación o degradación microbiana) y abióticos (fotodegradación, hidrólisis, oxidación y reducción). Una degradación efectiva está fuertemente influenciada por factores como las propiedades fisicoquímicas del suelo , la composición química y la estructura de los antibióticos, la naturaleza y el tipo de microorganismos y los factores ambientales (por ejemplo, pH, fuerza iónica…) (Pan y Chu, 2016; Koba et al., 2017). Debido a que hay muchos factores abióticos y bióticos diferentes que afectan a este proceso, las tasas de degradación en los suelos varían en un amplio rango, con vidas medias en el suelo entre < 1 y 3.466 días (Cycoń et al., 2019). Las fluoroquinolonas, los macrólidos y las tetraciclinas se caracterizan por una elevada persistencia, con valores altos de DT50, 1155 -3466 para ciprofloxacino y 990-1386 para norfloxacino, 408-990 para azitromicina y 578 en el caso de la tetraciclina (Walters et al. 2010), lo que indica una fuerte adsorción a los coloides del suelo (Cycoń et al., 2019). Por el contrario, la amoxicilina, según algunos autores es fácilmente degradable, con una vida media (DT50) de 0,43 a 0,57 días (Braschi et al., 2013). La persistencia de los antibióticos en el suelo se puede estimar mediante el coeficiente de sorción (Koc) (Cycoń et al., 2019), de modo que los antibióticos con valores de Koc > 4.000 l/kg son poco degradables (muy persistentes) y poco móviles, necesitando más de 60 días para la degradación del 50%, mientras que los que tienen un Koc <15 l/kg son muy móviles y se degradan fácilmente pudiendo clasificarse como compuestos de baja persistencia en suelos (DT50 < 5 días) (Crane et al., 2010). La persistencia relativamente baja de algunos antibióticos en los suelos puede estar relacionada con su baja afinidad por los componentes del suelo.

En los siguientes apartados se resumen los aspectos más relevantes de cada tipo de degradación.

1.5.1.1 Biodegradación

Este proceso es realizado por la comunidad microbiana nativa del suelo, que incluye bacterias, algas y hongos. Puede involucrar reacciones como la descarboxilación oxidativa y la hidroxilación, las cuales modifican los antibióticos, pudiendo los productos resultantes de la biodegradación ser incluso más tóxicos para los microorganismos del suelo que el antibiótico original (Pikkemaat et al., 2016). La duración de este proceso es muy variable, de pocos días a casi un año, dependiendo de las características del antibiótico, de las propiedades del suelo, de la temperatura y de la humedad (Sarmah et al., 2006; Pikkemaat et al., 2016). En un estudio realizado por Zhang et al. (2021) se obtuvieron porcentajes de biodegradación entre el 6 y el 14% para diferentes tetraciclinas, del 8-26% para varias sulfonamidas y del 8-24% para

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diferentes quinolonas, por lo que la biodegradación podría desempeñar un papel secundario en la disipación a corto plazo de los antibióticos.

1.5.1.2 Fotodegradación

Es un proceso clave de degradación abiótica impulsado por la luz solar, aunque su efecto se limita a la capa superficial del suelo debido a la baja penetración de la luz en profundidad (Thiele-Bruhn y Peters, 2007; Pikkemaat et al., 2016). Los antibióticos pueden degradarse directamente al absorber luz, un proceso conocido como fotodegradación directa (Zepp y Cline, 1977; Wammer et al., 2013; Tian et al., 2018). En contraste, la fotodegradación indirecta ocurre mediante la acción de especies oxidantes, como radicales hidroxilos, oxígeno singlete (estado excitado del oxígeno molecular generado fotoquímicamente o químicamente, que le permite reaccionar con muchas moléclas biológicas), superóxido, peróxido de hidrógeno, radicales peroxilo y materia orgánica disuelta en estado excitado triplete que producen los fotosensibilizadores bajo la luz (Li y Hu, 2016; Tian et al., 2018).

Las tasas de fotólisis directa de las fluoroquinolonas se vieron afectadas por el pH debido a que estos fármacos pueden existir en varias formas (forma catiónica, zwitteriónica o aniónica) según el pH de la solución (Wammer et al., 2013; Ge et al., 2015). Un estudio realizado por Yang et al. (2021) encontró que las vidas medias de seis fluoroquinolonas que experimentaban fotodegradación directa mediada por el sol variaban de 0,56 a 28,8 minutos en aguas superficiales (Yang et al., 2021). Sin embargo, Trovó et al. (2009) demostraron que las condiciones naturales son insuficientes para garantizar la eliminación ambiental completa, ya que estos procesos por si solos generan intermediarios persistentes y tóxicos.

1.5.1.3 Hidrólisis

Se trata de un proceso abiótico por el cual los compuestos se degradan en el medio ambiente (Biošić et al., 2017) debido a la interacción entre el compuesto orgánico y el agua (Basu, 2010), en la que ésta es capaz de romper los enlaces moleculares del compuesto. La temperatura y el valor de pH son los principales factores que contribuyen a la hidrólisis de los antibióticos (Yang et al., 2021). Los β-lactámicos (Harrower et al., 2021), los macrólidos (Tang et al., 2020) y las tetraciclinas (Zhong et al., 2022) demostraron ser susceptibles a la hidrólisis en condiciones ácidas o alcalinas a altas temperaturas, mientras que las sulfonamidas mostraron resistencia a este proceso (Pikkemat et al., 2016). La amoxicilina, uno de los antibióticos β-lactámicos, es inestable y se degrada fácilmente en sistemas acuosos debido a la hidrólisis en su anillo β-lactámico (Yang et al., 2021). Todo esto indica la variabilidad de la eficacia de este proceso en la degradación de los fármacos.

1.5.1.4 Condiciones redox

Las condiciones redox en los suelos se refieren al equilibrio entre las sustancias que pueden ganar o perder electrones. Estas condiciones son muy importantes para determinar el grado y la velocidad de biodegradación de los contaminantes, los cuales pueden sufrir transformaciones oxidativas o reductoras; entre ellos, los antibióticos son un ejemplo de compuestos que pueden ser susceptibles a este tipo de procesos. En general, para la mayoría de los contaminantes, las tasas de biodegradación aeróbica son mucho mayores que las de biodegradación anaeróbica (Maier y Gentry, 2015). Así, Yang et al. (2009) observaron que la disipación de oxitetraciclina en suelos está altamente influenciada por las condiciones redox,

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siendo más alta la degradación de este antibiótico en condiciones aeróbicas que en condiciones anóxicas. Además, las reacciones de oxidación-reducción de ciertos antibióticos pueden ser inducidas por la presencia de diferentes minerales en los suelos, como por ejemplo los óxidos de Mn para las fluoroquinolonas y la hematita para el ciprofloxacino (Chen et al., 2024).

1.5.2 Adsorción-desorción

La adsorción es el paso de un soluto de una fase acuosa a la superficie de una fase sólida, mientras que la desorción es el proceso inverso (Calvet, 1989). La capacidad de adsorción de un antibiótico sobre una en fase sólida, se expresa mediante el coeficiente de adsorción Kd, (Kd=qa/Ceq), que es la relación entre la concentración de antibiótico adsorbida a la fase sólida (qa en mg kg^{-1}) y la que permanece en la disolución de equilibrio (Ceq en mg L^{-1}). Este es uno de los procesos más importantes que permiten explicar el destino de los antibióticos y su comportamiento en el medio ambiente. Estas reacciones condicionan el transporte, pues cuanto más se adsorbe un compuesto, especialmente de manera irreversible, más limitada es su movilidad en el suelo, reduciendo la probabilidad de que alcance las aguas superficiales, subsuperficiales o subterráneas, o de que esté disponible para ser absorbido por las plantas y microorganismos. Esto significa que la interacción de los antibióticos con los suelos juega un papel clave en su presencia o ausencia en las aguas y en su entrada a la cadena trófica (Pikkemaat et al., 2016; Tasho and Cho, 2016).

La retención de antibióticos en los suelos depende de las características del suelo (pH, materia orgánica, arcillas, óxidos metálicos, etc.), así como de las características de los antibióticos (fotoestabilidad, biodegradación, solubilidad en agua, carga eléctrica, etc.) (Conde-Cid et al., 2020d). Entre los parámetros fisicoquímicos más influyentes en el destino de los antibióticos en el suelo están, la textura, el pH, las condiciones redox, y el contenido de coloides inorgánicos y orgánicos, entre otros (Sukul et al., 2008; Figueroa-Diva et al., 2010; Tamtam et al., 2011). La materia orgánica es uno de los principales componentes activos del suelo natural. Su cantidad y composición influyen significativamente en la adsorción de antibióticos en el suelo, los cuales compiten directamente por la superficie o los sitios de adsorción disponibles en los compuestos orgánicos (Wang y Wang, 2015).

Las partículas del suelo poseen una determinada carga superficial que depende parcial o significativamente de la naturaleza de las partículas y de las modificaciones en el pH (Ishiguro y Koopal, 2016). La mayoría de los antibióticos tienen tendencia a ionizarse y a mostrar multivalencia con los cambios en el pH del medio. Estos fármacos poseen diversas constantes de disociación ácida (pKa), las cuales están asociadas a diferentes grupos funcionales, y a medida que varía el pH de la solución, estos compuestos pueden adoptar valencias positivas, neutras o negativas (Wang y Wang, 2015). Autores como Graouer-Bacart et al. (2015) y Zhou et al. (2019) señalaron las fuerzas electrostáticas, las interacciones π-π, los puentes catiónicos y la complejación superficial como principales mecanismos de adsorción de antibióticos en el suelo.

1.5.2.1 Mecanismos de adsorción de los antibióticos en el suelo

Los antibióticos se pueden adsorber a la fase sólida del suelo a través de enlaces físicos y químicos, tales como las fuerzas de van der Waals, enlaces de hidrógeno, intercambio iónico, puente catiónico, intercambio de ligandos, partición hidrofóbica y transferencias de carga (Gothwal y Shashidhar, 2014). Un antibiótico puede tener varios mecanismos de adsorción en el que participan primero enlaces fuertes y progresivamente enlaces cada vez más débiles (Koskinen y Harper, 1990)

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- Fuerzas de Van der Waals: Se trata de fuerzas electrostáticas causadas por un momento dipolar fluctuante temporalmente que surge de un breve cambio de electrones orbitales a un lado de un átomo o molécula, lo que crea un cambio similar en átomos o moléculas adyacentes (Lützow et al., 2006). Los enlaces de van der Waals individuales son de corta duración, pero esta forma de enlace puede lograr una mayor permanencia si se forman muchos de estos enlaces a través de la sustancia adsorbente y la superficie, porque en un momento dado, un cierto número de enlaces de van der Waals pueden ser suficientes para evitar la desorción (Keil y Mayer, 2014). Son especialmente importantes en la adsorción de compuestos de alto peso molecular (Calvet, 1989; Koskinen y Harper, 1990).

- Enlaces de hidrógeno: Este enlace es una fuerza intermolecular que se produce entre un átomo de hidrógeno que actúa como donador y un átomo electronegativo, como nitrógeno, oxígeno o flúor, que actúa como aceptor (Centurion et al., 2023) y constituyen el principal mecanismo por el cual las moléculas polares no iónicas se adsorben a minerales arcillosos y sustancias húmicas (Calvet, 1989). El núcleo de hidrógeno electropositivo forma puentes entre dos átomos electronegativos. La energía de enlace para este tipo de uniones puede variar entre 5 y 40 KJ mol^{-1} (Li y Wu, 2015), lo que los hace algo débiles en comparación con los enlaces covalentes, sin embargo, son más fuertes que las fuerzas van der Waals a pesar de ser también interacciones dipolo-dipolo.

- Intercambio iónico: Es una reacción química en la que los iones móviles libres de un sólido, el intercambiador de iones, se intercambian por iones diferentes de carga similar en disolución (Kumar y Jain, 2013). El intercambio iónico se considera un método altamente efectivo y asequible de bajo costo para el tratamiento de aguas residuales (Zagorodni, 2006). Este proceso puede clasificarse en dos tipos principales según la naturaleza de los iones implicados: intercambio catiónico e intercambio aniónico. Por lo general, en el caso de los suelos se observa con mayor frecuencia el intercambio catiónico, frente al intercambio aniónico, ya que las superficies de las partículas del suelo, especialmente arcillas y materia orgánica, tienden a tener una carga neta negativa (Koskinen y Harper, 1990). Este mecanismo de retención implica la atracción electrostática de grupos funcionales catiónicos del antibiótico por un sitio con carga negativa en la superficie del suelo, que está ocupado por un catión metálico (Mortland, 1986). El pH del suelo es de gran importancia en este mecanismo porque determina la carga del antibiótico y la de los componentes con carga variable del suelo (Wang y Wang, 2015). En cuanto al intercambio aniónico, su importancia aumenta en suelos ácidos que tienen cargas dependientes del pH, ya que desarrollan cargas positivas a valores de pH ácido, en los grupos amina de la materia orgánica, la caolinita y minerales no cristalinos (aluminosilicatos y óxidos e hidróxidos de Fe y Al) (Koskinen y Harper, 1990). Cuanto mayor sea el valor de la capacidad de intercambio iónico mayor será el potencial para la eliminación de contaminantes del suelo (Rizwan et al., 2016; Ali et al., 2017).

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- Puente catiónico: Es un mecanismo de adsorción débil que implica la formación de un complejo de esfera interna entre un catión intercambiable y un grupo funcional aniónico o polar del antibiótico (Koskinen y Harper, 1990), formando complejos ternarios del tipo ácido húmico-catión multivalente-molécula de antibiótico o arcilla- catión multivalente-molécula de antibiótico. Esta interacción también está condicionada por el pH del medio debido a que este determina la carga tanto del antibiótico como de las superficies de las arcillas y las sustancias húmicas.

- Intercambio de ligandos: Consiste la formación de un complejo de esfera interna cuando un grupo funcional orgánico (como un hidroxilo o un carboxilato) desplaza un hidroxilo inorgánico o una molécula de agua de un ion metálico (principalmente Fe o Al) en la superficie de un mineral del suelo (Stingh et al., 2023). Este intercambio ocurre principalmente en suelos ácidos y suelos ricos en óxidos (Keil y Mayer, 2014) y forma enlaces muy fuertes que pueden persistir durante más de 100 años (Lützow et al., 2006).

- Partición hidrofóbica: Son fuerzas que mantienen la cohesión entre compuestos apolares inmersos en una solución o solvente polar, basadas en la incapacidad de las sustancias apolares de formar enlaces de hidrógeno con las moléculas de agua en una solución. Se conocen como "interacciones inespecíficas" dado que no se relacionan con la afinidad entre las moléculas de soluto, sino más bien con la tendencia de las moléculas de agua a mantener sus propias interacciones por medio de enlaces de hidrógeno. Este mecanismo es relevante en la adsorción de compuestos aromáticos sin grupos funcionales polares

- Transferencias de carga: Este mecanismo se produce entre un donante y un receptor de electrones, lo que implica una superposición parcial de los orbitales de sus respectivas moléculas para que se produzca el intercambio de electrones (interacciones π-π) (Calvet, 1989).

1.5.2.2 Propiedades de los antibióticos y de los suelos que afectan a la adsorción-desorción

Los procesos de adsorción tienen un papel decisivo en el comportamiento medioambiental y en el destino final de los antibióticos, por lo que se pueden aplicar a métodos de descontaminación. En relación con otros métodos tiene varias ventajas como la simplicidad, la confiabilidad, el bajo requerimiento de energía y la fácil recuperación del adsorbente (Choudhry et al., 2021). Este proceso en los suelos depende principalmente de las propiedades moleculares del antibiótico, las características del suelo y las propiedades de la fase líquida, especialmente el pH y la fuerza iónica (Calvet, 1989; Du and Liu, 2012; Wang and Wang, 2015; Carvalho and Santos, 2016).

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1.5.2.2.1. Propiedades moleculares del antibiótico

Los antibióticos son moléculas complejas que poseen varios grupos funcionales dentro de la misma molécula, presentan baja volatilidad, son generalmente bastante solubles en agua y son hidrófilos, teniendo un logKow bajo (Harrower et al., 2021). Las propiedades fisicoquímicas de los antibióticos varían significativamente según su clase estructural; entre ellas, las que presentan mayor influencia en los procesos de adsorción-desorción son:

Estructura electrónica: La estructura electrónica de una molécula está principalmente determinada por la naturaleza de sus grupos funcionales y átomos. Entre las propiedades moleculares fundamentales, asociadas a la estructura electrónica, se incluyen la polaridad, la polarizabilidad, la deslocalización de carga y la ionización (Calvet, 1989). La polaridad depende de la distribución de electrones, mientras que la polarizabilidad representa la facilidad con que la nube electrónica se deforma en un campo eléctrico. La descolocación de carga es consecuencia de la presencia de electrones π, y es importante en la adsorción a través de mecanismos como la transferencia de carga y enlaces de hidrógeno. Por último, la ionización determina la carga neta de la molécula. En relación con esto último, la mayoría de los antibióticos son bases o ácidos débiles y su ionización depende principalmente del pH, por lo cual, será la constante de disociación de ácidos (pKa) la que determinará la proporción que va a estar en forma aniónica, catiónica y/o zwitteriónica a un determinado valor de pH.

Coeficientes de partición: Se trata de las relaciones de concentraciones de un soluto entre dos fases inmiscibles, como ya se comentó en un apartado anterior. Desempeñan un papel importante a nivel ambiental, especialmente cuando abordan el destino, el transporte, la exposición y los efectos de concentraciones relativamente bajas de contaminantes orgánicos. Además, el coeficiente de partición se ve afectado significativamente por parámetros fisicoquímicos como la composición, el pH, el contenido de metales, de carbono orgánico y de nutrientes y la fuerza iónica (Chen y Zhou, 2014; Zhou et al., 2019; Chen et al., 2020). Concretamente, el coeficiente de partición octanol/agua (Kow), que describe la distribución de una sustancia entre una fase acuosa y una fase orgánica (n-octanol) en equilibrio, es ampliamente utilizado para estimar la adsorción de los compuestos orgánicos. Este coeficiente está altamente relacionado con el comportamiento, la movilidad y el destino en el medio ambiente, ya que es una de las aportaciones más importantes para la aplicación de modelos de transporte-difusión en suelos, sedimentos y en aguas superficiales y subsuperficiales (Mackay y Fraser, 2000).

Forma y tamaño de las moléculas: Esta puede influir en el proceso de adsorción, mediante el volumen molecular, que influye en la solubilidad en agua y, especialmente a través del área superficial, la cual afecta a algunas interacciones como pueden ser las mediadas por fuerzas de van der Waals (Calvet, 1989).

1.5.2.2.2 Características de los suelos que afectan al proceso de adsorción

Efecto del pH: El pH del suelo es un factor crucial que influye en la adsorción de los antibióticos, ya que puede alterar la carga superficial del suelo y el estado de disociación de estos fármacos, lo que a su vez afecta a los procesos de adsorción y a los mecanismos involucrados en el sistema suelo-disolución (Doretto y Rath, 2013; Jiang et al., 2020). La mayoría de los antibióticos tienen propiedades anfotéricas y presentan varios constantes de disociación ácida (pKa), por lo que, dependiendo del pH del medio, predominará una u otra especie (catiónica, zwitteriónica y/o aniónica). La adsorción de los antibióticos está fuertemente

controlada por la capacidad de numerosos compuestos para ionizarse según el pH del medio (Yeager y Halley, 1990), por ejemplo, los coeficientes octanol/agua de los compuestos ionizables varían considerablemente en un rango de pH cercano a la constante de disociación ácida (Lützhøf et al., 2000). De hecho, estudios previos han señalado que la adsorción de una gran variedad de antibióticos tiene una significativa dependencia del valor del pH, tanto en suelos (Boxall et al., 2002; Lertpaitoonpan et al., 2009; Chen et al., 2020; Conde-Cid et al., 2020c), como en arcillas aisladas (Porubcan et al., 1978; Figueroa et al., 2004; Gao y Pedersen, 2005; Wang et al., 2010), sedimentos (Wegst-Uhrich et al., 2014), óxidos e hidróxidos (Kahle y Stamm, 2007; Zhang y Huang, 2007; Hsu et al., 2018) y sustancias húmicas (Guo et al., 2016b; Liu et al., 2017).

Efecto de la MO: Tanto la cantidad como la calidad de la materia orgánica del suelo desempeñan un papel fundamental en los procesos de adsorción y desorción de diversos antibióticos. La materia orgánica, junto con los minerales arcillosos del suelo, es el principal componente del suelo que posee una capacidad de adsorción significativa a través de la formación de complejos, la adsorción por intercambio o la quelación (Lasota et al., 2020), por lo que la pérdida de materia orgánica del suelo, podría suponer un efecto negativo sobre la capacidad del suelo para retener contaminantes, entre ellos los antibióticos (Pignatello, 1998; Antilen et al., 2016; Lasota et al., 2020). Formando parte de la MO del suelo se encuentran los ácidos fúlvicos (AF), ácidos húmicos (AH) y huminas. Particularmente relevantes en la interacción entre los antibióticos y la materia orgánica del suelo son los AH, debido a sus propiedades surfactantes y su carácter multifuncional, lo que les permite establecer interacciones tanto con grupos funcionales polares como apolares de estos fármacos, favoreciendo la formación de agregados micelares (Antilen et al., 2016). En relación con lo comentado anteriormente, el pH tiene una gran influencia en estas interacciones MO-antibióticos, junto con el estado de ionización de estos últimos (Zhao et al., 2012; Chen et al., 2017)

Minerales del suelo: Las arcillas silicatadas, los aluminosilicatos y óxidos e hidróxidos no cristalinos son los minerales que más influyen en los procesos de adsorción de compuestos orgánicos, mientras que otro tipo de minerales, como los carbonatos, parecen tener un papel menos importante en este proceso (Calvet, 1989). Dentro de los distintos grupos de minerales también hay diferencias. En relación con las arcillas silicatadas, Xu et al. (2021) indicaron que los minerales 2:1 expandibles (montmorillonita) presentan una adsorción más fuerte, concretamente de enrofloxacina, que los minerales 1:1, no expandibles (caolinita) y lo atribuyeron a que, éstos últimos, poseen menor área de superficie y menor CIC. Duan et al. (2018) también compararon la adsorción de ciprofloxacino entre diferentes minerales silicatados (halloysita, turmalina y biotita), siendo la halloysita la que obtuvo los valores de adsorción más altos, atribuyéndolo también a su mayor superficie específica. Por otro lado, Hafner y Parikh, (2020) determinaron el grado en que diferentes óxidos de Fe y Mn, comunes en el suelo (birnesita, goetita, hematita) pueden unirse y transformar la monensina, obteniendo los valores más elevados de adsorción y transformación en la hematita.

Fuerza iónica: La fuerza iónica puede influir en la adsorción de antibióticos ionizados al cambiar el potencial interfacial y al competir por sitios de intercambio iónico (Ter Laak et al., 2006). La mayoría de las superficies del suelo presentan una carga neta negativa y a medida que la fuerza iónica aumenta, los cationes son atraídos electrostáticamente hacia estas

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superficies, desplazando así los compuestos orgánicos catiónicos adsorbidos (Brownawell et al., 1990) y disminuyendo la carga superficial negativa.

1.5.2.3. Descripción de los mecanismos de adsorción. Las isotermas de adsorción

La importancia del proceso de adsorción ha motivado a investigadores de diversas disciplinas a profundizar en su comprensión. Debido a ello, se han llevado a cabo numerosos estudios tanto experimentales como teóricos, entre los que destacan las isotermas de adsorción y sus ecuaciones asociadas por su capacidad para describir de forma detallada y completa los mecanismos de adsorción (Majd et al., 2022). En general, una isoterma de adsorción es una curva invaluable que describe el fenómeno que rige la retención (o liberación) o movilidad de una sustancia desde los medios porosos acuosos o entornos acuáticos a una fase sólida a una temperatura y pH constantes (Allen et al., 2004; Limousin et al., 2007). La información más importante para comprender correctamente el proceso de adsorción es la relacionada con el equilibrio de adsorción, el cual se alcanza cuando el adsorbato ha estado en contacto con el adsorbente durante suficiente tiempo, de modo que la cantidad que queda en la solución se mantiene constante porque está en equilibrio dinámico con la cantidad adsorbida en la superficie (Ghiaci et al., 2004; Kumar y Sivanesan, 2007).

En general, los modelos basados en regresión se reconocen como las herramientas más prácticas para estimar los parámetros de adsorción de antibióticos, ya que están programados explícitamente y requieren solo datos de entrada fácilmente disponibles (Jinshenghu et al., 2022).

Los modelos más utilizados en la literatura para describir las curvas de adsorción de antibióticos en suelos son el modelo Lineal, de Freundlich y el de Langmuir.

Modelo lineal

El modelo lineal consiste en una regresión lineal simple que representa una relación lineal entre el adsorbato adsorbido y la cantidad total de adsorbato presente en la disolución (Chen et al., 2022). Este modelo de isoterma describe un ajuste apropiado para la adsorción del adsorbato a concentraciones relativamente bajas, de modo que todas las moléculas de adsorbato están aisladas de sus vecinas más cercanas (Ruthven, 1984).

Modelo de Freundlich

La isoterma de Freundlich es aplicable al proceso de adsorción que ocurre en superficies heterogéneas (Ayawei et al., 2015a). Esta proporciona una expresión que define la heterogeneidad de la superficie y la distribución exponencial de los sitios activos y sus energías (Ayawei et al., 2015b).

Modelo de Langmuir

La isoterma de Langmuir es un modelo empírico que asume que la adsorción ocurre únicamente en un número limitado de sitios específicos y que la capa adsorbida es monocapa, es decir, tiene un espesor de una sola molécula (Langmuir, 1916).

Los tres modelos de adsorción se explican más detalladamente en el apartado de Material y Métodos.

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1.6 ESTRATEGIAS DE MITIGACIÓN DE LA CONTAMINACIÓN POR ANTIBIÓTICOS

Ante el problema global de la contaminación por antimicrobianos y su amenaza para la salud, es fundamental conocer la eficacia de las estrategias de eliminación existentes, así como estudiar su degradación en el medio ambiente. Dado que los procesos de tratamiento convencionales no pueden eliminar completamente los antibióticos, tecnologías avanzadas como la electrocoagulación (Tarpani y Azapagic, 2018), la filtración por membranas (Liao et al., 2020), el proceso oxidativo avanzado (Mehrjouei et al., 2014) o la adsorción (Han et al., 2019 ; Guillossou et al., 2019) han recibido gran atención y se han propuesto para el tratamiento de efluentes que contienen antibióticos (Tarpani y Azapagic, 2018; Ashiq et al., 2019; Chauhan et al., 2020). Si bien, procesos como la oxidación avanzada pueden descomponer las moléculas de antibióticos en compuestos más simples o incluso mineralizarlos por completo, su alto costo limita su aplicación (Mehrjouei et al., 2014). Sin embargo, ciertas tecnologías fisicoquímicas están demostrando ser una opción de tratamiento muy adecuado para contaminantes orgánicos (Wang et al., 2007; Homem y Santos, 2011). La adsorción destaca como una de las más prometedoras debido a su alta eficacia, sencillez de diseño y de operación, menor costo respecto al medio ambiente y la ausencia de subproductos altamente tóxicos (Imanipoor et al., 2021). Su eficiencia se ve muy afectada por el tipo de adsorbente, las propiedades del adsorbato y las composiciones de la corriente de desechos (Aksu y Tunç, 2005). La capacidad de los materiales adsorbentes está fuertemente influenciada por sus propias propiedades, incluyendo la interacción de cargas (donde las cargas opuestas favorecen la adsorción y cargas iguales la dificultan), así como por su área superficial, porosidad y diámetro de poro (Yu et al., 2016). Además de la capacidad de adsorción y de las propiedades fisicoquímicas de los materiales, factores como el costo del adsorbente, la facilidad de síntesis, la toxicidad y la reutilización también son factores clave para evaluar la idoneidad de un material adsorbente frente a otros (Eniola et al., 2019). Por ejemplo, se han propuesto adsorbentes como carbón activado, óxido de grafeno, zeolitas, hidrogeles o estructuras metalorgánicas (Yao et al., 2020; Chen et al., 2019; Premarathna et al., 2019; Cao et al., 2018; de Sousa et al., 2018; Wang et al., 2016), pero su alto costo o la inviabilidad del reciclaje (Hacıosmanoğlu et al., 2022), así como la necesidad de pretratamientos antes de su aplicación, entre otros, son algunos de los inconvenientes para su aplicación práctica. Entre los adsorbentes citados, los materiales carbonosos indicados anteriormente han sido ampliamente estudiados para abordar la contaminación ambiental, gracias a su alta área superficial, estructura, porosidad y fuertes interacciones (Yu et al., 2016); sin embargo, como ya se comentó, su alto costo de operación y regeneración representan desventajas (Zazouli et al., 2013), generando una demanda creciente de adsorbentes más económicos que no requieran pretratamientos costosos adicionales (Han et al., 2008). Así, por ejemplo, Putra et al. (2009) utilizaron bentonita para la retención de AMX, logrando un 88% de eficiencia en la eliminación de este antibiótico. En este contexto, los bioadsorbentes obtenidos de diversas fuentes, incluidos muchos materiales residuales que requieren reciclaje, representan una opción viable para la retención y eliminación de antibióticos contaminantes en distintos compartimentos ambientales (Núñez-Delgado et al., 2015a). Estudios previos han evaluado múltiples materias primas como bioadsorbentes de contaminantes, entre ellos subproductos de la viticultura (Rodríguez-Salgado et al., 2014), residuos y subproductos forestales (Gong et al., 2005; Abdel-Ghani et al., 2008; Gundogdu et al., 2009; Gichangi et al., 2012; Seco-Reigosa et al., 2013; Cutillas-Barreiro et al., 2014; Fernández-Calviño et al., 2017; Romar-Gasalla et al., 2018; Conde-Cid et al., 2019, 2021), residuos y subproductos de la industria alimentaria (Amarasinghe y Williams, 2007; Shaheen et al., 2013; Pandey et al., 2014; Fernández-Calviño et al., 2016; Romar-Gasalla et al., 2018; Banerjee et al., 2019; Conde-Cid et al., 2019, 2021) y

rocas de desecho de la minería (Sadeghalvad et al., 2016, 2017, 2018). Entre estos residuos y subproductos, en Galicia destacan la corteza de pino, la ceniza de roble y la concha de mejillón debido a su versatilidad y alta disponibilidad, lo que permite su uso en la biorremediación a gran escala de suelos y aguas. Además, el uso de estos materiales podría mejorar las propiedades fisicoquímicas y biológicas del suelo. En concreto, en estudios previos se demostró que la aplicación de concha de mejillón en los suelos mejora significativamente el pH del suelo y conduce a una disminución en el contenido de aluminio (Paz-Ferreiro et al., 2012), y que la aplicación de corteza de pino y concha de mejillón incrementa significativamente el crecimiento bacteriano, así como el fúngico en el caso de la corteza de pino (Fernández-Calviño et al., 2018).

A continuación, se describen las características y la disponibilidad de estos tres potenciales bioadsorbentes.

1.6.1 Corteza de pino

En España, en 2022 se cortaron 20 millones m^3 de madera con corteza, con Galicia aportando aproximadamente la mitad de la producción, alcanzando los 10.995.206 m^3 (MITECO, 2022). Las coníferas representaron el 58% del total, siendo *Pinus radiata*, *Pinus pinaster* y *Pinus sylvestris* las especies mayoritarias (MITECO, 2022). En el año 2023, en Galicia se llevaron a cabo aprovechamientos de madera de pino con un volumen total de 9,8 millones de metros cúbicos (Xunta de Galicia, 2024). En cuanto a las estadísticas de producción de corteza, estas son escasas y generalmente se estiman de manera indirecta a partir de la producción total de madera. Entre las especies forestales, el pino es la conífera con mayor capacidad de producir corteza, representando entre un 10% y un 22% del volumen total del tronco, según su tamaño y especie (FAO, 2002). La corteza de pino se convierte en un subproducto del procesamiento de la madera cuya eliminación representa un desafío debido al gran volumen generado. Por ello, se han desarrollado diversas investigaciones para explorar alternativas de reutilización, como puede ser su uso como adsorbente de contaminantes inorgánicos y orgánicos, gracias a su alto contenido de lignina, celulosa y hemicelulosa que le confieren un gran potencial para ello. Investigaciones anteriores estudiaron la corteza de pino como adsorbente de metales pesados (Argun et al., 2009; Gonçalves-Junior et al., 2012; Cutillas-Barreiro et al., 2014), pesticidas (Ratola et al., 2003a, b; Silva et al., 2004; Brás et al., 2005), colorantes (Leitch et al., 2006; Ahmad, 2009; Litefti et al., 2019), fenoles (Brás et al., 2004; Kumar et al., 2018) y, en menor medida, productos farmacéuticos (Braga et al., 2011; Silva et al., 2020, Conde-Cid et al., 2020a,b) obteniendo en la mayoría de los casos resultados satisfactorios.

1.6.2 Ceniza de roble

La biomasa lignocelulósica, como la madera, es un combustible abundante, renovable y neutro en carbono que se emplea comúnmente para la generación de energía térmica en calderas. Sin embargo, los subproductos de su combustión, como la ceniza, pueden representar un riesgo ambiental y requieren un tratamiento y reciclaje adecuados (Wang et al., 2017). La ceniza de madera es un material barato y ampliamente disponible. Aunque no existen datos referentes a España, se estima que en Galicia se producen anualmente entre 40.000 y 50.000 toneladas de ceniza (Someshwar, 1996; Núñez-Delgado et al., 2011; Pousada-Ferradás et al., 2011). No obstante, existen algunas posibles alternativas de reciclaje para este residuo como su uso como fertilizante para suelos forestales, devolviendo así parte de los nutrientes extraídos por los árboles (Merino et al., 2017). Además, el uso de este material, debido a su propiedad hidrófila, puede aumentar la capacidad de retención de agua, el contenido de humedad y la disponibilidad de nutrientes (Scheepers et al., 2016), así como su uso como adsorbente de bajo

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coste para la eliminación de contaminantes tanto en fase gaseosa como líquida (Lee et al., 2023). Investigaciones anteriores han demostrado que la ceniza de madera puede eliminar metales tóxicos como mercurio (Hg), arsénico (As), cadmio (Cd), plomo (Pb), níquel (Ni) y zinc (Zn) de varias muestras de agua (Wang y Wu, 2006; Núñez-Delgado et al., 2015b; Rivas-Pérez et al., 2016; Quintáns-Fondo et al., 2017; Calugaru et al., 2017; Pelalak et al., 2021). Las propiedades de la ceniza que influyen en su capacidad de adsorción son el área de superficie específica, la distribución del tamaño de partícula, el tamaño y el volumen de poro (Kilpimaa et al., 2013).

1.6.3 Acícula de pino

Las acículas de pino secas caídas constituyen un residuo forestal de biomasa lignocelulósica abundantemente disponible (Singh et al., 2017). Debido a su alto contenido en lignina, los residuos de pino se descomponen lentamente formando una cubierta que no permite el sotobosque y obstaculiza la capacidad de absorción de agua del suelo, alterando el nivel freático, al inhibir la recarga de agua subterránea por bloqueo de los poros del suelo, además de causar la acidificación del suelo (Stegelmeier et al., 1996; Tzamtzis et al., 2006; Bueno y Baruch, 2011; Dwivedi et al., 2016; Varma y Mondal, 2017). Por otra parte, esta acumulación de capas de acículas aumenta el riesgo de incendios que a su vez causan contaminación del aire (Dwivedi y Singh, 2016; Allen, 2019). Debido a su impacto en el medio ambiente, sería interesante buscarles usos alternativos, como podría ser su uso como adsorbentes de contaminantes edáficos. Un ejemplo es un estudio previo que analizó la capacidad de acículas de pino para adsorber cobre, obteniendo adsorciones de hasta 9,7 mg g^{-1} (Tyagi et al., 2022).

1.6.4 Hoja de eucalipto

Las hojas de eucalipto fueron utilizadas en numerosos estudios como adsorbente de distintos contaminantes. Nia et al. (2017) estudiaron la aplicación de hojas de eucalipto como adsorbente, en la eliminación de tinte de violeta de metilo de una solución acuosa, mientras que Khavidaki y Aghaie, (2012) utilizaron polvo de hojas de eucalipto como sorbente para eliminar iones de talio de soluciones acuosas. En un trabajo realizado por Alimohammadi et al. (2017) se estudió la capacidad de adsorción de mercurio y arsénico de las hojas de eucalipto. Dado que los eucaliptos son árboles cuya presencia en Galicia es muy amplia, especialmente en los últimos decenios, sus hojas serían adsorbentes de muy bajo coste.

1.6.5 Concha de mejillón

Galicia fue durante muchos años la primera productora de mejillón a nivel mundial. Posteriormente ocupó el segundo lugar detrás de China (Caballero-Míguez et al., 2009) y, actualmente, ocupa el tercer puesto, tras Dinamarca y China. Según datos de la Xunta de Galicia en 2022 se produjeron 220.000 toneladas de mejillón y su procesado en la industria conservera genera unas 70000 toneladas de concha. Galicia es la principal región productora de conservas de España, 8 de las 10 principales empresas conserveras tienen sus plantas en Galicia (Fernández-González et al., 2021). El atún en conserva es el producto principal, que supuso el 71,3% del volumen total en 2019, seguido por las conservas de sardinas (6,9%) y mejillones (4,2%) (Amigo-Dobaño et al., 2011). La concha de mejillón producida acaba principalmente

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en vertederos o en el fondo del mar, teniendo un impacto ambiental significativo. Ante la gran cantidad de conchas generadas, en las últimas décadas algunas fábricas han adoptado tratamientos industriales para convertirlas en un producto final de valor. Osakwe y Maduako (2004) utilizaron conchas de mejillón en polvo para la eliminación de colorantes y para la recuperación de aceites lubricantes, mientras que estudios más recientes utilizaron conchas de mejillón desechadas para enmendar suelos ácidos y en la restauración de minas, aditivos para alimentos animales y aditivos en materiales de construcción (Iglesias-Teixeira et al., 1997; Asensio-Fandiño et al., 2008; Peña-Rodríguez et al., 2010; Álvarez et al., 2012;). Además, más recientemente la concha de mejillón también se utilizó como adsorbente de fósforo, metales pesados y antibióticos, obteniendo en algunos casos buenos resultados (Paradelo et al., 2016; Conde-Cid et al., 2020 a,d).

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2. JUSTIFICACIÓN Y OBJETIVOS

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2. JUSTIFICACIÓN Y OBJETIVOS

Cada año se usan millones de kilogramos de antibióticos a nivel mundial para prevenir o tratar enfermedades tanto en animales como en humanos. Una elevada proporción de la dosis suministrada se excreta a través de la orina y las heces, terminando en las aguas residuales, en las fosas de purines o acumulándose en los lodos de EDAR, pudiendo entrar en el suelo a través del uso de estos residuos como fertilizantes. De hecho, ya se han detectado antibióticos en suelos agrícolas, ríos y fuentes de agua subterránea y superficial (Kay et al., 2005; Campagnolo et al., 2002; Batt et al., 2006; Conde-Cid et al., 2018).

La presencia de antibióticos en estos compartimentos ambientales se está convirtiendo en una preocupación mundial, siendo uno de los principales problemas, el aumento de cepas bacterianas resistentes, que pueden infectar a los humanos y ser extremadamente difíciles de combatir (Fisher y Scott, 2008; Organización Mundial de la Salud, 2015). Una vez introducidos en el suelo, estos contaminantes pueden sufrir degradación, lixiviación, absorción por la biota o procesos de adsorción/desorción por los diferentes compuestos orgánicos e inorgánicos del suelo, lo que afectaría su movilidad y transporte. Por ello, el conocimiento de estos procesos es de gran importancia para evitar la contaminación de las aguas superficiales y subterráneas, así como del medio ambiente en general. Concretamente, el proceso de adsorción es uno de los más importantes que permiten explicar el destino de los antibióticos y su comportamiento en el medio ambiente. Además, una medida que podría ayudar a minimizar el efecto indeseable de los antibióticos en el medio puede ser la retención de estos utilizando materiales bioadsorbentes. Uno de los adsorbentes más utilizado es el carbono activado (Liu et al., 2011), pero su alto coste y la dificultad de regeneración suponen dos grandes desventajas. En este sentido, podrían emplearse residuos o subproductos procedentes de la industria forestal y conservera, ambos sectores con una destacada presencia en Galicia. Ejemplos de estos materiales incluyen la corteza y acículas de pino, la ceniza de biomasa, las hojas de eucalipto y las conchas de mejillón, todos ellos bioadsorbentes de bajo coste y fácil disponibilidad.

El objetivo general de esta tesis es estudiar la capacidad de adsorción y desorción de tres antibióticos ampliamente utilizados en medicina humana, dos pertenecientes al grupo de los β-lactámicos, amoxicilina y cefuroxima, y uno del grupo de los macrólidos, la azitromicina por parte de diferentes suelos de Galicia. Además, también se estudiará la potencialidad que tienen varios residuos y subproductos, procedentes de las industrias forestal y alimentaria, en la retención de estos antibióticos.

Como objetivos específicos del proyecto de Tesis, podemos destacar los siguientes:

- Determinar el contenido de varios antibióticos de consumo humano en lodos EDAR de diferentes depuradoras de Galicia, tanto en los lodos sin tratar como los tratados por gestores de residuos. De igual forma, cuantificar la presencia de estos antibióticos en suelos que fueron enmendados con estos lodos, así como en la vegetación que crece en ellos. (Publicación I)

- Estudiar el proceso de adsorción y desorción de amoxicilina (AMX), cefuroxima (CFX) y azitromicina (AZM) en suelos, de manera individual (un único antibiótico presente en el sistema) y también en sistema competitivo (los tres antibióticos presentes

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simultáneamente en el sistema). (Publicación II, Publicación V, Publicación VIII y Publicación XI)

- Estudiar el proceso de adsorción y desorción de amoxicilina (AMX), cefuroxima (CFX) y azitromicina (AZM), en sistema simple y competitivo, por parte de materiales bioadsorbentes (concha de mejillón, ceniza de combustión de biomasa, corteza de pino, acícula de pino y hoja de eucalipto). (Publicación III, Publicación VI, Publicación IX y Publicación XI)

- Estudiar el proceso de adsorción y desorción de amoxicilina (AMX), cefuroxima (CFX) y azitromicina (AZM) de manera individual en mezclas de suelos con cada uno de los tres materiales bioadsorbentes que resultaron más eficientes en la adsorción los antibióticos estudiados (concha de mejillón, corteza de pino y ceniza de combustión de biomasa). (Publicación IV, Publicación VII y Publicación IX)

- Estudiar la competencia de adsorción y desorción de la amoxicilina (AMX) y el ciprofloxacino (CIP) en un sistema binario, tanto en suelos como en varios bioadsorbentes (ceniza de combustión de biomasa, corteza de pino y concha de mejillón. (Publicación X).

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3. MATERIAL Y MÉTODOS

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3. MATERIAL Y MÉTODOS

3.1 LODOS DE DEPURADORA Y LODOS TRATADOS POR GESTORES DE RESIDUOS

En una primera parte del trabajo se recogieron muestras de distinta naturaleza -lodos de estaciones depuradoras de aguas residuales (EDAR), lodos tratados por gestores de residuos, suelos que reciben estos lodos y cultivos-para analizar en ellas un amplio abanico de los antibióticos de consumo humano.

Se tomaron lodos de 30 estaciones depuradoras de aguas residuales de Galicia en primavera y otoño de 2019. Se seleccionaron EDAR de ciudades entre 65.000-248.000 habitantes (A Coruña, Ferrol, Pontevedra, Santiago de Compostela), de otras con una población próxima a los 17.000 habitantes (A Estrada, Boiro, Ribeira, Teo, Tui, Sanxenxo, Vilagarcía), y de pueblos más pequeños (A Guarda, Ares, Betanzos, Cedeira, Curtis, Miño, Ordes, Ribadeo, Ribadumia). En la segunda campaña de muestreo (otoño), se tomaron además muestras de EDAR adicionales de Cambados, Cangas, Moaña, Sada, Lugo, y 3 EDAR industriales más pertenecientes a la industria agroalimentaria, avícola y conservas de pescado. Todas las EDAR urbanas utilizan tanto tratamientos primarios (desarenado, tamizado, sedimentación) como secundarios (eliminación de materia orgánica), mientras que los tratamientos terciarios se aplican de forma diferente en cada EDAR (solo se aplica desinfección UV en todas ellas).

La legislación de la Comunidad Autónoma de Galicia exige que los lodos sean tratados antes de ser aptos para su uso como enmienda en suelos agrícolas. Los lodos de estas depuradoras fueron tratados por cuatro gestores de residuos que colaboraron en este trabajo, generado compost o mezclas compostadas al mezclarlos con diversos subproductos animales y vegetales. Por motivos de protección de datos, no es posible revelar los nombres de los gestores de residuos, de modo que serán identificados como gestor 1, gestor 2, gestor 3 y gestor 4.

El gestor 1 trata los lodos EDAR de: Betanzos, Ribeira, Tui, Ares, Cedeira, A Guarda, Lugo, Moaña, Sada y Cangas. Este gestor elabora dos tipos de mezclas: una que contiene estiércol de pollo, astillas y cal y otra que contiene lodos de cárnicas, concha de mejillón y cal.

El gestor 2 trata los lodos de las depuradoras EDAR de Miño, Ordes y Ribadeo. El tratamiento consiste en mezclar los lodos de las distintas EDAR con concha de mejillón, astillas, leche y restos de estómagos de animales (SANDACH). Pasadas 8 semanas desde que se realiza la mezcla, se obtiene un fertilizante que está registrado.

El gestor 3 solo trata los lodos de la depuradora de Ferrol, mezclándolos con lodos de conserveras y de industrias lácteas, restos de alimentos y astillas, dejando compostar esta mezcla hasta obtener un producto con buenas características organolépticas.

Por último, el gestor 4 trata lodos de depuradora de A Estrada, Santiago de Compostela, Sanxenxo, Vilagarcía, Boiro, dos de Teo y una depuradora Industrial.

Tanto los lodos de las estaciones depuradoras de aguas residuales (EDAR) como posteriormente las mezclas tratadas fueron muestreadas en las instalaciones de los gestores de residuos. Una vez en el laboratorio fueron liofilizados y almacenados a -4°C hasta su análisis.

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3.2 MUESTREO DE SUELOS Y VEGETACIÓN Y DESCRIPCIÓN DE LAS ÁREAS DE ESTUDIO

Para llevar a cabo el presente estudio se recogieron 59 muestras de suelos de cultivo que recibieron los lodos tratados por los gestores de residuos, tomando también muestras control (sin enmendar) de suelos de cultivo y forestales. En este estudio se seleccionaron fincas de maíz y de viñedo donde los gestores (1, 2 y 4) aplicaron los lodos, situadas en diferentes zonas de Galicia. Se recogieron 27 suelos de maíz: 10 en el municipio de Castro de Rei, que recibieron lodos tratados por el gestor 1; 5 fueron recogidos en Cospeito y recibieron lodos del gestor 2; en Arzúa se tomaron 12 suelos enmendados con lodos del gestor 4. También se muestrearon 33 suelos de viñedo en diferentes zonas de Galicia (Barbanza, Cambados, Valdeorras, Ribadavia), todos enmendados con lodos del gestor 4. En estas mismas fincas también se recogieron muestras de maíz (raíz y parte aérea) y muestras de viñedo (uvas). En todos los casos, los suelos tienen un historial de adición de lodos de depuradora tratados de al menos tres años.

En cada parcela se recogieron 10 sub-muestras en zigzag de la capa superficial del suelo (0-20cm), las cuales fueron posteriormente mezcladas para formar una muestra compuesta de cada una de las parcelas. Seguidamente, las muestras fueron transportadas al laboratorio, se secaron en una estufa a 40 °C hasta alcanzar peso constante. A continuación, se tamizaron con un tamiz de 2 mm de diámetro de luz y se almacenaron hasta su análisis.

En estas mismas parcelas se recogieron muestras de vegetación. En cada parcela de maíz, se tomaron muestras de tres plantas seleccionadas al azar, separando raíz, tallo, hoja y grano; de cada una de estas partes se elaboró una muestra compuesta con las tres plantas. En el caso de las parcelas de viñedo, también se recogieron tres plantas por parcela y varios racimos de cada planta para formar una muestra compuesta por parcela

De los 59 suelos muestreados, se seleccionaron 20 para llevar a cabo los ensayos de adsorción/desorción. Concretamente, se recogieron 18 muestras de suelo de cultivo previamente enmendadas con lodos de depuradoras tratados por gestores, de las cuales, 6 fueron bajo cultivo de maíz, (1 en Castro de Rei, Lugo, y 5 en Arzúa, A Coruña) y 12 bajo viñedos en las provincias de Ourense (3 en Valdeorras, 2 en O Ribeiro) y de Pontevedra (2 en Cambados, 3 en la zona das Rías Baixas y 2 en la zona del Barbanza). Además, se recogieron dos muestras de suelo, uno bajo viñedo y otro bajo maíz, en parcelas que no fueron previamente enmendadas con lodos (muestras control). Por otro lado, también se tomaron muestras de tres suelos forestales, bajo *Eucalyptus nitens*, *Quercus robur* y *Pinus radiata* en el ayuntamiento de Friol (Lugo). En la figura 8 se muestra la localización de las distintas zonas donde se recogieron los suelos utilizados en este trabajo.

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Figura 8. Localización de las zonas donde se recogieron las muestras de suelo utilizadas en los ensayos de adsorción/desorción.

Castro de Rei es un municipio de la provincia de Lugo, perteneciente a la comarca de la Terra Chá. Su altitud media se sitúa entre los 400 y 500 metros, aunque en la zona oriental, la orografía se vuelve más accidentada, destacando los Montes dos Millares y Pedrasalvas con 620 y 619 metros respectivamente. La temperatura media es de 12 °C y la precipitación anual es de 995 mm. Las rocas más abundantes son esquistos, cuarcitas, pelitas, areniscas y granitos. Los suelos son predominantemente ácidos y normalmente se clasifican en los grupos de los Umbrisoles o de los Cambisoles, en este último caso cuando no tiene un horizonte de diagnóstico A úmbrico porque no cumple los requisitos de profundidad o de color (WRB, 2022).

El municipio de Cospeito está situado en la provincia de Lugo, en la comarca da Terra Chá. Su relieve es principalmente llano, con pequeñas ondulaciones. La temperatura media anual es de 13°C y la precipitación anual total es de 1200 mm. En cuanto a su geología, las rocas más abundantes en esta zona son los esquistos, pizarras, areniscas y cuarcitas.

El municipio de Arzúa, situado en la provincia de A Coruña, se encuentra a una altitud de 389 m sobre el nivel del mar. La temperatura media de Arzúa es de 13 °C y la precipitación anual es de 1270 mm. En cuanto a la geología, este municipio ocupa la parte central de la serie de Ordes, en la que predominan los esquistos (de la serie de Ordes) y las anfibolitas. Estas últimas son rocas metamórficas de carácter básico que dan lugar a la formación de suelos normalmente bastante profundos, de texturas finas y con una buena fertilidad química dentro del contexto de Galicia, si bien son pobres en K. El tipo de suelo predominante sobre estas rocas puede ser más variado, pudiendo existir Andosoles cuando se trata de suelos jóvenes, Gleysoles en las zonas más llanas con problemas de hidromorfía, puede haber suelos con horizonte Bt (Acrisoles o Alisoles), Cambisoles, aunque predominarán los Umbrisoles (WRB, 2022).

La comarca de Valdeorras está situada en la provincia de Ourense, concretamente en la zona más oriental, cerca de la provincia de León y con una altitud de entre 300 y 1200 m. La temperatura media anual es de 11 °C y la precipitación total anual oscila entre los 850 y 1000 mm. La geología de esta comarca está formada mayoritariamente por pizarras, cuarcitas y

esquistos, lo que provoca la formación de suelos someros y ácidos, de tipo Leptosoles y Umbrisoles (WRB, 2022).

En cuanto a la comarca del Ribeiro, su altitud se sitúa entre los 75 y los 400 m de altitud. La temperatura media anual es de 14,5 °C y con una precipitación máxima anual de 950 mm. Las principales rocas presentes en estos suelos son el granito y el esquisto micáceo. Son suelos ácidos, bastante permeables, y de profundidad media. La mayoría de los suelos pertenecen al grupo de los Umbrisoles, aunque también puede haber Leptosoles en las zonas de mayor pendiente y Cambisoles (WRB, 2022).

La zona de las Rías Baixas presenta un clima oceánico de inviernos suaves y veranos cálidos con temperaturas por encima de los 9°C y lluvias principalmente concentradas en primavera, siendo la media anual de 1600mm. Predominan las rocas graníticas que dan lugar a la formación de suelos de textura gruesa, poco profundos, permeables y ácidos, pertenecientes mayoritariamente al grupo de Umbrisoles , Cambisoles y Leptosoles (WRB, 2022).

Cambados es un municipio de la provincia de Pontevedra, situado en la comarca do Salnés. Este municipio se encuentra a 16 metros de altitud. La temperatura a lo largo del año oscila entre los 7 °C y los 25 °C, y la precipitación promedio anual es de 1540 mm. Las parcelas en las que se recogieron las muestras para la realización de este estudio se sitúan en la parroquia de Vilariño. La zona se encuentra dentro del dominio magmático y de rocas graníticas (Grupo de Laxe). En este grupo se encuentran granitos de dos micas más o menos homogéneos y una serie de enclaves que han sufrido procesos metamórficos muy intensos, pudiendo ser considerados como migmatitas. De forma general, puede decirse que los suelos serán ácidos, de textura gruesa, permeables y con bajo contenido en nutrientes, perteneciendo principalmente a los grupos de los Umbrisoles, Cambisoles y también a los Leptosoles (WRB, 2022).

En la comarca del Barbanza las temperaturas medias anuales son de 14 °C, con amplitudes que van desde los 7 °C hasta los 20 °C, y una precipitación anual que oscila entre los 1000 mm en las zonas cercanas a la costa y los 2000 mm en las zonas del interior. Las muestras se recogieron en dos parcelas del municipio de Padrón que se encuentran sobre granito, por lo que las características de los suelos y su clasificación será la misma que en el caso de Cambados.

El municipio de Friol está situado en la provincia de Lugo a una altitud media de aproximadamente 478 m sobre el nivel del mar. El relieve es predominantemente suave, aunque en su sector occidental se eleva la sierra de la Cova da Serpe, alcanzando los 838 metros en el Monte da Cova. La temperatura media anual es de 10,7 °C y la precipitación anual es de 1365 mm, concentrándose principalmente en el invierno. Geológicamente, el municipio se asienta sobre formaciones de granito y dioritas del Precámbrico-Paleozoico, por lo que las características y clasificación de estos suelos coincide con la comentada anteriormente para estos materiales.

3.3 ANÁLISIS DE SUELOS

La determinación de las principales características fisicoquímicas de estas muestras se realizó siguiendo los métodos estándar de caracterización de suelos (Tan, 1996). Para llevar a cabo el análisis granulométrico y determinación de texturas se utilizó el Método Internacional de la Pipeta de Robinson previa separación de la fracción arena por tamizado (Guitián y Carballas, 1976). El pH del suelo se midió en agua (pH_{H2O}) y en KCl (pH_{KCl}) a partir de una relación suelo:líquido 1:2,5; el tiempo de contacto en agua fue de 10 minutos, mientras que en KCl fueron dos horas (Guitián y Carballás, 1976). El carbono orgánico total (CO) y el nitrógeno total del suelo (N) se determinaron con un equipo de análisis elemental LECO modelo TRUSPEC CHNS. Los cationes de cambio fueron desplazados de sus posiciones utilizando un

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extracto de NH_4Cl 1M (Peech et al., 1947) en una relación suelo:disolución 1:10, tras una noche de contacto y posterior filtración. El Ca, Mg y Al se determinaron por espectrofotometría de absorción atómica y Na y K mediante espectrofotometría de emisión. La suma de los cationes de cambio (Ca, Mg, Na, K y Al) constituye la capacidad de intercambio catiónica efectiva (CICe) (Kamprath et al., 1970). El Ca, Mg, Na, Al, Cr, As, Ni, Cd, Co, Fe, Mn, Cu, K, Zn y Hg totales siguiendo EPA 3051, que consiste en realizar una digestión ácida en un microondas con una disolución de HNO_3 al 65%, realizándose posteriormente la medición con ICP-MS. La determinación de Fe y Al no cristalinos totales (Fe_{ox} y Al_{ox}) se realizó mediante la extracción de estos elementos con oxalato amónico a pH 3 (Blakemore., 1978); el Fe y Al ligados a la materia orgánica (Fe_{pir} y Al_{pir}) se extrajeron con pirofosfato sódico a pH 10 (Bascomb, 1968) y se midieron con el espectrofotómetro de absorción atómica.

En la tabla 5 se muestran algunas de las propiedades fisicoquímicas de los suelos utilizados en este trabajo.

Tabla 5. Principales propiedades fisicoquímicas de los suelos: pH H_2O: pH en agua; pH KCl: pH en cloruro potásico 0,1M; CO: carbono orgánico; N: nitrógeno total; MO: materia orgánica.

Suelo	Arena	Limo	Arcilla	pH H_2O	pH KCl	CO	N	MO
	%	%	%			%	%	%
M1	49,57	27,93	22,50	8,02	7,76	4,98	0,33	8,58
M2	43,42	31,93	24,65	5,33	5,07	2,66	0,27	4,59
M3	43,57	34,00	22,43	5,65	5,17	2,58	0,25	4,44
M4	45,64	31,93	22,43	5,01	4,75	2,04	0,21	3,52
M5	63,35	24,29	12,36	6,11	5,97	7,67	0,63	13,21
M6	59,28	24,22	16,50	6,43	6,5	5,56	0,53	9,59
CM	61,35	18,14	20,50	5,38	5,1	3,09	0,23	5,33
VO1	61,40	25,47	13,14	5,60	4,2	0,63	0,06	1,09
VO2	48,05	29,51	22,44	5,70	5,0	1,00	0,11	1,72
VO3	43,50	33,59	22,90	5,60	4,8	1,61	0,18	2,78
VO4	47,50	34,29	18,22	5,87	4,62	1,94	0,11	3,35
VO5	49,57	26,29	24,14	6,04	4,8	1,77	0,10	3,06
VP1	68,60	17,17	14,23	5,60	4,9	3,69	0,26	6,36
VP2	50,16	29,59	20,25	6,10	5,5	4,38	0,37	7,55
VP3	60,62	21,03	18,35	6,10	5,3	2,83	0,23	4,88
VP4	65,54	13,39	21,07	5,47	4,2	6,58	0,49	11,35
VP5	58,22	20,39	21,38	5,70	4,9	4,81	0,40	8,30
VP6	67,42	10,14	22,43	7,05	6,5	2,43	0,14	4,19
VP7	67,50	20,29	12,22	7,27	6,45	3,56	0,22	6,14
CV	61,57	18,36	20,07	5,80	4,84	4,01	0,30	6,91
FP	69,35	17,78	12,86	4,88	4	7,70	0,48	13,27
FE	67,28	19,86	12,86	4,80	3,92	9,78	0,67	16,87
FR	69,35	15,78	14,86	4,68	4,09	7,15	0,49	12,32

Como se puede observar, estos suelos presentan un amplio rango para la mayoría de los parámetros, lo que permitirá estudiar la respuesta al aporte de antibiótico en diferentes condiciones edáficas. En todos ellos, la arena es la fracción más abundante, con valores entre un 43,42% y un 69,35% (Tabla 5). El contenido de limo oscila entre 10,14 y 34,29%, y el de arcilla entre 12,22 e 24,65%, clasificándose en la mayoría de los casos como suelos franco-arenosos segundo el método USDA. El pH en agua de los suelos varía desde 4,68 hasta 8,02,

y aunque es un rango muy amplio, más del 65% de los suelos tienen un pH comprendido entre 4,68 y 5,87. Estos valores más ácidos son los habituales en los suelos de Galicia, debido a que las altas precipitaciones favorecen el lavado de los cationes básicos y, además, el material parental es principalmente de naturaleza silícica. En el caso del pH en KCl, se observa que los valores son inferiores a los obtenidos para el pH en agua, indicando un predominio de las cargas negativas del suelo. También el contenido de materia orgánica presenta una alta variabilidad, oscilando entre 1,09% y 16,78% (Tabla 5).

Tabla 6. Cationes intercambiables y capacidad de intercambio catiónico de los suelos estudiados. Ca: calcio intercambiable; Mg: magnesio intercambiable; Na: sodio intercambiable; K: potasio intercambiable; Al: aluminio intercambiable; CICe: capacidad de intercambio catiónico efectiva; Fe_{ox}, Al_{ox}: Fe y Al no cristalinos: Fe_{pir} y Al_{pir}: Fe y Al ligados a la materia orgánica.

Solo	CICe	Ca	Mg	Na	K	Al	Fe_{ox}	Fe_{pir}	Al_{ox}	Al_{pir}
	$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
M1	42,81	39,44	2,07	0,60	0,69	0,01	4140	1501	5351	2111
M2	6,88	5,35	0,67	0,02	0,44	0,40	5745	3159	3401	2871
M3	7,48	6,18	0,77	0,00	0,28	0,24	5780	3315	3881	2717
M4	5,94	4,58	0,67	0,10	0,17	0,43	4545	2923	2896	1945
M5	20,84	18,81	1,60	0,08	0,33	0,02	6990	3719	11651	6179
M6	24,35	21,65	1,60	0,16	0,93	0,02	6525	3231	4991	4087
CM	6,63	4,85	0,39	0,13	0,38	0,89	3320	2561	3246	3483
VO1	5,96	2,85	0,73	1,39	0,31	0,68	1665	1273	634	1961
VO2	5,43	3,15	0,32	1,48	0,43	<0,06	2515	715	484	173
VO3	6,02	3,38	0,26	1,83	0,5	<0,06	2555	1047	894	1047
VO4	6,41	3,69	0,88	0,15	0,52	1,17	1980	1421	1601	1321
VO5	7,42	4,94	0,86	0,16	0,69	0,77	1790	1171	1556	1141
VP1	19,52	4,74	1,07	1,64	11,91	0,16	3045	2353	5739	4543
VP2	22,58	8,43	1,56	2,46	10,12	<0,05	3220	6309	4024	3897
VP3	37,23	6,33	1,48	18	11,4	<0,05	3460	4889	4294	3005
VP4	10,51	6,55	0,37	2,60	0,27	0,72	3540	6459	6624	14857
VP5	17,36	13,40	1,09	2,32	0,55	<0,10	2765	4519	3679	3965
VP6	9,09	8,11	0,51	0,08	0,37	0,02	1720	1225	3981	1703
VP7	12,32	10,53	1,04	0,44	0,27	0,04	1945	823	4576	1597
CV	8,60	5,73	0,83	0,17	1,13	0,74	2690	2129	3861	3573
FP	6,43	0,07	0,00	0,11	0,09	6,16	5410	9239	8184	10767
FE	6,59	0,05	0,00	0,12	0,12	6,29	5805	8249	10189	11347
FC	7,48	0,18	0,07	0,00	0,22	7,01	3055	6789	5619	9457

En la tabla 6 se muestran los cationes intercambiables de los suelos utilizados en este trabajo, su capacidad de intercambio catiónico (CICe) y la concentración de Al y Fe no cristalino total (Al_o, Fe_o) y ligado a la materia orgánica (Al_{pir}, Fe_{pir}). Al igual que en otras propiedades comentadas previamente, la capacidad de intercambio catiónico efectiva (CICe) presentó una amplia variabilidad entre los distintos suelos, oscilando entre 5,43 y 42,81 $cmol_c\ kg^{-1}$ (Tabla 6). De los cationes de cambio, el Ca es el más abundante en la mayoría de los suelos agrícolas, mientras que en el caso de los suelos forestales el Al es el catión predominante,

coincidiendo con los valores de pH más bajos. En cuanto a las formas no cristalinas de Fe y Al, se aprecia también un alto rango en los distintos suelos (Tabla 6); el Fe no cristalino total (Fe_{ox}) varía entre 1695-6990 mg kg^{-1}, mientras que el Al (Al_{ox}) se encuentra entre 484 y 11651 mg kg^{-1}. En relación con las formas ligadas a la materia orgánica (Fe_{pir} e Al_{pir}), los valores Fe_{pir} varían entre 715-9239 mg kg^{-1} mientras que los de Al_{pir} oscilan entre 173- 14857 mg kg^{-1}.

La nomenclatura empleada para algunos suelos presenta variaciones entre las distintas publicaciones utilizadas en esta tesis. En la tabla 7 se muestra la correspondencia entre las denominaciones utilizadas para cada suelo en las diferentes publicaciones.

Tabla 7. Equivalencia de los nombres de los suelos en las distintas publicaciones usadas en esta tesis.

Publicación II	Publicación IV	Publicación V	Publicación VII	Publicación VIII	Publicación IX	Publicación XI
M1						C
M2	C1		M1		C1	
M3	C2		M2		C2	
M4	C3		M3		C3	
VO3		VO2				
VO4		VO3				
VO5	VO	VO4	VO		V	VO
VP3	VP1					
VP4		VP3				
VP5	VP2					
VP6						VP2
VP7		VP4				VP3
FC		FR		FR		F

3.4 ANTIBIÓTICOS UTILIZADOS

Los tres antibióticos utilizados fueron suministrados por Sigma-Aldrich (Barcelona, España): amoxicilina (AMX): pureza ≥95%; cefuroxima (CFX): pureza ≥ 95%; azitromicina (AZM): puro.

3.5 ANÁLISIS DE LOS BIOADSORBENTES

3.5.1 Residuos de la industria forestal y alimentaria

Los materiales residuales de la industria forestal y alimentaria que se utilizarán para conocer su potencial para adsorber los antibióticos a estudio son los siguientes:

1. Cenizas de biomasa, procedentes de la combustión de leña de roble, proporcionadas por el hospital Polusa (Lugo).
2. Corteza de pino, se utilizó un producto comercial de la marca Geolia (Madrid, España).
3. Acícula de pino (*Pinus radiata*), obtenida en una plantación situada en el municipio de Friol (Lugo).
4. Hoja de eucalipto (*Eucalyptus nitens*), obtenida en una plantación situada en el municipio de Friol (Lugo).

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5. Concha de mejillón sin calcinar, suministrada por la empresa Abonomar S.L., situada en la Isla de Arousa (Pontevedra).

Todos ellos fueron secados, triturados y pasados por tamiz de <2mm.

1. Ceniza de biomasa

2. Corteza de pino

3. Acícula de pino

4. Hoja de eucalipto

5. Concha de mejillón

Figura 9: Bioadsorbentes procedentes de la industria forestal y agroalimentaria utilizados en esta tesis.

La caracterización fisicoquímica de estos materiales se realizó siguiendo los mismos métodos que se describieron para los suelos (apartado 3.2). En la tabla 8 se muestran las propiedades de los diferentes bioadsorbentes utilizados en esta Tesis.

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Tabla 8. Características de los materiales bioadsorbentes. Ca_e, Mg_e, Na_e, K_e, Al_e: elementos en el complejo de intercambio; Sat. Al: saturación de Al en el complejo de intercambio; CICe: capacidad de intercambio catiónico efectiva; X_T: contenido total del elemento (X); Alo, Feo: Al y Fe no cristalinos. Valores promedio (n=3), con coeficientes de variación siempre <5%.

Parámetro	Unidades	Hoja de eucalipto	Corteza de pino	Acícula de pino	Ceniza de roble	Concha de mejillón
C	%	53,05	48,70	50,31	13,23	11,43
N	%	1,35	0,08	1,08	0,22	0,21
C/N		39,18	608,75	46,76	60,14	55,65
pH_{agua}		4,88	3,99	3,68	11,31	9,39
pH_{KCl}		4,81	3,42	3,51	13,48	9,04
Ca_e	$cmol_c\ kg^{-1}$	7,95	5,38	2,13	95,0	24,75
Mg_e	$cmol_c\ kg^{-1}$	8,53	2,70	7,15	3,26	0,72
Na_e	$cmol_c\ kg^{-1}$	1,36	0,46	1,42	12,17	4,37
K_e	$cmol_c\ kg^{-1}$	12,93	4,60	11,09	250,65	0,38
Al_e	$cmol_c\ kg^{-1}$	0,13	1,78	2,15	0,07	0,03
CICe	$cmol_c\ kg^{-1}$	30,90	14,92	23,94	361,15	30,25
Sat Al	%	0,42	11,93	8,98	0,02	0,10
Available-P	$mg\ kg^{-1}$	262,84	70,45	217,95	462,83	54,17
Na_T	$mg\ kg^{-1}$	242,31	68,92	271,54	2950	5174,00
Mg_T	mg/kg	840,96	473,55	653,40	26171	980,66
Al_T	$mg\ kg^{-1}$	80,58	561,50	246,95	14966	433,24
K_T	$mg\ kg^{-1}$	4464,10	737,84	4123,44	99515	202,07
Ca_T	$mg\ kg^{-1}$	2262,96	2318,81	538,96	136044	280168
Cr_T	$mg\ kg^{-1}$	0,13	1,88	0,74	36,28	4,51
Mn_T	$mg\ kg^{-1}$	614,92	30,19	356,28	10554	33,75
Fe_T	$mg\ kg^{-1}$	43,13	169,78	47,27	12081	3535
Co_T	$mg\ kg^{-1}$	0,03	0,20	0,38	17,25	1,02
Ni_T	$mg\ kg^{-1}$	2,17	1,86	0,93	69,25	8,16
Cu_T	$mg\ kg^{-1}$	2,80	<LD	3,81	146,33	6,72
Zn_T	$mg\ kg^{-1}$	7,66	6,98	5,78	853,00	7,66
As_T	$mg\ kg^{-1}$	0,02	<LD	0,02	8,36	1,12
Cd_T	$mg\ kg^{-1}$	0,0	0,13	0,05	19,93	0,07
Al_o	$mg\ kg^{-1}$	45,0	315,0	169,0	8323	178,33
Fe_o	$mg\ kg^{-1}$	77,0	74,0	15,0	4233	171,0

Como se puede observar, también los bioadsorbentes tienen unas propiedades muy variables entre ellos. El pH en agua oscila entre valores muy ácidos, como el que presenta la acícula y la corteza de pino (pH = 3,68 y 3,99, respectivamente), hasta valores alcalinos, como el de la ceniza y la concha (pH = 11,31 y 9,04, respectivamente). El carbono orgánico también presenta un amplio rango, desde el 13,23% en la ceniza hasta más del 50% en las hojas de eucalipto y en las acículas de pino. En cuanto al N, oscila entre el 0,08% en la corteza y el 1,35% en la hoja de eucalipto.

La CICe también varía mucho dependiendo del tipo de bioadsorbente, con valores entre 14, 92 $cmol_c\ kg^{-1}$ en la corteza de pino y 361,15 $cmol_c\ kg^{-1}$ en la ceniza. En la corteza y la concha, el catión de cambio predominante es el Ca, con valores mucho más altos en la concha (24,75 frente a 5,38 $cmol_c\ kg^{-1}$). En cambio, en la ceniza, la hoja de eucalipto y la acícula de pino, el catión predominante es el K, especialmente en la ceniza, que presenta concentraciones tan altas como 250,65 $cmol_c\ kg^{-1}$.

También destacan los altos valores de P disponible en la ceniza (462,83 $mg\ kg^{-1}$), seguida de la hoja de eucalipto y la acícula de pino (siempre superiores a 200 $mg\ kg^{-1}$). Los metales

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pesados totales son claramente más elevados en la ceniza en comparación con los otros bioadsorbentes, presentando además las mayores concentraciones de Fe y Al no cristalino (Fe_{ox}, Al_{ox}) (8323 y 4233 mg kg^{-1}, respectivamente), seguida de la concha de mejillón, que presenta valores mucho menores (178,33 y 171,0 mg kg^{-1}, respectivamente).

3.5.2. Mezclas de suelos y bioadsorbentes

Se añadieron corteza de pino, concha de mejillón y ceniza de biomasa en proporciones de 12 t ha^{-1} y 48 t ha^{-1} a algunos de los suelos citados anteriormente que presentaron los valores de adsorción más bajos, con el objetivo de evaluar el efecto de estos bioadsorbentes sobre la capacidad de adsorción de los suelos. Para la preparación de las diferentes mezclas suelo/bioadsorbente se pesaron 100g de suelo en botes de polipropileno de 500 mL de capacidad y se le añadió la cantidad correspondiente de material bioadsorbente para obtener las dosis establecidas (12 t ha^{-1} y 48 t ha^{-1}), teniendo en cuenta una profundidad efectiva del suelo de 20 cm y una densidad aparente de 1,2 g cm^{-3}. Estas mezclas se agitaron durante 72 h en un agitador rotatorio y a temperatura ambiente (25 ± 2 °C).

En la tabla 9 se muestran los valores de pH de estos suelos sin bioadsorbente y con la adición de cada uno de los bioadsorbentes en las dos dosis utilizadas (12 t ha^{-1} y 48 t ha^{-1}).

Tabla 9. Valores de pH en el suelo original y después de añadir cada uno de los bioadorbentes (A: ceniza; MS: concha de mejillón; PB: corteza de pino) en dosis de 12 t ha^{-1} y 48 t ha^{-1}.

Mixture (12 t/ha)	pH	Mixture (48 t/ha)	pH
VP1	6,1	VP1	6,1
VP1-A	6,53	VP1-A	7,20
VP1-MS	6,07	VP1-MS	6,05
VP1-PB	5,67	VP1-PB	5,45
VP2	5,7	VP2	5,7
VP2-A	6,41	VP2-A	7,28
VP2-MS	6,13	VP2-MS	6,84
VP2-PB	5,87	VP2-PB	6,39
VO	6,04	VO	6,04
VO-A	6,11	VO-A	7,81
VO-MS	6,06	VO-MS	5,92
VO-PB	5,73	VO-PB	5,35
C1	5,33	C1	5,33
C1-A	6,40	C1-A	6,93
C1-MS	5,79	C1-MS	5,29
C1-PB	5,16	C1-PB	4,92
C2	5,65	C2	5,65
C2-A	5,97	C2-A	7,04
C2-MS	5,70	C2-MS	5,76
C2-PB	5,51	C2-PB	5,24
C3	5,01	C3	5,01
C3-A	6,11	C3-A	6,93
C3-MS	6,06	C3-MS	5,46
C3-PB	5,73	C3-PB	4,79

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En general, se puede observar que la ceniza (y a veces la concha) provocan un aumento de pH del suelo original, mientras que la corteza lo disminuye; el efecto es más evidente en la dosis más alta de bioadsorbente añadida.

3.6 ENSAYOS DE ADSORCIÓN-DESORCIÓN

Para conocer que capacidad de adsorción de los tres antibióticos (AMX, CFX y AZM) presentan los suelos y materiales bioadsorbentes, se realizaron ensayos de adsorción/desorción tipo batch (Figura 10). Este análisis se realizó tanto de manera individual, añadiendo un solo antibiótico, como en condiciones competitivas, incorporando los tres antibióticos de forma simultánea.

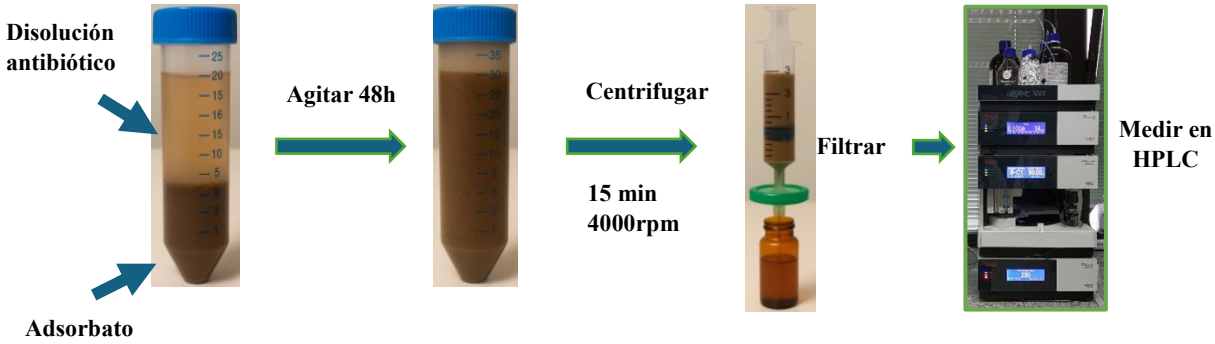

Figura 10: Ensayo de adsorción/desorción tipo batch llevado a cabo en esta tesis.

3.6.1 Adsorción/desorción individual

3.6.1.1. Muestras de suelo

Se estudió el proceso de adsorción y desorción en el equilibrio de los tres antibióticos (AMX, CFX y AZM) en los materiales y suelos citados anteriormente, procediendo de la siguiente manera: se pesaron 2 gramos de suelo en tubos de centrífuga de polipropileno y se suspendió en disoluciones individuales de concentraciones crecientes de cada uno de los antibióticos, utilizando una relación suelo disolución de 2:5. En el caso de la CFX y AMX las concentraciones utilizadas variaron entre 2,5 y 50 µmol L^{-1}, mientras que en el caso de la AZM el rango estuvo entre 2,5 y 600 µmol L^{-1}, todas ellas utilizando $CaCl_2$ 0,005 M como fondo iónico. Las diferencias en las concentraciones añadidas de los distintos antibióticos se deben a la alta capacidad de adsorción de la azitromicina (AZM) en comparación con los otros antibióticos, por lo que para evaluar con mayor precisión la influencia de las propiedades fisicoquímicas del suelo sobre el proceso de adsorción es necesario incrementar la concentración de este antibiótico. Las suspensiones se agitaron durante 48 horas en un agitador rotatorio en la oscuridad y a temperatura ambiente (25 ± 2 °C). Transcurrido este tiempo de contacto, las suspensiones se centrifugaron a 4000 rpm durante 15 minutos en una centrífuga modelo 5810R (Eppendorf, Hamburgo, Alemania) y los sobrenadantes resultantes se pasaron a través de filtros de jeringa de nylon (0,45 µm de tamaño de poro) y se guardaron a 4 °C hasta análisis. En ellos se midió el pH con un pHmetro (modelo MultiMeter MM41, Crison, Barcelona, España) y se determinó la concentración de antibiótico mediante HPLC-UV (ver apartado 3.6). La cantidad de antibiótico adsorbida al suelo se determinó por diferencia entre la cantidad inicial de antibiótico añadida y la cantidad de antibiótico presente en la disolución de equilibrio transcurrido el período de 48 h. A continuación, para estudiar la desorción, se pesaron

los residuos de suelo sometidos previamente al proceso de adsorción para determinar la cantidad de disolución ocluida y se suspendieron en 5 ml de $CaCl_2$ 0,005 M. Estas suspensiones se agitaron, centrifugaron, filtraron y analizaron de igual manera que en el caso de la adsorción. Todos los experimentos se llevaron a cabo por triplicado.

3.6.1.2 Materiales bioadsorbentes

Además de en los suelos, también se estudió el proceso de adsorción y desorción de los tres antibióticos (AMX, CFX y AZM) en los materiales bioadsorbentes. En el caso de la cefuroxima y amoxicilina se utilizaron 5 materiales bioadsorbentes (concha de mejillón, ceniza de combustión de biomasa, corteza de pino, hoja de eucalipto y acícula de pino), mientras que para la azitromicina se usaron 3 materiales bioadsorbentes (concha de mejillón, ceniza de biomasa y corteza de pino). El procedimiento utilizado fue similar al descrito para los suelos (apartado 3.5.1.1), pero con pequeñas modificaciones. En este caso se pesaron 0,5 gramos de bioadsorbente y se añadieron 10 ml de disolución creciente de antibiótico, mientras que las restantes condiciones fueron las mismas que en los suelos.

3.6.1.3 Suelos enmendados con materiales bioadsorbentes

También se estudió el proceso de adsorción y desorción de los tres antibióticos (AMX, CFX y AZM) en suelos enmendados con tres bioadsorbentes (concha de mejillón, ceniza de combustión de biomasa y corteza de pino). En el caso de la AMX y AZM los bioadsorbentes fueron añadidos a 4 suelos seleccionados en una relación de 48 t ha^{-1}, mientras que en la CFX se eligieron 6 suelos a los que se añadieron los tres bioadsorbentes en proporciones de 12 t ha^{-1} y 48 t ha^{-1}.

El procedimiento utilizado para el estudio del proceso de adsorción y desorción en estas mezclas fue idéntico al utilizado en el caso de los suelos (apartado 3.5.1.1).

3.6.2 Adsorción/desorción competitiva

3.6.2.1. Muestras de suelo

Se estudió el proceso de adsorción y desorción competitivo entre los tres antibióticos (AMX, CFX y AZM) y también entre la amoxicilina (AMX) y el ciprofloxacino (CIP). Para estos ensayos se seleccionaron 6 suelos con distinto pH y con diferente contenido en materia orgánica.

La competencia se estudió mediante sistemas ternarios, añadiendo simultáneamente los tres antibióticos (AMX, CFX y AZM). Además, también se estudió la competencia entre la AMX y CIP en un sistema binario, al tratarse de dos antibióticos ampliamente utilizados en medicina humana. Las concentraciones utilizadas en el sistema ternario variaron entre 2,5 y 50 µmol L^{-1} , mientras que en el sistema binario oscilaron entre 25 y 400 µmol L^{-1} de cada uno de los antibióticos. La relación suelo: líquido fue de 2:5 en ambos sistemas. Los ensayos se realizaron de igual manera y en las mismas condiciones que lo descrito en el caso de los sistemas simples (apartado 3.5.1.1).

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3.6.2.2 Materiales bioadsorbentes

Por último, se estudió la competencia de adsorción también en un sistema ternario (AMX, CFX y AZM) y en uno binario (AMX y CFX) en tres materiales bioadsorbentes (concha de mejillón, ceniza de combustión de biomasa y corteza de pino). Las concentraciones iniciales utilizadas en el sistema ternario variaron entre 2,5 y 50 µmol L^{-1}, mientras que en el sistema binario oscilaron entre 25 y 400 µmol L^{-1}. La relación adsorbente: disolución fue de 0,5:10 en ambos sistemas. Las condiciones utilizadas fueron las mismas que las descritas para los experimentos de adsorción y desorción competitiva en suelos (apartado 3.5.2.1).

3.7. CUANTIFICACIÓN DE CEFUROXIMA, AMOXICILINA Y AZITROMICINA

La cuantificación de los antibióticos se realizó mediante HPLC-UV. Para ello se utilizó un cromatógrafo líquido ThermoFisher equipado con una bomba cuaternaria HPG-3400, un muestreador automático WPS-3000, un compartimento termostatizado para la columna TM-120 y un detector ultravioleta-visible UVD170U. Se utilizó una columna Luna C18 (150 mm de largo; 4,6 mm de diámetro interno; 5 µm de tamaño de partícula) proporcionada por Phenomenex (Madrid, España) y una pre-columna (4 mm de largo; 2 mm de diámetro interno; 5µm de tamaño de partícula) empaquetada con el mismo material que el de la columna. El volumen de inyección fue de 50 µL y el flujo de 1,5 mL min^{-1}.

En el caso de la cefuroxima, la fase móvil consistió en acetonitrilo (fase A) y ácido fosfórico 0,01M (fase B). Se utilizó un gradiente lineal que varió de un 5% a un 32% de fase A y de 95% a 68% de fase B durante 10,5 minutos. A continuación, reestablecieron las condiciones iniciales (5% de fase A y 95% de fase B) en 2 minutos y se mantuvieron durante 2,5 minutos. El tiempo total de análisis fue de 15 minutos, con un tiempo de retención para este antibiótico de 8,69 minutos. La longitud de onda utilizada para la detección fue de 212 nm.

En cuanto a la amoxicilina, la fase móvil consistió en acetonitrilo (fase A) y ácido fosfórico 0,01M (fase B). Se utilizó un gradiente lineal que tuvo una variación desde un 5% a un 32% de fase A y desde un 95% a 85% de fase B durante 4 minutos. A continuación, se reestablecieron las condiciones iniciales (5% de fase A y 95% de fase B) en 2 minutos y mantenidas durante otros 2 minutos. El tiempo de análisis total fue de 10 minutos, con un tiempo de retención para la amoxicilina de 3,9 minutos. La longitud de onda utilizada para la detección fue de 230 nm.

En el caso de la azitromicina, la fase móvil consistió en acetonitrilo (fase A) y fosfato monopotásico 0,025M (fase B). Se utilizó un gradiente lineal que tuvo una variación desde un 5% a 70% de fase A y desde 95% a 30% de fase B durante 18 minutos. Luego, las condiciones iniciales (5% de fase A y 95% de fase B) fueron reestablecidas en 2 minutos y mantenidas durante 3 minutos. El tiempo de análisis total fue de 22 minutos, con un tiempo de retención de 9,8 minutos. La longitud de onda utilizada para la detección fue de 210 nm.

3.8. TRATAMIENTO DE LOS DATOS, MODELIZACIÓN Y ESTADÍSTICA

A partir de los datos obtenidos de los experimentos de adsorción y desorción en el equilibrio se construyeron las curvas de adsorción y desorción, en las cuales se representó la cantidad de antibiótico adsorbida por el suelo (q_e, µmol kg^{-1}) frente a la concentración de antibiótico presente en la disolución en el equilibrio (C_{eq}, µmol L^{-1}).

Estas curvas se describieron utilizando los modelos Lineal (Ec. 1), de Freundlich (Ec. 2) y de Langmuir (Ec. 3).

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$$q_e = K_F C_{eq} \qquad \text{(Ec. 1)}$$

$$q_e = K_F C_{eq}^n \qquad \text{(Ec. 2)}$$

$$q_e = \frac{q_m K_L C_{eq}}{1+K_L C_{eq}} \qquad \text{(Ec. 3)}$$

donde q_e (µmol kg^{-1}) y C_{eq} (µmol L^{-1}) son las concentraciones de antibiótico presentes en el suelo y en la disolución en el equilibrio, respectivamente; K_d (L kg^{-1}) es el coeficiente de distribución; K_F (L^n μmol^{1-n} kg^{-1}) es el coeficiente de afinidad de Freundlich; n (adimensional) es el índice de linealidad de Freundlich; K_L (L μmol^{-1}) es un parámetro de Langmuir relacionado con la energía de adsorción; y q_m (µmol kg^{-1}) es la capacidad máxima de adsorción de Langmuir.

Además, la adsorción también se expresó como la cantidad de antibiótico adsorbida (µmol kg^{-1}) para cada una de las concentraciones iniciales utilizadas, así como el porcentaje que representa esta cantidad adsorbida con respecto a la cantidad añadida. En cuanto a la desorción, también se expresó en µmol kg^{-1} para cada una de las concentraciones iniciales utilizadas y como el porcentaje que representa la cantidad desorbida con respecto a la cantidad previamente adsorbida.

En el caso de la adsorción competitiva en el sistema ternario (AMX, CFX y AZM) los datos experimentales también se describieron utilizando una modificación de los modelos Lineal (Ec. 4) y de Freundlich (Ec. 5) (Arias et al., 2006).

$$(q_{e\,AMX} + q_{e\,CFX} + q_{e\,AZM}) = Kd(C_{eq\,AMX} + C_{eq\,CFX} + C_{eq\,AZM}) \qquad \text{(Ec 4)}$$

$$(q_{e\,AMX} + q_{e\,CFX} + q_{e\,AZM}) = KF(C_{eq\,AMX} + C_{eq\,CFX} + C_{eq\,AZM})^n \qquad \text{(Ec 5)}$$

Finalmente, para el sistema binario (AMX y CIP) los datos experimentales de la adsorción competitiva se describieron utilizando una modificación del modelo de Freundlich (Ec 6) (Russell y Yost, 2021).

$$(q_{e\,AMX} + q_{e\,CIP}) = KF(C_{eq\,AMX} + C_{eq\,CIP})^n \qquad \text{(Ec 6)}$$

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4. RESULTADOS

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PUBLICACIÓN I

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Occurrence of Nine Antibiotics in Different Kinds of Sewage Sludge, Soils, Corn and Grapes After Sludge Spreading

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The huge worldwide use of antibiotics triggers the accumulation of these substances in sludge from wastewater treatment plants (WWTP) and the possible contamination of soils amended with it, as well as of crops growing in these soils. In this work we analyzed the presence of the antibiotics amoxicillin (AMO), cefuroxime (CEF), ciprofloxacin (CIP), clarithromycin (CLA), levofloxacin (LEV), lincomycin (LIN), norfloxacin (NOR), sulfadiazine (SUL), and trimethoprim (TRI), in sludge from different WWTPs in Galicia (NW Spain), as well as in sludge technically treated by waste-managers, in soils where treated sludge was applied, and in crops (corn and vineyard) growing in the amended areas. The antibiotics were quantified by means of high resolution HPLC-mass-chromatography. The results indicate that almost all the sludge samples contained antibiotics, being ciprofloxacin and levofloxacin the most abundant reaching maximum values of 623 and 893 ng/g, respectively. The sludge treatment significantly reduced the number and the concentrations of antibiotics. In 12% of the soil samples where sludge was applied, some antibiotics were detected, but always in small concentrations. Regarding the crops, no antibiotic was detected in the roots, stalk, leaves and grain of corn, neither in grapes sampled in vineyards. It can be concluded that the treatments currently applied in the WWTPs under study are not totally effective in removing antibiotics from the sludge, although the findings of this research suggest that the additional specific treatment of the sludge derived from these WWTPs is effective in reducing the risk of environmental pollution due to a variety of antibiotics, and specifically in the case of soils amended with these organic materials and crops growing on it.

Keywords: antibiotics, composting, crop soils, sludge treatment, wastewater treatment plants

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INTRODUCTION

Antibiotics have been widely used in human medicine since the discovering of penicillin in 1928, mainly to treat bacterial infections. Their consumption reaches globally up to 15.7 daily doses (DDDs) per 1,000 people (Sriram et al., 2021) with a slightly higher average for Europe, with 16.4 DDD per 1,000 inhabitants (ECDC, 2021). These pharmaceuticals are also provided as food supplement to animals for growth promotion, although this it is banned in Europe (Spielmeyer, 2018), and for animal health control which can lead to the soil persistence of these compounds after repeated application of manure and slurry (Gros et al., 2019). Globally, between the years 2000–2015, 42.3 billion DDD were consumed by humans and the antibiotics consumption rate increased 39%, being higher in high-income countries (Klein et al., 2018), with amoxicillin and amoxicillin/clavulanic acid being the most frequently consumed antibiotics in most countries (Aldeyab et al., 2020). Within the European Union, the antibiotics more consumed are the ones from the group of β-Lactam antibacterial molecules and penicillins (42.3%) and the general consumption pattern has not significantly change in the period 1997–2017 (Bruyndonckx et al., 2021). The country raking of antibiotic consumption in Europe is led by Greece, with Cyprus and Spain in second and third place, respectively (Bruyndonckx et al., 2021).

After its administration, these compounds are partially metabolized and then excreted, reaching the sewage systems and wastewater treatment plants (WWTP). The sludge generated in the WWTP has a great capacity for adsorbing antibiotics, favoring their accumulation, which has been previously described. Specifically, in a study carried out in China, 21 different antibiotics were detected with concentrations up to 5,800 ng g^{-1} in two WWTPs (Zhou et al., 2013), and in Sweden 9 different antibiotics were detected in 11 WWTPs (Östman et al., 2017). Some of the most relevant antibiotics present in WWTPs are: ciprofloxacin, appearing in 90% of the sampled WWTP effluents in a European survey (Loos et al., 2013); lincomycin, which is among the three main individual pollutants usually found in concentrations over 10 μg L^{-1} in the sewage influents in Korea (Behera et al., 2011); and clarithromycin, detected in 73% of sludge samples analyzed in Sweden (Östman et al., 2017) and in effluents from WWTPs in Switzerland (Mcardell et al., 2003). Other antibiotics like levofloxacin (Okuda et al., 2009), norfloxacin (Gao et al., 2012) and trimethoprim (Östman et al., 2017) have been detected as well in the sludge of different WWTPs. Amoxicillin has been detected both in wastewater (Henninger et al., 2001) and in WWTP sludge (Kimosop et al., 2016). Namely, the conventional treatments implemented in WWTPs are not efficient in removing antibiotics from both water and sludge, and additional treatments are required (Sabri et al., 2020). These compounds are considered among the most critical pharmaceuticals and personal care products (PPCPs) regarding environmental risk (Verlicchi and Zambello 2015).

According to the Spanish National Sludge Register, 1.2 million tons of sludge (dry matter) are produced annually in Spain and are considered residues, therefore specific residues regulations are

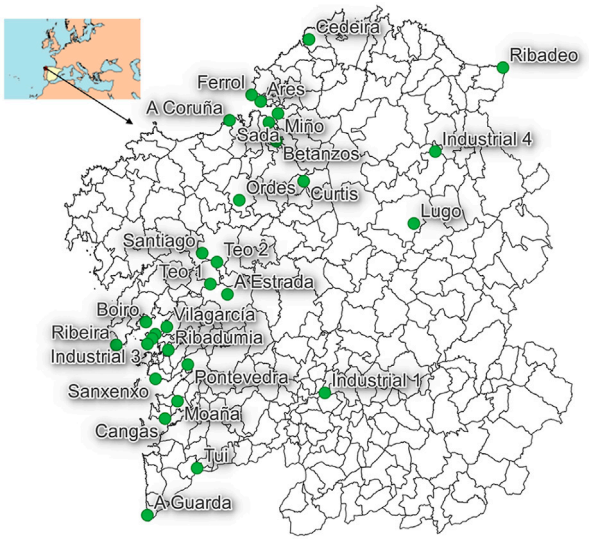

FIGURE 1 | Location of the wastewater treatment plants included in the study, all of them in Galicia (NW Spain).

applied related to their disposal and agricultural use (MAMRM, 2009). The sludge should be treated before field application, that in Spain represented around 80% of the sludge use (MAMRM, 2009). These treatments can be applied in the WWTP or after transfer to authorized residue managers. The current normative regulates the maximum concentration of heavy metals allowed in the sludge, meanwhile presence of antibiotics is not controlled. Despite the regulations regarding sludge management, antibiotics might end up in agricultural soils amended with the sludge, and therefore available to the crops growing in these soils (Verlicchi and Zambello 2015). The plants would tend to accumulate antibiotics in the roots, after uptake from the soil solution (Azanu et al., 2016). The presence of these pharmaceuticals has been identified as a new threat due to the application of sewage sludge in agriculture (Ivanová et al., 2018), generating risks for the consumers (Buta et al., 2021). Several studies have detected antibiotic uptake in crops like carrot and barley (Eggen et al., 2011), corn, green onion and cabbage (Kumar et al., 2005), lettuce and radish (Bassil et al., 2013) and grass, corn and wheat (Conde-Cid et al., 2018). It has been shown that different antibiotics may also impact on the soil microbial biomass (Santás-Miguel et al., 2020), and that their degradation in the soil environment is affected by variables such as soil pH, humic acids and salts (Rodríguez-López et al., 2022), which could substantially change after sludge spreading.

The aim of the current research is to screen the presence of antibiotics in sludge from different WWTPs in Galicia (NW Spain), as well as in sludge treated by residue managers, in soils where the treated sludge mixtures were applied, and in crops (corn and vine plants) growing on these soils. The study will focus on assessing the eventual success as regards the following aspects: 1) the eventual decrease in the amount of antibiotics present in sewage sludge subjected to technical treatment applied to these residues, and 2) after the spreading of the treated sludge, the

TABLE 1 | Treatments applied by the residue managers (RM) to the wastewater treatment plants (WWTP) sludge, by adding and mixing different by-products during certain time. Sp: spring sampling; Au: autumn sampling.

		WWTP	Additions	Time
RM 1	Mixture 1 (Sp)	Betanzos, Ribeira, Tui	Chicken manure, wood chips and lime	3 days
	Mixture 2 (Sp)	Ares, Betanzos, Cedeira	Sludge from meat industry, mussel shell and lime	
	Mixture 1 (Au)	A Guarda	Sludge from industrial WWTP, wood chips, chicken manure,	
	Mixture 2 (Au)	Cedeira, Lugo	crop substrate, compost, lime	
	Mixture 3 (Au)	Sada, Moaña, Cangas		
RM 2	Mixture 1	Miño, Ordes, Ribadeo	Sludge from industrial WWTP, animal rest (blood, stomach intestine, meat), mussel shell, wood chips, milk	8 weeks (Register fertilizer)
RM 3	Mixture 1	Ferrol	Sludge from cannery industry, dairy industry, food leftovers, wood chips	8 weeks
RM 4	Mixture 1	A Estrada, Teo 1-2, Santiago	Wood chips	2 weeks
	Mixture 2	Sanxenxo, Vilagarcía, Industrial 1		
	Mixture 3	Boiro, Vilagarcía, Industrial 1		

concentrations of antibiotics present in soils and crops would remain low. In addition, the research could shed further light on processes taking place and affecting the fate of these emerging pollutants in the environment, which is a matter of real concern as regards environmental and human health.

MATERIALS AND METHODS

Experimental Design

Sludge samples were collected from 30 different WWTPs in Galicia (NW Spain) (**Figure 1**) during two sampling campaigns, in spring and autumn of 2019. In the spring campaign, 21 urban WWTP, plus 1 industrial WWTP, were sampled. The urban WWTPs were placed in cities with 65,000–248,000 habitants (A Coruña, Ferrol, Pontevedra, Santiago de Compostela), as well as in villages with above 17,000 habitants (A Estrada, Boiro, Ribeira, Teo, Tui, Sanxenxo, Vilagarcía), and in smaller villages (A Guarda, Ares, Betanzos, Cedeira, Curtis, Miño, Ordes, Ribadeo, Ribadumia). In the second sampling campaign (autumn), 28 WWTP sludge samples were taken (the same as for the first sampling, except Cedeira, Ferrol and Teo 2), plus additional WWTPs from Cambados, Cangas, Moaña, Sada, Lugo, and 3 more industrial WWTPs. The industrial ones belong to the agri-food industry, poultry and canned fish production. Regarding configurations adopted in the treatment lines, all urban WWTPs use both primary (gritting, sieving, sedimentation) and secondary treatments (organic matter removal), while tertiary treatments are applied differently in each WWTP (only UV disinfection is applied is most of them, **Supplementary Table S1**).

Within Spain, the specific Galician legislation requires the sludge to be treated before being suitable to be used as an amendment in agricultural soils. The sludge generated from different WWTPs is processed by residue managers, generating compost or not composted mixtures. These processed sludge-derived materials were sampled in spring and autumn, from four different residue managers (RM) who produced different mixtures of WWTP sludges with several animal and vegetal by-products (**Table 1**). These treated mixtures were applied as fertilizers to several agricultural fields, where corn and vineyard were cultivated. Also soils and crops were sampled, as indicated below.

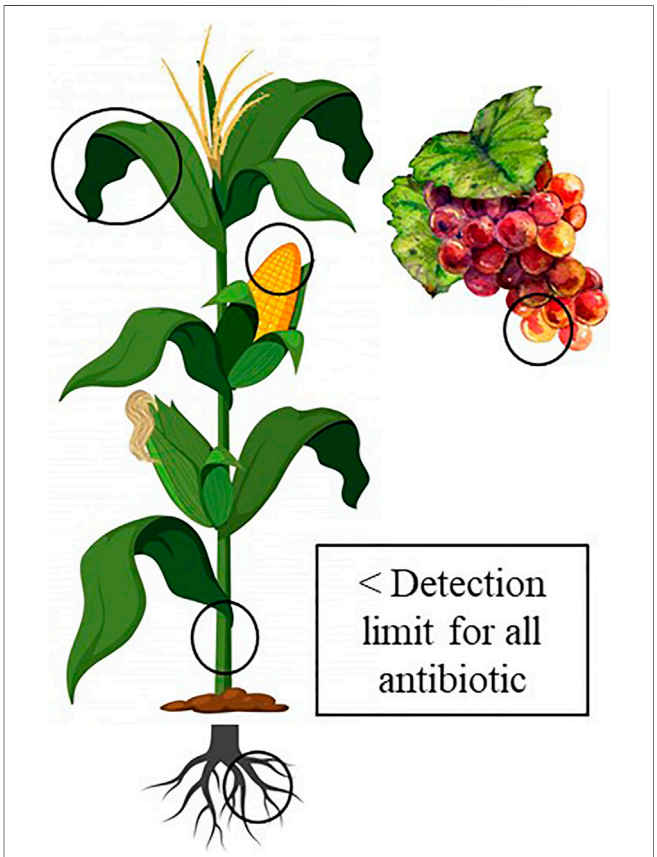

FIGURE 2 | Scheme of plant material sampling. It is shown that all the antibiotics studied in plant samples were below their detection limits using high performance HPLC-mass-chromatography.

Sludge, Compost, Soil, and Plant Sampling

The WWTP sludge and treated mixtures were sampled at the WWTPs and residue managers facilities, freeze-dried and stored at −4°C until analysis. Soils from amended fields, which had received that kind of fertilization during at least the last 3 years,

specifically with the treated mixtures from residue managers (RM) RM1, RM2 and RM4 were sampled at harvest time. A total of 59 amended soils were sampled, 27 under corn (10 amended by RM1, 5 by RM2, and 12 by RM4) and 32 under vineyard (all amended by RM4). The fields were located in different parts of Galicia with (according to the Köppen-Geigen classification) Csb climate (warm temperature with dry and warm summers) and characterized by high precipitations. In each field 10 subsamples were collected in a zigzag transect in the soil surface (depth 0–20 cm) and mixed as a composite sample per field. Soil was dry (at 40°C) until stable weight, sieved (by 2 mm mesh) and stored until analysis. At those same fields vegetal material was sampled at harvest time. In each corn field, three corn plants were randomly selected, and leaves, stem, grains, and roots were taken, making a composite sample per site (**Figure 2**). In the vineyard fields, three plants were also randomly selected, and several bunches of grapes were sampled from each plant making a composite sample per site. In the laboratory, the roots were washed with distilled water, and in stems and leaves the parts closer to the cob were selected for analysis, discarding the central nerve and the sides in the leaves.

Physicochemical Analyses of Soil and Sludge Samples

Standard procedures were used to carry out the analyses (Guitián Ojea and Carballas, 1976; Tan, 1996). Soil texture was determined using the international method of Robison Pipette. For both soil and sludge samples, pH values were obtained in water and KCl (1: 2.5), using a pH-meter CRISON, model 2001. Total C and N were determined by elemental analysis using a LECO equipment, model TRUSPEC CHNS. Extractable P was measured in soil samples using the Olsen method (Olsen and Sommers, 1982). Soil exchange cations (Ca, Mg, Na, K, Al) were extracted using NH_4Cl 1M (Peech et al., 1947) in 1:10 solution. Ca, Mg and Al were measured by atomic absorption spectrophotometry; Na and K were quantified using emission spectrophotometry. The sum of all this exchange cations was considered the effective cation exchange capacity (eCEC) (Kamprath., 1970). Non-crystalline Al and Fe (Blackmore, 1978) and organic Al and Fe (Bascomb, 1968) were measured by atomic absorption after specific extractions. In sludge samples, total element analysis was performed according to the EPA 3051 method, using ICP-MS for measuring Na, Mg, Al, K, P, Ca, Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Hg and Pb concentrations.

Chemicals, Reagents, and Stock Solutions for the Quantification of the Antibiotics

Amoxicillin, cefuroxime, ciprofloxacin, clarithromycin, levofloxacin, lincomycin, norfloxacin, sulfadiazine, and trimethoprim (purity >98%) and formic acid (purity >99% for analysis) were bought from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol HPLC grade were obtained from Merk (Darmstadt, Germany) and Milli-Q water was obtained from a Millipore system (Bedford, MA, USA) installed in the laboratory.

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For each antibiotic, an amount of 20 mg was accurately weight (±0.1 mg) and transferred to a 25 ml volumetric flask containing approximately 10 ml of solvent, the volume was finally made up to the graduation mark and the concentration was calculated taking in consideration the purity of the compound. Specifically, ciprofloxacin was dissolved in methanol:water:formic acid (50: 49.95:0.05), amoxicillin in water acidified with formic acid, and the rest of antibiotics in methanol. Each day of the analysis a new standard solution of amoxicillin was prepared as this pharmaceutical is very unstable and it degrades easily. The other standard solutions were stored in the dark at −18°C. These stock solutions were mixed and diluted several times with a mixture of methanol and water (50:50) to obtain a working standard solution mixture of antibiotics at 1 and 10 $\mu g\ ml^{-1}$.

Antibiotics Extraction Protocol

The extraction solvent was not the same for all matrices, however, independently of the matrix type, 2 g of sample was weight in a 15 ml Falcon tube. From roots, leaves and soils, the antibiotics were extracted with a mixture of acetonitrile:water: formic acid (50:49.95:0.05), whereas from grapes and stalk were extracted with acetonitrile acidified with 0.2% of formic acid, and from sewage sludge with acetonitrile. After the addition of the extraction solvent, the falcon tubes were shaken on a rotary shaker for 30 min and centrifuged at 4,500 rpm for 15 min. A portion of 1 ml of the supernatant was filtered through an Acrodisk Syringe Filter (Waters, USA) and transferred to a HPLC amber vial for analysis. For each batch of samples and type of matrix (soil, sludge, stalk, roots, leaves and grapes) a matrix-matched calibration curved with analyte-free sample was prepared. The concentration of each antibiotic in the calibration curved varied with the matrix but in average ranged from 10 to 1,000 $ng\ g^{-1}$. These calibration curves were used to identify and correctly quantify each of the antibiotics.

Analyses of the Antibiotics by HPLC-MS/MS

The samples were analyzed in an LC-MS/MS system from Bruker (Bremen, Germany) consisting of a UPLC® Elute with degasser, pump, oven, column and autosampler connected to an Evoq Elite triple quadrupole detector. Analytes were separated on an HPLC column Kinetex biphenyl (2.6 µm, 2.1 × 50 mm) from Phenomenex (Torrance, CA, USA). The temperature of the column was set up at 43°C, and the separation of the analytes was achieved on a gradient mode mixture of phase A (0.1% acidified water with formic acid) and phase B (acetonitrile 0.1% acidified with formic acid). The flow was maintained at 375 $\mu L\ min^{-1}$, the column temperature at 43°C, and the samples at 8°C. The mass spectrometer was calibrated as follows: the capillary voltage at 4,800 V, the cone pressure at 20 psi, the cone temperature at 300°C, the source pressure at 30 psi, the source temperature at 500°C, and the misting gas pressure at 60 psi. The injection volume was 15 µL. The MRM transitions monitored for each compound as well as the collision energy employed are compiled in **Table 2**.

TABLE 2 | Ionization mode, precursor and product ion and collision energy employed for pharmaceuticals quantification.

	Ionization mode	Precursor Ion	Product Ions	Collision energy
Amoxicillin	Positive	366.101	114.000	29
			208.100	15
Cefuroxime	Negative	422.9	207.0	15
			318.0	15
Ciprofloxacin	Positive	331.95	231.100	41
			288.100	31
Clarithromycin	Positive	749.846	158.200	33
			83.100	77
Levofloxacin	Positive	362.362	261.000	31
			318.200	21
Lincomycin	Positive	407.3	126.2	22
			359.2	12
Norfloxacin	Positive	320.0	302.0	15
			276.0	15
Sulfadiazine	Positive	252.1	156.0	12
			108.0	19
Trimethoprim	Positive	291.272	261.100	21
			81.000	59

TABLE 3 | Concentration (ng g^{-1}) of antibiotic in the sludge from the different WWTP at the spring (Sp) and autumn (Au) sampling time. Antibiotic detected: ciprofloxacin (CIP), clarithromycin (CLA), levofloxacin (LEV), lincomycin (LIN), norfloxacin (NOR), sulfadiazine (SUL), trimethoprim (TRI).

WWTP	CIP		CLA		LEV		LIN		NOR		SUL		TRI	
	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au	Sp	Au
A Coruña	<	<	<	<	<	<	<	<	<	<	<	<	9	<
A Estrada	<	<	<	<	<	<	<	<	<	<	<	<	<	<
A Guarda	599	<	<	<	<	<	<	<	<	<	<	<	<	<
Ares	105	<	63	<	<	<	<	<	<	<	<	<	21	<
Betanzos	<	<	<	<	<	<	<	<	<	<	<	<	24	<
Boiro	274	<	85	<	525	<	<	<	<	<	<	<	37	<
Cambados	-	<	-	<	-	<	-	<	-	<	-	<	-	<
Cangas	-	<	-	<	-	31	-	<	-	<	-	<	-	<
Cedeira	<	-	<	-	<	-	<	-	<	-	<	-	7	-
Curtis	<	49	<	<	<	18	<	<	<	<	<	<	<	<
Ferrol	<	-	<	-	<	-	<	-	<	-	<	-	<	-
Industrial 1	623	<	<	<	<	<	<	<	144	<	<	<	<	<
Industrial 2	-	<	-	<	-	<	-	<	-	<	-	<	-	<
Industrial 3	-	<	-	<	-	<	-	<	-	<	-	<	-	<
Industrial 4	-	<	-	<	-	<	-	<	-	<	-	<	-	<
Lugo	-	<	-	<	-	<	-	<	-	<	-	<	-	<
Miño	<	<	<	<	<	98	<	<	<	32	<	<	<	<
Moaña	-	1,573	-	<	-	92	-	<	-	<	-	<	-	<
Pontevedra	390	<	<	<	<	<	<	<	<	<	<	<	<	<
Ordes	<	<	256	<	118	<	<	<	<	<	<	<	<	<
Ribadeo	76	<	<	<	<	<	<	<	<	<	<	<	<	<
Ribadumia	<	<	<	<	<	<	<	<	<	<	<	<	<	<
Ribeira	150	<	<	<	137	56	<	<	<	<	<	<	114	20
Sada	-	<	-	<	-	<	-	<	-	<	-	15	-	27
Santiago	351	111	<	<	649	<	<	<	65	<	<	<	23	<
Sanxenxo	319	297	<	<	892	<	<	<	<	<	<	<	43	<
Teo 1	<	<	<	<	<	<	<	<	<	<	<	<	<	<
Teo 2	<	-	95	-	<	-	<	-	<	-	<	-	<	-
Tui	<	<	<	<	<	<	<	31	<	<	<	<	<	<
Vilagarcía	293	<	<	<	37	<	<	<	<	<	400	<	57	<

< indicates the antibiotic was below detection level and – indicates no data.

Statistical Treatment

Mean ± SD values of the general soil properties were used to compare the soils amended with different sludges and under different crops. These data were analyzed by performing a standard analysis of variance (ANOVA 1), and in the cases of significant F statistics, the Tukey's minimum significant different

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test was used to separate the means, using the statistical package SPSS 27.0 (IBM, USA). For the sludge and mixtures, the study of Pearson correlations ($p < 0.01$) among concentrations of antibiotics and total elements and physicochemical properties was performed using the R software package (R studio, version 4.1.0).

RESULTS AND DISCUSSION

Presence of Antibiotics in Sludge From WWTPs

Altogether in both sampling campaigns (spring and autumn of 2019) seven different antibiotics were detected in the WWTP sludge samples analyzed, with concentrations generally bigger in the first than in the second sampling date. In the first sampling 15 out of 22 of the sludge samples (68%) had at least one antibiotic detected, while 7 of the sludge samples had concentrations below the detection level for all the antibiotics analyzed (**Table 3**).

In the second sampling, which was performed in autumn, just 32% of the samples (9 out of 28) presented some antibiotic at detectable concentrations, even though one the samples showed the highest antibiotic concentration detected in the whole study, which reached 1,573 ng g^{-1} for ciprofloxacin (**Table 3**). Previous studies have shown that the presence of antibiotics in WWTP sludge has a seasonal pattern. In this regard, Golovko et al. (2014) found in water from WWTPs higher concentrations of different antibiotics in winter than in the rest of the year. In the northern hemisphere, antibiotics consumption peaks between January and March (Van Boeckel et al., 2014), which can lead to a total daily pharmaceutical load in the sludge as high as 1 kg day^{-1} in that season (Aydın et al., 2022). In the current study, the higher presence of antibiotics in spring than in autumn might be due to this seasonal consumption pattern.

The number of antibiotics detected in the present study was generally lower than values reported by other authors. Aydın et al. (2022) found ten different antibiotics in WWTP sludge in Turkey, with maximum concentrations detected in the same range that in the current study. In different works carried out in China, the maximum concentration of antibiotic detected in sewage sludge was higher than in our study. Zhou et al. (2013) detected 21 different antibiotics with maximum values up to 5,800 ng g^{-1}, 4-fold higher than our maximum; while Huang et al. (2020) described the presence of 24 different antibiotics in WWTP sludge with maximum concentration of the antibiotics reaching up to 11,000 ng g^{-1}, 7-fold higher than our maximum values. Ivanová et al. (2018) reported the presence of 15 different antibiotics in five WWTP in Slovakia, but the maximum concentration was relatively low, reaching 336 ng g^{-1} for azithromycin, antibiotic which was not detected in our study. On the other hand, Östman et al. (2017) detected only four different antibiotics in sludge samples in Sweden, but with the maximum concentration being 7-fold higher than for our samples. In the same geographic area of our study, the NW of Spain, Conde-Cid et al. (2018) found 8 different antibiotics in 42% of manure samples analyzed, but with maximum concentration values being more than 60-fold higher than those corresponding to our results for sludge.

The WWTP´s with greater number of antibiotics detected in our first sampling were Boiro, Santiago de Compostela and Vilagarcía (**Table 3**). Ciprofloxacin was the more frequently detected antibiotic in this sampling, present in 45% of the sampled sludge, with a maximum value of 623 ng g^{-1} detected in the industrial WWTP (**Table 3**). This same antibiotic showed the maximum concentration detected in our study, found in the second sampling, with a concentration as elevated as 1,573 ng g^{-1} in Moaña´s WWTP (**Table 3**). Several authors have described this antibiotic (ciprofloxacin) as one of the dominant emerging contaminants in all environmental compartments, detecting maximum values of 1,040 ng g^{-1} in sludge (Huang et al., 2020), or founding it in most of the WWTP effluents, with maximum concentration from 264 ng L^{-1} (Loos et al., 2013) to 2,733 96 ng L^{-1} (Nas et al., 2021). In the current study, the second most frequent antibiotic detected in the spring sampling was trimethoprim, which was present in 41% of the samples, with maximum values up to 114 ng g^{-1} in Ribeira´s WWTP sludge (**Table 3**). In a previous investigation this antibiotic was detected in 93% of the WWTP effluents analyzed in Europe (Loos et al., 2013). In the present work, the high frequency of trimethoprim detected could be related to the lack of a specific tertiary treatment (a sand filtration system) which has been described to reduce 60% of the amount of this antibiotic in activated sludge (Gobel et al., 2005). As shown in **Supplementary Table S1**, this filtration system was not present in any of the WWTPs of our study.

Among the less frequently detected antibiotics in the spring sampling, levofloxacin appeared just in 6 of the WWTPs (27%) but showed the highest antibiotic concentration detected at this sampling campaign, 892 ng g^{-1} in Sanxenxo´s WWTP. Other authors have included this antibiotic among the emerging compounds with highest concentration in sludge in WWTPs (Okuda et al., 2009), with values ranging from 204 ng g^{-1}–8,680 ng g^{-1}. Also, the difficulty to remove it by means of the treatments usually applied in WWTPs has been previously described (Martínez-Orgániz et al., 2021). The other antibiotics detected, clarithromycin, norfloxacin and sulfadiazine, were present in 4, 2 and 1 of the samples, respectively, with the maximum value found for the later (reaching 400 ng g^{-1}) in Vilagarcía´s WWTP. Contrarily to our results, other authors detected clarithromycin in 67% of the sludge samples (Aydın et al., 2022), and norfloxacin in 100% of the samples (Gao et al., 2012), with maximum values of 1,496 and 3,200 ng g^{-1} respectively. Loos et al. (2013), in a study of the WWTP effluents in Europe detected sulfadiazine only in 3% of the samples.

In the second sampling campaign of the current study (autumn) the most frequent antibiotic was levofloxacin, which was present in 5 samples (one of them present in the first sampling as well) with maximum values of 98 ng g^{-1} in Miño´s WWTP. Ciprofloxacin was present in 4 samples, showing the highest concentration found in this research, and was present as well in the first sampling in 2 of these WWTPs (**Table 3**). Norfloxacin and trimethoprim appeared in 2 samples, with maximum values of 42 and 27 ng g^{-1}, respectively, while

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TABLE 4 | Concentration (ng g^{-1}) of antibiotic in the sludge mixtures from the different residue managers (RM) at the spring and autumn sampling time. Antibiotic detected: ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR), lincomycin (LIN).

Mixtures Spring	CIP	LEV	NOR	LIN
RM1 – Mixture 1	<	<	551	<
RM1 – Mixture 2	<	<	<	<
RM3 – Mixture 1	<	<	587	<
RM4 – Mixture 1	286	369	<	<
RM4 – Mixture 2	391	289	<	<
RM4 – Mixture 3	394	393	<	<
Mixtures Autumn				
RM1 – Mixture 1	<	<	<	40
RM1 – Mixture 2	<	<	<	40
RM1 – Mixture 3	<	<	<	26
RM2 – Mixture 1	<	<	<	<
RM4 – Mixture 1	<	<	<	<
RM4 – Mixture 2	<	<	<	<
RM4 – Mixture 3	<	169	<	<

< indicates the antibiotic was below detection level.

sulfadiazine and lincomycin were present in just one sample (at 15 and 31 ng g^{-1} respectively). This last one antibiotic only appeared in this sampling, but was found in 37% of the WWTP effluents in an European survey (Loos et a., 2013).

Several previous studies have indicated that the primary and secondary treatments usually applied in WWTPs are not enough for removing many emerging pollutants, such as most antibiotics from both water effluents and sludge (Jelic et al., 2011; Verlicchi et al., 2012; Verlicchi and Zambello 2015; Nguyen et al., 2021), even though some tertiary treatments, like ozone disinfection, can provoke the degradation of macrolide antibiotics such as clarithromycin (Lange et al., 2006). In our study, only one of the WWTPs that presented this antibiotic in the sludge had a disinfection treatment, but was with UV and not ozone, which was not used in any of the WWTPs covered. In our samples the less frequent antibiotics were lincomycin and sulfadiazine. The latest belongs to the sulfonamides group, which have been reported to be easily biodegradable (García-Galán et al., 2011) and transformed during anaerobic sludge digestion (Gobel et al., 2005), a secondary treatment present in most WWTPs.

Presence of Antibiotics in the Sludge Treated by Residue Managers

The sludge treated by the different residue managers (RM) showed a reduced number of antibiotics, compared with the WWTP sludge not subjected to treatment. Specifically, the mixtures from RM1 presented norfloxacin (551 ng g^{-1}) in one of the mixtures from the spring sampling, and lincomycin in the three mixtures from the autumn sampling (maximum value 40 ng g^{-1}) (**Table 4**). The mixture from RM3 had, similarly to RM1, a high concentration of norfloxacin (587 ng g^{-1}), while no antibiotic was detected in the mixture from RM2. The three mixtures from RM4 sampled in spring showed two antibiotics, ciprofloxacin and levofloxacin, with maximum values of 394 ng g^{-1} and 393 ng g^{-1}, respectively, while in the autumn sampling only the mixture 3 presented levofloxacin (at 169 ng g^{-1}, **Table 4**).

As indicated by Nguyen et al. (2021) and Jelic et al. (2011), most of the treatments currently applied in WWTPs are not effective for removing antibiotics, some of which are accumulated in sludge, triggering the need of further sludge treatment to be carried out by residue managers. In the current work, this management consisted in the addition of other organic wastes (animal or vegetal) obtaining a final mixture, in some cases subjected to composting. The comparison between the concentrations of antibiotics detected in the sludge used by each RM and the resulting mixture (**Figure 3**), indicates the effectiveness of the residue management in the antibiotic removal. Specifically, RM1 successfully removed ciprofloxacin, clarithromycin, levofloxacin, sulfadiazine and trimethoprim from the sludge, even though two other antibiotics that were not detected in the sludge (lincomycin and norfloxacin) appeared later in the treated mixtures (**Figure 3**), the latter in high concentrations, which could be originated in the materials added by this RM. In this regard, it should be noted that the mixture 1, that showed high norfloxacin concentrations, had chicken manure included and poultry production industry uses norfloxacin for the prevention and therapy of infectious diseases in some countries (Al-Mustafa and Al-Ghamdi, 2000). However, in the European Union norfloxacin is used in human medicine, but it is not allowed as a veterinary medicine (Pereira et al., 2018), remaining unclear the origin of the norfloxacin in this mixture in the present work. Also to note that the same RM generates three other mixtures that presented small concentrations of lincomycin, without being this antibiotic present in the urban sludge used for the mixing. Sludge from an additional industrial WWTP was added to these mixtures, suggesting a possible industrial origin of the lincomycin, since this antibiotic has been detected in all the WWTPs analyzed in a study in an industrial city in Korea (Behera et al., 2011). However, in the current study we found it only in one of the urban samples and in none on the industrial ones analyzed, thus its origin also being unclear.

RM2 successfully removed levofloxacin and norfloxacin from the sludge. The sludge used by RM3 had no antibiotic detected, but the residue management introduced norfloxacin in the treated mixture. This residue manager, like RM1, added different sludge from industrial WWTPs that may introduce antibiotics that were no present in the urban sludge. On the other hand, RM4 managed to remove most of the antibiotics detected in the sludge, and decreases the amount of ciprofloxacin and levofloxacin, which showed high concentrations in the WWTP sludge (**Figure 3**). This RM carried out a composting process with wood chips. Previous studies reported the removal of levofloxacin from aqueous solutions with a biochar made of wood chips (Yi et al., 2016) and the persistence of ciprofloxacin in composted sludges (Khadra et al., 2019). The compost process is a well-known technology effective in the reduction of antibiotics from sludge, with ranges of removal 17%–100% (Verlicchi and Zambello 2015; Ezzariai et al., 2018). In general, in the current study the management executed by the different residue

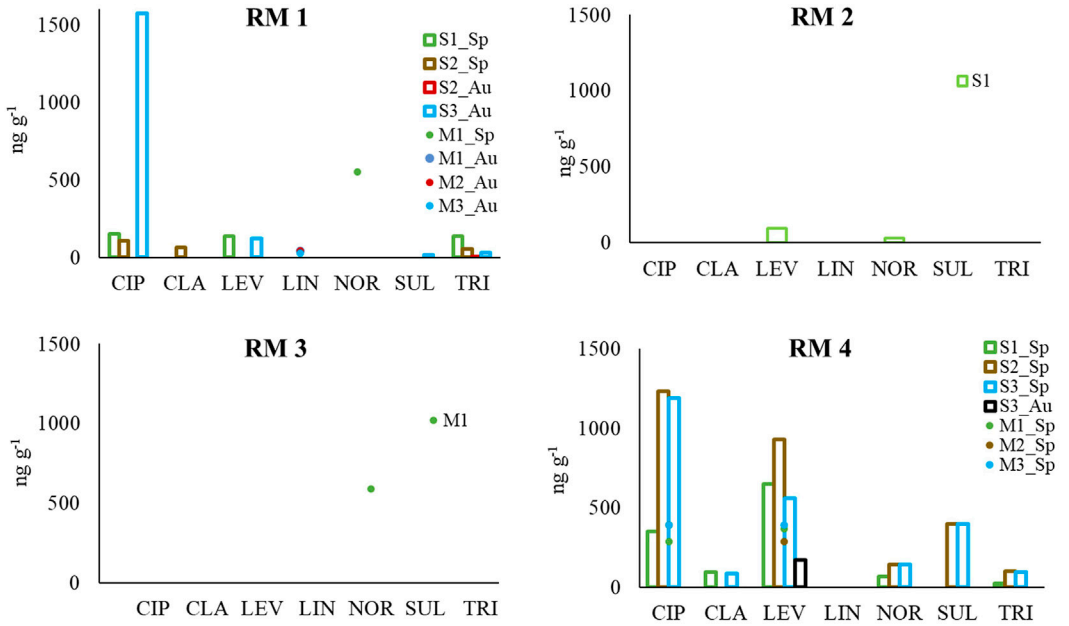

FIGURE 3 | Concentration (ng g^{-1}) of antibiotics present in the sludge (S) from wastewater treatment plants (sum of all antibiotics present in the sludge used for each mixture) and in the mixtures (M) generated by different residue managers (RM). Antibiotics detected: ciprofloxacin (CIP), clarithromycin (CLA), levofloxacin (LEV), lincomycin (LIN), norfloxacin (NOR), sulfadiazine (SUL), trimethoprim (TRI).

managers was successful in the removal or reduction of antibiotics concentrations from the WWTPs sludge.

Physicochemical Characteristics of Sludge and Mixtures

The physicochemical properties of the various sludges and mixtures differed widely, with ranges 5-12 for pH, 2.25%–57.47% for C, and 0.75%–60.85% for N (**Supplementary Table S2**). Similarly, the total elements contents showed great variation among the different sludge and mixture samples, with values (in mg kg^{-1}) between 107-9078 (for Na), 549-6395 (Mg), 143-26106 (Al), 0.6-25.1 (P), 343-8161 (K), 3-230 (Ca), 0-797 (Cr), 25-3595 (Mn), 133-48800 (Fe), 0.9-426 (Ni), 7-1229 (Cu), 0-1777 (Zn), 0.5-120 (As), 0-29 (Cd), 0-447 (Hg), and 0.4-110 (Pb). The WWTPs that presented the highest concentrations of total elements were Lugo (Na and P), Pontevedra (Cr, Fe, and Ni), Curtis (Cu and As), and Ferrol (Hg and Pb) (**Supplementary Table S2**). Regarding the sludge mixtures, the highest values for most of the elements were detected in mixtures from RM1 (Na, P, K, Ca, Cr, Mn, Ni, Cu, Zn, Cd, Hg and Pb), meanwhile a mixture from RM2 had higher concentration of Fe, and mixtures from RM4 showed the highest scores for Mg, Al, Cr and As (**Supplementary Table S2**).

Comparing with other studies, the values of pH were in the same range (Onchoke et al., 2022), while another study carried out in the USA with a lower number of WWTPs (Onchoke et al., 2018) showed narrower ranges for C (30–32%) and N (8–10%), and higher values of P (935–1705 mg kg^{-1}), and Ca (1,521–3,869 mg kg^{-1}), but the maximum values of Mg (709 mg kg^{-1}), K (679 mg kg^{-1}), Na (2,303 mg kg^{-1}), Al (12,857 mg kg^{-1}), Cr (20 mg kg^{-1}), Mn (1,103 mg kg^{-1}), Fe (22,688 mg kg^{-1}), Ni (19.8 mg kg^{-1}), Cu (550 mg kg^{-1}), Zn (772 mg kg^{-1}), As (13 mg kg^{-1}), Cd (0.5 mg kg^{-1}), Hg (0.6 mg kg^{-1}), and Pb (34 mg kg^{-1}) were significantly smaller than the maximum concentrations detected in our samples. Similarly, in a study in Poland Latosinska et al. (2021) reported that the maximum concentrations of Cu (196 mg kg^{-1}), Cr (2,760 mg kg^{-1}), Cd (12 mg kg^{-1}), and Ni (44 mg kg^{-1}) in sludge were smaller than in our samples, but Pb (427 mg kg^{-1}) and Zn (5,351 mg kg^{-1}) showed higher concentrations.

According to the Spanish regulation regarding the maximum concentration of heavy metals allowed in sludge to be suitable for field application (Royal Decree 1,310/1990), practically all the sludge samples from the spring sampling had values above the threshold allowed for Hg (16–25 mg kg^{-1}). The values detected in our sludge samples, with maximum of 447 mg kg^{-1}, far outweigh the results from other authors, which were in the range 0.6–6.1 mg kg^{-1} of total Hg (Mao et al., 2016; Onchoke et al., 2018). The management of the sludge did not manage to remove Hg in three of the spring mixtures (mixtures 1 and 2 from RM1, and RM3 mixture), as they presented Hg concentrations in the range 171–337 mg kg^{-1}. The mixture from RM3 was no applied to the field, but the mixtures from RM4 were spread, which supposes a clear pollution risk.

The fact that the sludge from the industrial WWTPs had Hg values conforming to the regulations, contrary to the urban ones, and that the samples with the highest concentrations belong to the WWTPs of the bigger cities, suggests a domestic origin of these elevated concentration of Hg, such as amalgams, food, laundry detergent, or other, like dentist business of pipe sediments

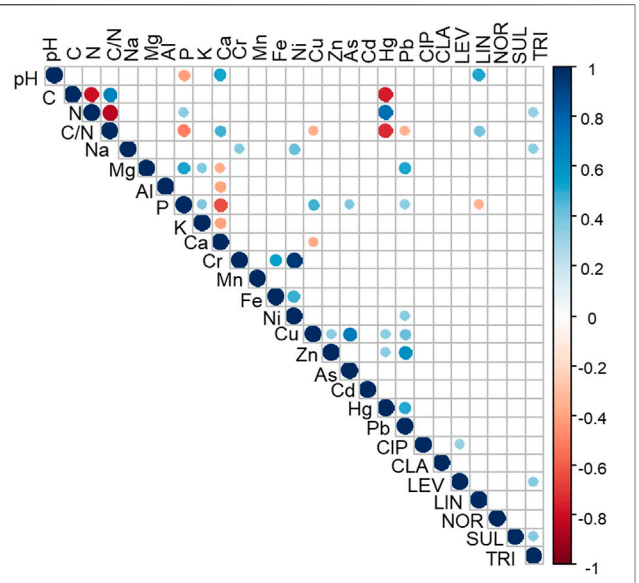

FIGURE 4 | Correlations among the sludge and sludge mixtures physicochemical properties, total element concentration and the sludge and sludge mixtures antibiotics concentrations. Blue and red dots show positive or negative (respectively) significant correlation Pearson coefficients ($p < 0.01$). Ciprofloxacin (CIP), clarithromycin (CLA), levofloxacin (LEV), lincomycin (LIN), norfloxacin (NOR), sulfadiazine (SUL), trimethoprim (TRI).

(Sörme et al., 2003). Previous studies correlated the high concentration of Hg detected in cattle in Galicia with the coal-fired power plants present in the area, that caused high Hg dispersion and deposition (López-Alonso et al., 2003), however nowadays these stations have been closed. The concentrations of the other heavy metals across the different WWTPs were among the Spanish regulation limits, with punctual exceptions.

The analysis of the correlations among antibiotics concentrations and chemical properties in sludge and sludge mixtures showed that lincomycin was correlated with pH (R = 0.52, $p < 0.001$), C/N ratio (R = 0.4, $p < 0.05$) and P content (R = -0.34, $p < 0.05$); while trimethoprim was correlated with N (R = -0.32, $p = 0.01$); and Na (R = 0.34, $p < 0.05$) (**Figure 4**). To note that parameters related to organic matter appear in the correlations, evidencing its marked influence on the retention of antibiotics the soil environment. Physicochemical properties are determinant in the adsorption of antibiotics in soils or bio-adsorbents (Cela-Dablanca et al., 2021, 2022), and similar adsorption mechanisms might take place in sludge. The concentrations of some of the antibiotics were positively correlated with each other: trimethoprim with levofloxacin (R = 0.37, $p < 0.01$) and with sulfadiazine (R = 0.37, $p < 0.05$), and ciprofloxacin with levofloxacin (R = 0.33, $p < 0.01$). In this regard, it should be noted that trimethoprim is used against different infections, from urinary to respiratory ones, and the antibiotic levofloxacin has emerged as an alternative for respiratory infections (Sarzynski et al., 2022). On the other hand, trimethoprim is sometimes administrated simultaneously with sulfadiazine to combat bacterial infections in animals, and ciprofloxacin and levofloxacin (both quinolone antibiotics) are used in a wide range of bacterial infections.

Impact on Agricultural Soils and Crops due to the Spreading of Treated Sludge

The physicochemical properties of the soils amended with the mixtures treated by the different residue managers are shown in **Table 5**. Regarding grain sizes and soil texture, the sand fraction clearly predominates, with a percentage above 45% in all cases. Soils amended with mixtures from RM1 (corn) and RM4 (vineyard) show high pH values (7.90 and 7.01, respectively), meanwhile the corn soils amended with mixtures from RM2 and RM4 had an acidic pH (5.32 and 5.53, respectively). The dominant exchange cation was Ca in all cases, and it is especially clear in the RM1-amended soils. These soils also presented the highest values of the other exchange cations, as well as the lowest values of exchangeable Al, coinciding with the highest pH. Also noteworthy are the high values of organic matter in the soils with the mixtures from RM1 (13.04%), while in the other soils the values were lower (between 6 and 6.7%) (**Table 5**). Non-crystalline Fe compounds (Fe_{ox} and Fe_{pir}) were more abundant in soils amended with RM2 and RM4 (corn). Non-crystalline Al compounds, however, showed higher levels in soils amended with RM1 mixtures.

Out of the 59 soils sampled, only seven soils showed detectable levels of antibiotics: two soils amended by RM2, and the other five by RM4 (**Table 6**). The two soils amended by RM2 had corn as crop, and one of them showed a concentration of 57 ng g^{-1} of amoxicillin, while the other one had 41 ng g^{-1} of clarithromycin. Regarding the soils amended by RM4, the three soil samples under corn contained the antibiotic cefuroxime, with maximum values of 276 ng g^{-1}, while the other two soils which were placed under vineyard showed small amounts of amoxicillin (**Table 6**). The mixture from RM2 did not show clarithromycin and the other two antibiotics were not detected in any of the sludge samples, nor for those coming directly from WWTPs, neither after the processing in the residue manager facilities (**Tables 3**, **4**). The presence of antibiotics in these soil samples might be due to their dispersion through contaminated WWTPs effluents (Zuccato et al., 2010) or to the persistence of antibiotics in the soil after several years of sludge application, even though long-term application of sludge in arable lands has been shown to not being a probable cause of antibiotics accumulation in soils (Rutgersson et al., 2020). On the other hand, the continues application of slurry and manure, especially swine slurry, has been demonstrated that contributes building up persistent antibiotic residues in the uppermost layers of the soil (Gros et al., 2019). No antibiotic was detected in any of the leaves, stem, grain, roots or grape samples (**Figure 2**). The post-WWTP management of the sludge is crucial to avoid soil contamination with antibiotics. Our results, with just 12% of the sampled soils presenting one antibiotic per sample, indicate the efficiency of the different management methods. Conde-Cid et al. (2018) studied the presence of veterinary antibiotics in soils amended with manure, detecting these compounds in 17% of the soil samples, with maximum values of 600 ng g^{-1}, doubling the maximum detected in the soils amended with the managed sludge in the current research. Composting have been proved to reduce antibiotics in both manure and sludge samples (Dolliver

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TABLE 5 | Physicochemical properties (average ± DE) of soil amended with mixtures from different residue managers (RM) and under corn and vineyard crops. OM (organic matter); eCEC (effective cation exchange capacity); Al_e (extractable Al); Fe ox (non-crystalline Fe); Fe pir (organic Fe); Al ox (non-crystalline Al); Al pir (organic Al). Different letters mean significant differences $p < 0.05$.

	Soil under corn		Soil under vineyard	
	RM1	RM2	RM4	RM4
pH (H_2O)	7.90 ± 0.10c	5.32 ± 0.28a	5.53 ± 0.42a	7.01 ± 0.13b
pH (KCl)	7.67 ± 0.07d	4.71 ± 0.18a	5.29 ± 0.50b	6.45 ± 0.19c
Sand (%)	53.95 ± 3.90b	46.24 ± 5.41a	49.16 ± 6.64ab	67.73 ± 2.34c
Silt (%)	28.53 ± 3.89b	32.99 ± 6.06c	28.36 ± 3.34b	15.63 ± 2.41a
Clay (%)	17.52 ± 3.29a	20.77 ± 1.69ab	22.48 ± 4.45b	16.64 ± 2.90a
Ca $cmol_{(+)}kg^{-1}$	47.28 ± 5.80c	5.30 ± 0.84a	8.85 ± 5.47ab	11.01 ± 2.77b
Mg $cmol_{(+)}kg^{-1}$	3.05 ± 0.86b	1.04 ± 0.09a	0.88 ± 0.35a	0.88 ± 0.31a
Na $cmol_{(+)}kg^{-1}$	0.70 ± 0.30b	0.20 ± 0.05a	0.05 ± 0.05a	0.15 ± 0.11a
K $cmol_{(+)}kg^{-1}$	1.38 ± 0.67b	0.19 ± 0.03a	0.42 ± 0.20a	0.47 ± 0.16a
Al $cmol_{(+)}kg^{-1}$	0.02 ± 0.01a	0.51 ± 0.35c	0.26 ± 0.24b	0.03 ± 0.01a
eCEC $cmol_{(+)}kg^{-1}$	52.43 ± 6.99b	7.23 ± 0.75a	10.46 ± 5.83a	12.54 ± 3.16a
Al_e (%)	0.05 ± 0.02a	7.23 ± 4.92c	3.50 ± 3.48b	0.27 ± 0.27a
P mg kg^{-1}	181.89 ± 27.47c	16.09 ± 1.85a	52.09 ± 12.27b	25.33 ± 9.99a
C (%)	7.56 ± 1.54b	3.53 ± 0.21a	3.56 ± 1.55a	3.90 ± 0.83a
OM (%)	13.04 ± 2.66b	6.08 ± 0.36a	6.13 ± 2.67a	6.72 ± 1.44a
N (%)	0.55 ± 0.15c	0.36 ± 0.03b	0.34 ± 0.12ab	0.24 ± 0.06a
C/N	14.07 ± 1.39b	9.85 ± 0.51a	10.26 ± 0.70a	16.66 ± 1.48c
Fe ox (mg kg^{-1})	4,929 ± 1339b	8,161 ± 2030d	6010 ± 646c	2,450 ± 488a
Fe pir (mg kg^{-1})	2,552 ± 585b	3,362 ± 319c	3,281 ± 224c	1,396 ± 267a
Al ox (mg kg^{-1})	7235 ± 2552a	1,632 ± 1170a	4,561 ± 2342b	4,126 ± 948b
Al pir (mg kg^{-1})	3,392 ± 1012b	1,409 ± 140a	3,189 ± 1135b	2,548 ± 633b

TABLE 6 | Concentration (ng g^{-1}) of antibiotic in soils under corn and vineyard crops and amended with compost/mixtures from the different residue managers (RM). Antibiotic detected: amoxicillin (AMO), cefuroxime (CEF), clarithromycin (CLA).

	RM	AMO	CEF	CLA
Soil under corn	RM2	57	<	<
		<	<	41
	RM4	<	150	<
		<	213	<
		<	276	<
Soil under vineyard	RM4	*	<	<
		*	<	<

*< indicates the antibiotic was below detection level and * indicated below quantification level.*

et al., 2008; Ezzariai et al., 2018). In the present study, the presence of antibiotics in the mixtures that were lacking in the initial sludge highlights the need of carefully selecting the materials to be combined with the sludge for the composting process. Indeed, the co-composting of contaminated sludges with agricultural wastes has been suggested as a good strategy for the sanitation of compost (Alvarenga et al., 2015). These latter authors mentioned, however, that the risk of heavy metals accumulation driven by the composting process should be considered.

In previous investigations, different crops growing in manure-applied soils have shown antibiotic uptake (Bassil et al., 2013), but in some cases the values were below the quantification limit (Kang et al., 2013). In Galicia, previous studies showed that 44% of the crops amended with manure had detectable concentrations of veterinary antibiotics (Conde-Cid et al., 2018), these crops including grass, corn and wheat samples, with maximum values detected in corn, reaching up to 600 ng g^{-1} for some veterinary antibiotics. The absolute absence of any antibiotic detected in plant material in the current research highlights the importance of the residue management before application to agricultural fields. In this sense, the spreading of manure, contrarily to what happens with sludge, is not regulated, and no management its required before field application in Spain, with less than 10% of the total animal manure being processed at the European level (Foged et al., 2011). It is possible that the situation in other regions and countries differ from this, although it means that we need to do further efforts regarding emerging pollutants monitoring. The impact of antibiotic contamination includes the presence of ARGs (antibiotic resistance genes) that has been reported in crops growing in soils where un-treated WWTP sludge was applied (Rahube et al., 2016), which is a real issue of concern as regards environmental and human health.

CONCLUSION

The treatments applied in the wastewater treatment plants facilities investigated in the current study were not effective in removing the variety of antibiotics used in human medicine that were determined, which were specifically accumulated in sludge. In fact, most of the sludge studied contained some antibiotics, especially in the spring sampling, which is related to the greater consumption of these drugs in certain months. The antibiotics most frequently detected and in the greatest quantity in these sludge materials were ciprofloxacin and levofloxacin. The lack of

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specific regulation regarding these emergent pollutants in sludge and soils makes this an environmental problem needing further efforts to be correctly addressed. Our findings suggest that additional treatments of the sludge derived from these WWTP, carried out by waste managers, are effective in reducing the risk of environmental pollution due to antibiotics, specifically in the case of soils amended with these organic materials, preventing their entry in the food chain through the crops that grow in those agricultural soils.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/sjss.2022.10741/full#supplementary-material

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Supplementary Material

Occurrence of nine antibiotics in different kinds of sewage sludge, soils, corn and grapes after sludge spreading

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TABLE S1. Treatment applied at the different urban wastewater treatment plants (WWTP). Primary treatments include removal of floating and suspended solids, sand, fat and oils, using roughing of solids, sand trap, degreaser, sedimentation and anaerobic digestion processes. Secondary treatments are biological treatments that remove organic matter, such as active mud, aerobic digestion, aerated lagoons, peat beds and biodiscs.

WWTP	Primary treatment	Secondary treatment	Tertiaty treatments			
			UV desinfection	Chlorination	Microfiltration	Thickener
A Coruña	X	X	X			X
A Estrada	X	X				
A Guarda	X	X	X			
Ares	X	X		X		
Betanzos	X	X	X		X	
Boiro	X	X	X			
Cambados	X	X		X		
Cangas	X	X	X	X		
Cedeira	X	X	X		X	
Curtis	X	X	X		X	X
Ferrol	X	X	X			
Lugo	X	X		X		
Miño	X	X	X			
Moaña	X	X	X			
Pontevedra	X	X	X			
Ordes	X	X				
Ribadeo	X	X				
Ribadumia	X	X	X			
Ribeira	X	X	X			
Sada	X	X	X		X	
Santiago	X	X				
Sanxenxo	X	X	X			
Teo 1	X	X		X		
Teo 2	X	X				
Tui	X	X	X			
Vilagarcía	X	X	X			

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TABLE S2. Physicochemical properties (% C and N) and element total concentration (mg kg^{-1}) of the sludge from the wastewater treatment plants (WWTP) and mixtures (M) treated for the different residue managers (RM), sampled in spring (Sp) and autumn (Au).

WWTP	Time	pH	C	N	C/N	Na	Mg	Al	P	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	As	Cd	Hg	Pb
A Coruña	Sp	7.9	5.4	34.8	0.16	1488	3629	9380	15.1	1391	18.0	43.9	218	17428	24.3	283	1179	16.0	1.2	410.5	101.1
A Coruña	Au	8.0	31.3	5.6	5.58	2098	5381	9252	19.8	1631	13.3	46.0	230	21655	27.6	345	1559	17.5	1.0	0.6	96.6
A Estrada	Sp	6.9	7.3	40.3	0.18	1057	3304	6924	15.8	5955	4.1	27.7	157	6132	24.4	458	622	4.3	1.1	120.5	28.4
A Estrada	Au	5.8	38.2	6.2	6.19	380	2991	9680	10.6	2446	4.0	30.9	192	8390	24.1	611	648	5.8	0.9	0.7	31.8
A Guarda	Sp	5.2	5.8	37.4	0.16	240	2421	13537	12.1	2699	3.2	32.6	93	9217	27.4	411	356	7.6	0.8	228.6	54.6
A Guarda	Au	5.7	39.2	6.7	5.83	1335	3265	6346	13.4	3334	4.6	15.2	64	4627	16.7	455	646	10.4	1.1	0.3	63.7
Ares	Sp	5.7	4.7	29.6	0.16	3035	4318	13650	13.6	3023	5.4	40.9	115	27884	22.5	404	664	12.2	0.6	219.9	53.2
Ares	Au	5.4	28.4	4.7	6.05	936	4240	15095	14.3	3186	5.1	32.8	138	27920	22.3	396	523	14.6	0.6	0.4	64.7
Betanzos	Sp	6.1	5.6	34.7	0.16	4513	4476	14890	14.1	2879	3.8	17.8	153	8381	18.2	118	265	7.9	0.3	142.8	34.2
Betanzos	Au	5.7	36.3	5.9	6.14	2273	3844	14617	15.1	2620	4.3	7.7	291	7396	13.1	129	236	8.6	0.4	0.3	33.4
Boiro	Sp	5.4	7.0	43.0	0.16	1405	4245	4554	18.5	7088	4.4	21.7	45	3292	12.7	200	417	9.9	0.7	124.3	29.0
Boiro	Au	5.7	39.8	7.2	5.57	1008	3732	5964	15.2	5072	3.3	21.4	51	4702	15.3	234	441	9.9	0.8	0.4	37.3
Cambados	Au	5.8	37.6	6.9	5.42	4564	4539	4713	16.1	3814	4.1	20.4	57	17377	13.0	515	574	19.9	1.0	0.3	28.5
Cangas	Au	5.7	39.0	6.8	5.74	3115	2619	2830	3.9	5126	55.4	7.0	383	2802	9.0	46	170	2.6	0.2	0.1	29.5
Cedeira	Sp	5.9	5.5	32.8	0.17	1212	3296	16394	16.7	1968	6.6	24.3	92	6979	18.2	243	377	6.6	0.5	167.2	39.6
Curtis	Sp	5.6	6.0	35.5	0.17	581	3633	8533	24.6	4258	4.8	80.1	272	42262	40.3	1229	497	120.4	0.6	219.8	52.9
Curtis	Au	5.7	35.2	6.3	5.56	424	3312	6312	17.4	4281	3.0	91.3	140	23411	22.2	692	353	95.2	0.3	2.4	42.1
Ferrol	Sp	7.5	3.5	26.5	0.13	868	4074	25803	15.6	1753	7.3	44.5	292	16022	27.1	442	857	32.0	0.9	446.6	110.4
Industrial 1	Sp	6.2	3.6	60.9	0.06	860	549	7626	9.5	1624	3.5	14.2	65	8843	7.5	28	368	1.3	0.2	16.5	2.9
Industrial 1	Au	6.1	57.5	4.5	12.64	608	557	8636	12.0	915	4.4	10.4	97	14412	7.2	25	213	5.4	0.0	0.0	2.4
Industrial 2	Au	6.0	39.8	7.2	5.50	4350	2006	13452	19.6	4816	3.9	0.0	48	2192	3.8	25	53	0.6	0.0	0.1	4.3
Industrial 3	Au	6.2	13.4	0.7	18.03	4620	1891	143	0.6	343	230.4	2.1	26	133	0.9	7	0	0.5	0.0	0.0	0.4
Industrial 4	Au	6.6	29.4	3.7	8.03	4417	1237	3130	6.6	1645	90.9	7.1	3595	2578	11.9	14	103	2.8	0.7	0.4	38.8

Lugo	Au	7.8	29.7	5.3	5.60	440	6395	16625	25.1	3103	12.6	168.3	149	9899	21.6	197	809	16.7	0.8	0.6	101.5
Miño	Sp	5.8	6.1	35.4	0.17	435	3240	15064	13.9	4047	3.2	17.1	114	8829	13.7	199	298	9.4	0.6	169.1	40.3
Miño	Au	6.2	35.6	6.3	5.65	493	3344	17120	14.5	4245	4.2	38.3	129	11083	17.2	288	580	9.8	0.6	0.2	48.8
Moaña	Au	5.7	36.3	6.4	5.71	5068	3436	13809	8.5	3443	30.2	30.5	436	9784	32.1	230	1777	12.2	0.6	0.4	52.7
Pontevedra	Sp	6.0	4.0	37.7	0.11	8478	4434	5410	13.8	1486	11.7	439.9	85	39393	299.0	458	494	3.9	4.9	358.6	85.9
Pontevedra	Au	5.7	35.9	3.6	9.98	7263	4183	4894	18.9	1514	10.2	797.0	79	48800	426.0	492	397	6.0	2.0	0.4	73.9
Ordes	Sp	6.5	6.8	39.1	0.17	667	4881	8342	22.4	7421	5.1	27.4	118	7333	19.9	354	336	6.3	0.6	199.7	48.2
Ordes	Au	6.1	39.4	7.0	5.61	527	3839	5731	17.8	6171	3.9	22.5	92	5925	16.3	286	339	6.1	0.4	0.6	45.0
Ribadeo	Sp	5.2	6.4	38.8	0.16	1250	3162	5411	19.1	2841	5.5	27.0	90	24360	15.7	222	418	9.4	0.7	185.1	44.4
Ribadeo	Au	5.5	37.7	6.7	5.66	870	3017	5061	17.2	2423	5.8	35.8	111	22036	20.9	206	723	7.5	0.7	0.3	62.2
Ribadumia	Sp	7.4	6.2	32.9	0.19	2350	2590	26106	23.7	4043	3.4	42.0	64	5358	14.2	248	551	12.2	2.4	52.9	11.9
Ribadumia	Au	5.7	35.8	6.9	5.17	3229	2559	12762	17.6	4565	3.4	20.4	104	3914	15.8	251	380	16.5	0.9	0.4	11.0
Ribeira	Sp	5.5	5.6	35.2	0.16	9078	5172	4560	22.5	4022	6.2	27.8	58	32872	36.4	228	477	12.8	0.7	274.8	67.0
Ribeira	Au	5.8	37.6	6.0	6.28	7124	4198	14748	18.6	2374	9.9	19.0	60	6944	48.6	380	708	10.2	2.0	0.4	62.6
Sada	Au	5.5	38.5	7.2	5.38	1392	3540	6569	14.4	4874	6.8	7.4	71	5022	12.9	304	410	32.3	28.9	0.3	28.3
Santiago	Sp	12.4	2.3	20.9	0.11	142	1920	3299	4.6	566	138.8	25.9	144	34648	21.6	125	201	2.1	0.2	134.4	32.4
Santiago	Au	12.4	18.2	1.9	9.59	107	1791	2123	4.4	390	193.0	14.7	292	26429	8.2	105	273	1.7	0.1	0.1	28.2
Sanxenxo	Sp	5.2	6.6	38.4	0.17	793	4723	8317	19.5	6911	4.4	22.9	81	5950	15.8	305	412	11.3	0.7	145.1	34.6
Sanxenxo	Au	5.6	35.9	6.8	5.32	1008	4958	5608	22.2	6591	6.0	21.7	97	5148	17.6	315	320	11.4	0.9	0.1	36.5
Teo 1	Sp	6.1	7.3	41.6	0.18	692	4179	7884	15.1	4314	5.0	44.6	82	5595	38.4	538	538	6.9	1.4	272.0	66.2
Teo 1	Au	5.8	41.9	7.4	5.64	590	3227	5303	12.7	4440	3.7	41.8	66	3900	28.0	568	526	7.6	0.8	0.7	61.2
Teo 2	Au	5.4	7.3	41.0	0.18	461	3470	6408	12.3	3810	3.1	28.8	78	4365	27.7	448	510	6.4	1.0	208.7	50.2
Tui	Sp	7.6	4.7	31.6	0.15	1343	3309	15470	17.3	2411	12.4	61.2	1164	14730	32.2	436	1634	13.5	2.2	300.1	71.3
Tui	Au	8.1	30.4	4.6	6.59	5847	2856	5376	5.3	3163	65.3	13.9	517	5372	19.9	82	203	3.6	0.4	0.2	80.6
Vilagarcía	Sp	5.5	7.0	40.7	0.17	3182	4333	10113	19.1	8161	5.4	23.7	85	4813	12.5	307	469	18.4	3.1	176.6	41.7
Vilagarcía	Au	5.7	40.7	7.8	5.23	2947	3451	9335	16.7	4960	6.1	18.2	48	3496	10.7	245	396	22.3	4.2	0.3	37.5
RM1 - M1	Sp	8.1	2.5	29.1	0.09	863	3613	17383	15.4	2496	10.1	112	561	15733	186.3	470	1216	11.9	4.6	337.4	82.1
RM1 - M2	Sp	10.1	2.2	30.2	0.07	1449	4199	7669	16.1	6439	3.3	15.5	46	4900	11.3	189	342	9.5	0.7	170.9	41.0
RM3 - M1	Sp	7.2	2.0	24.8	0.08	2794	4465	9086	15.0	6326	4.8	20.3	70	6410	14.2	222	591	6.9	3.5	247.1	58.6

RM4 - M1	Sp	6.00	17.8	2.5	7.21	931	2571	10339	8.3	2192	14.7	82.6	247	14063	42.6	158	222	5.4	0.5	0.3	27.4
RM4 - M2	Sp	6.94	17.4	2.3	7.57	1852	3762	13542	14.2	3717	7.3	18	213	10331	25.5	304	336	10.2	2.5	0.4	55.3
RM4 - M3	Sp	7.64	19.3	2.8	6.93	1546	3075	11699	12.2	3729	6.9	39.5	202	7939	11.9	195	231	7.1	1.2	0.3	29.4
RM1 - M1	Au	9.1	30.0	2.9	10.51	1579	2928	7641	9.2	5447	41.1	65.3	261	7433	10.1	113	207	4.4	0.4	0.2	30.3
RM1 - M2	Au	11.8	27.9	1.8	15.28	6640	4037	6388	7.1	3192	63.0	36.1	912	7817	10.6	88	324	4.5	0.8	0.2	23.3
RM1 - M3	Au	12.4	23.5	2.3	10.23	1847	3943	3034	5.9	5810	63.9	22.6	274	2411	4.6	70	396	5.1	1.8	0.1	12.8
RM2 - M1	Au	7.0	33.9	3.8	8.91	4727	1506	5135	7.9	4537	9.4	129	249	79945	54.1	216	257	6.3	1.6	0.2	24.3
RM4 - M1	Au	7.4	17.6	2.4	7.26	1686	3727	20519	12.2	4064	6.0	202	442	26867	91.2	203	275	16.8	0.8	0.3	37.7
RM4 - M2	Au	7.2	22.5	2.5	8.87	746	4960	18126	5.4	3034	2.4	24.2	402	25414	32.2	54	88	6.3	0.2	0.0	10.3
RM4 - M3	Au	7.7	22.5	2.6	8.59	2491	4058	21049	14.3	3974	36.7	210	458	30000	81.5	270	338	7.7	1.4	0.4	52.3

PUBLICACIÓN II

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Retention of the Antibiotic Cefuroxime onto Agricultural and Forest Soils

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Abstract: Antibiotics in wastewater, sewage sludge, manures, and slurries constitute a risk for the environment when spread on soils. This work studies the adsorption and desorption of the antibiotic cefuroxime (CFX) in 23 agricultural and forest soils, using batch-type experiments. Our results show that the adsorption values were between 40.75 and 99.57% in the agricultural soils, while the range was lower (from 74.57 to 93.46%) in forest soils. Among the Freundlich, Langmuir, and Linear models, the Freundlich equation shows the best fit for the adsorption results. In addition, agricultural soils with higher pH are the ones that present the highest adsorption. Further confirmation of the influence of pH on adsorption is given by the fact that Freundlich's K_F parameter and the Linear model K_d parameter shows a positive correlation with pH and with the exchangeable Ca and Mg values, which are known to affect the charges of the soil colloids and the formation of cationic bridges between adsorbents and adsorbate. In addition, Freundlich's n parameter shows a positive and significant correlation with the organic matter content, related to the high adsorption taking place on forest soils despite their pH < 5. Regarding desorption, in most cases, it is lower than 1%, which indicates that CFX is adsorbed in a rather irreversible way onto these soils. Overall, these results can be considered relevant regarding their potential impact on environmental quality and public health.

Keywords: adsorption; desorption; emerging pollutants; soils

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1. Introduction

Antibiotics have been widely used for the treatment of infectious diseases, both in veterinary and human medicine. Regarding antibiotics for human use, Kovalakova [1] indicates that their worldwide consumption increased by around 65% between 2000 and 2015, when expressed in defined daily doses (DDD). In the case of Europe, 3821 tons of antibiotics were used in human medicine in 2014, of which 9% were consumed in Spain [2]. To note that the most recent data available indicate that "in 2019, the average total (community and hospital sector combined) consumption of antibacterials for systemic use in the EU/EEA was 19.4 DDD per 1000 inhabitants per day (country range: 9.5–34.1), evidencing that during the period 2010–2019, a statistically significant decrease was observed for the EU/EEA overall" [3].

Once the antibiotics are administered, a significant proportion (between 28% and 90%, depending on the type of antibiotic) is excreted as parent compounds or metabolites through feces and urine [4,5]. After their excretion, these compounds reach the environ-

ment, and the subsequent potential impact has been causing increasing concerns in recent years, as previously occurred for some inorganic pollutants [6,7].

In fact, Kim et al. [8] indicate that since the beginning of the 21st century a growing number of studies has revealed the presence of a wide range of persistent pharmaceuticals in soil and water, and its relevance has been confirmed [9]. The main entry of human-medicine antibiotics into the environment takes place through wastewater and sewage sludge, after domestic use. In fact, it has been reported that the direct application of wastewater in irrigation, or the application of sewage sludge as fertilizer in agricultural soils, can be the main source of antimicrobials to the edaphic environment [10–13], as wastewater treatment plants usually do not eliminate these pollutants [14–17]. Excessive concentrations of these drugs in the soil could affect food quality, the safety of crop production, and the health of the environment, bearing in mind that these contaminants move through environmental compartments and the food chain, especially after being absorbed by plants [18–20].

Once the antibiotics reach soils, they are subjected to different physicochemical and biological processes in the soil-water system, mainly adsorption/desorption, degradation, transportation, and uptake by crops, depending on the properties of the antibiotic, the characteristics of the soil, and climatic conditions. The magnitude of each of these processes will strongly determine the fate and persistence of these compounds in the environment [21,22].

Among antibiotics, cefuroxime (CFX) is a compound belonging to the cephalosporin group, and it has become one of the most consumed worldwide [23]. It is frequently used to treat diseases like pneumonia, otitis, sinusitis, and urinary tract infection [24]. Regarding its presence in the environment, in a study dealing with antibiotics in treated wastewater, Latrou et al. [25] found that CFX was the one with the highest concentrations, reaching around 6.6 μg L^{-1}. Moreover, regarding CFX in the environment, Li et al. [26] found that the application of manure increased the levels of this and other antibiotics in greenhouse soils. Furthermore, as regards its effects on microbiota in the environment, Orlewska et al. [27] reported a low initial resistance of soil microbial communities to CFX, but these authors concluded that despite a short-term negative effect, the balance of the soil ecosystem could be clearly disturbed. The chemical structure of the antibiotic CFX (shown in Figure 1) has relevance as regards its potential interactions with various soil compounds [22], which would need further attention, and should need additional discussion.

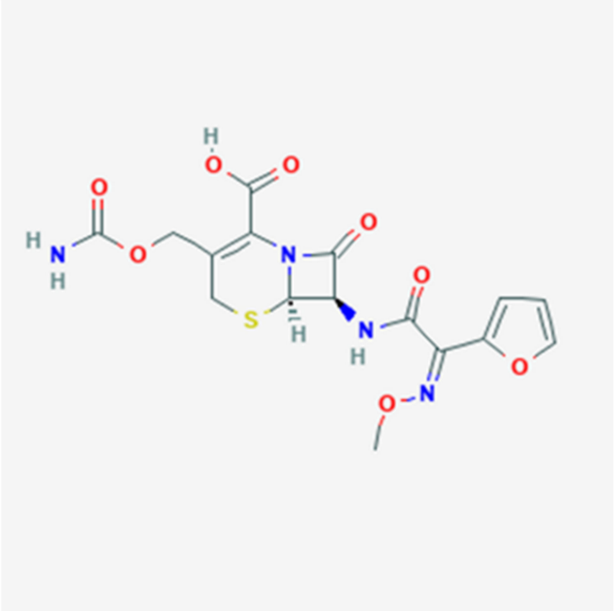

Figure 1. Chemical structure of the antibiotic cefuroxime.

Overall, there is a lack of studies providing results on CFX retention/release on/from soils, and additional research would be needed to elucidate the fate of this compound in the soil, and other environmental compartments.

Taking all this background into account, the main objective of the current study is to evaluate the eventual influence of soil characteristics on the behavior of CFX in the edaphic environment. For this, the adsorption and desorption of the antibiotic were studied in 23 forest and agricultural soils presenting a variety of physicochemical properties. The results of this research could be relevant to increase the knowledge on the evolution and potential repercussions of this pollutant in the environment, and specifically in the edaphic system, from which it could undergo transport towards water bodies and enter the food chain.

2. Materials and Methods

2.1. Soils

A total of 23 soils were sampled in different areas of Galicia (NW Spain). Table S1 (Supplementary Materials) shows some basic details on these sampling areas. Within these samples, 20 corresponded to agricultural soils as follows: Thirteen vineyard soils, with five in Ourense province—VO1 to VO5, and 8 in Pontevedra province—VP1 to VP8, while the other 7 were corn (or maize) soils—M1 to M7. In addition, the three forest soils sampled were: One of them under *Pinus*—FP, another one under oak (*Q. robur*)—FC, and the third one under *Eucalyptus*—FE.

In each plot, 10 sub-samples were collected in a zig-zag, in the surface layer of the soil (0–20 cm), and these sub-samples were then grouped to make a composite sample for each of the plots. Subsequently, in the laboratory, they were dried at 40 °C, sieved through a 2 mm mesh, and stored until analysis.

2.2. Soils Analyses

Different physicochemical parameters were determined in the soil samples. Specifically, these parameters were: Soil texture, pH values in water and in KCl, total C, total N, exchangeable Ca, Mg, Na, K and Al, effective cation exchange capacity (eCEC), available phosphorus, total non-crystalline Fe and Al, and finally, Fe and Al bound to organic matter. The methods used for the characterization of the soils are indicated in the Supplementary Materials, as well as the results obtained (Table S2).

2.3. Adsorption and Desorption Experiments

The adsorption and desorption experiments were carried out using batch-type tests performed with the different soil samples. Specifically, amounts of 2 g of soil (taken from those previously dried at 40 °C and sieved through a 2 mm mesh) were mixed with 5 mL of solutions containing different concentrations of the antibiotic (2.5, 5, 10, 20, 30, 40, or 50 μmol L^{-1}) and 0.005 M $CaCl_2$ as background electrolyte. The suspensions were shaken for 48 h (time enough for reaching equilibrium, as demonstrated by kinetic studies previously performed—data not shown) in the dark, using a rotary shaker, and then centrifuged at 4000 rpm for 15 min, using a Model 5810R Centrifuge (Eppendorf, Hamburg, Germany). The pH values of the equilibrium solutions were determined with a pH meter (MultiMeter MM41 model, Crison, Barcelona, Spain). The resulting supernatants were filtered through 0.45 μm nylon syringe filters (Fisher scientific, Madrid, Spain), and the antibiotic concentration of the filtered liquids was determined by HPLC-UV.

2.4. Chemical Reagents and Quantification of CFX

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Cefuroxime (purity ≥ 95%) was supplied by Sigma-Aldrich (Barcelona, Spain). Phosphoric acid (85% extra pure) and acetonitrile (purity ≥ 99.9%) used for HPLC were supplied by Fisher Scientific (Madrid, Spain), while Ca_2Cl (95% purity) was provided by Panreac (Barcelona, Spain). All solutions used during HPLC determinations were prepared with milliQ water (Millipore, Madrid, Spain). The HPLC determinations were carried out with a

Model LPG 3400 SD equipment (Thermo Fisher Scientific, Waltham, MA, USA). A Luna C18 column (150 mm × 4.6 mm, 5 µm) and a precolumn (4 mm × 2 mm, 5 µm), packed with the same material as the column, was also used; both provided by Phenomenex (Madrid, Spain). During the HPLC analysis, the injection volume was 50 µL, and the flow rate was 1.5 mL min^{-1}, with the mobile phase being acetonitrile (phase A) and 0.01 M phosphoric acid (phase B). A linear gradient was used, varying from 5% to 32% of phase A, and from 95% to 68% of phase B. The initial conditions were re-established in 2 min and maintained for 2.5 min. The total analysis time was 15 min, with a retention time of 8.69 min. The wavelength used for detection was 212 nm. The amounts of antibiotic adsorbed were calculated by the difference between the concentrations in the added solutions and those in the solutions at equilibrium.

Once the adsorption experiments were carried out, desorption was also studied, which determines the degree of reversibility of the process. For this, 5 mL of 0.005 M $CaCl_2$ (without antibiotic) were added to the soil materials resulting from the adsorption process, and then the same process used for adsorption was repeated.

All determinations corresponding to adsorption and desorption experiments were made in triplicate. Selected chromatograms are presented in Figure S1 (Supplementary Materials).

2.5. Fitting to Adsorption Models and Statistical Treatment

The experimental data obtained in the adsorption tests were adjusted to the Freundlich (1), Langmuir (2), and Linear (3) models [6,7].

$$q_e = K_F C_{eq}{}^n \quad (1)$$

$$q_e = \frac{q_m K_L C_{eq}}{1 + K_L C_{eq}} \quad (2)$$

$$Kd = q_e / C_{eq} \quad (3)$$

where q_e is the amount of antibiotic retained by the soil; C_{eq} is the concentration of antibiotic present in the solution at equilibrium; K_F is the Freundlich parameter related to the adsorption capacity; n is a parameter of the Freundlich model related to the nonlinearity of the adsorption; K_L is the Langmuir adsorption constant; and q_m is the maximum adsorption capacity according to the Langmuir model. K_d is the partition coefficient in the linear model.

For the adjustments of the adsorption experiments to the Langmuir, Freundlich, and Linear models, as well as for performing statistical correlation analyses, the SPSS Statistics 21 software was used.

3. Results and Discussion

3.1. Characteristics of the Soil Samples

The physicochemical properties of the soils used are shown in Table S2 (Supplementary Materials). Regarding the particle size distribution, most soils are sandy loam, in correspondence with the dominance of silicic original material, with sand contents between 43.42% and 69.35%, and the highest percentages generally corresponding to forest soils. The clay fraction ranges between 12.36 and 24.5%, with agricultural soils showing higher values. The range of pH in water (pH_{H2O}) is very wide, going from 4.68 to 8.02, with the most acidic values corresponding to forest soils. There is also a high variability in the organic C content, ranging between 0.63 and 9.78%, while the N content ranges between 0.06 and 0.67%. For both C and N, the highest levels correspond to forest soils.

The effective cationic exchange capacity (eCEC) values also show a wide oscillation (Table S2, Supplementary Materials), between 5.43 and 42.81 $cmol_c$ kg^{-1}, with Ca being the cation that dominates in most agricultural soils, while Al dominates in forest soils. The non-crystalline forms of Fe and Al also show high variability. Specifically, total non-crystalline Fe (Fe_{ox}) is between 1695 and 6990 mg kg^{-1}, with the highest values generally corresponding to soils on amphibolite, while total non-crystalline Al (Al_{ox}) oscillates between 484 and

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11,651 mg kg^{-1}, with the lowest values corresponding to soils on schist/slate. Regarding Fe bound to organic matter (Fe_{pir}), the range goes from 715 to 9239 mg kg^{-1}, while Al bound to organic matter (Al_{pir}) ranges from 484 to 14,857 mg kg^{-1}, with generally higher values in forest soils (FP, FE, and FC), and in those with more organic carbon (M5 and VP4) within agricultural soils.

In fact, previous studies have shown that these soil characteristics are very relevant as regards retention/release of a variety of pollutants [7], and specifically antibiotics [12], which will be further discussed below.

3.2. Adsorption of CFX and Modeling

3.2.1. Adsorption Data

Table S3 (Supplementary Materials) shows CFX adsorption (in μmol kg^{-1} and percentage) as a function of the concentration of antibiotic added.

In the soils with corn cultivation, when the highest dose of CFX was added, the adsorption values oscillate between 71.36 and 123.91 μmol kg^{-1} (Table S3, Supplementary Materials), while in the vineyard soils on schist and slate adsorption is between 101.05 and 122.86 μmol kg^{-1}, and in vineyard soils on granite is between 54.39 and 125 μmol kg^{-1}. Forest soils show a scarce oscillation, between 109.9 and 116.71 μmol kg^{-1}. Analyzing the data expressed as a percentage, a large part of the soils adsorbed more than 75% of the CFX added. In this sense, the range of variation was small in all vineyard soils on schist and slate, and in forest soils (from 79.26 to 96.24%, and between 74.57 and 93.46%, respectively), while a wide oscillation was observed within corn soils (ranging 49.49–99.2%) and vineyard soils on granite (ranging 40.75–99.57%). In other words, some of the soils under study adsorb almost 100% of the maximum concentration of the antibiotic added, while others do not adsorb even half of that concentration.

In the agricultural soils of the current study, whose pH values vary between 5.01 and 8.02, the differences in adsorption would be mainly related to these pHs. In fact, those soils having the highest pH (M1, M5, VP6, and VP7) show higher adsorption. Similar results were reported by Fakhri and Adami [28] when studying the adsorption of various cephalosporine antibiotics on magnesium oxide nanoparticles and carbon nanotubes, finding higher adsorption when the pH of the medium was >8. pH is one of the parameters having the highest influence on antibiotic-adsorbent interactions, since it simultaneously affects the chemical speciation of antibiotics and adsorbent surfaces [29]. Both antibiotics and soil components, have functional groups that can undergo protonation/deprotonation reactions, depending on the pH of the solution. The CFX molecule is amphoteric, having two dissociation constants (pka_1 3.15 and pka_2 10.97), which can have a positive or negative charge, or be in the form of a zwitterion, depending on the pH of the medium [30].

At pH above 10.97, CFX is in anionic form, while at pH below 3.97, it is in cationic form, and between both pH values, it is a zwitterion. Regarding the soils in this study, since most have a pH above 5.5, the components with variable charge (1:1 clays, organic matter, and non-crystalline minerals) will preferably be negatively charged. At these pH values, CFX would be as a zwitterion, meaning the positively charged amino groups of the antibiotic would interact electrostatically with the negatively charged adsorbent surfaces of the soils, while the anionic groups (COO-) of CFX would bind to these surfaces through a cationic bridge [31]. The higher the pH of the soil, the more negative charges would be produced in its components, which would explain the influence of pH on these adsorption processes.

In the case of forest soils, the adsorption of CFX is generally above 70% (Figure S1, Supplementary Materials), despite having a clearly acidic pH (<5). This relevant score for adsorption could be related to the high content of organic matter in these soils (between 12.32 and 16.86%), much higher than that of the agricultural soils of the study. Organic components have a pH-dependent charge and are highly involved in antibiotic adsorption processes [32], giving that at pH < 5, the –COOH groups of organic matter

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begin to dissociate [33], and then could interact electrostatically with the positively charged groups of CFX.

3.2.2. Fitting to Adsorption Models

Figure 2 shows the adsorption curves of the 23 soils used in this work, plotting the amount of antibiotic adsorbed versus its concentration in the equilibrium solution.

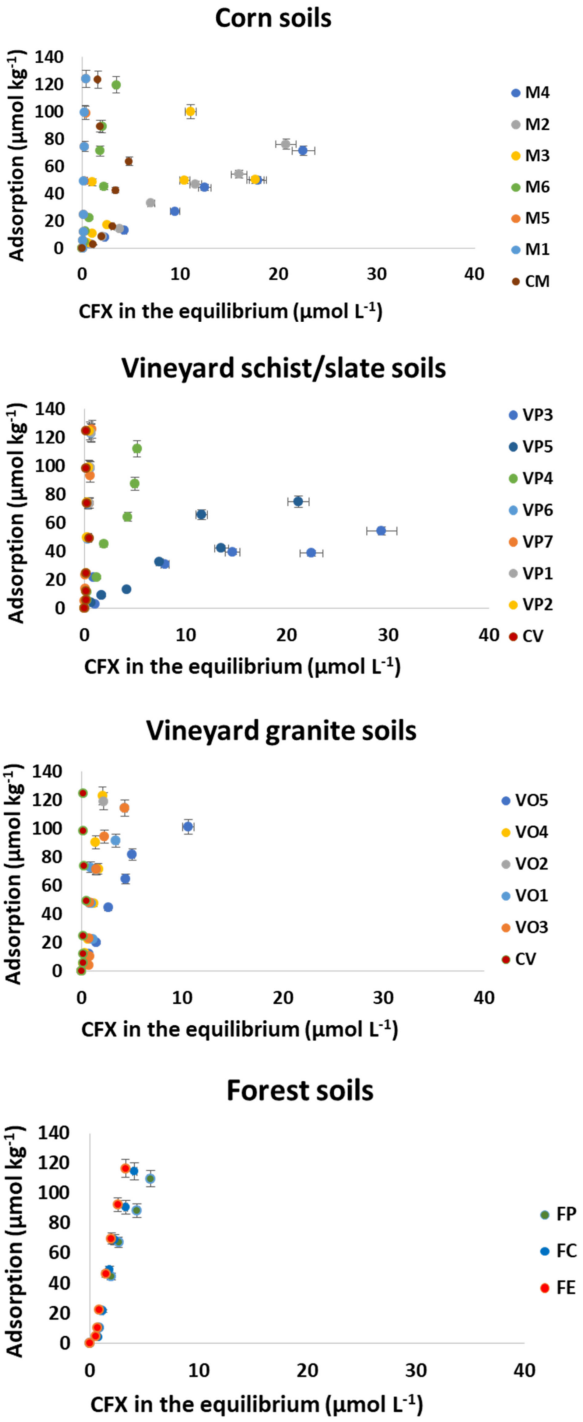

Figure 2. Adsorption curves for CFX on the various soils were studied. M, maize (corn) soils; VO, vineyard soils (Ourense province); VP, vineyard soils (Pontevedra province); F, forest soils; CM and CV, control soils in maize and vineyard areas. Average values (n = 3), with coefficients of variation always <5%.

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In addition, Table 1 shows the parameters of the adsorption equations obtained from the adjustments of the experimental data to the Freundlich, Langmuir, and Linear models. The adsorption data of most of the soils adjusted better to the Freundlich equation, as indicated by the values of the coefficient of determination obtained for most of the soils (R^2 above 0.9). In the Linear and Langmuir models, R^2 higher than 0.9 was obtained in a smaller number of soils. In addition, this last model was the most affected by high errors (mean squared errors—MSE) associated with the fitting, making it the least adequate for the soils in this study. The values of the distribution coefficient (K_d) of the linear model show wide variation in corn soils (3.08–247.33 L kg^{-1}) and vineyard soils on granite (2.01–201.92 L kg^{-1}), while the range is lower in vineyard soils on schist and slate (11.48–51.39 L kg^{-1}), and especially in forest soils (20.43–33.43 L kg^{-1}). To be noted that, using mean squared errors, only the MSE of different fits to the same data should be compared, as it will depend on the magnitude of the data (larger values tend to have higher variability). Future more detailed treatment of these data could be made using more sophisticated, weighted nonlinear regression, as well as modeling comparison based on likelihood ratio tests and/or Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC).

Table 1. Fitting of adsorption results to the parameters of the Freundlich, Langmuir, and Linear models. K_F ($L^n \mu mol^{1-n} kg^{-1}$); n (dimensionless); K_L (L μmol^{-1}); q_m ($\mu mol\ kg^{-1}$); K_d (L kg^{-1}). -: error values too high for fitting.

	Freundlich					Langmuir					Linear Model		
Soil	K_F	**Error**	n	**Error**	R^2	K_L	**Error**	q_m	**Error**	R^2	K_d	**Error**	R^2
M1	478.866	152.237	1.575	0.29	0.893	-	-	-	-	-	247.337	29.375	0.797
M2	5.133	0.958	0.881	0.07	0.988	0.013	0.008	341.167	174.072	0.989	3.699	0.122	0.983
M3	22.027	11.843	0.41	0.23	0.57	-	-	-	-	-	4.735	1.169	0.328
M4	3.234	1.002	0.983	0.11	0.979	-	-	-	-	-	3.084	0.113	0.978
M5	470.333	140.326	1.655	0.3	0.917	-	-	-	-	-	233.145	26.194	0.816
M6	32.85	8.286	1.013	0.25	0.894	-	-	-	-	-	33.257	2.798	0.894
CM	-	-	-	-	-	-	-	-	-	-	-	-	-
VO1	36.079	9.37	0.782	0.21	0.808	-	-	-	-	-	27.873	3.461	0.779
VO2	40.898	10.846	0.83	0.28	0.746	-	-	-	-	-	35.283	4.958	0.728
VO3	32.17	13.084	0.855	0.35	0.603	-	-	-	-	-	27.53	4.932	0.593
VO4	42.584	6.548	1.378	0.26	0.943	-	-	-	-	-	51.391	3.805	0.917
VO5	21.681	4.085	0.685	0.1	0.948	0.113	0.037	193.418	37.659	0.957	11.48	1.085	0.863
VP1	228.797	25.578	1.589	0.18	0.971	-	-	-	-	-	157.328	13.55	0.888
VP2	327.646	63.461	1.54	0.23	0.941	-	-	-	-	-	201.923	19.545	0.861
VP3	10.877	3.319	0.458	0.1	0.895	0.15	0.095	58.473	11.744	0.876	2.018	0.266	0.693
VP4	20.2	5.829	0.944	0.19	0.947	-	-	-	-	-	18.56	1.088	0.947
VP5	10.842	3.289	0.459	0.1	0.896	0.15	0.094	58.46	11.649	0.878	2.017	0.265	0.696
VP6	207.294	19.519	1.451	0.15	0.975	-	-	-	-	-	157.181	11.182	0.923
VP7	164.232	5.17	1.099	0.05	0.996	-	-	-	-	-	156.031	3.176	0.993
CV	-	-	-	-	-	-	-	-	-	-	-	-	-
FP	21.07	3.307	0.978	0.11	0.971	-	-	-	-	-	20.43	0.876	0.971
FE	24.905	2.618	1.323	0.1	0.986	-	-	-	-	-	33.435	1.836	0.954
FC	20.149	3.368	1.251	0.14	0.971	-	-	-	-	-	26.738	1.548	0.949

The K_d values are linked to soil characteristics, as indicated by the statistical results that show positive and significant correlation ($p < 0.05$) with pH in water (r = 0.50; R^2 = 0.25), with the concentrations of exchangeable Ca and Mg (r = 0.58 and 0.43, R^2 = 0.34 and 0.18, respectively), and with eCEC (r = 0.57; R^2 = 0.32). To note that bivariate correlations were calculated using the Pearson's correlation method, in which the obtained "r" values indicate the degree of correlation (values closer to 1 indicate that more relation exists between the parameters) and "p" is the level of significance. The squared values of r (R^2, coefficients of determination) indicate the fraction of the variability explained by the respective soil property.

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Regarding the Freundlich model, to consider the higher the values of the K_F parameter, the greater the adsorption capacity of the adsorbent [34]. In the present study, K_F values also show high variability in corn soils (3.23–478.86 L^n μmol^{1-n} kg^{-1}) and VP vineyards (0.84–327.64 L^n μmol^{1-n} kg^{-1}), with lower oscillation for vineyard soils on schist and slate and for forest soils (21.6–42.58 and 20.14–24.90 L^n μmol^{1-n} kg^{-1}, respectively). Considering the whole set of soils used in this work, K_F showed a positive and significant correlation ($p < 0.05$) with the same soil parameters as K_d, specifically with pH in water (r = 0.56; R^2 = 0.31), pH and in KCl (r = 0.56; R^2 = 0.31), concentrations of exchangeable Ca (r = 0.71; R^2 = 0.50), exchangeable Mg (r = 0.45; R^2 = 0.20), and with eCEC (r = 0.49; R^2 = 0.24).

Kodešová et al. [35] also found high correlations among these soil properties and values of te Freundlich's K_F coefficient corresponding to adsorption of various types of antibiotics onto soils. As previously indicated, these results show the importance of pH in the adsorption processes, mainly due to affecting the charge of both antibiotics and soil components, as well as the possible intervention of cationic bridges. The values of Freundlich's n parameter (related to the reactivity and heterogeneity of the active sites of the sorbent) [36,37] were highly variable for the different soils. The highest oscillations were observed in crop soils (between 0.011 and 1.655 in corn soils, between 0.21 and 1.589 in granite vineyard soils, and between 0.685 and 1.378 in vineyard soils on schist and slate), while the variability was lower, and the values were relatively high, in forest soils (from 0.978 to 1.323).

A good fit to the Freundlich model indicates that, at low adsorbate concentrations, the amount adsorbed clearly increases with a rise in the concentration of the sorbate in solution [37]. When the values of the n parameter are between 0 and 1, they indicate that the adsorption is carried out in a heterogeneous matrix, where the highest energy sites are occupied first. Being closer to zero, n values indicate a higher degree of heterogeneity in the adsorption surface [36,38]. When the values of the n parameter are >1, it is considered indicative of deviation from the Freundlich equation, due to factors, such as the possible existence of irreversible reactions, as precipitation [39], or the presence of cooperative adsorption [36].

When $n = 1$, the Freundlich equation would be equivalent to a linear equation. In the present study, values of $n < 1$ are obtained in 57% of the soils (Table 1). This parameter is positively and significantly correlated with the soil organic matter content (r = 0.44 and $p < 0.05$), indicating that CFX adsorption would be stronger in soils with more organic matter. This could explain the high adsorption of the antibiotic shown by forest soils, despite their low pH, since their high organic matter contents create adsorption sites by dissociating certain functional groups, such as carboxylic, even at pH < 5 [33].

3.3. Desorption

Table 2 shows the amounts of CFX desorbed (in $\mu mol\ kg^{-1}$ and percentage), as a function of the concentration of antibiotic added. Desorption values were low, never exceeding 1.87 $\mu mol\ kg^{-1}$, regardless of the concentrations of antibiotic added. In maize (corn) and vineyard soils on schist and slates, the maximum desorption value was 0.53 $\mu mol\ kg^{-1}$. The score was slightly higher in vineyard soils on granite (0.73 $\mu mol\ kg^{-1}$). Forest soils showed the maximum desorption values among the whole set of samples in the study (1.87 $\mu mol\ kg^{-1}$).

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Table 2. CFX desorption, expressed in μmol kg^{-1} (and in a percentage, between brackets), from the various studied soils, as a function of the concentration of antibiotic added (C_0, in μmol L^{-1}). M, maize (corn) soils; VO, vineyard soils (Ourense province); VP, vineyard soils (Pontevedra province); F, forest soils. Average values (n = 3), with coefficients of variation always < 5%.

	C_0 (μmol L^{-1})						
Soil	**2.5**	**5**	**10**	**20**	**30**	**40**	**50**
M1	0 (0)	0.37 (1.5)	0.37 (0.8)	0.39 (0.8)	0.51 (0.7)	0.51 (0.5)	0.53 (0.4)
M2	0 (0)	0.36 (3.0)	0.35 (1.4)	0.36 (0.7)	0.39 (0.5)	0.36 (0.4)	0.48 (0.4)
M3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.11 (2.7)
M4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VP1	0.39 (6.64)	0.41 (3.35)	0.43 (1.77)	0.50 (1.02)	0.49 (0.67)	0.59 (0.60)	0.59 (0.48)
VP2	0.38 (6.45)	0.38 (3.15)	0.41 (1.67)	0.46 (0.92)	0.66 (0.89)	0.65 (0.66)	0.73 (0.59)
VP3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VP4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VP5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VP6	0 (0)	0 (0)	0.37 (1.55)	0.34 (0.69)	0.47 (0.64)	0.42 (0.43)	0.49 (0.40)
VP7	0.44 (7.98)	0.42 (3.07)	0.39 (1.66)	0.33 (0.67)	0.27 (0.37)	0.30 (0.33)	0.51 (0.40)
VP8	0.36 (6.27)	0.35 (2.90)	0.33 (1.34)	0.11 (0.23)	0.32 (0.43)	0.31 (0.31)	0.46 (0.37)
FP	0.14 (3.12)	0.33 (3.22)	0.73 (3.33)	1.13 (2.53)	1.22 (1.81)	0.41 (0.47)	0 (0)
FE	0.11 (2.32)	0.26 (2.42)	0.58 (2.58)	1.17 (2.54)	0.96 (1.38)	0.66 (0.72)	0.49 (0.42)
FC	0.15 (3.53)	0.35 (3.50)	1.05 (4.85)	1.87 (3.79)	0.77 (1.11)	0.58 (0.64)	0.33 (0.29)

A statistical test focusing on eventual relations among the maximum desorption values and the properties of the different soils showed a significant and positive correlation with sand content (r = 0.45, $p < 0.05$), and a negative correlation with clay content (r = −0.43, $p < 0.05$). Therefore, clays would have an important role in the intensity of the adsorption, reducing the reversibility of the process.

Analyzing desorption data presented in percentage (Table 2), CFX desorption was always below 8% (and below 1% in most cases), evidencing that adsorption has low reversibility in the soils of the current study, as previously observed in other Galician soils for tetracycline antibiotics [40], and contrary to what was found for sulfonamides, in which desorption exceeded 50% in some cases [41]. The marked differences among adsorption and desorption scores indicate the relevance of hysteresis affecting these processes.

4. Conclusions

In the agricultural soils included in this study, the adsorption of the antibiotic cefuroxime (CFX) was dependent on the pH of the soil, with the highest adsorption corresponding to those soils having higher pH values. In the case of forest soils, which had a very acidic pH, their high organic matter contents would explain the high CFX adsorption obtained. Regarding desorption, it was below 1% in most cases, indicating the low reversibility of the

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adsorption process, especially in soils with a higher proportion of clay. Adsorption data fitted better to the Freundlich than to the Linear and Langmuir models. The correlations of Freundlich's K_F parameter and the K_d parameter of the Linear model with soil characteristics indicate that the most important factors in adsorption are pH and the concentrations of exchangeable Ca and Mg, probably due to their influence on the electric charges of colloids and the formation of cationic bridges. These results can be considered relevant in terms of the evaluation of the possible evolution of the antibiotic CFX when it reaches the soil as a contaminant, affecting its retention/release. Therefore, there is a probability that it will be mobilized into water bodies or absorbed by plants, entering the food chain, with have consequent environmental and public health repercussions.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11104663/s1, Table S1: Basic details corresponding to the 23 soils sampled for this study. M, maize (corn) soils; VO, vineyard soils (Ourense province); VP, vineyard soils (Pontevedra province); F, forest soils; CM and CV, control soils in maize and vineyard areas; Table S2: Values corresponding to the basic parameters determined in the various studied soils. M, maize (corn) soils; VO, vineyard soils (Ourense province); VP, vineyard soils (Pontevedra province); F, forest soils; CM and CV, control soils in maize and vineyard areas. Average values (n = 3), with coefficients of variation always <5%; Table S3: CFX adsorption, expressed in μmol kg−1 (and in a percentage, between brackets), onto the various studied soils, as a function of the concentration of antibiotic added. M, maize (corn) soils; VO, vineyard soils (Ourense province); VP, vineyard soils (Pontevedra province); F, forest soils; CM and CV, control soils in maize and vineyard areas. Average values (n = 3), with coefficients of variation always <5%; Figure S1: Selected chromatograms corresponding to the quantification of the antibiotic cefuroxime.

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Supplementary Material

Retention of the antibiotic cefuroxime onto agricultural and forest soils

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Basic data regarding sampling areas

Table S1 shows data on the soils sampled for this study in different areas of Galicia (NW Spain). The 20 agricultural soils sampled were as follows: 13 vineyard soils, with 5 in Ourense province – VO1 to VO5- and 8 in Pontevedra province –VP1 to VP8-, and with the other 7 being corn (or maize) soils –M1 to M7-). In addition, the 3 forest soils sampled were: one of them under *Pinus* –FP-, another one under oak (*Q. robur*) –FC-, and the third one under *Eucaliptus* –FE-.

Table S1. Basic details corresponding to the 23 soils that were sampled for this study. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas.

Code	Crop/Trees	Parent material	Mean temperature (°C)	Annual rainfall (mm)
M1	Corn	Schists, quartzite, pelite, sandstones and granites	12	995
M2 M3 M4 M5 M6	Corn	Schist and amphibolite	13	1270
M7	Corn	Granite	13.4	1587
VO1	Vineyard	Schist	11	850-1000
VO2		Slate		
VO3		Slate		
VO4 VO5	Vineyard	Granite and mica-schists	14.5	950
VP1 VP2 VP3	Vineyard	Granite	13.8	1540
VP4 VP5	Vineyard	Granite	14	1000-2000
VP6 VP7	Vineyard	Granite	13.8	1540
VP8	Vineyard	Granite	13.4	1587
FP FE FC	Pine, Eucalyptus, Oak	Granite and gneiss	11.2	1389

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Quantification of soil parameters

Table S2 shows the results of the determinations of the basic parameters carried out on the 23 soils under study.

Regarding methods, soil texture was determined using the International Method of the Robinson Pipette. The pH values in water and pH in KCl (soil:liquid ratio 1:2.5) were measured with a CRISON-2001 pH meter (Crison, Spain). The determination of total C and N was carried out by elemental analysis, with a LECO CHN-100 equipment (Leco Corporation, St. Joseph, MI, USA). The exchangeable cations (Ca_e, Mg_e, Na_e, K_e and Al_e) were displaced using a 1M NH_4Cl solution in a 1:10 soil:solution ratio, and were then determined by atomic absorption spectrophotometry (Ca, Mg and Al) or atomic emission spectrophotometry (Na and K) with a Perkin Elemer AAnalyst 200 equipment (Perkin Elmer, USA). The sum of these exchangeable cations is considered the effective cation exchange capacity (eCEC). The available phosphorus was extracted using 0.5 M $NaHCO_3$ and determined by the phosphomolybdic complex method, using a UV-visible spectrophotometer (UV-1201, Shimadzu, Japan). To determine the total non-crystalline Fe and Al contents, an extraction was carried out with ammonium oxalate buffered at pH 3, stirring for 4 hours in the dark; five drops of 0.25% superfloc were added to the resulting extract, then centrifuged at 2000 rpm for 10 minutes; it was then filtered, and the supernatant is diluted 1:5; Fe and Al in non-crystalline form were measured by atomic absorption spectrophotometry. The determination of Fe and Al bound to organic matter (Fe_{pir} and Al_{pir}) was carried out by extraction with sodium pyrophosphate at pH 10; the extraction was carried out by shaking 1 g of sample with 100 mL of sodium pyrophosphate for 16 hours; the samples were diluted in half with distilled water and finally Fe and Al were measured in by atomic absorption spectrophotometry.

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Table S2. Values corresponding to the basic parameters determined in the various soils studied. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas. Average values (n=3), with coefficients of variation always < 5%.

Soil	pH_{H20}	OC	OM	N	Sand	clay	eCEC	Ca	Mg	Na	K	Al	Fe_{ox}	Fe_{pir}	Al_{ox}	Al_{pir}
				%						$cmol_c\ kg^{-1}$					$mg\ kg^{-1}$		
M1	8.02	4.98	8.58	0.33	49.57	22.50	42.81	39.44	2.07	0.60	0.69	0.01	4140	1501	5351	2111
M2	5.33	2.66	4.59	0.27	43.42	24.65	6.88	5.35	0.67	0.02	0.44	0.40	5745	3159	3401	2871
M3	5.65	2.58	4.44	0.25	43.57	22.43	7.48	6.18	0.77	0.00	0.28	0.24	5780	3315	3881	2717
M4	5.01	2.04	3.52	0.21	45.64	22.43	5.94	4.58	0.67	0.10	0.17	0.43	4545	2923	2896	1945
M5	6.11	7.67	13.21	0.63	63.35	12.36	20.84	18.81	1.60	0.08	0.33	0.02	6990	3719	11651	6179
M6	6.43	5.56	9.59	0.53	59.28	16.50	24.35	21.65	1.60	0.16	0.93	0.02	6525	3231	4991	4087
CM	5.38	3.09	5.33	0.23	61.35	20.50	6.63	4.85	0.39	0.13	0.38	0.89	3320	2561	3246	3483
VO1	5.6	0.63	1.09	0.06	61.40	13.14	5.96	2.85	0.73	1.39	0.31	0.68	1665	1273	634	1961
VO2	5.7	1.00	1.72	0.11	48.05	22.44	5.43	3.15	0.32	1.48	0.43	<0.06	2515	715	484	173
VO3	5.6	1.61	2.78	0.18	43.50	22.90	6.02	3.38	0.26	1.83	0.5	<0.06	2555	1047	894	1047
VO4	5.87	1.94	3.35	0.11	47.50	18.22	6.41	3.69	0.88	0.15	0.52	1.17	1980	1421	1601	1321
VO5	6.04	1.77	3.06	0.10	49.57	24.14	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141
VP1	5.6	3.69	6.36	0.26	68.60	14.23	19.52	4.74	1.07	1.64	11.91	0.16	3045	2353	5739	4543
VP2	6.1	4.38	7.55	0.37	50.16	20.25	22.58	8.43	1.56	2.46	10.12	<0.05	3220	6309	4024	3897
VP3	6.1	2.83	4.88	0.23	60.62	18.35	37.23	6.33	1.48	18	11.4	<0.05	3460	4889	4294	3005
VP4	5.5	6.58	11.35	0.49	65.54	21.07	10.51	6.55	0.37	2.60	0.27	0.72	3540	6459	6624	14857
VP5	5.7	4.81	8.30	0.40	58.22	21.38	17.36	13.40	1.09	2.32	0.55	<0.10	2765	4519	3679	3965
VP6	7.05	2.43	4.19	0.14	67.42	22.43	9.09	8.11	0.51	0.08	0.37	0.02	1720	1225	3981	1703
VP7	7.27	3.56	6.14	0.22	67.50	12.22	12.32	10.53	1.04	0.44	0.27	0.04	1945	823	4576	1597
CV	5.8	4.01	6.91	0.30	61.57	20.07	8.60	5.73	0.83	0.17	1.13	0.74	2690	2129	3861	3573
FP	4.88	7.70	13.27	0.48	69.35	12.86	6.43	0.07	0.00	0.11	0.09	6.16	5410	9239	8184	10767
FE	4.8	9.78	16.87	0.67	67.28	12.86	6.59	0.05	0.00	0.12	0.12	6.29	5805	8249	10189	11347
FC	4.68	7.15	12.32	0.49	69.35	14.86	7.48	0.18	0.07	0.00	0.22	7.01	3055	6789	5619	9457

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Experimental data on adsorption

These data are shown in Table S3, corresponding to CFX adsorbed onto the different soils studied, expressed as amounts and as percentage values.

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Table S3. CFX adsorption, expressed in μmol kg^{-1} (and in percentage between brackets), onto the various soils studied, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas. Average values (n=3), with coefficients of variation always < 5%.

Soil	CFX added (μmol/L)	CFX adsorbed, concentration and %	Soil	CFX added (μmol/L)	CFX adsorbed, concentration and %
M1	0	0 (0)	VO1	0	0 (0)
	2.5	5.88 (94.12)		2.5	5.06 (81.01)
	5	12.10 (96.81)		5	10.66 (85.26)
	10	24.52 (98.09)		10	22.16 (88.65)
	20	49.43 (98.87)		20	47.41 (94.81)
	30	74.29 (99.06)		30	72.71 (96.95)
	40	99.24 (99.24)		40	91.35 (91.35)
	50	123.88 (99.10)		50	114.26 (91.42)
M2	0	0 (0)	VO2	0	0 (0)
	2.5	5.87 (93.95)		2.5	5.28 (84.54)
	5	12.08 (96.61)		5	10.67 (84.86)
	10	24.53 (98.12)		10	22.43 (89.72)
	20	49.39 (98.79)		20	47.36 (95.67)
	30	74.36 (99.15)		30	70.89 (94.99)
	40	98.98 (98.96)		40	91.41 (91.41)
	50	123.91 (99.12)		50	118.89 (95.59)
M3	0	0 (0)	VO3	0	0 (0)
	2.5	3.13 (55.94)		2.5	4.28 (68.54)
	5	8.63 (62.39)		5	10.48 (83.89)
	10	14.51 (60.17)		10	23.17 (92.67)
	20	33.06 (65.42)		20	47.86 (92.73)
	30	46.98 (61.94)		30	71.29 (95.06)
	40	54.18 (57.74)		40	94.23 (94.23)
	50	76.13 (59.41)		50	114.14 (91.31)
M4	0	0 (0)	VO4	0	0 (0)
	2.5	4.14 (73.67)		2.5	5.12 (90.91)
	5	10.90 (80.31)		5	12.70 (93.56)
	10	17.49 (73.27)		10	22.47 (93.17)
	20	48.35 (94.74)		20	47.29 (94.05)
	30	49.77 (65.62)		30	71.63 (94.45)
	40	50.09 (53.38)		40	90.32 (96.24)
	50	99.91 (78.36)		50	122.87 (95.89)
M5	0	0 (0)	VO5	0	0 (0)
	2.5	3.25 (58.34)		2.5	4.52 (80.34)
	5	7.86 (57.07)		5	11.82 (85.80)
	10	13.25 (54.98)		10	20 (84.18)
	20	27.08 (53.58)		20	44.37 (86.93)
	30	44.47 (58.92)		30	64.55 (85.54)
	40	50.02 (52.77)		40	81.69 (86.62)
	50	71.35 (55.96)		50	101.06 (79.26)

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Soil	CFX added (μmol/L)	CFX adsorbed, concentration and %	Soil	CFX added (μmol/L)	CFX adsorbed, concentration and %
M6	0	0 (0)	VP1	0	0 (0)
	2.5	4.96 (88.23)		2.5	5.85 (93.69)
	5	12.78 (93.65)		5	12.08 (96.66)
	10	22.25 (92.25)		10	24.44 (97.75)
	20	44.97 (88.98)		20	49.21 (98.41)
	30	71.23 (93.92)		30	73.68 (98.24)
	40	89.14 (94.51)		40	98.58 (98.58)
	50	119.39 (93.17)		50	123.30 (98.64)
M7	0	0 (0)	VP2	0	0 (0)
	2.5	2.75 (49.48)		2.5	5.85 (93.56)
	5	8.60 (62.77)		5	11.98 (96.30)
	10	16.03 (67.12)		10	24.47 (97.87)
	20	42.51 (83.29)		20	49.60 (98.70)
	30	63.52 (84.16)		30	74.23 (98.96)
	40	89.30 (96.78)		40	98.83 (98.83)
	50	123.40 (96.78)		50	124.26 (98.92)
FP	0	0 (0)	VP3	0	0 (0)
	2.5	4.64 (74.57)		2.5	2.96 (52.65)
	5	10.19 (82.31)		5	9.40 (68.26)
	10	22.07 (88.30)		10	21.73 (90.11)
	20	44.80 (90.05)		20	30.97 (60.99)
	30	67.64 (91.09)		30	39.53 (51.86)
	40	88.64 (89.09)		40	38.63 (40.76)
	50	109.88 (88.78)		50	54.40 (42.67)
FE	0	0 (0)	VP4	0	0 (0)
	2.5	4.63 (75.13)		2.5	5.55 (88.79)
	5	10.63 (85.45)		5	11.72 (93.80)
	10	22.44 (90.65)		10	21.90 (87.61)
	20	46.16 (92.32)		20	45.15 (90.31)
	30	69.91 (93.21)		30	64.23 (85.63)
	40	92.54 (93.47)		40	87.43 (87.43)
	50	116.71 (93.36)		50	111.96 (89.57)
FC	0	0 (0)	VP5	0	0 (0)
	2.5	4.29 (68.58)		2.5	3.87 (69.39)
	5	10.15 (82.43)		5	9.5 (68.62)
	10	21.55 (87.51)		10	13.51 (56.58)
	20	49.26 (90.84)		20	32.40 (63.48)
	30	68.89 (92.31)		30	42.11 (55.53)
	40	90.77 (91.68)		40	65.75 (69.36)
	50	114.65 (91.72)		50	74.86 (58.71)

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Soil	CFX added (μmol/L)	CFX adsorbed, concentration and %
VP6	0	0 (0)
	2.5	5.81 (93.49)
	5	11.99 (96.43)
	10	24.20 (97.77)
	20	48.81 (98.10)
	30	73.22 (98.60)
	40	97.94 (98.44)
	50	122.66 (98.62)
VP7	0	0 (0)
	2.5	5.50 (97.67)
	5	13.54 (98.26)
	10	23.63 (98.47)
	20	49.94 (98.33)
	30	73.82 (98.30)
	40	93.34 (98.47)
	50	125.57 (98.49)
VP8	0	0 (0)
	2.5	5.76 (93.07)
	5	11.99 (95.95)
	10	24.50 (97.99)
	20	48.91 (97.35)
	30	73.66 (99.20)
	40	98.45 (99.43)
	50	124.47 (99.57)

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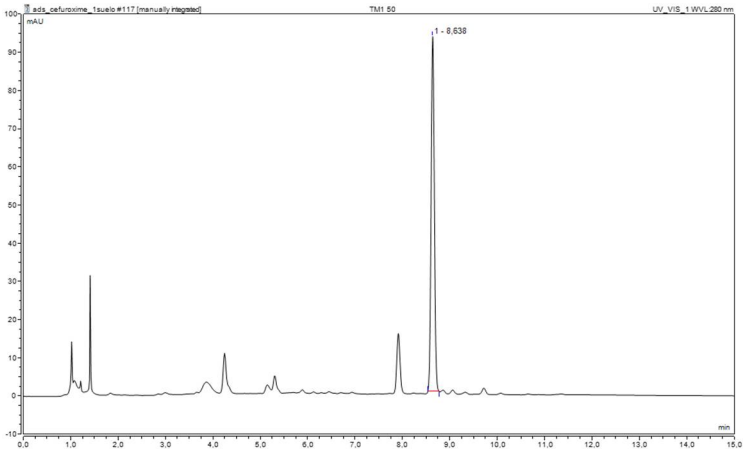
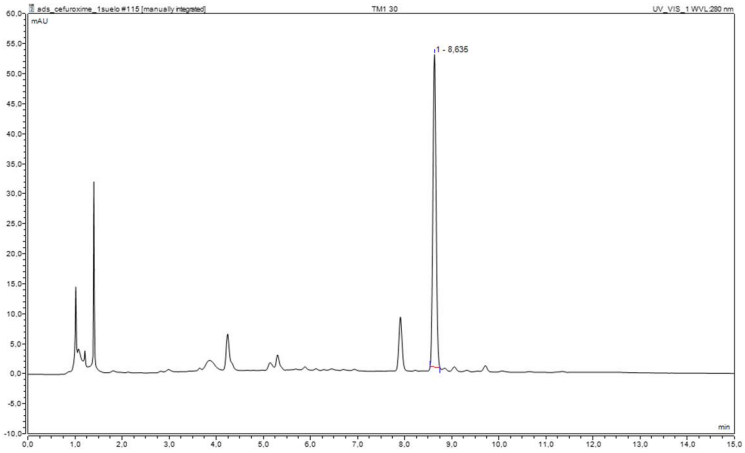
Soil M2. Concentration of CFX added 50 μmol L^{-1}



Soil M4. Concentration of CFX added 30 μmol L^{-1}



Soil VP4. Concentration of CFX added 20 μmol L^{-1}

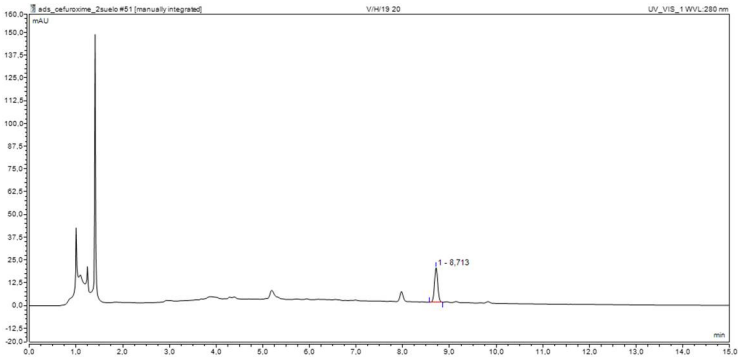
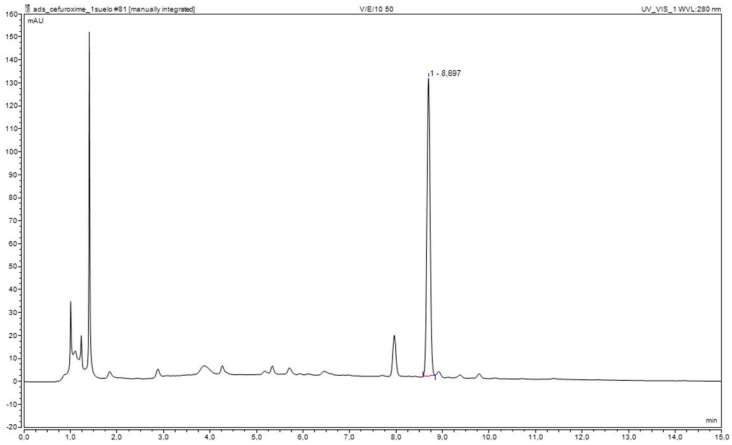
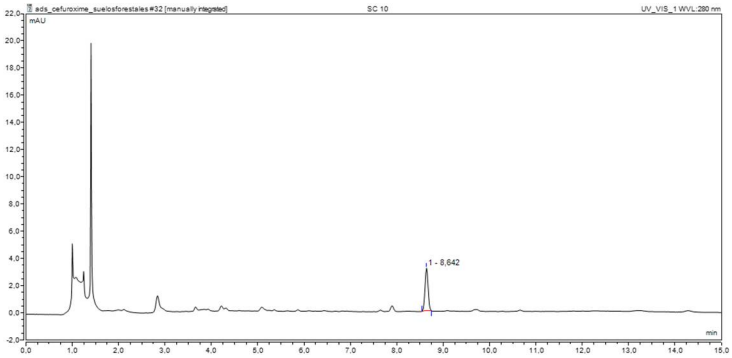


Figure S1. Selected chromatograms corresponding to the quantification of the antibiotic cefuroxime.

Soil VP3. Concentration of CFX added 50 μmol L^{-1}



Soil FC. Concentration of CFX added 10 μmol L^{-1}



Soil FP. Concentration of CFX added 50 μmol L^{-1}

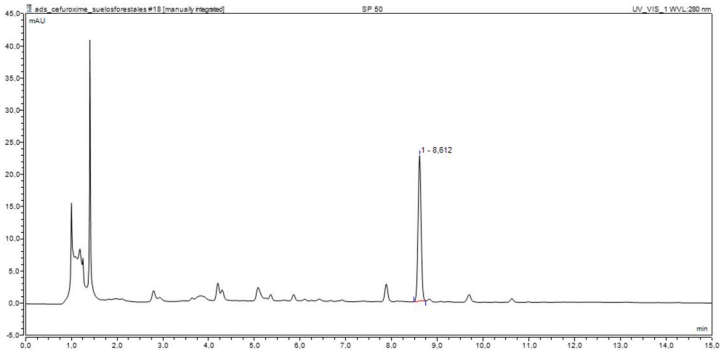


Figure S1 (continuation). Selected chromatograms corresponding to the quantification of the antibiotic cefuroxime.

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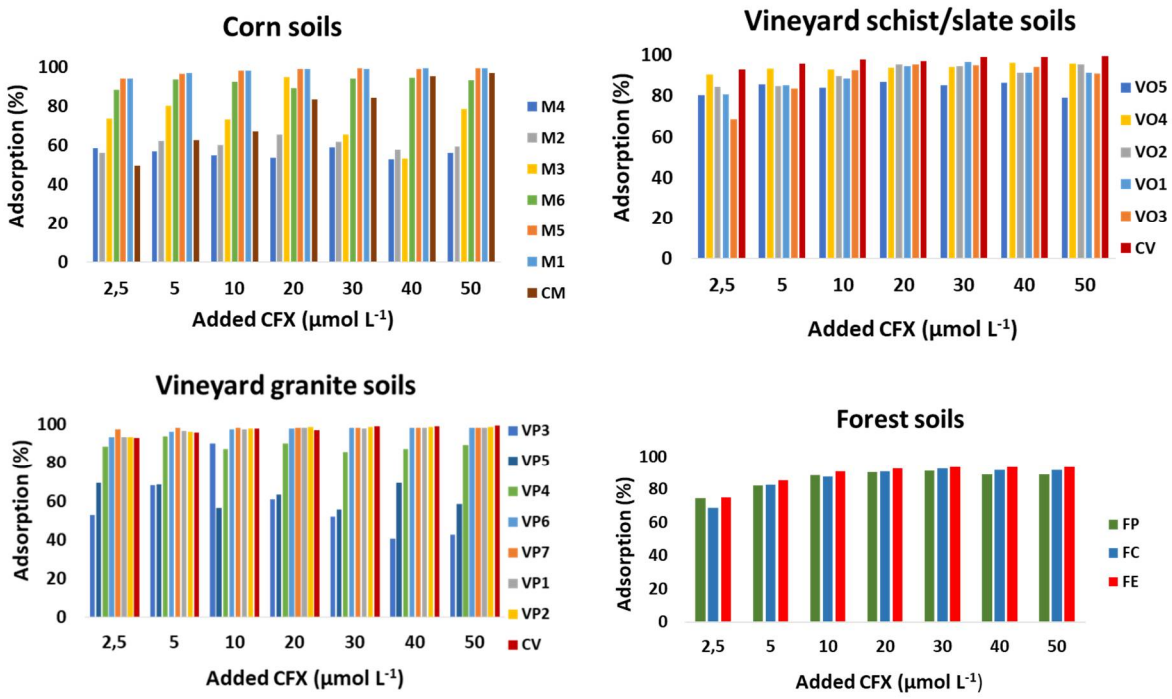

Figure S3. Percentage CFX adsorption on the various soils studied as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas. Average values (n=3), with coefficients of variation always < 5%.

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PUBLICACIÓN III

Cela-Dablanca, Raquel[1]., Nebot, Carolina[2]., Rodríguez-López, Lucía[3]., Fernández-Calviño, David[3]., Arias-Estévez, Manuel[3]., Núñez-Delgado, Avelino[1]., Fernández-Sanjurjo, María J[1]., & Álvarez-Rodríguez, Esperanza[1]. (2021). **Efficacy of different waste and by-products from forest and food industries in the removal/retention of the antibiotic cefuroxime**. *Processes*, *9*(7), 1151. https://doi.org/10.3390/pr9071151

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Article

Efficacy of Different Waste and By-Products from Forest and Food Industries in the Removal/Retention of the Antibiotic Cefuroxime

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Abstract: Environmental pollution due to antibiotics is a serious problem. In this work, the adsorption and desorption of the antibiotic cefuroxime (CFX) were studied in four by-products/residues from the forestry and food industries. For this, batch-type experiments were carried out, adding increasing concentrations of CFX (from 0 to 50 μmol L^{-1}) to 0.5 g of adsorbent. The materials with a pH higher than 9 (mussel shell and wood ash) were those that presented the highest adsorption percentages, from 71.2% (23.1 μmol kg^{-1}) to 98.6% (928.0 μmol kg^{-1}). For the rest of the adsorbents, the adsorption was also around 100% when the lowest concentrations of CFX were added, but the percentage dropped sharply when the highest dose of the antibiotic was incorporated. Adsorption data fitted well to the Langmuir and Freundlich models, with R^2 greater than 0.9. Regarding desorption, the materials that presented the lowest values when the highest concentration of CFX was added were wood ash (0%) and mussel shell (2.1%), while pine bark and eucalyptus leaves presented the highest desorption (26.6% and 28.6%, respectively). Therefore, wood ash and mussel shell could be considered adsorbents with a high potential to be used in problems of environmental contamination by CFX.

Keywords: antibiotics; eucalyptus leaves; mussel shell; pine bark; pine needles; retention/release; wood ash

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1. Introduction

From 2000 to 2015, the worldwide consumption of antibiotics in humans and in veterinary medicine increased by almost 65% [1,2], due to the increase in the world population, and to the higher demand for protein, which intensified animal production, requiring a higher use of antibiotics [3,4].

Among these drugs, cephalosporins, belonging to the group of beta-lactams, are widely used in the treatment of bacterial infections, as they have good tolerance and few side effects [5,6]. In human medicine, they are used mainly in specific infections of the human genital tract, as well as in serious infections such as meningitis [7,8]. In veterinary medicine, cephalosporins are widely used in infections of the respiratory tract and mammary glands [8–10].

Among the second-generation cephalosporins, cefuroxime (CFX) is the most prescribed, representing more than 50% of the total administration of cephalosporins in most European countries [11]. These antimicrobials are poorly absorbed by the intestine and a significant proportion (up to 90%) are excreted through feces and urine as the parent

compound [12–14], being incorporated into wastewater in the case of humans, and passing to slurry pits or directly into the environment in the case of farm animals. These wastewaters reach treatment plants, but many of these facilities have not been designed to eliminate antibiotics [14,15], removing between 20 and 90% of the pollutants, either through its accumulation in sewage sludge [16], or by degradation processes, which affect antibiotics such as penicillin [17]. However, other antimicrobials such as cephalosporins, fluoroquinolones, and tetracyclines are more resistant to natural degradation [17,18].

As an example, a study conducted in Greece on the presence of antibiotics in wastewater detected high concentrations of amoxicillin, clarithromycin, CFX, and ciprofloxacin [19]. The incorporation of antibiotics into the soil, through wastewater or fertilization with sewage sludge or livestock manure, can cause the appearance of bacterial resistance, contamination of underground or surface water bodies, and the passage of these pollutants to the food chain, both through drinking water and animal or vegetable products, as different crops can absorb the antibiotics present in the soils [20], constituting a threat to human health [21].

Recently, Cela-Dablanca et al. [22] studied the retention of CFX in various soils, finding adsorption values between 40.8% and 99.6% (between 54.39 and 125 μmol kg^{-1}) in those devoted to agricultural production, and between 74.6% and 93.5% (between 109.9 and 116.71 μmol kg^{-1}) in forest soils. However, taking into account that the presence of antibiotics both in soils and other environmental compartments is considered a matter of real concern, growing research is focusing on the design of systems and procedures to remove/retain these molecules, with special emphasis on wastewater, which is often used as irrigation waters [2,23], and also in soils [24–26].

The methods generally used for the removal of antibiotics include advanced oxidation, biological technology, and membrane separation, but they can be excessively expensive and even produce toxic by-products [14,24,27]. Adsorption technologies have the advantages of being relatively simple, low-cost, long-lasting, and renewable, and they do not generate toxic by-products [25,26]. The most common adsorbents include activated carbon, mineral materials, and biological materials [13]. Within these, the most used for the removal of antibiotics is activated carbon, but its high cost and difficulty of regeneration are considered relevant disadvantages [28]. In view of that, it would be clearly interesting to determine the adsorption capacity and potential effectivity of alternative low-cost adsorbent materials. In this line, bio-adsorbents derived from a wide variety of sources, many of them residual materials that need to be recycled, can be a viable option for the retention/removal of antibiotics present as contaminants in environmental compartments [29].

In view of the above background, this work focused on studying the adsorption/desorption characteristics of the antibiotic CFX when it interacts with different residues/by-products generated by the forestry industry (eucalyptus leaf, pine bark, pine needles, and wood ash), and of a waste from the food industry (mussel shell), in order to evaluate their potential suitability to be used in processes of removal/retention of this antibiotic, which could be very relevant for environmental protection and preservation of public health.

2. Materials and Methods

2.1. Sorbent Materials

The following materials were used: (a) four residues and by-products derived from the forestry industry, specifically eucalyptus leaves from plantations in the province of Lugo (Spain), pine bark (a commercial product of Geolia, Madrid), pine needles (from plantations located in the province of Lugo, Spain), and wood ash from a combustion boiler in Lugo (Spain); and (b) a waste/by-product from the food industry: crushed mussel shell (<1 mm in diameter), provided by Abonomar S.L. (Pontevedra, Spain). It should be noted that Galicia is one of the geographic areas with a higher production related to the forest industry, as well as in relation to mussel processing, which generates high amounts of by-products and wastes needing recycling.

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The characterization of these sorbents was carried out following the methods detailed in the Supplementary Material.

2.2. Chemical Reagents

The CFX used (purity $\geq$ 95%) was supplied by Sigma-Aldrich (Barcelona, Spain). Phosphoric acid (85% extra pure) and acetonitrile (purity $\geq$ 99.9%) used for HPLC were supplied by Fisher Scientific (Madrid, Spain), and Ca_2Cl (95% purity) by Panreac (Barcelona, Spain). To carry out HPLC determinations, all solutions were prepared with milliQ water (Millipore, Madrid, Spain).

2.3. Adsorption and Desorption Experiments

Batch-type experiments were carried out to study the adsorption/desorption of CFX on/from the different bio-adsorbents. For this, 0.5 g of bio-adsorbent was weighed, and 10 mL of a solution with different concentrations of the antibiotic (2.5, 5, 5, 10, 20, 30, 40, and 50 μmol L^{-1}) was added, also containing 0.005 M of $CaCl_2$ as a background electrolyte. The suspensions were shaken for 48 h in the dark by means of a rotary shaker (this time being enough to reach equilibrium, according to previous kinetic tests, data not shown). These suspensions were then centrifuged at 4000 rpm for 15 min. The resulting supernatants were filtered through 0.45 μm nylon syringe filters, and the antibiotic concentration in the filtered liquids was determined by HPLC-UV with LPG 3400 SD equipment (Thermo-Fisher, Waltham, MA, USA).

For this, a Luna C18 column (150 mm long, 4.6 mm internal diameter, 5 μm particle size) was used, provided by Phenomenex (Madrid, Spain), as well as a pre-column (4 mm long, 2 mm in diameter, 5 μm particle size) packed with the same material as the column. The injection volume was 50 μL and the flow rate was 1.5 mL min^{-1}. The mobile phase consisted of acetonitrile (phase A) and 0.01 M of phosphoric acid (phase B). A linear gradient was used varying from 5% to 32% of phase A and from 95% to 68% of phase B. The initial conditions were re-established in 2 min and maintained for 2.5 min. The total analysis time was 15 min, with a retention time of 8.69 min, and the wavelength used for detection was 212 nm. The amounts of antibiotic adsorbed were calculated by the difference between the concentrations initially present in the added solutions and those remaining in the solutions at equilibrium. All determinations were made in triplicate. Figure S1 (Supplementary Material) shows some selected chromatograms corresponding to the quantification of CFX.

Once the adsorption experiments were carried out, desorption was studied, allowing the evaluation of the reversibility of the process. For this, a volume of 10 mL of 0.005 M of $CaCl_2$ (without antibiotic) was added to the material resulting from the adsorption process, and then the subsequent procedure carried out for adsorption was repeated. All determinations were made in triplicate.

2.4. Data Treatment

The experimental data obtained in the adsorption/desorption tests were adjusted to the Freundlich (Equation (1)), Langmuir (Equation (2)), and Linear (Equation (3)) models.

$$q_e = K_F C_{eq}{}^n \quad (1)$$

$$q_e = \frac{q_m K_L C_{eq}}{1 + K_L C_{eq}} \quad (2)$$

$$Kd = q_e / C_{eq} \quad (3)$$

where q_e is the amount of antibiotic retained by the bio-sorbent (calculated as the difference between the concentration added and that remaining in the equilibrium solution); C_{eq} is the concentration of antibiotic present in the solution at equilibrium; K_F is the Freundlich constant related to the adsorption capacity; n is a parameter of the Freundlich model associated with the degree of heterogeneity of the adsorption; K_L is the Langmuir adsorption

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constant; q_m is the maximum adsorption capacity according to the Langmuir model; and K_d is the partition coefficient in the linear model.

The SPSS Statistics 21 software was used to carry out the adjustment of the data derived from the adsorption experiments to the Langmuir, Freundlich, and Linear models, as well as statistical correlation studies among parameters of the bio-adsorbent materials and adsorption.

3. Results and Discussion

3.1. Characteristics of the Sorbent Materials

The bio-adsorbents used in this study showed some marked differences in their physicochemical properties (Table 1). The pH in water ranged from highly acidic values, such as that of pine needles (pH = 3.68), to clearly alkaline values, such as that of wood ash (pH = 11.31). The total C content also presented a wide range, going from 13.26% in wood ash to more than 50% in eucalyptus leaves and pine needles. Regarding the total N content, it ranged between 0.08% in pine bark and 1.35% in eucalyptus leaves.

Table 1. Characteristics of the bio-adsorbent materials. C: total carbon; N: total nitrogen; Ca_e, Mg_e, Na_c, K_e, Al_e: elements in the exchange complex; Sat. Al: Al-saturation in the exchange complex; eCEC: effective cation exchange capacity; X_T: total content of the element (X); Al_o, Fe_o: noncrystalline Al and Fe. Average values (n = 3), with coefficients of variation always <5%.

Parameter	Unit	Eucalyptus Leaves	Pine Bark	Pine Needles	Wood Ash	Mussel Shell
C	%	53.05	48.70	50.31	13.23	11.43
N	%	1.35	0.08	1.08	0.22	0.21
C/N		39.18	608.75	46.76	60.14	55.65
pH_{water}		4.88	3.99	3.68	11.31	9.39
pH_{KCl}		4.81	3.42	3.51	13.48	9.04
Ca_e	$cmol_c\ kg^{-1}$	7.95	5.38	2.13	95.0	24.75
Mg_e	$cmol_c\ kg^{-1}$	8.53	2.70	7.15	3.26	0.72
Na_e	$cmol_c\ kg^{-1}$	1.36	0.46	1.42	12.17	4.37
K_e	$cmol_c\ kg^{-1}$	12.93	4.60	11.09	250.65	0.38
Al_e	$cmol_c\ kg^{-1}$	0.13	1.78	2.15	0.07	0.03
eCEC	$cmol_c\ kg^{-1}$	30.90	14.92	23.94	361.15	30.25
Sat Al	%	0.42	11.93	8.98	0.02	0.10
Available-P	$mg\ kg^{-1}$	262.84	70.45	217.95	462.83	54.17
Na_T	$mg\ kg^{-1}$	242.31	68.92	271.54	2950	5174.00
MgT	mg/kg	840.96	473.55	653.40	26,171	980.66
Al_T	$mg\ kg^{-1}$	80.58	561.50	246.95	14,966	433.24
K_T	$mg\ kg^{-1}$	4464.10	737.84	4123.44	99,515	202.07
Ca_T	$mg\ kg^{-1}$	2262.96	2318.81	538.96	136,044	280,168
Cr_T	$mg\ kg^{-1}$	0.13	1.88	0.74	36.28	4.51
Mn_T	$mg\ kg^{-1}$	614.92	30.19	356.28	10,554	33.75
Fe_T	$mg\ kg^{-1}$	43.13	169.78	47.27	12,081	3535
Co_T	$mg\ kg^{-1}$	0.03	0.20	0.38	17.25	1.02
Ni_T	$mg\ kg^{-1}$	2.17	1.86	0.93	69.25	8.16
Cu_T	$mg\ kg^{-1}$	2.80	<LD	3.81	146.33	6.72
Zn_T	$mg\ kg^{-1}$	7.66	6.98	5.78	853.00	7.66
As_T	$mg\ kg^{-1}$	0.02	<LD	0.02	8.36	1.12
Cd_T	$mg\ kg^{-1}$	0.0	0.13	0.05	19.93	0.07
Al_o	$mg\ kg^{-1}$	45.0	315.0	169.0	8323	178.33
Fe_o	$mg\ kg^{-1}$	77.0	74.0	15.0	4233	171.0

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The effective cation exchange capacity (eCEC) values also varied greatly depending on the type of bio-adsorbent, from 23.94 $cmol_c\ kg^{-1}$ for pine needles to 361.15 $cmol_c\ kg^{-1}$ for wood ash. In pine bark and mussel shell, the predominant exchangeable cation was Ca^{2+}, while it was K^+ in wood ash, eucalyptus leaves, and pine needles. In addition, noteworthy are the high values of available P present in wood ash, followed by eucalyptus leaves

and pine needles (always higher than 200 mg kg^{-1}). The total contents of heavy metals were clearly higher in wood ash compared to the other bio-adsorbents, also presenting the highest concentrations of noncrystalline Fe and Al (Fe_o, Al_o), followed by mussel shell (Table 1).

3.2. Adsorption and Desorption of CFX

3.2.1. Adsorption

Figure 1 shows the adsorption curves of CFX for the different adsorbents. Table S1 (Supplementary Material) shows the values of the adsorbed amounts and adsorption percentages as a function of the concentration of antibiotic added. The maximum adsorption values were between 551.62 μmol kg^{-1} of pine bark and 927.95 μmol kg^{-1} of wood ash.

A statistical analysis showed that the maximum adsorption values for CFX were significantly and positively correlated with the eCEC of the adsorbents ($r = 0.927$, $p < 0.05$), with its Ca contents ($r = 0.918$, $p < 0.05$), its K contents ($r = 0.927$, $p < 0.05$), and its Na contents ($r = 0.903$, $p < 0.05$).

Comparing CFX adsorption data for the five adsorbents, wood ash showed the highest adsorption percentages, retaining practically 100% of the amount added, even for the highest concentration (50 μmol L^{-1}). For pine bark and pine needles, percentage adsorption values decreased as the CFX concentration added increased, going from 100% (for the lowest concentration added) to around 55% (for the highest concentration added). Eucalyptus leaves also presented a higher CFX adsorption percentage (94.26%) when low concentrations of the antibiotic were added, compared with a value of 67.29% reached when higher CFX concentrations were used, which is probably due to the progressive saturation of the adsorption sites. In the case of mussel shell, adsorption percentages varied to a lesser extent depending on the concentration of antibiotic added, ranging between 71.19% and 85.02%.

Comparing the results obtained for the different adsorbents, it is clear that the materials with the highest pH (mussel shell and wood ash) are those with the highest adsorption values, especially when the highest concentrations of the antibiotic are added. Similarly, Fakhri and Adami [30] pointed out that the parameter that most influences the adsorption of cephalosporins is pH, as it simultaneously affects the chemical speciation of the antibiotics and the adsorbent surfaces, making the adsorption of cephalosporins low at $pH < 6$, while a basic or alkaline medium favors the adsorption processes for these compounds. These same authors also studied the adsorption of CFX in different materials (magnesium oxide nanoparticles and carbon nanotubes), finding that sorbents with $pH > 8$ presented a higher adsorption, as has occurred in the present study with wood ash. The acid dissociation constant (pK_a) is an important parameter that determines the mobility/retention of organic compounds, and therefore their distribution in environmental compartments [8,31]. Cephalosporins have at least two dissociation constants ($pK_{a1} = 3.15$ and $pK_{a2} = 10.97$) [32,33], and their molecules can have a positive or negative charge, as well as behave as a zwitterion, depending on the pH of the medium. At pH above 10.97, they are in anionic form, while they are cations when the pH is below 3.15, and they will be zwitterions between these two pH values. In view of that, CFX would be a zwitterion in all the adsorbents used in this work (except wood ash), so the positively charged groups (NH_4^+) of the antibiotic will interact electrostatically with the surfaces of the negatively charged adsorbents, which are more abundant in adsorbents with a higher pH, while the anionic groups (COO^-) of CFX will bind to these surfaces through a cationic bridge [34]. In the case of wood ash, the pH value is higher than that of the pK_{a2} value of the antibiotic (10.97), so CFX would be negatively charged, as would certain components of the ash, specifically noncrystalline Fe and Al minerals (Table 1), favoring adsorption taking place through a cationic bridge, an interaction that will be also favored by the high concentration of changeable Ca^{2+} present in the ash.

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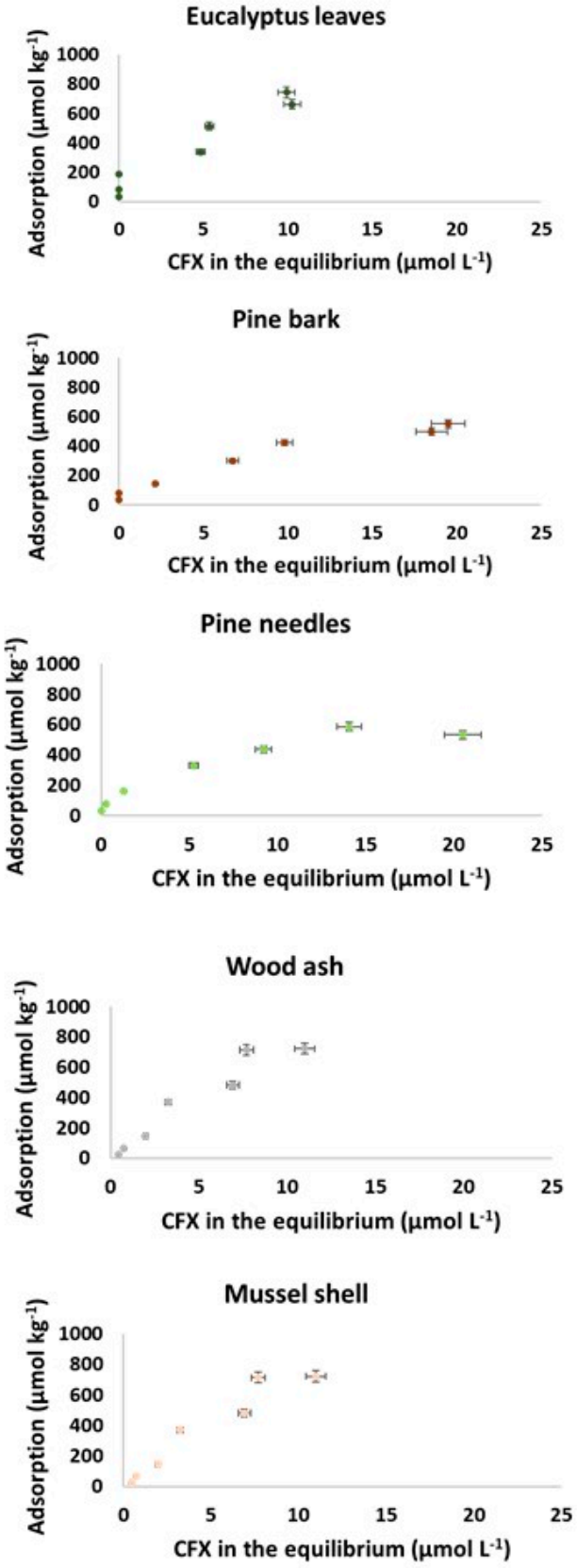

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Figure 1. Adsorption curves for CFX and the five sorbent materials used.

When all five adsorbents were considered together, significant correlations were obtained between the amount of CFX adsorbed when the highest concentration of this

antibiotic was added and the exchangeable cations (with values of r = 0.918, 0.903, and 0.927, for Ca, Na, and K, respectively; $p < 0.05$). This suggests their participation in bonds taking place by means of cationic bridges.

For three of the materials used in this study (wood ash, pine bark, and mussel shell), Conde-Cid et al. [35,36] studied in previous works their adsorption capacity for three tetracycline antibiotics (oxytetracycline, chlortetracycline, and tetracycline) and for three sulfonamides (sulfadiazine, sulfamethazine, and sulfachloropyridazine). In the case of tetracyclines, a high adsorption capacity was also obtained for wood ash, with pine bark also showing a high capacity to retain tetracyclines (unlike what was observed in the present study for CFX and pine bark), while the mussel shell was not suitable for the removal of these substances. Regarding the results of sulfonamide adsorption on those materials, Conde-Cid et al. [36] also obtained results showing differences in relation to the current work, with pine bark retaining practically 100% of the added sulfonamide, while wood ash and mussel shell were not effective in retaining these antibiotics.

Several authors have studied the adsorption of different cephalosporins on other materials [37–41]. For example, activated carbon obtained from different plant remains showed an adsorption capacity higher than 80% for cephalexin present in an aqueous solution [38,39,41]. In a study by Samarghandi et al. [42], the natural zeolites used retained 28% of the added cephalexin at pH 7, but the adsorption increased to 89% when these zeolites were coated with manganese oxide nanoparticles. Biochar made from pine wood showed a very high adsorption for different antibiotics and, therefore, can be considered promising in terms of treating contamination by these substances [14,43,44]. This kind of biochar reaches and even exceeds the adsorption capacity of powdered activated carbon biochar, a product that is commercially available but is very expensive [14,44]. It should be noted that, in the present study, residual materials such as mussel shell and especially wood ash showed high potentials to adsorb CFX, without performing additional modification treatments.

3.2.2. Modeling of Adsorption Data

The Langmuir isotherm assumes that the adsorption is homogeneous and in a single layer, with no interaction among the molecules of the sorbate. It is recognized that each sorbate molecule occupies a site, and no further adsorption can take place on it [45]. Unlike Langmuir's model, the Freundlich's model assumes that adsorption can occur in multiple layers, and that the adsorption sites are heterogeneous, with those with the highest energy being the first to be occupied, which means that there may be different functional groups involved in adsorption on the surface of the sorbent, with the intervention of different binding energies [45].

In the present study, the experimental adsorption data obtained were adjusted to the Freundlich (Equation (1)), Langmuir (Equation (2)), and Linear (related to the Henry's isotherm equation) (Equation (3)) models. Table 2 shows the parameters of the adsorption equations obtained from the adjustments to the three models. Figure 2 shows graphically the fitting to the adsorption models used.

Table 2. Fitting of the adsorption data to the Freundlich, Langmuir, and Linear models. K_F (L^n μmol^{1-n} kg^{-1}); K_L (L μmol^{-1}); n (dimensionless); q_m (μmol kg^{-1}); K_d (L kg^{-1}). -: standard error values too high for fitting.

	Freundlich					Langmuir					Linear Model		
Adsorbent	K_F	**Error**	n	**Error**	R^2	K_L	**Error**	q_m	**Error**	R^2	K_d	**Error**	R^2
Eucalyptus leaves	66.94	18.00	0.72	0.13	0.955	0.067	0.056	862.05	458.17	0.951	36.41	2.45	0.929
Pine bark	110.86	27.94	0.54	0.09	0.965	0.096	0.038	817.67	140.82	0.971	30.41	2.70	0.867
Pine needles	161.98	27.35	0.43	0.07	0.967	0.202	0.067	700.09	75.87	0.974	34.25	4.31	0.748
Wood ash	-	-	-	-	-	-	-	-	-	-	597.15	181.51	0.145
Mussel shell	93.25	34.91	0.77	0.18	0.909	0.077	0.067	1223.47	666.70	0.923	56.83	5.09	0.884

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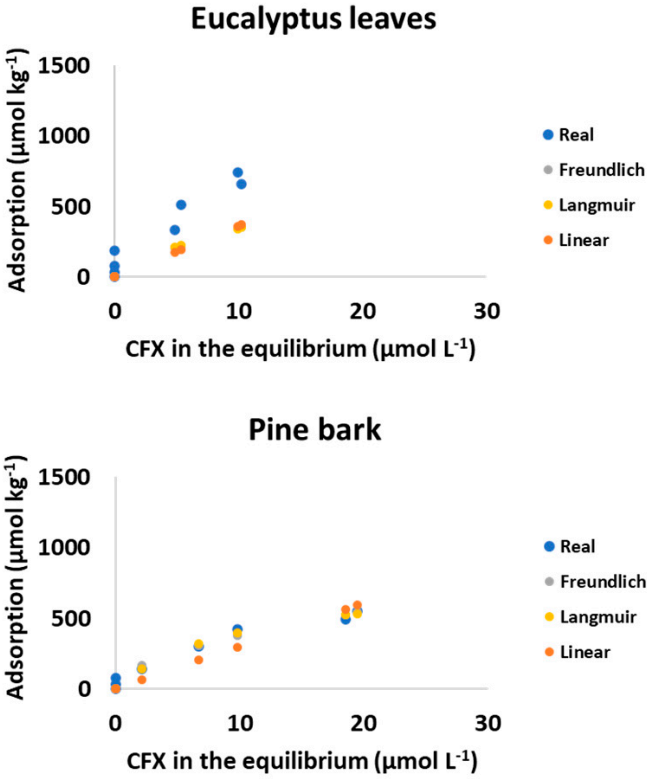

Figure 2. Adsorption curves for CFX also including data of fitting to the adsorption models used.

For the linear model, the R^2 values were lower than 0.90 in all materials (with the exception of eucalyptus leaves, R^2 = 0.929), with R^2 being very low in the case of wood ash. On the contrary, in the Freundlich and Langmuir models, R^2 values were higher than 0.9, with the exception of wood ash (bearing in mind that for this material, it was not possible to adjust both models, due to the existence of too high an error). In fact, CFX adsorption onto wood ash did not fit any model, as it adsorbed 100% of the concentrations of CFX added, which prevents the adjustment of the experimental data to the adsorption models used.

The Freundlich's K_F parameter, related to the adsorption capacity, varied between 66.9 and 162.0 L^n μmol^{1-n} kg^{-1}. The dimensionless Freundlich's n parameter, related to the heterogeneity of the active sites of the sorbent [46], showed values between 0.43 and 0.77, with the highest corresponding to mussel shell. All the adsorbents used in this study presented values of $n < 1$, which indicates a heterogeneous adsorption surface, where the highest energy sites are occupied first. This means that the adsorption energy decreases exponentially as the available surface is smaller [46,47]. It is also relevant that, the closer n is to zero, the more heterogeneous the adsorption surface will be [46,48,49].

3.2.3. Desorption

Table 3 shows data corresponding to CFX desorption from the different adsorbents, expressed in µmol kg^{-1} (and as a percentage, between parentheses). The adsorbents presenting the highest desorption values were eucalyptus leaves (ranging between 6.4 and 212.2 µmol kg^{-1}) and pine bark (ranging between 8.4 and 157.0 µmol kg^{-1}). Regarding the rest of the adsorbents (pine needles, mussel shell, and wood ash), the desorbed quantities did not exceed 20.7 µmol kg^{-1} in any case.

With desorption data expressed as percentages, it is confirmed that eucalyptus leaves and pine bark were the materials showing the highest desorption, with values ranging between 19.5 and 28.6% for eucalyptus leaves, and between 25.9 and 59.1% for pine bark. In the rest of the materials, desorption was less than 20.6% for pine needles, 12.3% for mussel shell, and 0% for wood ash. The lower desorption values of mussel shell and wood ash

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correspond to the presence of higher contents of noncrystalline Al and/or Fe compounds (Al_o, Fe_o) in these bio-adsorbents (Table 1).

Table 3. CFX desorption, expressed in μmol kg^{-1} (and in a percentage, between brackets), for the five different sorbents, as a function of the concentration of antibiotic added (C_0, in μmol L^{-1}). -: no data. Average values (n = 3), with coefficients of variation always <5%.

	C_0 (μmol L^{-1})						
Sorbent	**2.5**	**5**	**10**	**20**	**30**	**40**	**50**
Eucalyptus leaves	6.4(19.8)	16.3(20.2)	41.1(22.2)	65.9(19.5)	141.1(27.5)	164.5(24.9)	212.2(28.6)
Pine bark	8.4(25.9)	36.2(44.8)	84.0(59.1)	133.3(44.5)	157.0(37.1)	141.8(28.6)	146.5(26.6)
Pine needles	0(0)	15.4(20.5)	20.7(13.0)	19.5(5.9)	-	-	-
Wood ash	0(0)	-	-	0(0)	-	0(0)	0(0)
Mussel shell	0.3(12.2)	0.6(8.4)	1.6(10.7)	1.5(3.9)	1.4(2.9)	1.5(2.1)	1.5(2.1)

In three of these materials (oak ash, pine bark, and mussel shell), previous works studied the desorption of other groups of antibiotics, specifically three tetracyclines and three sulfonamides. For the former, Conde-Cid et al. [35] also obtained a low desorption from wood ash, but (unlike what was observed in the present study with CFX) these authors found that pine bark adsorbed tetracyclines in a way that was practically irreversible, while desorption from mussel shell was high (up to 44% of what was adsorbed). In the case of sulfonamides, Conde-Cid et al. [36,50] (2021, 2020) reported that only pine bark retained irreversibly high concentrations of these antibiotics, while wood ash and mussel shell had a low adsorption capacity and desorbed a high percentage of what was previously retained.

Considering together data on CFX adsorption and desorption for the five different adsorbents used, wood ash presented the best results, with the highest adsorption and the lowest desorption values. In previous studies, this material was also found to be very effective for the irreversible adsorption of tetracyclines [35]. Mussel shell also has potential utility for retaining CFX present in polluted media. However, pine bark could not be recommended for the adsorption of CFX, as it had low adsorption and high desorption, especially for the highest concentrations added, despite the fact that in previous studies, it showed great effectiveness to strongly retain tetracyclines and sulfonamides [35].

4. Conclusions

Among the five sorbents evaluated, the most effective for the adsorption of the antibiotic cefuroxime (CFX) were wood ash and mussel shell. Both materials were those with the highest pH values and were also characterized by their richness in noncrystalline compounds. Both sorbents would be of high interest for being used in CFX retention/removal processes, which would contribute to their recycling. The rest of the materials studied (pine bark, pine needles, and eucalyptus leaves) could not be recommended for retention/removal of this antibiotic, as they showed low adsorption and high desorption when the highest concentrations of CFX were added.

CFX adsorption generally showed a good fit to the Langmuir isotherm, and especially to the Freundlich model. Furthermore, in these materials, the Freundlich's n values were always lower than 1, which would indicate the relevance of heterogeneous adsorption sites, with those with the highest energy being the first to be occupied. Future additional studies could focus on delving into the retention mechanisms of CFX and other antibiotics (especially cephalosporins) in the sorbents that showed better results in the current work. Likewise, the influence of the simultaneous presence of several antibiotics, or of antibiotics and other contaminants, both organic and inorganic, could be evaluated, as well as the impact of modifying the values of different variables that could affect the final effectivity in the retention/release of the pollutants. Globally, the results of this study can be considered relevant at an environmental level, in relation to the potential promotion of waste and by-product recycling, protection against pollution, and its potential repercussions on aspects that affect public health.

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Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9071151/s1, Table S1: Adsorption amounts (μmol kg^{-1}) and percentages for the five adsorbents used, after adding the different initial concentrations (C_0) of the antibiotic CFX; Figure S1. Selected chromatograms corresponding to the detection of CFX after adding various concentrations of the antibiotic to the different adsorbents used in the study.

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Supplementary Materials: Efficacy of Different Waste and By-Products from Forest and Food Industries in the Removal/Retention of the Antibiotic Cefuroxime

Raquel Cela-Dablanca [1], Carolina Nebot [2], Lucia Rodríguez López [3], David Fernández-Calviño [3], Manuel Arias-Estévez [3], Avelino Núñez-Delgado [1,*], María J. Fernández-Sanjurjo [1] and Esperanza Álvarez-Rodríguez [1]

Characterization of the sorbent materials

The values corresponding to pH in water and pH in KCl (solid:liquid ratio 1:2.5) were measured with a CRISON-2001 pH-meter (Crison, Barcelona, Spain). Total C and N were determined by means of elemental analysis, using a LECO CHN-100 equipment (Leco Corporation, St. Joseph, MI, USA). The exchangeable cations (Ca_e, Mg_e, Nae, K_e and Al_e) were displaced using a 1 M NH_4Cl solution in a 1:10 soil: solution ratio, and were then quantified by means of atomic absorption spectrophotometry, using a Perkin Elemer AAnalyst 200 apparatus (Perkin Elmer, Inc, USA). The effective cation exchange capacity (eCECe) was determined as the sum of these five exchangeable cations. Available phosphorus was extracted using 0.5 M $NaHCO_3$ and quantified using a UV-visible spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). To determine total non-crystalline Fe and Al, a specific extraction was performed using ammonium oxalate buffered at pH 3, stirred for 4 h in the dark, then adding 5 drops of 0.25% superfloc to the resulting extract, then centrifuging at 2000 rpm for 10 min, filtering and diluting the supernatant to 1:5. Finally, Fe and Al in non-crystalline form (Fe_o and Al_o) were quantified by atomic absorption spectrophotometry. Total contents of different elements (Na_T, Mg_T, Al_T, K_T, Ca_T, Cr_T, Mn_T, Fe_T, Co_T, Ni_T, Cu_T, Zn_T, As_T and Cd_T) were determined following the EPA 3051 method, performing an acid digestion in a microwave with a 65% HNO_3 solution, and subsequent determination with ICP-MS.

Data on adsorption

Table S1 shows relevant data on amounts adsorbed and percentage adsorption, for the various sorbent materials and added concentrations of cefuroxime used in the experiments.

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Table S1. Adsorption amounts (Ads, in μmol kg^{-1}) and percentages (Ads, %) for the five adsorbents used, after adding the different initial concentrations (C_0) of the antibiotic CFX.

Adsorbent	C_0 (μM)	C_{eq} (μM)	Ads (μmol kg^{-1})	Ads (%)
Eucalyptus leaves	1.62	0	32.43	100
	4.04	0	80.76	100
	9.25	0	185.06	100
	21.71	4.83	337.50	77.7
	30.98	5.34	512.86	82.8
	43.35	10.26	661.75	76.3
	47.07	9.92	743.05	78.9
Pine bark	1.62	0	32.43	100
	4.04	0	80.76	100
	9.25	2.15	142.13	76.8
	21.71	6.72	299.70	69.0
	30.98	9.80	423.57	68.4
	43.35	18.52	496.61	57.3
	47.07	19.49	551.62	58.6
Pine needles	1.62	0	32.43	100
	4.04	0.28	75.06	92.94
	9.25	1.29	159.33	86.10
	21.71	5.25	329.14	75.80
	30.98	9.22	435.28	70.24
	43.35	14.07	585.53	67.54
	47.07	20.51	531.28	56.43
Wood ash	1.62	0.25	27.34	84.32
	4.04	0.42	72.29	89.51
	9.25	0.70	170.99	92.40
	21.71	0.69	420.31	96.80
	30.98	0.80	603.76	97.43
	43.35	0.73	852.39	98.32
	47.07	0.68	927.95	98.56
Mussel shell	1.62	0.47	23.09	71.20
	4.04	0.73	66.12	81.87
	9.25	1.98	145.43	78.59
	21.71	3.25	369.19	85.03
	30.98	6.91	481.38	77.68
	43.35	7.70	712.88	82.23
	47.07	10.99	721.65	76.65

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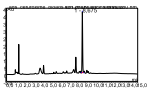
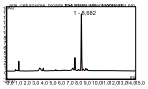
Chromatograms

Figure S1 shows selected chromatograms corresponding to the detection and quantification of cefuroxime in the experiments performed.

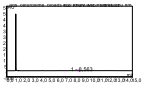
Adsorption on mussel shell. Concentration of CFX added 20 μmol/L



Adsorption on mussel shell. Concentration of CFX added 50 μmol/L



Adsorption on wood ash. Concentration of CFX added 10 μmol/L



Adsorption on pine bark. Concentration of CFX added 50 μmol/L

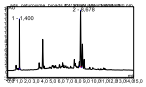


Figure S1. Selected chromatograms corresponding to the detection of CFX after adding various concentrations of the antibiotic to the different adsorbents used in the study.

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PUBLICACIÓN IV

Cela-Dablanca, Raquel[1]., Míguez-González, Ainoa[1]., Rodríguez-López, Lucía[2]., Barreiro, Ana[1]., Arias-Estévez, Manuel[2]., Fernández-Sanjurjo, María J[1]., Álvarez-Rodríguez, Esperanza[1] & Núñez-Delgado, Avelino[1]. (2024). **Removal of Cefuroxime from Soils Amended with Pine Bark, Mussel Shell and Oak Ash**. *Processes*, *12*(7), 1335. https://doi.org/10.3390/pr12071335

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Removal of Cefuroxime from Soils Amended with Pine Bark, Mussel Shell and Oak Ash

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Abstract: The global increase in antibiotics consumption has caused hazardous concentrations of these antimicrobials to be present in soils, mainly due to the spreading of sewage sludge (or manure or slurry) and wastewater, and they could enter the food chain, posing serious risks to the environment and human health. One of these substances of concern is cefuroxime (CFX). To face antibiotics-related environmental pollution, adsorption is one of the most widely used techniques, with cost-effective and environmentally friendly byproducts being of clear interest to retain pollutants and increase the adsorption capacity of soils. In light of this, in this work, three low-cost bioadsorbents (pine bark, oak ash, and mussel shell) were added to different soil samples (at doses of 12 and 48 t/ha) to study their effects on the adsorption of CFX. Specifically, batch experiments were carried out for mixtures of soils and bioadsorbents, adding a range of different antibiotic concentrations at a fixed ionic strength. The results showed that the addition of pine bark (with pH = 3.99) increased the adsorption to 100% in most cases, while oak ash (pH = 11.31) and mussel shell (pH = 9.39) caused a clearly lower increase in adsorption (which, in some cases, even decreased). The Freundlich and linear models showed rather good adjustment to the experimental data when the bioadsorbents were added at both doses, while the Langmuir model showed error values which were too high in many cases. Regarding desorption, it was lower than 6% for the soils without bioadsorbents, and there was no desorption when the soils received bioadsorbent amendments. These results show that the addition of appropriate low-cost bioadsorbents to soils can be effective for adsorbing CFX, helping in the prevention of environmental pollution due to this emerging contaminant, which is a result of clear relevance to environmental and human health.

Keywords: adsorption; antibiotics; byproducts; desorption; soil

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1. Introduction

Pharmaceuticals are broadly used worldwide, generating high concentrations of substances such as antibiotics in wastewaters, which can be released to different environmental compartments [1]. The massive use of antibiotics has caused their widespread presence in the environment, with derived pollution detected in soils, sediments, sludges, water, plants, and aquatic animals [2,3]. The time required for the degradation of antibiotics in the environment depends on many factors, such as the concentration and chemical structure of the pollutant, as well as the characteristics of the affected soils or sediments (including the humic acid content, humidity, pH, temperature, sorption capacity, presence of inorganic matter, and availability of oxygen and microorganisms that support the biodegradation

process) [4,5]. We note that between 20% and 97% of any dose of most antibiotics administered to humans and animals is excreted as active substances [6,7]. Specifically, the incomplete absorption of antibiotics by humans entails the discharge of large quantities of these pharmaceuticals in municipal wastewater treatment plants (WWTPs) [1] which, together with their inefficiency at removing antibiotics [8], is the main cause of this pollution problem. These WWTP coexist with and contact relevant amounts of microorganisms and antibiotic residues for prolonged periods of time, triggering the additional problem of increasing antibiotic resistance [9–11]. The increase in antibiotic-resistant bacteria (ARB) is a major problem for public health due to the fact that infections caused by these resistant microbes are difficult to treat, with COVID-19 deriving an additional rise in antibiotic consumption [12], also affecting the general levels of antimicrobial resistance (AMR) [13].

The removal of antibiotics in WWTPs by means of conventional treatments is limited to 50–80%, depending on the antibiotics' physicochemical properties [14–16]. In this regard, the antibiotic CFX has been detected in wastewater at μg/L and ng/L levels [17] as well as in surface waters, with the highest concentration reaching 210 μg/L for wastewater from the pharmaceutical industry and hospitals around the world [18,19]. A study carried out in multiple urban wastewaters in Germany showed CFX concentrations up to 6196 ng/L and 1957 ng/L in influent and effluent samples, respectively [20].

The spreading of wastewater and sewage sludge is a potential way to introduce pharmaceutical compounds into waterbodies and soils [21,22]. The use of reclaimed wastewater for the irrigation of crops is an important source of ARB and antibiotic resistant genes (ARGs) in agricultural soils [23]. Furthermore, it is quite common that annual fertilization with repeated applications of inappropriate doses of sewage sludge is carried out to provide soil nutrients and organic matter [24,25]. In Europe, for many years, more than 50% of produced sewage sludge is used in agriculture [26–29]. However, the application of sewage sludge to soils may pose a problem for the environment, due to their contents including a wide variety of toxic pollutants [29–32]. Furthermore, emerging pollutants pose an important risk if they are transferred to groundwater because it is a source of drinking water [33–35], and degradation will likely be extremely limited in these aquatic systems [36,37].

Antibiotic residues have different persistence times and transport types in agricultural soils, affected by factors such as adsorption, degradation, and lixiviation [38,39]. Their dissipation rates, expressed as half-lives, can range from 18.6 to 21.3 days for sulfonamide antibiotics, whereas for tetracyclines, they can be up to 1–2 months, and for enrofloxacin, they can be approximately 7–8 months [40]. Conde-Cid et al. [41] observed no degradation for three sulfonamides in the dark, while under simulated sunlight, after 2 h of exposure, sulfachloropyridazine and sulfadiazine degraded by 90%, and sulfamethazine degraded by 80% after 8 h. Rodríguez-López et al. [42] studied the degradation of clarithromycin, ciprofloxacin, and trimethoprim under different conditions, observing that after 1 h of exposure to light radiation, 85% of the ciprofloxacin degraded. They also reported that claritromycin degradation was pH-dependent, obtaining the highest degradation percentage (30%) at pH = 4, whereas trimethoprim reached less than 5%. A study carried out in Chinas's Xuanwu Lake found four cephalosporins degraded abiotically in the surface water in the dark, with half-lives between 2.7 and 18.7 days, while the time decreased by up to 2.2–5 days under exposure to simulated sunlight [43].

Adsorption is widely used to remove antibiotics due to its simple design and because it is environmentally friendly, economic, and versatile [44]. Its efficiency depends on the type of adsorbent, adsorbate properties, and waste stream (in the case of waste liquids) composition [45,46]. Depending on the pH of the medium and the pK_a of the pollutant, sorption is also affected by the charges of the molecular forms of the sorbates, (i.e., neutral, cationic, anionic, or zwitterionic) [47]. Furthermore, developing cost-effective and environmentally friendly treatment techniques for the remediation of specific contaminants (such as antibiotics) present in soils is imperative [48,49]. The need to deal with agricultural and industrial wastes or byproducts has drawn additional attention to the adsorption field,

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due to the eco-friendly approach and advantages of using these materials, such as their easy access, high availability, and low cost compared with synthetic or other high-cost materials [50]. Some examples were shown by Ahmed et al. [51], whereas other specific previous studies showed that the addition of pine bark to soils increased the retention of three sulfonamides [52]. Also, in a previous work, we studied the effectiveness of the byproducts pine bark, oak ash, and mussel shell as bioadsorbents for the antibiotics amoxicillin, ciprofloxacin, and trimethoprim, with good results [53], and other studies focused on the retention of CFX in soils [54] and onto the same byproducts [55], although no research was performed dealing with the retention or release of CFX on or from soils amended with these sorbents.

In light of the above background, the objective of this work was to characterize the potential of three bioadsorbents (pine bark, oak ash, and mussel shell) to retain or remove the antibiotic cefuroxime (CFX) after the amendment of six different soils at two doses (12 and 48 t/ha). The results of the investigation could be of relevance regarding the management of soils contaminated with this emerging pollutant as well as the recycling of byproducts, potentially facilitating sustainability and a circular economy.

2. Materials and Methods

2.1. Soils and Bioadsorbents

To carry out this research, six different soil samples were selected, with all of them coming from different agricultural areas in Galicia (northwest Spain). These soils are devoted to vineyards (named VP1, VP2, and VO), and corn production (denoted as C1, C2, and C3). In a previous work [54], we studied CFX adsorption onto 23 different soils, and we selected among them the 6 soils with the lowest CFX adsorption capacities for use in the current research. The selected soils were amended with various bioadsorbents to assess the eventual modification of CFX adsorption onto these soils. The methods used for their characterization are presented in the Supplementary Materials, with the results shown in Table S1.

Three byproducts were used as bioadsorbents, with two of them coming from the forestry industry—pine bark, provided by Geolia (Madrid, Spain), and oak ash from a combustion boiler in Lugo (Spain)—while the third one was from the canning industry: mussel shell, provided by Abonomar S.L (Pontevedra, Spain).

Table 1 shows the results corresponding to the characterization of different parameters for the three bioadsorbents. The pH values were determined using a soil-to-liquid ratio of 1:2.5. The contact time for the pH in water was 10 min, while for the pH in KCl, it was 2 h [55]. The measurements were made with a CRISON pH meter, model 2001 (Crison, Barcelona, Spain). The available phosphorous was determined by the Olsen method [56]. To carry out this method, 2.5 grams of bioadsorbent was weighted, and 50 mL of the extracting solution (sodium bicarbonate at pH = 8.5) was added. This mixture was subjected to shaking for 30 min before utilizing visible spectophotometry for P quantification. Determination of the total C and N was carried out using an elemental analysis method with a LECO TRUSPEC CHNS instrument (Leco, St. Joseph, MI, USA). The exchangeable cations (Ca_e, Mg_e, Na_e, K_e, and Al_e) were displaced using a 1 M NH_4Cl solution in a 1:10 sorbent:solution ratio, with subsequent quantification by means of atomic absorption spectrophotometry using a Perkin Elemer AAnalyst 200 apparatus (Perkin Elmer, Waltham, MA, USA). The total contents of the elements analyzed were determined through the EPA 3051 method using acid digestion with a 65% HNO_3 solution. The measurements were performed by means of an ICP-MS (Perkin-Elmer, USA). To determine the total non-crystalline Fe and Al contents, a specific type of extraction was performed using ammonium oxalate buffered at a pH level of 3, followed by stirring for 4 h in the dark, adding 5 drops of 0.25% superfloc to the resulting extract, centrifuging at 2000 rpm for 10 min, and filtering and diluting the supernatant to a 1:5 ratio. Finally, Fe and Al in non-crystalline form (Fe_o and Al_o) were quantified with atomic absorption spectrophotometry (Perkin-Elmer, USA).

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Table 1. Characteristics of the bioadsorbent materials. Ca_e, Mg_e, Na_e, K_e, and Al_e = elements in the exchange complex; Sat. Al = Al saturation in the exchange complex; eCEC = effective cation exchange capacity; XT = total content of the element (X); and Alo and Feo = non-crystalline Al and Fe, respectively. Average values (n = 3) with coefficients of variation always <5%.

Parameter	Unit	Pine Bark	Oak Ash	Mussel Shell
C	%	48.70	13.23	11.43
N	%	0.08	0.22	0.21
C/N		608.75	60.14	55.65
pH_{water}		3.99	11.31	9.39
pH_{KCl}		3.42	13.48	9.04
Ca_e	$cmol_c\ kg^{-1}$	5.38	95.0	24.75
Mg_e	$cmol_c\ kg^{-1}$	2.70	3.26	0.72
Na_e	$cmol_c\ kg^{-1}$	0.46	12.17	4.37
K_e	$cmol_c\ kg^{-1}$	4.60	250.65	0.38
Al_e	$cmol_c\ kg^{-1}$	1.78	0.07	0.03
eCEC	$cmol_c\ kg^{-1}$	14.92	361.15	30.25
Sat Al	%	11.93	0.02	0.10
Available P	$mg\ kg^{-1}$	70.45	462.83	54.17
Na_T	$mg\ kg^{-1}$	68.92	2950	5174.00
Mg_T	mg/kg	473.55	26,171	980.66
Al_T	$mg\ kg^{-1}$	561.50	14,966	433.24
K_T	$mg\ kg^{-1}$	737.84	99,515	202.07
Ca_T	$mg\ kg^{-1}$	2318.81	136,044	280,168
Cr_T	$mg\ kg^{-1}$	1.88	36.28	4.51
Mn_T	$mg\ kg^{-1}$	30.19	10554	33.75
Fe_T	$mg\ kg^{-1}$	169.78	12,081	3535
Co_T	$mg\ kg^{-1}$	0.20	17.25	1.02
Ni_T	$mg\ kg^{-1}$	1.86	69.25	8.16
Cu_T	$mg\ kg^{-1}$	<LD	146.33	6.72
Zn_T	$mg\ kg^{-1}$	6.98	853.00	7.66
As_T	$mg\ kg^{-1}$	<LD	8.36	1.12
Cd_T	$mg\ kg^{-1}$	0.13	19.93	0.07
Al_o	$mg\ kg^{-1}$	315.0	8323	178.33
Fe_o	$mg\ kg^{-1}$	74.0	4233	171.0

Other details related to additional previously published characteristics are provided in the Supplementary Materials.

These bioadsorbents were added to soil samples in doses of 12 and 48 t/ha. The mixtures reached the pH values that are shown in Table S2 (Supplementary Materials).

2.2. Chemicals and Reagents

The antibiotic cefuroxime (CFX) was provided by Sigma Aldrich (Barcelona, Spain) and was of >95% purity. Phosphoric acid (85% extra pure) was supplied by Acros Organics (Barcelona, Spain), acetonitrile (purity $\geq$ 99.9%) was supplied by Fisher Scientific (Madrid, Spain), and $CaCl_2$ (95% purity) was supplied by Panreac (Barcelona, Spain). All of the reagents needed for the quantification of antibiotics were of HPLC grade.

2.3. Adsorption and Desorption Experiments

Batch experiments were carried out to study the adsorption capacity of the mixtures of soil and bioadsorbents. To accomplish this, 5 mL of a 0.005 M $CaCl_2$ solution (which served as the background electrolyte to maintain a constant ionic strength) received the appropriate amount of antibiotics to achieve different specific CFX concentrations (0, 2.5, 5, 10, 20, 30, 40, and 50 µmol/L). Then, each of these solutions was added to 2 g of the corresponding mixtures of soil and bioadsorbent. Afterward, the samples were subjected to shaking for 48 h and centrifugated at 4000 rpm for 15 min, with the resulting supernatants being passed

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through 0.45 µm nylon-type syringe filters. Previous kinetic studies demonstrated that 48 h is enough time to reach equilibrium.

Quantification of the antibiotic concentrations was performed using HPLC equipment, with all the details provided in the Supplementary Materials.

Figure S1 shows some select chromatograms corresponding to the quantification of CFX.

After the adsorption phase, the desorption experiments were carried out. For this, 5 mL of $CaCl_2$ was added to the remaining post-adsorption materials. The samples were shaken for 48 h, centrifugated, filtered, and analyzed in the same conditions as those during the adsorption process.

2.4. Modeling and Statistics

The experimental adsorption data were fitted to the Freundlich (Equation (1)), Langmuir (Equation (2)), and linear (Equation (3)) models [57]:

$$q_e = K_F C_{eq^n} \quad (1)$$

$$q_e = \frac{q_m K_L C_{eq}}{1 + K_L C_{eq}} \quad (2)$$

$$K_d = q_e / C_{eq} \quad (3)$$

where q_e is the amount of CFX retained (concentration added minus concentration in the equilibrium); C_{eq} is the CFX concentration in equilibrium; K_F is the Freundlich adsorption capacity parameter; n is a parameter related to heterogeneity in adsorption; K_L is the Langmuir adsorption constant; q_m is the Langmuir maximum adsorption capacity; and K_d is the linear model's partition coefficient.

All of the experimental determinations were made in triplicate.

The fitting of the experimental data to the Langmuir, Freundlich, and linear models was performed by means of SPSS Statistics 21 software.

The studies carried out for this research can be seen as the first steps within an overall broader strategy which will need further future works to be completed, both with classic and new scientific tools [58,59].

3. Results and Discussion

3.1. CFX Adsorption

Figures 1 and 2 show the adsorption curves corresponding to the different soils amended with doses of 12 and 48 t/ha of each of the three bioadsorbents.

Both figures show that, overall, mussel shell (pH = 9.39) was the sorbent with the greatest positive effect on the increase in CFX adsorption for any of the soils receiving both doses of the amendments. In fact, with this byproduct, the concentrations in the antibiotic's equilibrium (expressed in µmol/L) were the lowest, being associated with the highest adsorption levels (expressed in µmol/kg). In contrast, pine bark (pH = 3.99) showed the weakest effects as an amendment intended to increase CFX adsorption for the soils treated with the three byproducts.

Compared with previous studies [54,60] focused on retention of the same antibiotic on equivalent soils and byproducts but performed for soils and sorbents separately (not mixing the soils and sorbents, which was performed for the first time in the present study), the following comments can be presented. Regarding the adsorption of CFX onto the bioadsorbents (without soil), based on a previous publication by Cela-Dablanca et al. [60], the influence of the pH level was confirmed, with oak ash (pH = 11.31) showing the highest adsorption values (927.95 µmol/kg when a concentration of 50 µmol/L was added), followed by mussel shell (pH = 9.39), with maximum adsorption of 721.65 µmol/kg when a concentration of 50 µmol/L was added, and finally pine bark (pH = 3.99), which presented the lowest adsorption (551.62 µmol kg^{-1} when a concentration of 50 µmol/L was added). Furthermore, in another previous study [54] focused on CFX adsorption by soils (without

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bioadsorbents) with different pH values, the authors confirmed that soils with an alkaline pH presented the highest CFX adsorption, with scores oscillating between 74.86 µmol kg^{-1} (for soil with pH = 5.7) and 123.88 µmol/kg (for soil with pH = 8.02).

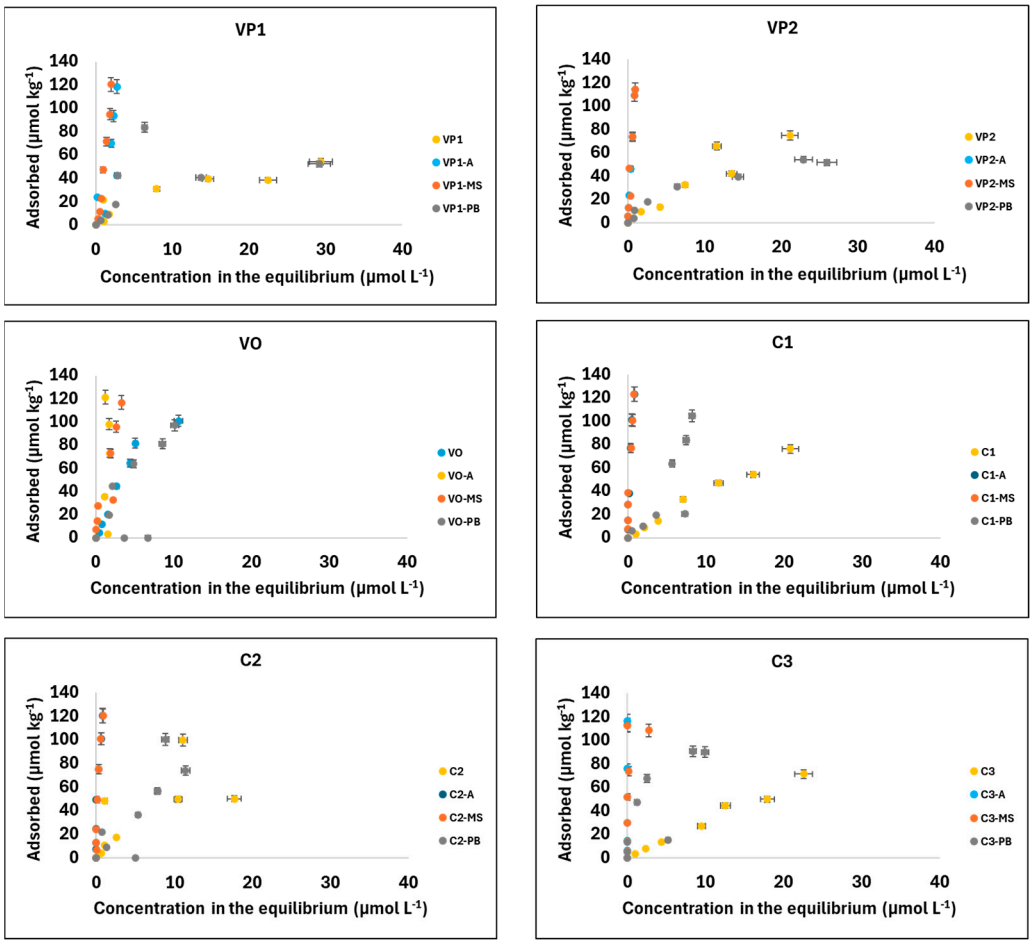

Figure 1. Adsorption curves for CFX corresponding to soils without and with bioadsorbents (amended with a dose of 12 t/ha). Average values (n = 3) are with coefficients of variation always lower than 5%. Bars indicate standard deviation. C = corn soils; VO and VP = vineyard soils; A = oak ash; MS = mussel shell; and PB = pine bark.

Figures 3 and 4 show the CFX adsorption (as a percentage) for the different antibiotic concentrations added to the soils (2.5–50 µmol/L) without and with the amendment of the three bioadsorbents, which were applied in doses of 12 t/ha (Figure 3) and 48 t/ha (Figure 4).

As shown in Figure 3, when the bioadsorbents were added to soils at a dose of 12 t/ha, the behaviors were different as a function of the byproduct used. Specifically, the addition of mussel shell and oak ash generated an increase in CFX adsorption, reaching values of 100% retention in most cases. However, when pine bark was added to soils, the adsorption increase was significantly lower, and adsorption even decreased in some cases.

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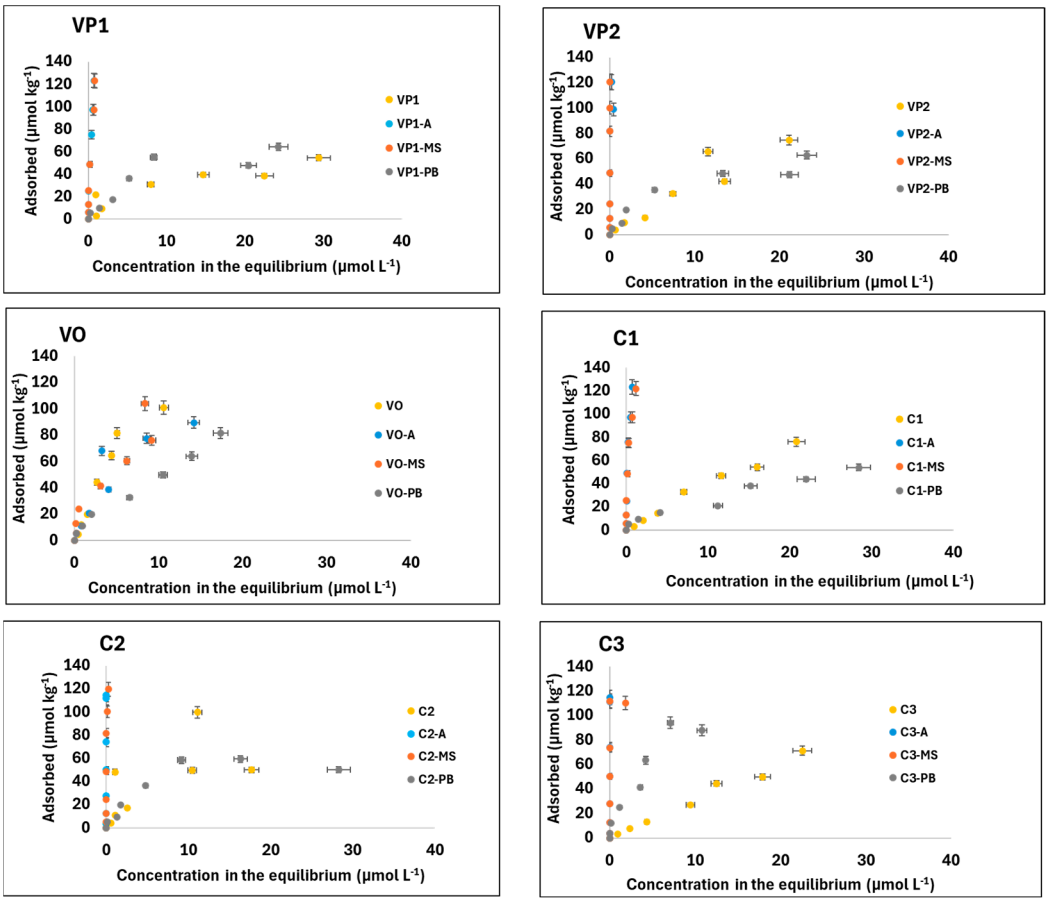

Figure 2. Adsorption curves for CFX corresponding to soils without and with bioadsorbents (amended at a dose of 48 t/ha). Average values (n = 3) are with coefficients of variation always lower than 5%. Bars indicate standard deviation. C = corn soils; VO and VP = vineyard soils; A = oak ash; MS = mussel shell; and PB = pine bark.

Despite this, in a previous work carried out for other antibiotics (three sulfonamides), Conde-Cid et al. [52] found that adding pine bark to soils increased the adsorption capacity to 99.6%. Once again, it should be noted that mussel shell and oak ash present alkaline pH values (9.39 and 11.31, respectively), while pine bark has a pH level of 3.99. This would suggest that CFX adsorption is pH-dependent. These results were confirmed by the abovementioned previous studies performed separately for soils and bioadsorbents [54,60]. We note that the dissociation constant (pK_a) is a parameter having marked relevance regarding the environmental fate of organic compounds [4]. In this sense, the pH level affects the behavior of antibiotics when they are cationic, anionic, or neutral molecules, and it also affects the activity of functional groups in a solution [61]. According to El-Shaboury et al. [62], Lin et al. [63], and Ribeiro and Schmidt [64], cephalosporin structures generally have two or more ionization centers. In the case of CFX, the dissociation constants are pka_1 = 3.15 and pka_2 = 10.97 [64]. At pH values below 3.15, CFX will be in its cationic form, and above pH 10.97, it will be in its anionic form. In the pH range 3.15–10.97 (in which our samples were placed), CFX would be zwitterionic. In this case, the positive charges of the antibiotic (NH_4^+) will bind to the negative charges of the sorbents due to electrostatic interactions, while the negative charges of CFX (COO^-) could make use of a cationic bridge with negative charges present on the surface of the sorbent materials [65]. Calcium was the predominant cation in most samples used in this study, and it would be the most probable binding element acting as a cationic bridge between the adsorbents and the adsorbate. Soils amended with the bioadsorbents used here generally reach a pH level above 5.5, with the highest values corresponding to amendments with mussel shell and the lowest ones

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associated with pine bark (which has a pH lower than that of the used soils), particularly for those at doses of 48 t/ha (Table S2, Supplementary Materials). The variable charge components of the soils used in this study (mainly kaolinite, non-crystalline Al and Fe minerals, and organic matter) and non-crystalline minerals of the bioadsorbents (extremely abundant in the case of oak ash) would exhibit a negative charge at these pH values. This negative charge in the soil and bioadsorbent mixture would be greater for higher pH levels (soil with mussel shell and soil with oak ash), favoring electrostatic interactions between these negative charges and the positively charged groups of CFX, or through cationic bridges with the anionic groups of this antibiotic [65]. In contrast, the soils amended with pine bark showed lower CFX adsorption since the pH level was lower than those of the other mixtures, as the high amount of organic matter they provided would present few negative charges. Additionally, pine bark had a lower content of non-crystalline minerals, which provide a negative charge at pH > 6, and a lower Ca content to establish cationic bridges between CFX and the soils amended with this byproduct. All of this would explain why this bioadsorbent was less effective in CFX retention than the other two. Furthermore, in acidic conditions, free H^+ in the medium could contribute to the formation of hydrogen bonds between the adsorbent's surface and the antibiotic [66].

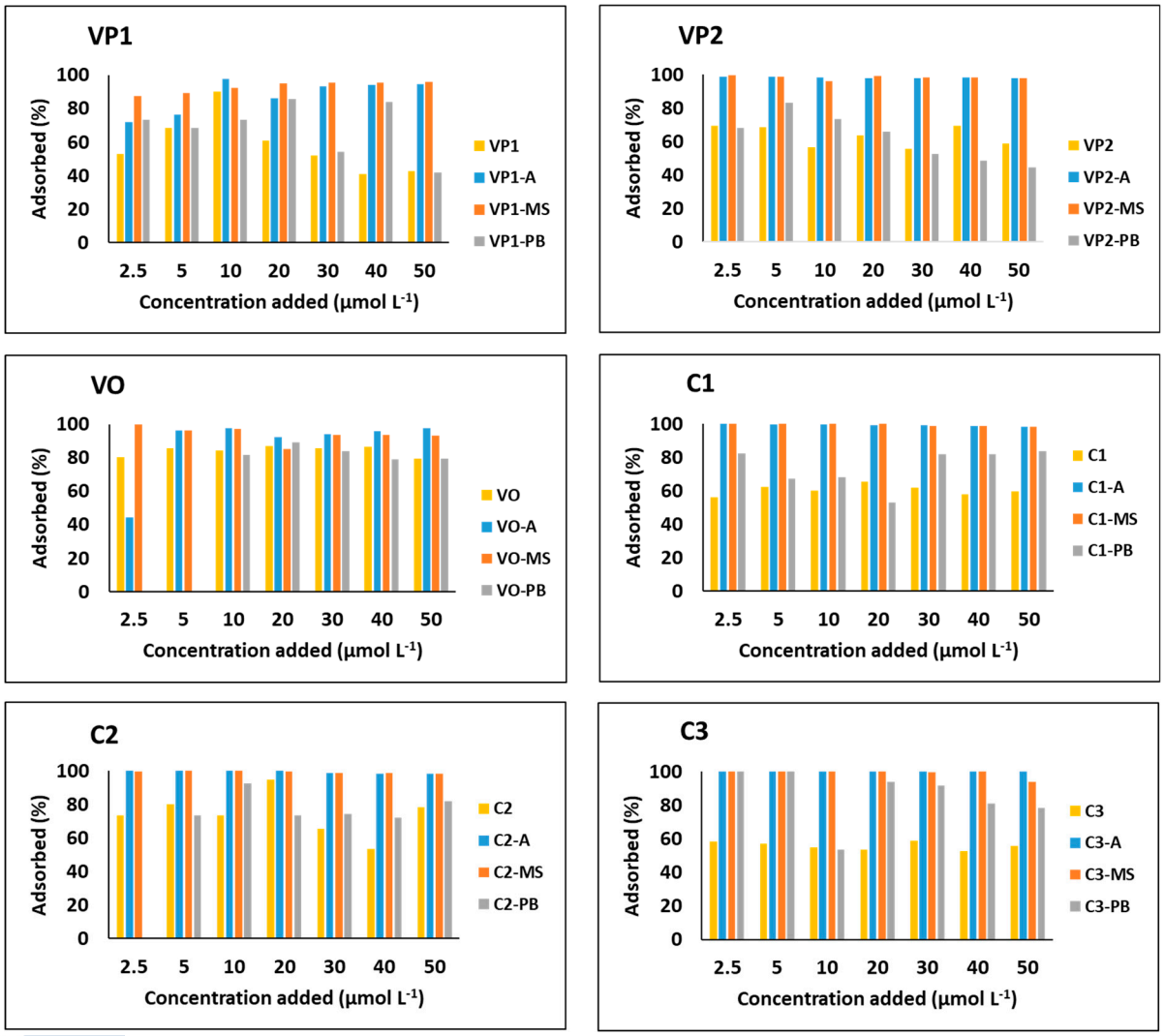

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Figure 3. Adsorption of CFX (as a percentage) by soils without and with bioadsorbents amended at a dose of 12 t/ha as a function of the concentration of antibiotics added. Average values (n = 3) are with coefficients of variation always lower than 5%. C = corn soils; VO and VP = vineyard soils; A = oak ash; MS = mussel shell; and PB = pine bark.

Figure 4 shows the CFX adsorption by soils without bioadsorbents and amended with the three bioadsorbents at a dose of 48 t/ha.

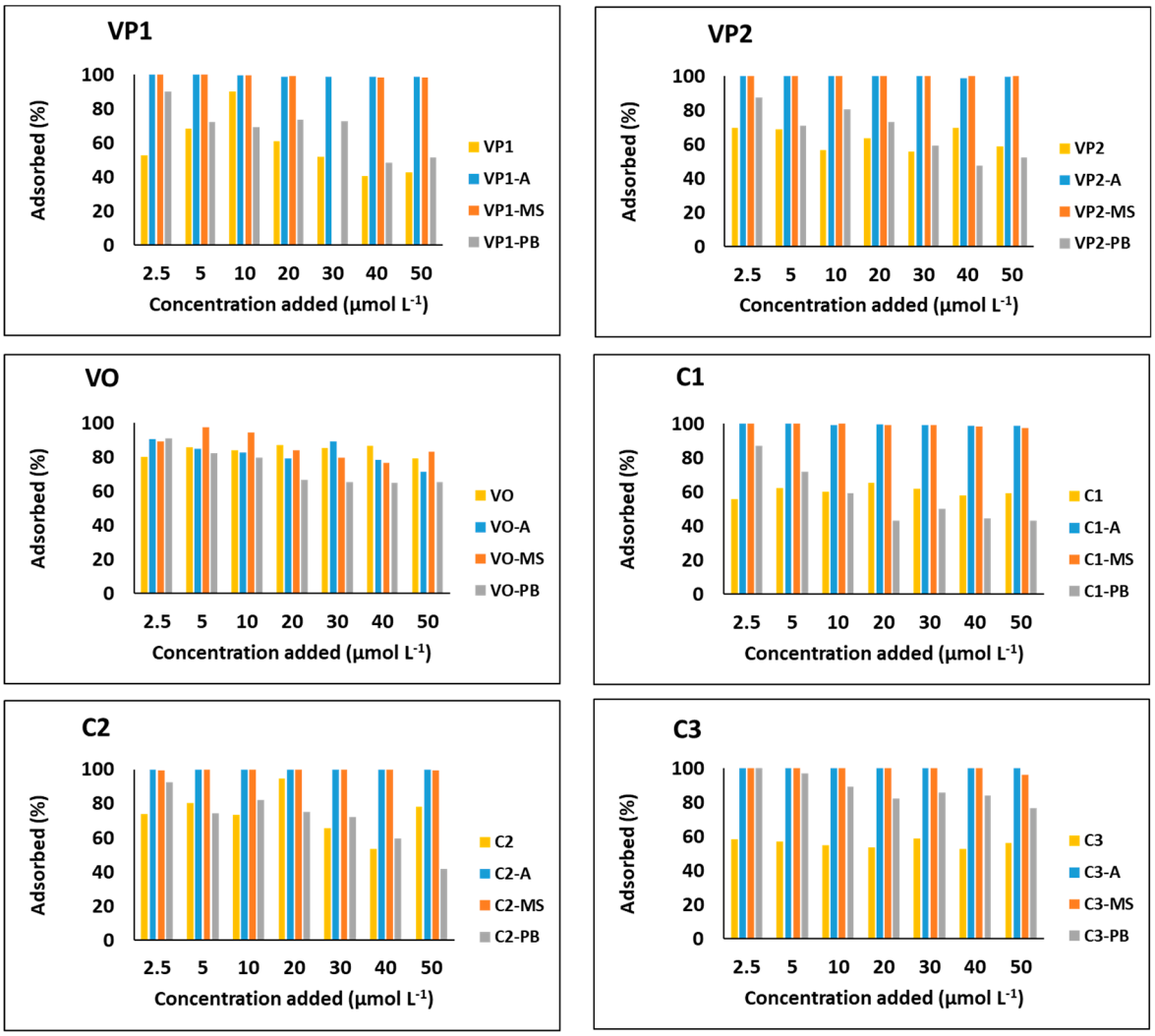

Figure 4. Adsorption of CFX (as a percentage) by soils without and with bioadsorbents amended at a dose of 48 t/ha as a function of the concentration of antibiotics added. Average values (n = 3) are with coefficients of variation always lower than 5%. C = corn soils; VO and VP = vineyard soils. A = oak ash; MS = mussel shell; and PB = pine bark.

The data obtained corresponding to the addition of the bioadsorbents to the six soils at a dose of 48 t/ha (Figure 4) confirmed an equivalent behavior to that found for the dose of 12 t/ha. The addition of bioadsorbents with alkaline pH levels (oak ash and mussel shell) was associated with the highest increase in adsorption, while the addition of pine bark (with an acidic pH) caused a lower increase (and decreased adsorption in several cases). These results would confirm that CFX adsorption onto these sorbents depends on the pH level. In this regard, a previous study dealing with the adsorption of one cephalosporin (cefixime) by a porous, aluminum-based metal–organic framework-aminoclay nanocomposite reported an increase in antibiotic adsorption when the pH oscillated from 3 to 7, obtaining the maximum adsorption at a pH level of seven [67]. To further evidence the potential relation, statistical analyses were performed, obtaining a significant and positive correlation between adsorption and pH ($r = 0.440$, $p < 0.05$). In a previous study in which the adsorption of amoxicillin by mixtures equivalent to those used here was studied, it was observed that the organic matter content also influenced the adsorption of the antibiotic [68]. Furthermore, previous studies focused on CFX adsorption for soils varying within a wide range of pH

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level (4.62–8.02) and organic matter contents (1.09–16.87), showing a clear influence of these two parameters on retention of the antibiotic [54]. However, in the current research, all soil samples had a moderate organic matter content, with the pH level being the most influential characteristic affecting CFX adsorption for the mixtures of soil and bioadsorbent. In addition to the pH level, other properties of the bioadsorbent materials, such as the higher exchangeable Ca content in mussel shell and oak ash and the non-crystalline Al and Fe contents in oak ash, would contribute to explaining the higher increase in CFX adsorption caused by these two materials compared with pine bark [60].

3.2. Adsorption Isotherms for Soils and Mixtures of Soil and Bioadsorbents

The fitting of the experimental adsorption results to adsorption equations was evaluated for the Freundlich, Langmuir, and linear models. Table 2 shows the results of the parameters of the adjustments, considering soils without and with bioadsorbents added at a dose of 12 t/ha.

Table 2. Parameters of adsorption isotherms for cefuroxime in soils without and with bioadsorbents at a dose of 12 t/ha (K_F expressed in $L^n\ kg^{-1}\ \mu mol^{1-n}$; n = dimensionless; K_L expressed in $L\ \mu mol^{-1}$; qm expressed in $\mu mol\ kg^{-1}$ K_d expressed in $L\ kg^{-1}$; - = error value too high for fitting). C = corn soils; VO and VP = vineyard soils; A = oak ash; MS = mussel shell; and PB = pine bark.

	Freundlich					Langmuir					Linear Model		
Sample	**K_F**	**Error**	**n**	**Error**	**R^2**	**K_L**	**Error**	**qm**	**Error**	**R^2**	**K_d**	**Error**	**R^2**
VP1	10.877	3.319	0.458	0.104	0.895	0.15	0.095	58.473	11.744	0.876	2.018	0.266	0.693
VP1-A	22.781	17.403	1.345	0.826	0.661	-	-	-	-	-	30.783	5.082	0.648
VP1-MS	41.676	3.52	1.483	0.142	0.982	-	-	-	-	-	52.343	3.516	0.932
VP1-PB	22.04	10.863	0.308	0.195	0.49	-	-	65.554	20.877	0.608	-	-	-
VP2	6.365	3.11	0.815	0.18	0.907	-	-	225.213	188.987	0.912	3.838	0.323	0.892
VP2-A	120.162	2.58	0.909	0.048	0.996	0.129	0.118	1053.162	875.508	0.995	122.871	2.444	0.994
VP2-MS	122.858	12.836	0.959	0.235	0.916	-	-	-	-	-	124.267	9.223	0.916
VP2-PB	10.765	1.548	0.499	0.049	0.98	0.13	0.03	67.645	5.521	0.983	2.345	0.241	0.811
C1	5.135	0.958	0.881	0.068	0.988	0.013	0.008	341.167	174.058	0.989	3.699	0.122	0.983
C1-A	137.408	7.386	0.52	0.054	0.979	2.742	0.896	174.487	24.936	0.973	169.606	18.516	0.824
C1-MS	122.905	14.431	0.329	0.126	0.904	-	-	211.46	172.105	0.848	169.582	18.416	0.826
C1-PB	-	-	-	-	-	-	-	-	-	-	9.249	1.523	0.671
VO	21.681	4.085	0.685	0.096	0.948	0.113	0.037	193.42	37.656	0.975	11.48	1.085	0.863
VO-A	52.829	16.977	-	-	0.359	-	-	-	-	-	43.309	11.013	0.314
VO-MS	33.059	15.12	0.979	0.456	0.823	-	-	-	-	-	32.377	3.568	0.823
VO-PB	-	-	1.285	0.754	0.495	-	-	-	-	-	7.981	1.72	0.487
C2	22.027	11.843	0.410	0.225	0.57	-	-	80.688	28.959	0.6	4.735	1.169	0.328
C2-A	132.409	31.22	0.511	0.408	0.77	-	-	-	-	-	161.564	22	0.727
C2-MS	131.114	11.877	0.490	0.104	0.94	3.548	1.866	153.98	29.786	0.94	160.503	18.222	0.807
C2-PB	-	-	1.324	0.65	0.671	-	-	-	-	-	7.276	1.218	0.655
C3	3.219	0.817	0.985	0.088	0.985	-	-	-	-	-	3.084	0.095	0.985
C3-A	-	-	-	-	-	-	-	-	-	-	-	-	-
C3-MS	-	-	-	-	-	-	-	-	-	-	-	-	-
C3-PB	37.349	18.209	0.330	0.258	0.661	-	-	83.349	29.002	0.624	9.619	1.828	0.517

The results indicate that when the bioadsorbents were added at a dose of 12 t/ha, the Freundlich model showed the best adjustment, because 45% of the samples obtained an R^2 value > 0.9, whereas in the case of the Langmuir and linear models, the values decreased to 25% and 20%, respectively.

The Freundlich affinity coefficient (K_F) values ranged between 2.396 and 137.408 $L^n\ kg^{-1}\ \mu mol^{1-n}$, while the n values oscillated between 0.308 and 1.693. Some authors postulated that when n < 1, adsorption is carried out in a heterogeneous matrix, with the highest energy sites being occupied first [69]. Meanwhile, when n > 1, a deviation from the Freundlich equation would occur, such as the eventual existence of irreversible reactions like precipitation [70] or cooperative adsorption [71]. The values of the distribution constant of the linear model (K_d) ranged between 2.018 and 169.606 $L\ kg^{-1}$, whereas those of the Langmuir adsorption constant (K_L) were between 0.013 and 3.548 $L\ \mu mol^{-1}$.

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Table 3 shows the results of the parameters of the adjustments to the adsorption models considering soils without and with bioadsorbents added at a dose of 48 t/ha. In this case, 62.5% of the samples obtained an R^2 value >0.9 when adjusted to the Langmuir model. This percentage decreased to 54% and 25% when adjusted to the Freundlich and linear models, respectively (Table 3). In this regard, Jafari et al. [72] studied the adsorption of two cephalosporins (cephalexin and cefixime) onto multi-walled carbon nanotubes and obtained a better adjustment to the Freundlich model for the adsorption of cefixime, while cephalexin adsorption fit better to the Langmuir equation.

Table 3. Parameters of adsorption isotherms for cefuroxime in soils without and with bioadsorbents at a dose of 48 t/ha (K_F expressed in $L^n\ kg^{-1}\ \mu mol^{1-n}$; n = dimensionless; K_L expressed in $L\ \mu mol^{-1}$; qm expressed in $\mu mol\ kg^{-1}$ K_d expressed in $L\ kg^{-1}$; - = error value too high for fitting). C = corn soils; VO and VP = vineyard soils; A = oak ash; MS = mussel shell; and PB = pine bark.

	Freundlich					Langmuir					Linear Model		
	K_F	Error	n	Error	R^2	K_L	Error	qm	Error	R^2	K_d	Error	R^2
VP1	10.877	3.319	0.458	0.104	0.895	0.150	0.095	58.473	11.744	0.876	2.018	0.266	0.693
VP1-A	148.757	10.363	0.714	0.094	0.979	0.848	0.567	308.951	140.343	0.972	174.349	9.055	0.958
VP1-MS	129.954	8.905	0.592	0.102	0.973	1.635	1.073	200.034	63.88	0.961	149.227	11.307	0.933
VP1-PB	14.658	4.505	0.455	0.112	0.879	0.157	0.071	76.091	12.693	0.918	2.922	0.463	0.606
VP2	6.365	3.11	0.815	0.18	0.907	-	-	-	-	-	3.838	0.323	0.892
VP2-A	-	-	-	-	-	-	-	-	-	-	-	-	-
VP2-MS	-	-	-	-	-	-	-	-	-	-	-	-	-
VP2-PB	13.248	2.902	0.473	0.078	0.947	0.169	0.056	70.367	7.659	0.962	2.79	0.334	0.754
C1	5.133	0.958	0.881	0.068	0.988	0.013	0.008	341.167	174.058	0.989	3.699	0.122	0.983
C1-A	140.678	12.98	0.511	0.091	0.951	3.499	1.444	161.637	27.525	0.949	183.897	20.641	0.814
C1-MS	112.159	8.731	0.414	0.107	0.935	2.905	1.403	151.631	26.71	0.935	116.45	15.809	0.737
C1-PB	5.254	1.399	0.691	0.088	0.972	0.023	0.015	134.903	61.572	0.961	2.036	0.121	0.937
VO	21.681	4.085	0.685	0.096	0.948	0.113	0.037	193.42	37.656	0.975	11.48	1.085	0.863
VO-A	22.164	5.979	0.550	0.123	0.887	0.169	0.089	128.494	30.869	0.909	7.728	1.082	0.71
VO-MS	23.636	6.952	0.591	0.147	0.918	-	-	162.823	89.866	0.9	10.285	0.928	0.872
VO-PB	8.756	1.668	0.764	0.073	0.986	0.018	0.016	326.384	235.677	0.976	4.728	0.196	0.971
C2	22.027	11.843	0.41	0.225	0.57	-	-	80.688	28.959	0.6	4.735	1.169	0.328
C2-A	-	-	-	-	-	-	-	-	-	-	-	-	-
C2-MS	53.205	27.298	-	-	0.402	-	-	-	-	-	571.086	153.561	0.229
C2-PB	18.948	5.349	0.361	0.104	0.84	0.261	0.107	67.821	8.438	0.93	2.675	0.593	0.304
C3	3.219	0.817	0.985	0.088	0.985	-	-	-	-	-	3.084	0.095	0.985
C3-A	-	-	-	-	-	-	-	-	-	-	-	-	-
C3-MS	-	-	-	-	-	-	-	-	-	-	-	-	-
C3-PB	26.541	5.886	0.544	0.112	0.94	0.158	0.085	150.68	40.312	0.944	10.352	1.104	0.824

The values of the K_F parameter ranged between 3.219 and 148.757 $L^n\ kg^{-1}\ \mu mol^{1-n}$. These scores are similar to those previously obtained for another antibiotic (amoxicillin) in equivalent soil-bioadsorbent mixtures [68]. Focusing on the data, the K_F values were significantly higher when mussel shell or oak ash was added, indicating stronger adsorption when the soils were amended with these alkaline adsorbents. Regarding Freundlich's n values, they were lower than one in all cases, suggesting the presence of heterogeneous adsorption sites and a nonlinear and concave curve, which was frequently interpreted. This suggests that the sites with high adsorption energy are occupied first, indicating chemical adsorption [71,73]. The K_d values ranged between 2.018 and 571.086 $L\ \mu mol^{-1}$, while the Langmuir adsorption constant (K_L) ranged between 0.013 and 10.917 $L\ kg^{-1}$. These values are lower than those previously obtained for amoxicillin in equivalent mixtures [68].

3.3. Comparison with Previously Studied Bioadsorbents

Other authors previously studied the capacity of different bioadsorbents to retain or remove CFX. Awwad et al. [74] used activated carbon and a micelle-clay complex to retain this antibiotic, achieving adsorption values of 26.31 mg g^{-1} and 31.25 mg g^{-1}, respectively, for a CFX-added concentration of 20 mg L^{-1}. Ma et al. [75] studied CFX adsorption with a cationic *Entermorpha prolifera* polysaccharide-based hydrogel, obtaining adsorptions of 5 mg g^{-1} for a concentration of 50 mg L^{-1} and 25–30 mg g^{-1} for a concentration of

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400 mg L^{-1}. In addition, different bioadsorbents have been previously studied regarding their adsorption of other antibiotics of the cephalosporins group. Among them, biochar derived from fiber residues of palm oil were used to remove cephalexin, obtaining adsorption values of 57.47 mg g^{-1} [76]. Cephalexin adsorption was also studied for natural zeolite and zeolite coated with manganese oxide nanoparticles, reaching adsorption values of 16.1 and 24.5 mg g^{-1}, respectively [77]. When comparing these results with the data obtained in the current study, the bioadsorbents used here showed lower adsorption capacities in the specific experimental conditions of the present research (which are not identical to those of the other investigations). However, expressed as a percentage, adsorption was above 80% for the mussel shell and oak ash in most cases. We note that the adsorbent materials evaluated in the referred previous research are not low-cost materials. In light of this, in case of requiring better adsorption performance associated with the low-cost byproducts employed here, one might suggest assessing the effect of carrying out different chemical modifications in this regard. Table 4 shows the results corresponding to each of the compared bioadsorbents.

Table 4. Comparison of antibiotics' adsorption capacities for various bioadsorbents.

Bioadsorbent	Antibiotic	Initial Concentration	Adsorption (mg g^{-1})	Reference
Activated carbon	Cefuroxime	20 mg L^{-1}	26.31	Awwad et al., 2015 [74]
Micelle-clay complex	Cefuroxime	20 ppm	31.25	Awwad et al., 2015 [74]
Entermorpha prolifera polysaccharide-based hydrogel	Cefuroxime	50 mg L^{-1}	5	Ma et al., 2022 [75]
Entermorpha prolifera polysaccharide-based hydrogel	Cefuroxime	400 mg L^{-1}	25–30	Ma et al., 2022 [75]
Fiber residues of palm oil	Cephalexin	20 mg L^{-1}	57.47	Acelas et al., 2021 [76]
Natural zeolite	Cephalexin	10 mg L^{-1}	16.1	Samarghandi, 2015 [77]
MnO zeolite	Cephalexin	10 mg L^{-1}	24.5	Samarghandi, 2015 [77]
Pine bark	Cefuroxime	21 mg L^{-1}	0.037	This study
Mussel shell	Cefuroxime	21 mg L^{-1}	0.052	This study
Oak ash	Cefuroxime	21 mg L^{-1}	0.052	This study

3.4. Desorption

Desorption was always lower than 10% for the soils without bioadsorbents and decreased to 0% when the bioadsorbents were added, indicating strong retention. Similar results were previously obtained for amoxicillin in equivalent mixtures, where desorption decreased from 17% to 6% after adding bioadsorbent amendments to the soils [68].

4. Conclusions

The two bioadsorbents we investigated which had alkaline pH levels (mussel shell and oak ash) were highly effective at increasing CFX adsorption after being applied to the studied soils, resulting in strong and practically irreversible retention. The bioadsorbent amendment at 12 t/ha was sufficient to achieve these effects and did not require higher doses. In contrast, the bioadsorbent having an acidic pH, pine bark, was less effective, in some cases (high CFX concentrations and a dose of 48 t/ha of the bioadsorbent) even causing a decrease in CFX adsorption compared with the unamended soil. These facts suggest that among the different characteristics of the soils and bioadsorbents studied here, the pH level would be the one with the highest influence on adsorption of the antibiotic CFX. These results could be useful to manage soils and other environmental compartments affected by pollution episodes due to the antibiotic CFX, which can clearly be seen as relevant. For future research, it would be interesting to carry out equivalent experiments focusing on the efficacy of the bioadsorbents studied here and other low-cost

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bioadsorbents to retain combinations of different antibiotics simultaneously present in the medium. In addition, complementary studies to shed further light on the retention and release mechanisms would be of relevance, as well as investigating low-cost procedures to increase the efficacy of raw adsorbents in cases where they would be needed.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/pr12071335/s1. Table S1: Values corresponding to the basic parameters determined in the soils. C = corn soils; VO = vineyard soils (Ourense province); and VP = vineyard soils (Pontevedra province). Average values (n = 3) with coefficients of variation always <5%. Table S2: pH values of soils amended with the bio-adsorbents in doses of 12 and 48 t/ha. C = corn soils; VO = vineyard soils (Ourense province); VP = vineyard soils (Pontevedra province); A = ash; MS = mussel shell; and PB = pine bark. Figure S1: Example chromatograms corresponding to CFX adsorption onto soils amended with bio-adsorbents.

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SUPPLEMENTARY MATERIAL

Removal of Cefuroxime from Soils Amended with Pine Bark, Mussel Shell and Oak Ash

Raquel Cela-Dablanca[1,*], Ainoa Míguez-González[1], Lucía Rodríguez-López[2], Ana Barreiro[1], Manuel Arias-Estévez[2], María J. Fernández-Sanjurjo[1], Esperanza Álvarez-Rodríguez[1], Avelino Núñez-Delgado[1]

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Results of the characterization of the soils and misxtures soil+bioadsorbents used in this research

Table S1. Values corresponding to the basic parameters determined in the soils. C: corn soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province). Average values (n=3), with coefficients of variation always < 5%.

Soil	pH H_2O	OC	OM	N	Sand	Clay	eCEC	Ca	Mg	Na	K	Al	Fe ox	Fe pir	Al ox	Al pir
VP1	6.1	2.83	4.88	0.23	60.62	18.35	37.23	6.33	1.48	18	11.4	0	3460	4889	4294	3005
VP2	5.7	4.81	8.3	0.4	58.22	21.38	17.36	13.4	1.09	2.32	0.55	0	2765	4519	3679	3965
VO	6.04	1.77	3.06	0.1	49.57	2.414	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141
C1	5.33	2.66	4.59	0.27	43.42	24.64	6.88	5.35	0.67	0.02	0.44	0.4	5745	3159	3401	2871
C2	5.65	2.57	4.44	0.25	43.56	22.43	7.47	6.18	0.77	0	0.28	0.24	5780	3315	3881	2717
C3	5.01	2.04	3.52	0.21	45.64	22.43	5.94	4.58	0.67	0.1	0.17	0.43	4545	2923	2896	1945

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Table S2. pH values of soils amended with the bio-adsorbents in doses of 12 and 48 t/ha. C: corn soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); A: ash; MS: mussel shell; PB: pine bark

Mixture (12 t/ha)	pH	Mixture (48 t/ha)	pH
VP1	6.1	**VP1**	6.1
VP1-A	6.53	**VP1-A**	7.20
VP1-MS	6.07	**VP1-MS**	6.05
VP1-PB	5.67	**VP1-PB**	5.45
VP2	5.7	**VP2**	5.7
VP2-A	6.41	**VP2-A**	7.28
VP2-MS	6.13	**VP2-MS**	6.84
VP2-PB	5.87	**VP2-PB**	6.39
VO	6.04	**VO**	6.04
VO-A	6.11	**VO-A**	7.81
VO-MS	6.06	**VO-MS**	5.92
VO-PB	5.73	**VO-PB**	5.35
C1	5.33	**C1**	5.33
C1-A	6.40	**C1-A**	6.93
C1-MS	5.79	**C1-MS**	5.29
C1-PB	5.16	**C1-PB**	4.92
C2	5.65	**C2**	5.65
C2-A	5.97	**C2-A**	7.04
C2-MS	5.70	**C2-MS**	5.76
C2-PB	5.51	**C2-PB**	5.24
C3	5.01	**C3**	5.01
C3-A	6.11	**C3-A**	6.93
C3-MS	6.06	**C3-MS**	5.46
C3-PB	5.73	**C3-PB**	4.79

We have previously published additional details corresponding to aspects such as FTIR analyses, surface area and pore size analysis, XRD, and microstructural characterizations of the bio-adsorbents (see References list).

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Quantification of CFX and example chromatograms

The quantification of antibiotic concentrations was performed using an HPLC Thermo-Fisher Model LPG 3400 SD (USA) equipment. A Luna C18 column (150 mm long; 4.6 mm internal diameter; 5 µm particle size) provided by Phenomenex (Madrid, Spain) was used, along with a pre-column (4 mm long; 2 mm internal diameter; 5µm particle size). The injection volume was 50 µL and the flow rate was 1.5 mL min^{-1}. The mobile phase consisted of acetonitrile (phase A) and 0.01 M of phosphoric acid at pH=2 (phase B). A linear gradient was used varying from 5% to 32% of phase A and from 95% to 68% of phase B. The initial conditions were re-established in 2 min and maintained for 2.5 min. The total analysis time was 15 min, with a retention time of 8.69 min, and the wavelength used for detection was 212 nm. The amounts of antibiotic adsorbed were calculated by the difference between the concentrations of the solutions initially added and the concentrations of the solutions at equilibrium. All the determinations were made in triplicate.

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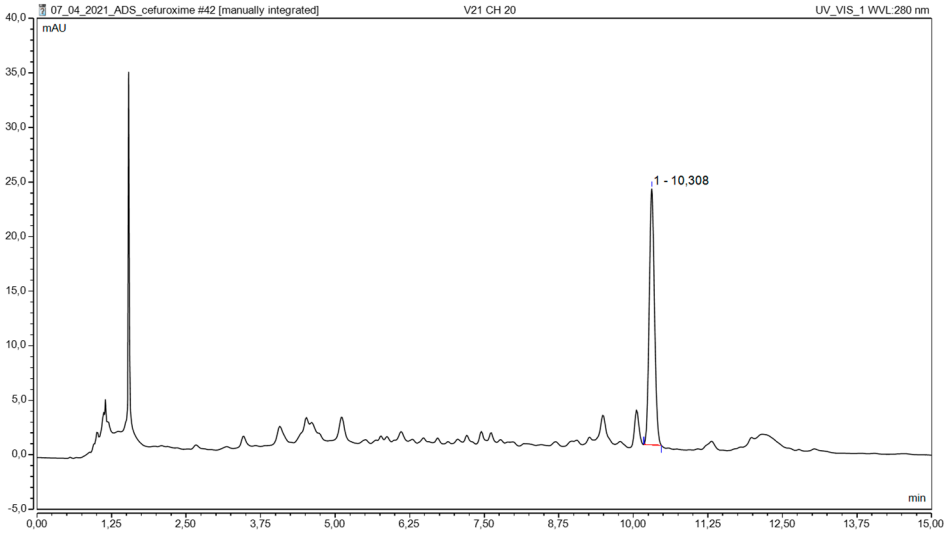
VP2 soil amended with pine bark in a dose of 12 t/ha; CFX concentration added 20 µmol L^{-1}



C1 soil amended with pine bark in a dose of 12 t/ha; CFX concentration added 20 µmol L^{-1}

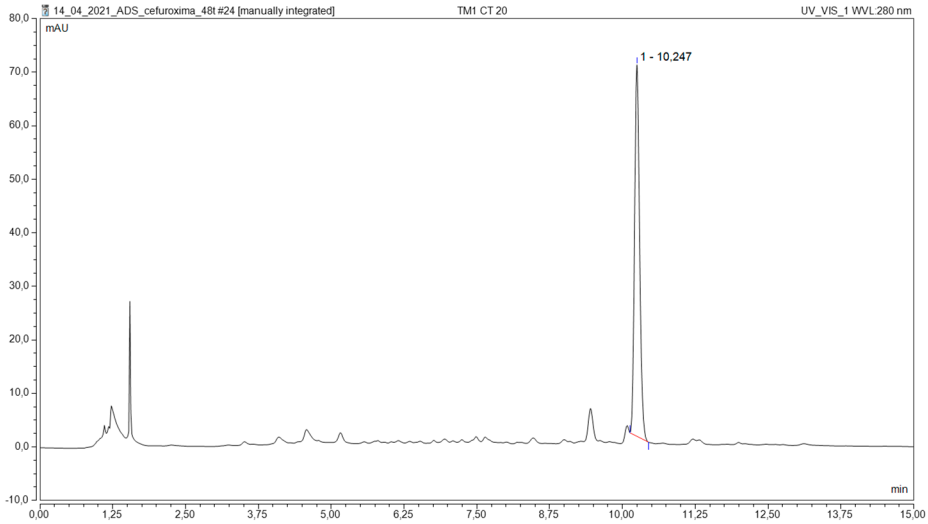


Figure S1. Example chromatograms corresponding to CFX adsorption onto soils amended with bio-adsorbents

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PUBLICACIÓN V

Cela-Dablanca, Raquel[a]., Barreiro, Ana[a]., Rodríguez-López, Lucía[b]., Santás-Miguel, Vanesa[b]., Arias-Estévez, Manuel[b]., Núñez-Delgado, Avelino[a]., Álvarez-Rodríguez, Esperanza[a]., & Fernández-Sanjurjo, María J[a]. (2022). **Relevance of sorption in bio-reduction of amoxicillin taking place in forest and crop soils**. *Environmental Research*, *208*, 112753. https://doi.org/10.1016/j.envres.2022.112753

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Relevance of sorption in bio-reduction of amoxicillin taking place in forest and crop soils

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ABSTRACT

The fate of antibiotics reaching soils is a matter of concern, given its potential repercussions on public health and the environment. In this work, the potential bio-reduction of the antibiotic amoxicillin (AMX), affected by sorption and desorption, is studied for 17 soils with clearly different characteristics. To carry out these studies, batch-type tests were performed, adding increasing concentrations of AMX (0, 2.5, 5, 10, 20, 30, 40, and 50 μmol L^{-1}) to the soils. For the highest concentration added (50 μmol L^{-1}), the adsorption values for forest soils ranged from 90.97 to 102.54 μmol kg^{-1} (74.21–82.41% of the amounts of antibiotic added), while the range was 69.96–94.87 μmol kg^{-1} (68.31–92.56%) for maize soils, and 52.72–85.40 μmol kg^{-1} (50.96–82.55%) for vineyard soils. When comparing the results for all soils, the highest adsorption corresponded to those more acidic and with high organic matter and non-crystalline minerals contents. The best adjustment to adsorption models corresponded to Freundlich's. AMX desorption was generally <10%; specifically, the maximum was 6.5% in forest soils, and 16.9% in agricultural soils. These results can be considered relevant since they cover agricultural and forest soils with a wide range of pH and organic matter contents, for an antibiotic that, reaching the environment as a contaminant, can pose a potential danger to human and environmental health.

1. Introduction

The environment is facing several threats, from climate change to air, water, and soil pollution. In recent times, a special attention is being paid to compounds called "emerging pollutants" (Taheran et al., 2018; Bilal et al., 2019), which include chemicals found in pharmaceuticals, personal care products, pesticides, industrial and household products, metals, surfactants, industrial additives and solvents. In some cases, these pollutants may cause severe damage on both the environment and human health (Petrisor, 2004). The current growth of the pharmaceutical industry increases the risk of spreading these compounds into the environment. Within pharmaceuticals, antibiotics, (which are increasingly used in medicine for the treatment of infectious diseases), become pollutants when spread into environmental compartments (Carvalho and Santos, 2016; Ezzariai et al., 2018). In fact, the number of studies that reveal the presence and effects of these compounds in soils, water and crops is increasing (Conde-Cid et al., 2018; Santás-Miguel et al., 2020; Beltrán et al., 2021).

Among antibiotics, amoxicillin (AMX) is a broad-spectrum antimicrobial included in the penicillin group, from the β-lactam subclass, and one of the most widely used in human-medicine in the primary care setting. In Europe AMX supposed 40% of the total prescribed antibiotics in 2017 (Buckler et al., 2018), and worldwide its market value was at 4.4 billion $ (Watson, 2018). The ample use of this antibiotic is due to, among other reasons, its effectiveness against a wide variety of gram-positive, and additionally some gram-negative bacteria, compared to penicillin (Sodhi et al., 2021). Due to its habitual use in medicine and the fact that between 80 and 90% of this antibiotic is excreted due to its poor absorption (Kidak and Doğan, 2018), its presence in the environment is considered an emerging problem, even though under the framework of OECD (European Centre for Disease Prevention and Control) tests, AMX did no present acute toxicity for denitrifying bacteria in soil, earthworms, and plants (Litskas et al., 2018). In fact, it can cause significant risks to human health, as well as environmental

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damage, such as the proliferation of antibiotics-resistant bacteria, ecotoxicity problems or their entry into the food chain.

Specifically, AMX is an antibiotic poorly absorbed in the intestine and, due to its low degradation into the body, it remains as an active compound in feces and urine (Andreozzi et al., 2005; Jara et al., 2007) and may end up being incorporated into wastewater, reaching Wastewater Treatment Plants (WWTP) (where available). In these plants, wastewater is subjected to purification processes that in most cases do not eliminate all of this and other antibiotics, while sludge can also accumulate these pollutants (Wang and Wang, 2016; Yang et al., 2016; Gogoi et al., 2018). In fact, Andreozzi et al. (2005) indicate that amoxicillin can appear in WWTP effluents in concentrations of up to 120 ng L^{-1}. As for sludge, they are frequently used as amendments in agricultural soils and in certain forested areas with pastoral exploitation, and despite the fact that in some countries it is mandatory that they undergo treatment for the elimination of pollutants before being incorporated into the soil, these treatments are not always totally effective in the case of antimicrobials. To note that the relatively high affinity of AMX to the sludge solid phase has been demonstrated in previous works (Andreozzi et al., 2004). Therefore, there is an obvious risk that, through the routes mentioned above, AMX and other antibiotics can reach the soil and be absorbed by plants from the soil solution (Rocha et al., 2021), entering the food chain, as shown by Azanu et al. (2016) for carrots and lettuce, with AMX levels between 14 and 45 ng g^{-1} being detected in those crops. In addition, AMX is an antibiotic highly used in veterinary (Binh et al., 2007; Yang et al., 2020a), with marked risks of entering soils and waterbodies when manures and slurries containing it are spread as fertilizers, as happens with other pollutants (Núñez-Delgado et al., 2002).

When AMX reaches the soil, it can interact with some edaphic components and/or with active molecules contained in by-products present on the soil surface, allowing a variable degree of retention of the pollutant by means of sorption mechanisms (Kim et al., 2012; Mohammed et al., 2020; Pandey et al., 2021), as happens with other contaminants (Seco-Reigosa et al., 2013) and other antibiotics (Conde-Cid et al., 2019, 2020; Cela-Dablanca et al., 2021a). Analyses of sorption mechanisms are crucial for environmental risk assessments, and the European Medicine Agency has included the quantification of pharmaceuticals sorption into the guidelines for human medicinal products (EMEA, 2006). The sorption, mobility and bioavailability of antibiotics will be mainly determined by the molecule's properties and soil physical-chemical characteristics, but also by meteorological factors. Several antibiotics, AMX among them, have been describe as highly mobile in soils and could therefore be rapidly transported to surface water (Kim et al., 2012).

Within the soil properties, soil organic matter favours the sorption of antibiotics to the soil particles since it provides negative charges and a high contact surface that can increase the sorption capacity, depending on the quantity and composition of this fraction (Thiele-Bruhn and Aust, 2004). On the other hand, soil pH can modify the charges of the antibiotics if, as for amoxicillin, they have zwitterion character. In forest soils, amoxicillin has been found to be more persistent than recently developed β-lactam antibiotics, even though the persistence was considered low ($t_{1/2}$ less than a day) (Braschi et al., 2013). The relatively high degree of sorption of AMX could be related to its zwitterion character, as happens with some fluoroquinolones and tetracyclines (Carrasquillo et al., 2008; ter Laak et al., 2006). In addition, Kim et al. (2012) found that the degree of AMX sorption was reduced onto two arable soils when decreasing pH and increasing soil organic matter content.

Also clearly interesting is the study of biodegradation of antibiotics (and specifically of AMX) present in environmental compartments as pollutants (Reis et al., 2020; Yang et al., 2020b), and the eventual effects of sorption processes on the availability of these antibiotics to be biodegraded (Wu et al., 2009; Wegst-Uhrich et al., 2014; Zhu et al., 2021).

Taking into account the previous background, this research aims to give new details on the retention of AMX in different agricultural and forest soils, as well as on the influence of the physical-chemical properties of the soils on the fate of the antibiotic in that environment. Previous works have analysed the sorption mechanisms in a limited number of arable soils, but data regarding soils under different agricultural and forestry exploitations is missing. The authors of this work have no notice on equivalent research performed for this antibiotic and with this diversity of soils, while its importance is clear at the level of potential environmental and public health repercussions.

2. Materials and methods

2.1. Soil sampling

Seventeen soils were used in this study, sampling in a variety of geographic areas in Galicia (Northwest Spain), with some basic details presented in Table S1 (Supplementary Material).

These soils were within a set previously used, characterized and commented in Cela-Dablanca et al. (2021a). Among the 17 soils here used, 14 were from agricultural areas: 8 vine soils (4 in the province of Ourense, VO1 to VO4, and 4 in the province of Pontevedra, VP1 to VP4); meanwhile the other 6 agricultural soils were maize (corn) soils (M1 to M6). The other 3 soils were forest (F) soils from areas dominated by *Pinus* (FP), oak (*Q. robur*) (FR), or *Eucalyptus* (FE). The agricultural soils were managed by annual addition of WWTP sludge, during at least 3 consecutive years.

In each sampling zone, a composite sample was performed mixing ten sub-samples that were taken in surface (0–20 cm depth). Once at the laboratory, and previously to be stored for further study, all soil samples were dried (at 40 °C) and sieved through a 2 mm mesh.

2.2. Soil analyses

The following physico-chemical characteristics were measured in all soil samples: pH (in H_2O), total C and N, clay and sand contents, effective cation exchange capacity, exchangeable Ca, Mg, Na, K and Al, non-crystalline Fe and Al contents, and Fe and Al bound to organic matter. The methods are detailed in the Supplementary Material, with Table S2 showing the results obtained.

2.3. Experiments on adsorption and desorption

Batch-type experiments were used to carry out adsorption and desorption studies on the whole set of soil samples. The procedure was as that shown in Cela-Dablanca et al. (2021a), and it is included in Supplementary Material. Adsorption and desorption studies were performed using batch-type experiments, where 2 g of soil were mixed with 5 mL of solutions containing 2.5, 5, 10, 20, 30, 40, or 50 μmol L^{-1} of AMX, as done in previously researches for the beta-lactam antibiotic cefuroxime (Cela-Dablanca et al., 2021b) and for various sulfonamide antibiotics (Conde-Cid et al., 2021).

2.4. Reagents and determination of AMX

Amoxicillin (with ≥95% of purity) was provided by Sigma-Aldrich (Barcelona, Spain). Phosphoric acid (being 85% extra pure) and acetonitrile (with ≥99.9% of purity) were from Fisher Scientific (Madrid, Spain), and Ca_2Cl (with purity of 95%) was from Panreac (Barcelona, Spain). MilliQ water (Millipore, Madrid, Spain) was used for preparing all solutions needed for HPLC quantification procedures.

HPLC analyses were performed as indicated in Cela-Dablanca et al. (2021a, 2021b), with details included in Supplementary Material. Also, Fig. S1 (Supplementary Material) shows some example chromatograms. Concentrations of the antibiotic added minus concentrations in the equilibrium solutions gave the amounts adsorbed. After adsorption, desorption experiments were carried out, thus informing on the eventual hysteresis of the process. The procedure consisted in adding 5 mL of

0.005 M $CaCl_2$ (with no antibiotic) to the soil materials derived from the adsorption phase, then repeating the process carried out for adsorption. All these procedures were performed in triplicate.

2.5. Modeling and statistics

Data from the adsorption experiments were fitted to various models: Linear, Langmuir, and Freundlich. More details are shown in Supplementary Material.

The software used for fitting models and for statistical analyses of Pearson correlations ($p < 0.05$) was SPSS 21.

3. Results and discussion

3.1. Soil characteristics

Table S2 (Supplementary Material) shows the physicochemical characteristics of the soils used. The soil pH ranged between 4.68 and 8.02, with the most acidic values corresponding to forest soils, and most of the agricultural soils being in the 5–6 range. The total C and N contents showed a high variability in the agricultural soils, with values ranging between 0.63-7.67% and 0.06–0.63%, respectively. The forest soils have the highest amount of total C (7.15–9.78%) and N (0.48–0.67%). Sand contents ranged between 43.42% and 69.35%, with the highest values generally corresponding to forest soils. The clay fraction ranged between 12.22% and 24.65%, with agricultural soils showing higher values. The effective cation exchange capacity (eCEC) values also showed a wide oscillation, between 5.94 and 42.81 $cmol_c$ kg^{-1}, with Ca being the cation that dominated in most crop soils, while Al dominated in forest soils. The non-crystalline forms of Fe and Al also showed high variability. Specifically, total non-crystalline Fe (Fe_o) was between 1665 and 6990 mg kg^{-1}. Regarding Fe bound to organic matter (Fe_{pir}), the range went from 823 to 9239 mg kg^{-1}, while Al bound to organic matter (Al_{pir}) ranged from 1047 to 14,857 mg kg^{-1}, with generally higher values in forest soils and in those with more carbon and organic matter contents within agricultural soils.

Previous papers dealing with AMX adsorption onto soils are scarce, which makes somehow difficult to compare the characteristics of the soils here used with that corresponding to those included in other investigations with a coincident focus. Just as comparison, Jodeh et al. (2012) used a soil with pH 7.14 and organic carbon content 7.34% in a study on the leachate of pharmaceuticals (including amoxicillin) through soil columns. Kim et al. (2012) studied sorption characteristics of three antibiotics (including amoxicillin) in two different soil types (pH 5.64–8.13, and organic matter percentage 1.83–0.82, respectively), while Braschi et al. (2013) used two soils with pH 8.2–5.0 and total organic carbon 7.7–21.8 g kg^{-1} (so 0.77–2.18 expressed as percentage values). As can be seen, these soils have values within the range of those corresponding to the current research.

3.2. Adsorption of AMX

AMX adsorption (which is expressed in μmol kg^{-1}, and in percentage), is represented as a function of the AMX concentration added in Table S3 (Supplementary Material). Specifically, Table S3 shows that the amount adsorbed increases as the added concentration is higher, while the total percentage adsorbed decreases in most cases. This is due to the fact that the adsorption sites in the soils become progressively saturated as higher concentrations of antibiotic are added.

The maximum values of AMX adsorption are in the range of 69.96–94.87 μmol kg^{-1} in maize/corn soils, between 73.47 and 80.81 μmol kg^{-1} in vine soils developed on granite, and between 90.97 and 102.54 μmol kg^{-1} in forest soils. In vineyard soils on slate and schists, the adsorption values are in a wider range, oscillating between 43.41 and 85.40 μmol kg^{-1} (Table S3).

AMX adsorption percentages are generally high, even for the maximum concentration of antibiotic added (50 μmol L^{-1}), with AMX adsorption values ranging between 61 and 93% in crop soils with maize/corn, between 87 and 99% in vineyard soils on granite, between 72 and 95% in vineyard soils on slate and schists, and between 74 and 82% in forest soils (Table S3).

The adsorption of antibiotics onto soils is dependent on the characteristics of these molecules (polarity, hydrophobicity, and molecular structure) and on soil characteristics (organic matter, pH, clays, and non-crystalline minerals) (Zhi et al., 2019). When comparing for the different soils AMX adsorption, some clear differences are observed. Specifically, when the maximum concentration of AMX (50 μmol L^{-1}) is applied, the maximum adsorption percentages correspond to one maize soil (M2, with 92.6%), and to one vineyard soil (VP3, with 82.5%), as well to the three forest soils (82.4, 81.8, and 74.2%, for FP, FE, and FR, respectively). These soils have in common a pH ≤ 5.5, organic matter content higher than 4.5%, and a sum of Fe and Al extracted with oxalate (which estimates low crystallinity minerals) above 15,000 mg kg^{-1}.

Putra et al. (2009) found that AMX adsorption on carbon and bentonite is strongly affected by pH. Githinji et al. (2011) reported a decrease in AMX adsorption as the pH of the adsorbent increased from 3.5 to 8.5, while other studies described a decrease in the maximum adsorption for pH > 5 when the adsorbent was peel shell pistachio (Mohammed et al., 2020) or activated charcoal (Shahrouzi et al., 2019). At very acidic pH values (<5.0), the amine groups of the antibiotic can become protonated, acquiring a positive charge, while at less acidic and basic pH the carboxyl groups are deprotonated, creating negative charges (see the molecular structure of AMX in Fig. S1, Supplementary Material).

Within a certain pH range, antibiotics can behave as a zwitterion, showing negative and positive charges at the same time. In the case of AMX, the compound has three pK_a values and its molecular charge suffers modifications depending on the pH, due to the presence of different functional groups, such as carboxyl (with $pK_{a1} = 2.68$), amino ($pK_{a2} = 7.49$) and phenolic ($pK_{a3} = 9.63$) (Homsirikamol et al., 2016). At pH < pK_{a1}, the positive charge of the amino group confers a charge on the molecule that is also positive, while at pH values between pK_{a1} and pK_{a2} the molecule behaves as a zwitterion with deprotonated carboxyl groups and protonated amino groups. On the other hand, at pH between pK_{a2} and pK_{a3} deprotonated carboxyl and amine groups are predominant, and, finally, at pH > pK_{a3} phenolic groups are also deprotonated (Pezoti et al., 2016). Soils with clearly acidic pH (specifically, pH < 5), may have a negative charge on clays with a permanent charge, and a positive charge on components of variable charge (1:1 clays, organic matter, non-crystalline components), while at pH > 5, negative charges will generally predominate, rising as the pH increases.

For the soils used in the present work, two clear pH ranges can be differentiated: a) pH < 5 in forest soils, and b) pH between 5 and 6.5 in most agricultural soils (with the exception of a corn soil that reaches a value of 8 and a vineyard soil that has pH = 7.27). Most of the pH of these soils lie between the pK_{a1} and pK_{a2} of AMX, values for which the antibiotic behaves like a zwitterion. A variety of mechanisms could be involved in the adsorption processes of AMX in the soil, including electrostatic interactions, cationic bridges and H bonds (Anastopoulos et al., 2020). In the forest soils of the present study, which have pH < 5, electrostatic interactions can occur between the positively charged variable charge components of the soil (organic matter and non-crystalline components, very abundant in these soils) with the deprotonated carboxyl groups of AMX, and interactions between negatively charged soil components (2:1 clays) with the protonated amine groups of the antibiotic. With pH values between 5 and 6.5, soils will preferentially present a negative charge, which can interact with the protonated amine groups of AMX. However, at pH > 6 negative charges will predominate in soils and antibiotics, favoring adsorption through a cationic bridge. In addition, adsorption decreases with rising pH due to the increase of OH^- ions in the solution, ions that can compete with the COO^- groups of the antibiotic for the adsorption sites in the soil

(Shahrouzi et al., 2019).

In the present work, the soils with the highest AMX adsorption have pH < 5.5 and high organic matter and non-crystalline minerals contents (Table S2, Supplementary Material), which at these pH values will have a positive charge, so adsorption would occur preferentially between the deprotonated carboxyl groups of AMX and the positive charges of these variable charge components of the soil.

3.3. Modeling adsorption

Adsorption curves for the 17 soils studied are shown in Fig. 1, where the amounts of AMX adsorbed are plotted versus its equilibrium concentrations. Data on adsorption (as a function of the AMX concentration added), are shown in Table S3 (Supplementary Material) expressed as amount and percentages. The fitted curves are non-linear for most of the soil samples, and the adsorption of the antibiotic rises with the increase in concentration, and remains constant after the equilibrium. The adsorption capacity differs according to the type of soil.

Table 1 also shows the fitting of adsorption parameters for three models (Freundlich, Langmuir, and Linear) assessed. Taking into account the coefficient of determination (R^2) values and the errors associated, AMX adsorption would show a better fit to the Freundlich model in most soils (only M2 does not adjust to this model). Even though the R^2 values for the Langmuir model are not low (>0.72) the errors associated are too high to allow the adjustment. The Linear model has smaller errors that the Langmuir model, but the coefficients of determination are the lowest of the three models. In 94% of the samples that fit the Freundlich model, the R^2 values are higher than 0.89. Other authors also obtained a better fit for this antibiotic with the Freundlich model (with R^2 values > 0.8), while with the Langmuir model the R^2 values were <0.4 (Kim et al., 2012).

A better adjustment to the Freundlich's model, is indicative of clear increase of the amount adsorbed as a function of the growth of the concentration of the adsorbate present in solution (this when the concentrations of that adsorbate are low), while, at high concentrations, the amount adsorbed trends to a constant value (Proctor and Toro-Vazquez, 1996). This model assumes heterogeneity in the energy of the surface sites and that there is no specified limit for maximum adsorption. A good fit to the Langmuir model would indicate that the adsorption is in a monolayer (Foo and Hameed, 2010), with a finite number of identical and specific adsorption sites, so that the amount adsorbed increases until reaching a limit value, corresponding to the coating of the surface by one layer, being the maximum predictable adsorption. Contrary to our results, other authors found that amoxicillin sorption fits the Sips model, which suggests, like the Langmuir model, that sorption of AMX occurs predominantly by chemisorption in a monolayer (Espina de Franco et al., 2017).

The values of the distribution coefficient (K_d) in the Linear model are smaller for the maize and forest soil samples than for the vineyard soils (Table 1), and are lower than those obtained in previous studies for cefuroxime (Cela-Dablanca et al., 2021a, 2021b) and for tetracyclines (Conde-Cid et al., 2019), but higher than those obtained for sulfonamides (Conde-Cid et al., 2020). This would indicate that the interactions involved in the adsorption of AMX onto these soils are stronger than those of sulfonamides, but weaker than those of tetracyclines and cefuroxime. When K_d values were statistically analysed, a significant and positive correlation was found with the Freundlich's K_F ($r = 0.597$, $p < 0.01$), and with the Langmuir's K_L ($r = 0.495$, $p < 0.05$) (data not shown).

The values of the Freundlich affinity coefficient (K_F), associated with the relative adsorption capacity, are (similarly to K_d) smaller for the maize and forest soil samples and higher for the vineyard ones, especially for the soils over granite (VP) (Table 1). The K_F coefficient is related to the adsorption capacity and the values obtained are generally lower than those obtained in previous studies for cefuroxime (Cela-Dablanca et al., 2021a, 2021b) and for tetracyclines (Conde-Cid et al., 2019), but much higher than those obtained for sulfonamides (Conde-Cid et al., 2020). Compared with a study where natural phosphates from the soil mineral phase were analysed (Bouyarmane et al., 2015) the K_F coefficients for AMX are much higher in the soils used in the current research. When performing a statistical analysis, K_F shows a significant and positive correlation with the K content ($r = 0.712$, $p < 0.01$), with q_m ($r = 0.600$, $p < 0.01$), with the Freundlich's n parameter ($r = 0.482$, p

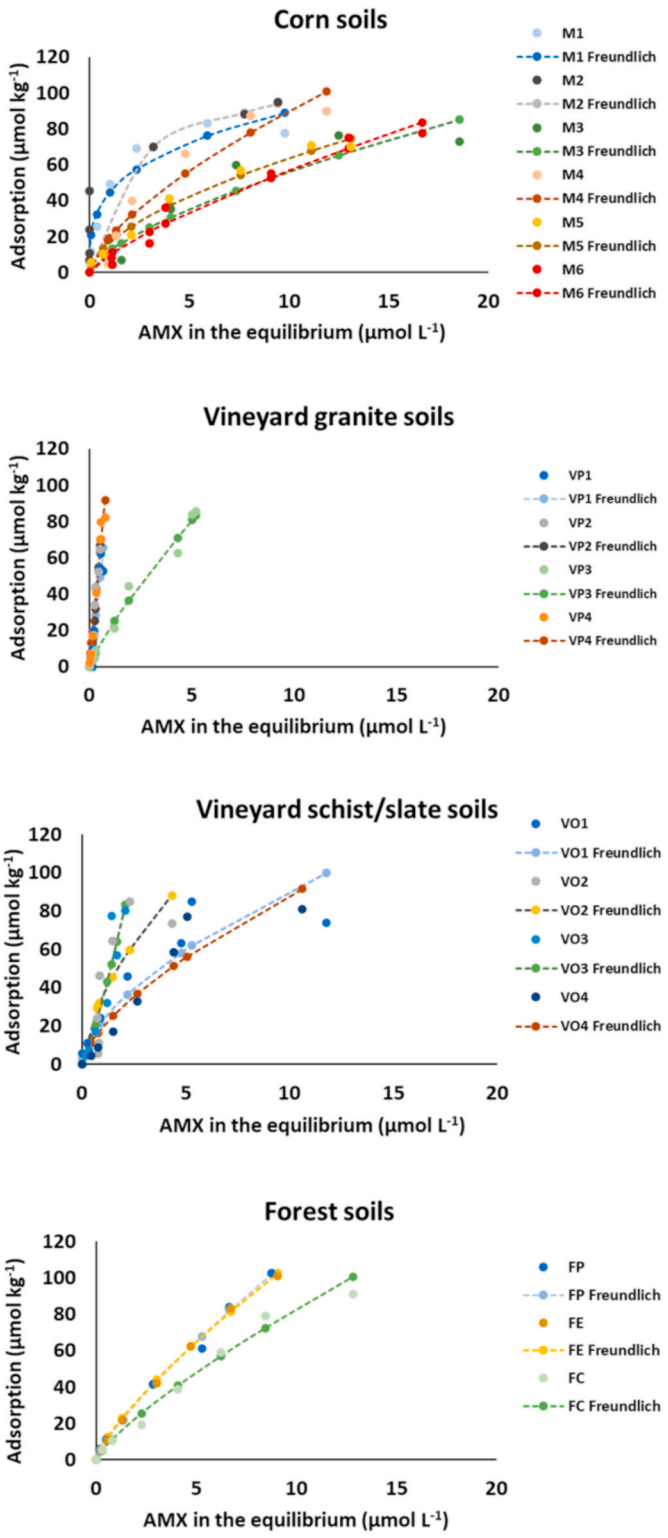

Fig. 1. Adsorption curves for AMX onto the various types of soils studied. Dash lines show the Freundlich isotherm for each soil sample.

Table 1
Fitting of adsorption results to the parameters of the Freundlich, Langmuir and Linear models. K_F ($L^n \mu mol^{1-n} kg^{-1}$); n (dimensionless); K_L ($L\ \mu mol^{-1}$); q_m ($\mu mol\ kg^{-1}$); K_d ($L\ kg^{-1}$). -: error values too high for fitting.

Soil	Freundlich					Langmuir					Linear		
	K_F	Error	n	Error	R^2	K_L	Error	q_m	Error	R^2	K_d	Error	R^2
M1	44.17	4.64	0.306	0.06	0.94	1.24	0.22	89.53	3.94	0.99	10.72	2.30	0.37
M2	–	–	–	–	–	–		112.70	48.88	0.72	11.27	3.79	0.78
M3	11.81	4.22	0.68	0.14	0.90	0.07	0.04	140.85	40.68	0.94	5.06	0.57	0.81
M4	19.17	4.63	0.67	0.11	0.93	0.12	0.05	161.81	34.49	0.96	9.30	0.95	0.84
M5	16.51	2.04	0.59	0.05	0.99	0.13	0.03	115.37	10.94	0.99	6.28	0.49	0.90
M6	9.82	2.42	0.76	0.10	0.97	0.05	0.02	180.07	50.51	0.98	5.34	0.34	0.94
VO1	22.85	2.45	0.60	0.06	0.98	0.21	0.03	125.68	9.15	0.99	10.35	0.83	0.89
VO2	35.47	8.76	0.62	0.23	0.68	0.34	0.32	144.26	78.10	0.72	23.55	4.22	0.53
VO3	34.18	6.48	1.20	0.32	0.89	–	–	–	–	–	37.5	3.37	0.88
VO4	19.29	5.08	0.66	0.14	0.90	0.13	0.07	150.203	43.32	0.94	9.68	1.15	0.79
VP1	99.53	20.66	1.09	0.28	0.84	–	–	–	–	–	93.63	0.67	0.48
VP2	150.34	34.64	1.33	0.25	0.90	–	–	–	–	–	3.49	0.26	0.91
VP3	21.03	3.11	0.83	0.10	0.98	0.08	0.06	293.44	165.57	0.98	16.37	0.68	0.97
VP4	116.05	9.89	0.93	0.13	0.97	–	–	–	–	–	120.34	5.63	0.97
FP	17.74	1.70	0.81	0.05	0.99	–	–	–	–	–	12.27	0.41	0.98
FE	18.81	0.57	0.77	0.02	1.00	0.06	0.01	278.39	34.52	1.00	12.07	0.45	0.98
FR	13.44	1.89	0.79	0.07	0.99	0.05	0.02	269.63	67.59	0.99	8.49	0.38	0.97

< 0.05), and with K_d ($r = 0.597$, $p < 0.05$) (data not shown).

Freundlich's n values are related to the heterogeneity of the adsorbent's active sites (Foo and Hameed, 2010). In the soil samples object of this study, n values are, in most cases, lower than 1, coinciding with that obtained by Githinji et al. (2011), which would indicate that the adsorption is non-linear. When n is between 0 and 1, it is indicative of adsorption taking place in a heterogeneous matrix, with the highest energy sites being the first to be occupied. The closer to zero is n, the more heterogeneous the adsorption surface will be, with adsorption being predominantly of chemical type (Foo and Hameed, 2010; Kong et al., 2012). When n is above 1 (just in three or our vineyard samples), it would indicate a deviation from the Freundlich model, which could be caused by irreversible reactions, such as precipitation (Elbana et al., 2018), or cooperative adsorption processes taking place (Foo and Hameed, 2010).

3.4. Desorption of AMX

Fig. 2 represents the sorption and desorption of AMX (in percentage), from the different soils as a function of the concentration of added antibiotic. Numerical values are presented in Tables S3 and S4 (Supplementary Material). Desorption amounts increase with higher concentrations of antibiotic added (Table S4, supplementary material), although the trend is not clear when expressed as percentage desorption (Fig. 2). When comparing desorption values among the set of soils, some differences are observed. Specifically, when the highest concentration of AMX is added, forest soils are those with the lowest desorption (with a maximum = 6.51 $\mu mol\ kg^{-1}$), followed by granite vineyard soils (maximum = 9.72 $\mu mol\ kg^{-1}$) and those on slate and schists (maximum = 13.21 $\mu mol\ kg^{-1}$), and the maximum desorption values corresponding to maize (corn) soils (18.49 $\mu mol\ kg^{-1}$) (Table S4, Supplementary Material). The fact that forest soils present less desorption than agricultural soils indicates that those having lower pH and higher organic matter and non-crystalline minerals contents (Table S2, supplementary material) adsorb antibiotics with enhanced retention intensity.

Desorbed AMX is generally less than 10%, in all samples in the case of forest soils, results that were also obtained in these same soils for the desorption of cefuroxime (Cela-Dablanca et al., 2021a, 2021b). These desorption values are higher than those found in previous studies for tetracyclines (Conde-Cid et al., 2019), but they are notably lower than those found for sulfonamides, which reached up to 50% (Conde-Cid et al., 2020). For other antibiotics that present higher sorption under basic pH soil conditions, like the macrolide tylosin, higher desorption levels in forest vs. crop soils have been described (Jeong et al., 2012).

The results obtained in the present study for AMX desorption indicate that, in most cases, the adsorption process is not easily reversible.

3.5. Environmental significance

A wide variety of antibiotics end up in agricultural soils coming from different sources. In the case of amoxicillin the origin can be animal or human, since it is used both in veterinary and human medicine. Antibiotics reach the soils through the repetitive use as fertilizers of WWTP sludge or slurry from animal farms. If the soil sorption intensity is weak, antibiotics get released/leached into surface run-off or groundwater (Díaz-Cruz et al., 2003). Soils can act as a filter, by adsorbing and accumulating antibiotics in the soil particles and avoiding/reducing contamination of water courses, if the sorption mechanisms and their intensities are strong. Protection of water from contamination with antimicrobials is recognized by FAO as the first step to reduce their impact on the environment (FAO, 2018).

It is relevant that even for the same antibiotic the adsorption mechanisms differ among different types of soils (Wang and Wang, 2015). The capacity of soil to sorb amoxicillin has been proved, but in a reduced number of soil types. For the analysis of the sorption mechanisms, the isotherm modeling is of key relevance. Sorption on amoxicillin is complex due to the zwitterion character of the molecule at the most common range of soil pH. This study shows that the amoxicillin sorption-desorption processes depend on soil characteristics such as pH and soil organic matter. The positive impact of low soil pH values on antibiotic sorption mechanisms has been described for other antibiotics, like sulfonamides (Park and Huwe, 2016) or trimethoprim (Zhang et al., 2014). Our research findings are significant for soils subjected to antibiotic pollution. The fact that forest soils show less desorption than agricultural soils, that are usually more exposed to these pollutants, should be considered when the risk of antibiotic contamination is assessed, both for soils and water courses. The development of agroforestry cropping strategies could be interesting because the forest area would act as a buffer mitigating antibiotic loss from agroecosystems (Chu et al., 2010). The results in the present study help to understand the dynamics of amoxicillin in different soil types.

4. Conclusions

In the present study, the soils showing the highest amoxicillin (AMX) adsorption capacities are those with both the most acidic pH (<5.5) and high organic matter and low-crystallinity minerals contents, these characteristics corresponding to all forest soils and two crop soils. This

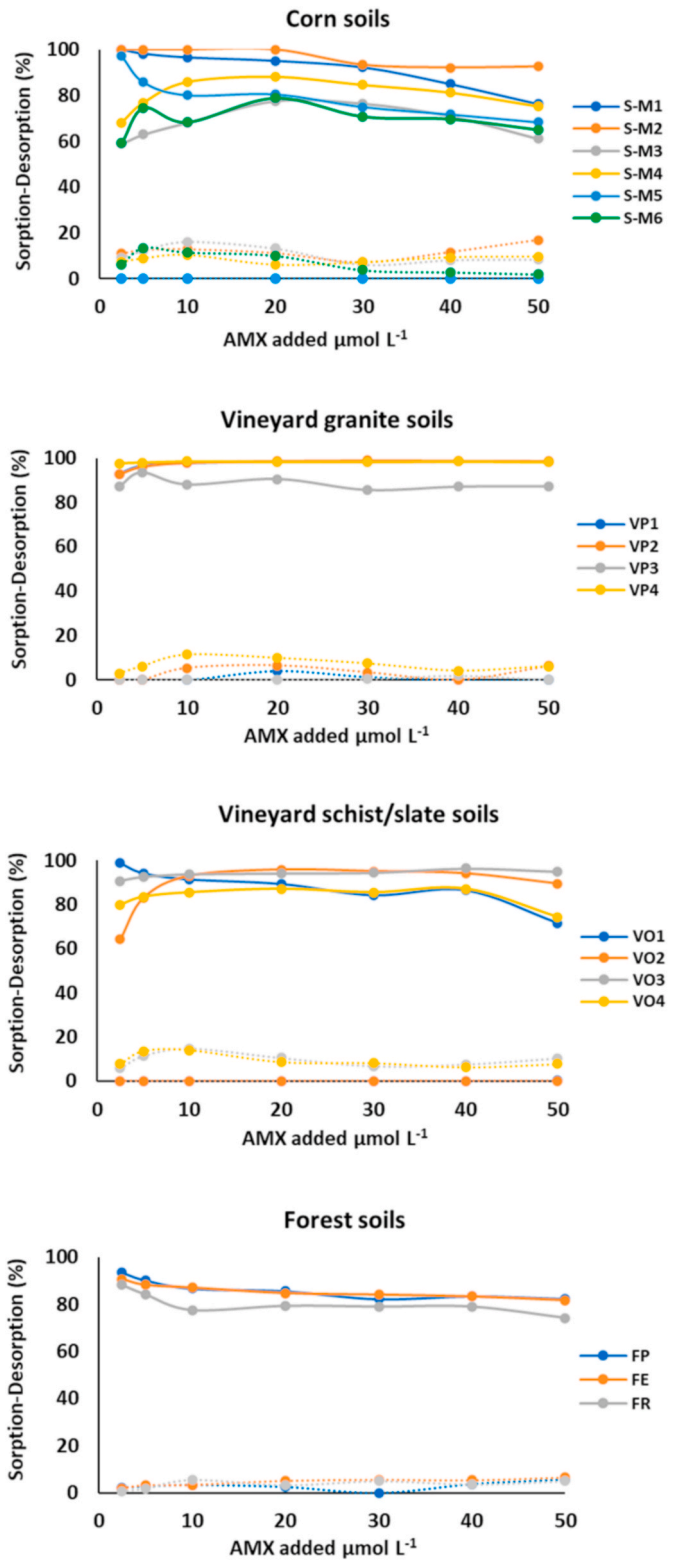

Fig. 2. Sorption and desorption (%) of each soil. Solid lines = Sorption; Dash lines = Desorption.

would indicate that adsorption occurs preferentially by interactions between the deprotonated carboxyl groups of AMX and the positive charges of these variable charge components of the soil. The Freundlich's model is the one that best explains the adsorption of AMX onto these soils, with *n* values suggesting the dominance of heterogeneous adsorption sites. AMX desorption is generally less than 10%, indicating a certain irreversibility of the adsorption process. Desorption is clearly lower in forest soils compared to agricultural soils, which would indicate a greater adsorbent-sorbate binding force in soils with more acidic pH and with higher organic matter and non-crystalline minerals contents. The results obtained in this work can be considered relevant regarding the evaluation of the risks associated to the incorporation of AMX into soils, in terms of its retention potential and probabilities of mobilization towards water bodies or entry into the food chain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.112753.

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Supplementary Material

Relevance of sorption in bio-reduction of amoxicillin taking place in forest and crop soils

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Data on sampling areas

Table S1 shows data on the soils sampled for this study in different areas of Galicia (NW Spain). Some of them are vineyard soils sampled in Ourense province (VO) and in Pontevedra province (VP). Others are maize/corn soils (M). In addition, the three forest (F) soils sampled were: one of them under *Pinus* (FP), another one under oak (*Q. robur*) (FR), and the third one under *Eucaliptus* (FE).

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Table S1. Basic details corresponding to the 19 soils that were sampled for this study. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas

Code	Crop/Trees	Parent material	Mean temperature (°C)	Annual rainfall (mm)
M1	Corn	Schists, quartzite, pelite, sandstones and granites	12.0	995
M2 M3 M4 M5 M6	Corn	Schist and amphibolite	13.0	1270
CM	Corn	Granite	13.4	1587
VO1 VO3	Vineyard	Schist Slate	11.0	850-1000
VO4 VO5	Vineyard	Granite and mica-schists	14.5	950
VP1 VP2	Vineyard	Granite	13.8	1540
VP4	Vineyard	Granite	14.0	1000-2000
VP7	Vineyard	Granite	13.8	1540
CV	Vineyard	Granite	13.4	1587
FP FE FR	Pine, Eucalyptus, Oak	Granite and gneiss	11.2	1389

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Determination of soil parameters

Regarding methods, pH in water (soil:liquid ratio 1:2.5) was measured with a CRISON-2001 pH meter (Crison, Spain). The determination of C and N were carried out by elemental analysis, with a LECO CHN-100 equipment (Leco Corporation, St. Joseph, MI, USA). Sand and clay contents were determined using the International Method of the Robinson Pipette. The exchangeable cations (Ca_e, Mg_e, Na_e, K_e and Al_e) were displaced using a 1M NH_4Cl solution in a 1:10 soil:solution ratio, and were then determined by atomic absorption spectrophotometry (Ca, Mg and Al) or atomic emission spectrophotometry (Na and K) with a Perkin Elemer AAnalyst 200 equipment (Perkin Elmer, USA). The sum of these exchangeable cations is considered the effective cation exchange capacity (eCEC). To determine the total non-crystalline Fe and Al contents (Fe_o and Al_o), an extraction was carried out with ammonium oxalate buffered at pH 3, stirring for 4 hours in the dark; five drops of 0.25% superfloc were added to the resulting extract, then centrifuged at 2000 rpm for 10 minutes; it was then filtered, and the supernatant is diluted 1:5; Fe and Al in non-crystalline form were measured by atomic absorption spectrophotometry. The determination of Fe and Al bound to organic matter (Fe_{pir} and Al_{pir}) was carried out by extraction with sodium pyrophosphate at pH 10; the extraction was carried out by shaking 1 g of sample with 100 mL of sodium pyrophosphate for 16 hours; the samples were diluted in half with distilled water and finally Fe and Al were measured in by atomic absorption spectrophotometry.

Table S2 shows the results of the determinations of the basic parameters carried out on the 19 soils under study. The range of pH in water (pH_{H2O}) was very wide, going from 4.68 to 8.02, with the most acidic values corresponding to forest soils. There was also a high variability in the carbon (C) content, ranging between 0.63 and 9.78%, while the nitrogen (N) content ranged between 0.06 and 0.67%. For both C and N, the highest levels corresponded to forest soils. Sand contents ranged between 43.42% and 69.35%, with the highest values generally corresponding to forest soils. The clay fraction ranged between 12.22 and 24.65%, with agricultural soils showing higher values. The effective cation exchange capacity (eCEC) values also showed a wide oscillation, between 5.94 and 42.81 $cmol_c\ kg^{-1}$, with Ca being the cation that dominated in most agricultural soils, while Al dominated in forest soils. The non-crystalline forms of Fe and Al also showed high variability. Specifically, total non-crystalline Fe (Fe_o) was between 1665 and 6990 $mg\ kg^{-1}$, with the highest values generally corresponding to soils on amphibolite, while total non-crystalline Al (Al_o) oscillated between 634 and 11651 $mg\ kg^{-1}$, with the lowest values corresponding to soils on schist/slate. Regarding Fe bound to organic matter (Fe_{pir}), the range went from 823 to 9239 $mg\ kg^{-1}$, while Al bound to organic matter (Al_{pir}) ranged from 1047 to 14857 $mg\ kg^{-1}$, with generally higher values in forest soils (FP, FE and FR), and in those with more carbon and organic matter (M5 and VP3) within agricultural soils.

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Table S2. Values corresponding to the basic parameters determined in the various soils studied. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas. Average values (n=3), with coefficients of variation always < 5%

Soil	pH_{H20}	C	OM	N	Sand	clay	eCEC	Ca_e	Mg_e	Na_e	K_e	Al_e	Fe_o	Fe_{pir}	Al_o	Al_{pir}
				%			$cmol_c\ kg^{-1}$						$mg\ kg^{-1}$			
M1	8.02	4.98	8.58	0.33	49.57	22.50	42.81	39.44	2.07	0.60	0.69	0.01	4140	1501	5351	2111
M2	5.33	2.66	4.59	0.27	43.42	24.65	6.88	5.35	0.67	0.02	0.44	0.40	5745	3159	3401	2871
M3	5.65	2.58	4.44	0.25	43.57	22.43	7.48	6.18	0.77	0.00	0.28	0.24	5780	3315	3881	2717
M4	5.01	2.04	3.52	0.21	45.64	22.43	5.94	4.58	0.67	0.10	0.17	0.43	4545	2923	2896	1945
M5	6.11	7.67	13.21	0.63	63.35	12.36	20.84	18.81	1.60	0.08	0.33	0.02	6990	3719	11651	6179
M6	6.43	5.56	9.59	0.53	59.28	16.50	24.35	21.65	1.60	0.16	0.93	0.02	6525	3231	4991	4087
CM	5.38	3.09	5.33	0.23	61.35	20.50	6.63	4.85	0.39	0.13	0.38	0.89	3320	2561	3246	3483
VO1	5.6	0.63	1.09	0.06	61.40	13.14	5.96	2.85	0.73	1.39	0.31	0.68	1665	1273	634	1961
VO2	5.6	1.61	2.78	0.18	43.50	22.90	6.02	3.38	0.26	1.83	0.5	<0.06	2555	1047	894	1047
VO3	5.87	1.94	3.35	0.11	47.50	18.22	6.41	3.69	0.88	0.15	0.52	1.17	1980	1421	1601	1321
VO5	6.04	1.77	3.06	0.10	49.57	24.14	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141
VP1	5.6	3.69	6.36	0.26	68.60	14.23	19.52	4.74	1.07	1.64	11.91	0.16	3045	2353	5739	4543
VP2	6.1	4.38	7.55	0.37	50.16	20.25	22.58	8.43	1.56	2.46	10.12	<0.05	3220	6309	4024	3897
VP3	5.5	6.58	11.35	0.49	65.54	21.07	10.51	6.55	0.37	2.60	0.27	0.72	3540	6459	6624	14857
VP4	7.27	3.56	6.14	0.22	67.50	12.22	12.32	10.53	1.04	0.44	0.27	0.04	1945	823	4576	1597
CV	5.8	4.01	6.91	0.30	61.57	20.07	8.60	5.73	0.83	0.17	1.13	0.74	2690	2129	3861	3573
FP	4.88	7.70	13.27	0.48	69.35	12.86	6.43	0.07	0.00	0.11	0.09	6.16	5410	9239	8184	10767
FE	4.8	9.78	16.87	0.67	67.28	12.86	6.59	0.05	0.00	0.12	0.12	6.29	5805	8249	10189	11347
FR	4.68	7.15	12.32	0.49	69.35	14.86	7.48	0.18	0.07	0.00	0.22	7.01	3055	6789	5619	9457

OM: organic matter content, resulting of multiplying C content by the factor 1.72

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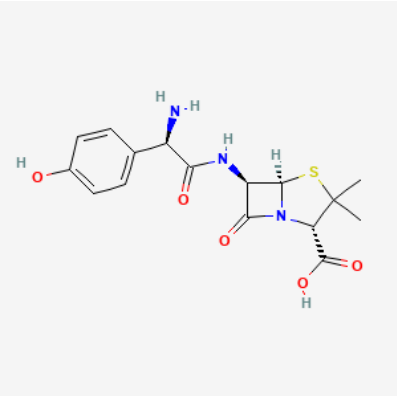
Adsorption and desorption experiments

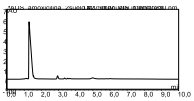
Adsorption and desorption studies were performed using batch-type experiment, where 2 g of soil were mixed with 5 mL of solutions containing 2.5, 5, 10, 20, 30, 40, or 50 µmol L^{-1}, of AMX, as well as 0.005 M $CaCl_2$ to act as background electrolyte, then shaken 48 h in the dark, and centrifuging for 15 min at 4000 rpm. The supernatants were filtered through 0.45 µm and the concentration of AMX determined using HPLC.

Quantification of AMX

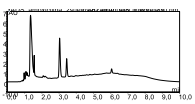
The HPLC determinations were carried out with a Model LPG 3400 SD equipment (Thermo-Fisher, USA). A Luna C18 column (150 mm long, 4.6 mm internal diameter, 5 µm particle size) and a pre-column (4 mm long, 2 mm diameter; 5 µm particle size) packed with the same material as the column were also used, both provided by Phenomenex (Madrid, Spain). During the HPLC analysis, the injection volume was 50 µL and the flow rate was 1.5 ml min^{-1}, with the mobile phase being acetonitrile (phase A) and 0.01 M phosphoric acid (phase B). A linear gradient was used, varying from 5% to 15% of phase A, and from 95% to 85% of phase B. The initial conditions were re-established in 2 minutes and maintained for 2 minutes. The total analysis time was 10 minutes, with a retention time of 3.3 minutes. The wavelength used for detection was 230 nm. Some example chromatograms are included in Figure S1 (Supplementary Material).



Soil M2; AMX concentration added 50 µM



Soil VP4; AMX concentration added 40 µM



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Soil FP; AMX concentration added 10 µM

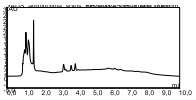

Figure S1. Molecular structure of AMX, and selected chromatograms corresponding to AMX adsorption onto soils

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Fitting to adsorption models

The data obtained in the adsorption experiments were adjusted to the Freundlich (Eq. 1), Langmuir (Eq. 2) and Linear (Eq. 3) models.

$$q_e = K_F C_{eq}{}^n \quad \text{Eq. 1}$$

$$q_e = \frac{q_m K_L C_{eq}}{1+K_L C_{eq}} \quad \text{Eq. 2}$$

$$Kd = q_e / C_{eq} (L\ kg^{-1}) \quad \text{Eq. 3}$$

Where q_e is the amount of AMX retained onto the soil; C_{eq} is the concentration of AMX in the solution at equilibrium; K_F is the Freundlich parameter related to the adsorption capacity; n is a parameter of the Freundlich model related to the intensity of the adsorption; K_L is the Langmuir adsorption constant; and q_m is the maximum adsorption capacity according to the Langmuir model. K_d is the partition coefficient in the linear model.

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Table S3. AMX adsorption, expressed in µmol kg^{-1} (and in percentage between brackets), from the various soils studied, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas. Average values (n=3), with coefficients of variation always < 5%

Soil	AMX added (µmol/L)	AMX adsorbed, concentration and %	Soil	AMX added (µmol/L)	AMX adsorbed, concentration and %
M1	2.5	5.44 (100)	VO1	2.5	5.40 (98.9)
	5	11.55 (98.2)		5	11.10 (94.3)
	10	25.30 (96.5)		10	23.97 (91.5)
	20	48.98 (95.0)		20	45.83 (89.4)
	30	69.25 (92.2)		30	63.25 (84.2)
	40	83.23 (85.0)		40	84.76 (86.5)
	50	77.77 (76.3)		50	74.01 (71.5)
M2	2.5	7.02 (100)	VO2	2.5	5.43 (98.8)
	5	10.73 (100)		5	11.11 (94.0)
	10	23.67 (100)		10	24.07 (91.8)
	20	45.20 (100)		20	46.21 (89.7)
	30	70.05 (93.3)		30	64.28 (84.8)
	40	88.02 (92.1)		40	85.11 (86.4)
	50	94.87 (92.6)		50	73.47 (71.0)
M3	2.5	4.15 (59.0)	VO3	2.5	4.53 (64.2)
	5	6.79 (63.0)		5	7.28 (67.6)
	10	16.12 (68.1)		10	16.97 (71.7)
	20	35.22 (77.5)		20	32.02 (70.5)
	30	59.63 (76.4)		30	56.82 (72.8)
	40	76.21 (70.9)		40	77.36 (72.0)
	50	73.08 (61.2)		50	80.31 (67.2)
M4	2.5	4.80 (68.1)	VO4	2.5	4.54 (64.4)
	5	8.28 (76.8)		5	8.81 (81.7)
	10	20.33 (85.9)		10	16.84 (71.1)
	20	40.04 (88.1)		20	32.86 (72.3)
	30	65.98 (84.6)		30	58.65 (75.2)
	40	87.21 (81.2)		40	76.89 (71.6)
	50	89.75 (75.1)		50	80.81 (67.6)
M5	2.5	5.34 (97.2)	VP1	2.5	5.14 (93.6)
	5	10.09(85.7)		5	9.68 (81.8)
	10	21.00 (80.2)		10	19.81 (75.6)
	20	41.11 (80.6)		20	43.49 (84.4)
	30	56.89 (75.0)		30	54.47 (71.8)
	40	70.62 (71.7)		40	62.29 (63.3)
	50	69.96 (68.3)		50	52.72 (51.0)

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Table S3
(Continuation)

Soil	AMX added (µmol/L)	AMX adsorbed, concentration and %	Soil	AMX added (µmol/L)	AMX adsorbed, concentration and %
M6	2.5	4.17 (59.1)	VP2	2.5	3.19 (58.1)
	5	8.04 (74.6)		5	7.34 (62.1)
	10	16.17 (68.3)		10	17.42 (66.5)
	20	35.87 (78.9)		20	33.86 (65.7)
	30	55.25 (70.8)		30	43.75 (57.7)
	40	74.91 (69.7)		40	52.20 (53.0)
	50	77.72 (65.1)		50	64.60 (62.5)
M7	2.5	5.02 (71.2)	VP3	2.5	4.80 (87.4)
	5	8.34 (77.4)		5	9.21 (77.9)
	10	18.84 (79.6)		10	20.93 (79.9)
	20	30.77 (67.7)		20	44.31 (86.0)
	30	58.83 (75.4)		30	62.46 (82.4)
	40	81.15 (75.5)		40	83.86 (85.2)
	50	79.10 (66.2)		50	85.40 (82.6)
FP	2.5	5.81 (93.5)	VP4	2.5	4.98 (70.6)
	5	11.25 (90.2)		5	6.98 (64.8)
	10	21.65 (86.7)		10	16.92 (71.5)
	20	41.71 (85.7)		20	40.76 (89.7)
	30	60.93 (82.3)		30	69.88 (89.6)
	40	83.97 (83.5)		40	79.47 (74.0)
	50	102.54 (82.4)		50	82.25 (68.8)
FE	2.5	5.70 (90.8)	CV	2.5	4.94 (91.35)
	5	11.03 (88.3)		5	11.55 (98.7)
	10	21.86 (87.2)		10	23.59 (90.0)
	20	42.13 (84.8)		20	49.70 (96.4)
	30	62.31 (84.2)		30	56.09 (74.3)
	40	83.02 (83.4)		40	49.68 (51.0)
	50	100.74 (81.8)		50	43.41 (42.6)
FR	2.5	5.49 (88.4)			
	5	10.38 (84.4)			
	10	19.37 (77.6)			
	20	38.89 (79.5)			
	30	59.15 (79.1)			
	40	78.76 (79.1)			
	50	90.97 (74.2)			

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Table S4: AMX desorption, expressed in µmol kg^{-1} (and in percentage between brackets), from the various soils studied, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; CM and CV: control soils in maize and vineyard areas. Average values (n=3), with coefficients of variation always < 5%

Soil	AMX added (µmol/L)	AMX desorbed, concentration and %	Soil	AMX added (µmol/L)	AMX desorbed, concentration and %
M1	0	0 (0)	VO1	0	0 (0)
	2.5	0 (0)		2.5	0 (0)
	5	0 (0)		5	0 (0)
	10	0 (0)		10	0 (0)
	20	0 (0)		20	0 (0)
	30	0 (0)		30	0 (0)
	40	0 (0)		40	0 (0)
	50	0 (0)		50	0 (0)
M2	0	0 (0)	VO2	0	0 (0)
	2.5	0.77 (10.9)		2.5	0 (0)
	5	1.34 (12.5)		5	0 (0)
	10	3.03 (12.8)		10	0 (0)
	20	5.03 (11.1)		20	0 (0)
	30	5.21 (7.2)		30	0 (0)
	40	11.50 (11.6)		40	0 (0)
	50	18.49 (16.9)		50	0 (0)
M3	0	0 (0)	VO3	0	0 (0)
	2.5	0.38 (9.3)		2.5	0.26 (5.7)
	5	0.83 (12.2)		5	0.83 (11.3)
	10	2.60 (16.1)		10	2.51 (14.8)
	20	4.64 (13.2)		20	3.34 (10.4)
	30	3.62 (6.1)		30	3.83 (6.7)
	40	6.15 (8.1)		40	5.81 (7.5)
	50	6.11 (8.4)		50	8.27 (10.3)
M4	0	0 (0)	VO4	0	0 (0)
	2.5	0.36 (7.4)		2.5	0.349 (7.7)
	5	0.74 (8.9)		5	1.18 (13.4)
	10	2.12 (10.4)		10	2.33 (13.8)
	20	2.45 (6.1)		20	2.82 (8.6)
	30	4.74 (7.2)		30	4.78 (8.2)
	40	8.14 (9.3)		40	4.82 (6.3)
	50	8.68 (9.7)		50	6.21 (7.7)

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Table S4
(Continuation)

Soil	AMX added (µmol/L)	AMX desorbed, concentration and %	Soil	AMX added (µmol/L)	AMX desorbed, concentration and %
M5	0	0 (0)	VP1	0	0 (0)
	2.5	0 (0)		2.5	0 (0)
	5	0 (0)		5	0 (0)
	10	0 (0)		10	0 (0)
	20	0 (0)		20	1.81 (4.2)
	30	0 (0)		30	0.79 (1.4)
	40	0 (0)		40	0 (0)
	50	0 (0)		50	0 (0)
M6	0	0 (0)	VP2	0	0 (0)
	2.5	0.26 (6.2)		2.5	0 (0)
	5	1.08 (13.4)		5	0 (0)
	10	1.85 (11.4)		10	0.38 (5.4)
	20	3.59 (10.0)		20	0.89 (6.6)
	30	2.08 (3.8)		30	0.60 (3.4)
	40	2.14 (2.9)		40	0.03 (0.2)
	50	1.51 (1.9)		50	1.61 (6.2)
M7	0	0 (0)	VP3	0	0 (0)
	2.5	0.28 (5.7)		2.5	0.68 (9.6)
	5	1.10 (13.2)		5	0.83 (7.8)
	10	2.77 (14.7)		10	3.7 (15.6)
	20	3.43 (11.2)		20	6.06 (13.5)
	30	5.64 (9.6)		30	8.66 (11.2)
	40	1.82 (2.2)		40	9.61 (8.9)
	50	6.58 (8.3)		50	13.19 (11.3)
FP	0	0 (0)	VP4	0	0 (0)
	2.5	0.05 (2.2)		2.5	0.15 (3.0)
	5	0.12 (2.7)		5	0.43 (6.2)
	10	0.29 (3.3)		10	1.94 (11.4)
	20	0.43 (2.5)		20	4.06 (10.0)
	30	0 (0)		30	5.21 (7.5)
	40	1.24 (3.7)		40	3.36 (4.2)
	50	2.29 (5.6)		50	5.11 (6.2)
FE	0	0 (0)	CV	0	0 (0)
	2.5	0.04 (1.9)		2.5	0 (0)
	5	0.14 (3.2)		5	0 (0)
	10	0.29 (3.4)		10	0 (0)
	20	0.86 (5.1)		20	0.80 (1.6)
	30	1.39 (5.5)		30	0 (0)
	40	1.80 (5.3)		40	0 (0)
	50	2.65 (6.5)		50	0 (0)

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Table S4
(Continuation)

Soil	AMX added (µmol/L)	AMX desorbed, concentration and %
FR	0	0 (0)
	2.5	0.02 (0.7)
	5	0.07 (1.6)
	10	0.43 (5.5)
	20	0.50 (3.2)
	30	1.19 (5.0)
	40	1.12 (3.5)
	50	1.84 (4.9)

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PUBLICACIÓN VI

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Potential of low-cost bio-adsorbents to retain amoxicillin in contaminated water

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ABSTRACT

Sewage sludge as agricultural amendment is the main route of human-medicine antibiotics to enter soils. When reaching environmental compartments, these compounds can cause significant risks to human and ecological health. Specifically, the antibiotic amoxicillin (AMX) is highly used in medicine, and the fact that more than 80% of the total ingested is excreted increases the chances of causing serious environmental and public health problems. As the use of low-cost bio-adsorbents could help to solve these issues, this research focuses on the retention of AMX onto four by-products of the forestry industry (eucalyptus leaf, pine bark, pine needles, and wood ash) and one from food industry (mussel shell). To carry out this study, batch-type tests were performed, where increasing concentrations of the antibiotic (0, 2.5, 5, 10, 20, 30, 40 and 50 μmol L^{-1}) were added to samples of 0.5 g of each bio-adsorbent. Eucalyptus leaf, pine needle and wood ash showed adsorption scores higher than 80%, while it was up to 39% and 48% for pine bark and mussel shell, respectively. For pine bark, wood ash and mussel shell, adsorption data showed good adjustment to the Freundlich and Linear models, while pine needles and eucalyptus leaf did not fit to any model. There was not desorption when the maximum concentration of AMX (50 μmol L^{-1}) was added. Overall, eucalyptus leaf, pine needles and wood ash can be considered good bio-adsorbents with high potential to retain AMX, which has significant implications regarding their eventual use to reduce risks of environmental pollution by this antibiotic.

1. Introduction

As antibiotics are highly used in the treatment and prevention of infectious diseases, both in humans and in animals, these compounds and related metabolites are often detected in waters, sediments, soils, and organisms (Radjenović et al., 2009; Rosal et al., 2010; Zhou et al., 2011). In general, its presence in the environment may cause harmful effects (Orozco-Hernández et al., 2019; Santás-Miguel et al., 2020; Shen et al., 2019).

Among these biocides, amoxicillin (AMX) is a broad spectrum β-lactam antibiotic, classified as one of the ten most commonly used drugs due to its high demand in livestock and human medicine treatments (Merlin et al., 2011). More than 80% of AMX is excreted through feces and urine after 2 h since ingestion, with subsequent presence in water and wastewater. In fact, most of the antibiotics that reach the environment come from pharmaceutical industries, hospital effluents, and excretions from humans and farm animals (Sophia et al., 2016; Abazari et al., 2019). For instance, Henninger et al. (2001) found AMX concentrations between 28 and 82.7 μg L^{-1} in wastewater from a hospital. In fact, the main route of human medicine antibiotics to enter the environment is through wastewater. After intake for therapeutic use, antibiotics are largely excreted as original compounds and partly as derived metabolites. After reaching Wastewater Treatment Plants (WWTP), antibiotics are subjected to different treatments, which are not always totally effective in the elimination of emerging pollutants (Krzeminski et al., 2019).

These treatments generate sludge, which is commonly used as amendments in agricultural plots, thus constituting a route of entry for antibiotics into the soil. Soil pollution by antibiotics has a negative impact (Li, 2014; Mackuľak et al., 2019), with an additional risk of

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contamination of surface and ground water, as well as entering the food chain, which can take place through drinking water and food since many crops could absorb these pollutants from soil. In this regard, is noteworthy that the environmental risk assessment of antibiotics persistent in the environment is a challenge, whicht is accentuated due to their movement patterns through different compartments (Yi et al., 2019; Najafpoor et al., 2019).

Regarding the removal of this kind of emerging pollutants, one of the first steps to be considered are WWTPs treatments, as some of them include different antibiotic elimination techniques. The most popular methods are biological and chemical treatments. Specifically, electrocoagulation, photo- and electro-chemical catalytic degradation, and advanced oxidation methods have shown to be useful in the case of AMX present in wastewater (Flamur et al., 2015; Liu et al., 2015; Mohammad et al., 2015; Padilla-Robles et al., 2015); however, the cost of these methods is high. As an alternative, adsorption is considered one of the most effective techniques for removing antibiotics from effluents (Homem and Santos, 2011). This method is efficient, simple to design and operate, as well as inexpensive (Han et al., 2008).

In addition, once in the soil, adsorption/desorption processes are of main importance in conditioning the entry of antibiotic residues into surface- and groundwater, as well as into crops, largely controlling their passage to the food chain (Christou et al., 2017; Harrabi et al., 2018; Zhou et al., 2018). In addition, the behavior of AMX in the edaphic environment is highly conditioned by the pH of the medium, which affects the ionization of the compound and the surface charge of the soil colloids. AMX has amphoteric properties due to the existence in its structure of three functional groups: NH_2, –COOH and –OH (Anastopoulos et al., 2020). The charge of the AMX molecule changes gradually as a function of pH, due to the pK_a of these functional groups. The carboxyl group has a $pK_{a1} = 2.68$, the amine group has a $pK_{a2} = 7.49$ and the phenolic hydroxyl group has a $pK_{a3} = 9.63$ (Homsirikamol et al., 2016). Therefore, in the pH range between 3 and 6, AMX is in its zwitterionic form, whereas at pH values below and above this range, the AMX molecule is mainly positively and negatively charged, respectively.

Currently, activated carbon has become one of the most common adsorbents (Liu et al., 2011), but its high cost of operation and regeneration represent disadvantages (Zazouli et al., 2013), creating a growing demand for other more economical adsorbents that do not require any additional costly pretreatment (Han et al., 2008). For example, Putra et al. (2009) used bentonite for the retention of AMX, achieving 88% efficiency in the elimination of this antibiotic. Also to be noted, the use of effective bio-adsorbents that are produced in abundance in the vicinity of the area where they are to be applied, would facilitate their supply and recycling, reducing costs. Specifically, in Galicia (NW Spain) there are abundant residues and by-products from the food industry, such as mussel shell, and from the forestry industry (such as pine bark, pine needles, eucalyptus leaves, and wood ash), which would be interesting candidates to study their capacity to adsorb AMX.

Considering this scenario, a first-time study was performed dealing with AMX adsorption and desorption on/from four by-products of the forestry industry (eucalyptus leaf, biomass combustion ash, pine bark and pine needles), and one by-product of the food industry (mussel shell). As all five are low-cost materials, their possible effective use in the retention of AMX present in the environment as pollutant would have a clear interest, both in terms of environmental protection, and promotion of recycling by-products within the precepts of the circular economy.

2. Materials and methods

2.1. Chemical reagents

AMX (with purity 95% or higher) was from Sigma-Aldrich (Barcelona, Spain). Acetonitrile and phosphoric acid (with purities of 99.9% and 85%, respectively) were from Fisher (Madrid, Spain), while Panreac (Barcelona, Spain) provided $CaCl_2$ (with purity 95%). Millipore (Madrid, Spain) provided milliQ water used to prepare solutions needed to perform HPLC analyses.

2.2. Sorbent materials

The materials used as sorbents were: i) eucalyptus leaves sampled at forest exploitations situated in Lugo (Spain), pine bark provided by Geolia (Spain), pine needles sampled at forest exploitations situated in Lugo (Spain), and wood ash sampled at combustion boiler situated in Lugo (Spain), all four considered by-products from forestry industries; and ii) mussel shell (ground at <1 mm), from Abonomar S.L. (Pontevedra province, Spain), which is a by-product generated at food industries. As further details, it should be noted that eucalyptus leaves are from *Eucalyptus nitens*, pine bark and pine needles are from *Pinus pinaster*, and mussel shells are from *Mityllus galloprovincialis*. The age of the mussels variated and was not defined, derived from many different shells used in the cannery industry. The age of *Eucalyptus nitens* was 7 years, while it is not known for *Pinus pinaster*. All bio-adsorbents were ground until achieving particle size <1 mm and then were stored in the lab, at room temperature (around 20 °C), until further analyses and use.

Supplementary Material includes the methodology used to characterize all five sorbent materials. In addition, complementary details have been previously published (Conde-Cid et al., 2019; Quintáns-Fondo et al., 2019).

2.3. Adsorption and desorption experiments

Batch experiments were performed to study the adsorption/desorption of AMX on/from all five bio-adsorbent materials. For this, 0.5 g of bio-adsorbent were weighed, and mixed with 10 mL of a solution with different concentrations of the antibiotic (2.5, 5, 10, 20, 30, 40, 50 μmol L^{-1}), also containing 0.005 M $CaCl_2$ as background electrolyte. The suspensions were shaken for 48 h in the dark using a rotary shaker (as 48 h was enough to reach equilibrium according to previous kinetic tests, data not shown). These suspensions were then centrifuged at 4000 rpm for 15 min. The resulting supernatants were filtered through 0.45 μm nylon syringe filters and the antibiotic concentration in the filtered liquids was determined by HPLC-UV with LPG 3400 SD equipment (Thermo-Fisher, USA).

More details regarding HPLC equipment and quantification are included in Supplementary Material, as well as selected chromatograms (Figure S1).

After adsorption, desorption was studied in those cases where the maximum AMX concentration (50 μmol L^{-1}) had been added, to evaluate the reversibility of the process. To do that, the material resulting from the adsorption process was added with a volume of 10 mL of 0.005 M $CaCl_2$ (with no antibiotic), then repeating the procedure performed for adsorption. Triplicate determinations were carried out in all cases.

2.4. Data treatment

Experimental adsorption data were adjusted to the Freundlich (Eq. (1)), Langmuir (Eq. (2)) and Linear (Eq. (3)) models (Ayawei et al., 2017).

$$q_e = K_F C_{eq^n} \quad (1)$$

$$q_e = \frac{q_m K_L C_{eq}}{1 + K_L C_{eq}} \quad (2)$$

$$Kd = q_e / C_{eq} (Lkg^{-1}) \quad (3)$$

In these equations q_e is the amount of AMX retained onto the sorbent, which was the concentration added minus that remaining in the equilibrium solution; C_{eq} is the AMX concentration in the solution at

Table 1
AMX adsorption, expressed in μmol kg^{-1} and in percentage, for the five different sorbents, as a function of the concentration of antibiotic added (C_0 in μmol L^{-1}). -: no data. Average values (n = 3), with coefficients of variation always <5%. C_{eq}: concentration in the equilibrium; Ads: quantity adsorbed; -: no data.

	C_0 (μM)	C_{eq} (μM)	Ads (μmol kg^{-1})	Ads (%)
Eucalyptus leaves	0	0	0	0
	2.46	0	49.12	100
	5.37	0	107.37	100
	10.93	0	206.22	100
	18.34	–	–	–
	29.13	0	560.19	100
	38.62	0	757.31	100
	52.07	12.42	762.56	76.15
Pine bark	0	0	0	0
	2.46	2.4	1.12	2.29
	5.37	5.2	3.37	3.14
	10.93	10.8	2.49	1.19
	18.34	14.58	75.18	20.49
	29.13	22.51	132.38	22.72
	38.62	23.66	299.20	38.73
	52.07	36.77	294.23	29.38
Pine needles	0	0	0	0
	2.46	0	48.16	100
	5.37	–	–	–
	10.93	0	214.30	100
	18.34	–	–	–
	29.13	0	582.60	100
	38.62	0	772.46	100
	52.07	0	1001.32	100
Wood ash	0	0	0	0
	2.46	0.31	41.99	81.19
	5.37	1.19	83.49	77.76
	10.93	1.98	178.90	81.84
	18.34	–	–	–
	29.13	4.67	470.40	83.97
	38.62	4.78	663.52	87.61
	52.07	5.36	934.21	89.71
Mussel shell	0	0	0	0
	2.46	1.52	18.35	38.10
	5.37	3.08	46.71	42.64
	10.93	7.33	70.55	32.92
	18.34	11.32	132.50	38.29
	29.13	16.66	226.69	42.80
	38.62	19.05	391.53	50.69
	52.07	27.00	501.46	48.15

equilibrium; K_F is a parameter representing the Freundlich's adsorption capacity; n is the Freundlich's parameter related to the degree of heterogeneity in adsorption; K_L is a parameter known as the Langmuir adsorption constant; q_m is the Langmuir's maximum adsorption capacity; and K_d is a parameter of the linear model known as partition coefficient. The fitting of adsorption data to the Freundlich, Langmuir, and Linear models was carried out by means of the SPSS 21 statistical package, also used to study statistical correlations involving adsorption results and parameters of the bio-adsorbents.

3. Results and discussion

3.1. Adsorption/desorption of AMX

Table 1 shows the adsorption values for the various bio-adsorbents, expressed in μmol kg^{-1} and in percentage, depending on the concentration of antibiotic added. For the highest AMX concentration added, pine needles showed the highest adsorption value (1001 μmol kg^{-1}), followed by wood ash (934 μmol kg^{-1}), eucalyptus leaf (762 μmol kg^{-1}), mussel shell (501 μmol kg^{-1}), while pine bark presented the lowest adsorption capacity (294 μmol kg^{-1}).

Expressed as percentage, eucalyptus leaf, pine needles and wood ash showed high adsorption scores (higher than 76%), while pine bark and mussel shell displayed values that did not exceed 39% and 51%, respectively.

AMX adsorption data corresponding to the different bio-adsorbents were statistically analyzed. Firstly, a normality test was carried out, showing that all the bio-adsorbents (except pine bark) presented a normal distribution. Data for pine bark was log-transformed to get them normalized. Then, an ANOVA analysis (with Tukey test) was performed finding that there are no significant differences (at $p < 0.05$) among the various bio-adsorbents, considering all the concentrations together. However, when comparing data corresponding to the maximum concentration of antibiotic added (50 μmol L^{-1}), significant differences were found for the adsorption of the different bio-adsorbents, obtaining the following sequence: pine bark^a < mussel shell^b < eucalyptus leaves^c < wood ash^d < pine needles^e (different letters indicate significant differences for adsorption, at $p < 0.05$). Finally, the different concentrations of antibiotic added were compared, considering all the bio-adsorbents together. The result of this analysis showed that there are significant differences (at $p < 0.05$) among the various concentrations, resulting the following sequence (for concentrations expressed in μmol L^{-1}): $2.5^a < 5^a < 10^{ab} < 20^{abc} < 30^{abc} < 40^{bc} < 50^c$.

In Table S1 (Supplementary Material), it is shown that pine needles and eucalyptus leaves had an acid pH (3.68 and 4.88, respectively), while wood ash and mussel shell showed alkaline values (11.31 and 9.04, respectively).

It is important to bear in mind that amoxicillin has three different pk_a values, related to the carboxyl ($pk_{a1} = 2.68$), amino ($pk_{a2} = 7.49$) and phenolic ($pk_{a3} = 9.63$) functional groups (Homsirikamol et al., 2016). At pH < 2.68 the positive charge of the amino group predominates, whereas at pH values between 2.68 and 7.49 AMX behaves as a zwitterion with both deprotonated carboxyl groups and protonated amino groups, while at pH between 7.49 and 9.63 deprotonated carboxyl and amino groups predominate (with negative charge), and, finally, at pH > 9.63, phenolic groups are also deprotonated (Pezoti et al., 2016) and the molecule acquires a high negative charge.

The five bio-adsorbent materials used in this study have very different acid-base characteristics, favoring also different adsorption mechanisms. Wood ash has a very alkaline pH (11.31), and non-crystalline Fe and Al contents much higher than the other four bio-adsorbents (Table S1, Supplementary Material), all of them being variable charge components, which will have a high negative charge at that pH. At pH 11.31, AMX will have deprotonated amino, carboxyl and phenol groups, also acquiring a high negative charge. Therefore, AMX adsorption onto wood ash would be through a cationic bridge (such as Ca^{2+}) between the negatively charged part of the antibiotic and the surface of the bio-adsorbent, also negatively charged, forming ternary complexes (Gu et al., 2007), and this interaction would be favored by the high concentration of exchangeable Ca in the ash (Table S1, Supplementary Material). The adsorption mechanism would be similar in the case of mussel shell, where its lower adsorption values would be due to the lower amount of non-crystalline minerals and exchangeable Ca. In fact, the pH of mussel shell is not so alkaline (9.39), being lower than the pk_{a3} of AMX, causing that the negative charge density of the antibiotic is not so high under these conditions. Conde-Cid et al. (2019) also found high adsorption scores for three tetracycline antibiotics (oxytetracycline, chlortetracycline and tetracycline) onto wood ash, much higher than onto mussel shell.

In the other bio-adsorbents (pine needles, pine bark, and eucalyptus leaves) the pH is very acidic (3.68, 3.99 and 4.88, respectively), between the pk_{a1} and pk_{a2} values of AMX, causing that the molecules of the antibiotic behave as a zwitterion, with protonated amino groups and deprotonated carboxyl groups. The high adsorption of the antibiotic onto pine needles and eucalyptus leaves can be related to their high organic carbon content (50 and 53%, respectively) (Table S1, Supplementary Material), with a large number of carboxyl groups that can dissociate and present negative charge at pH > 3 (Edwards et al., 1996). At these pH values, AMX will have protonated amino groups that can bind through electrostatic interactions to the carboxyl groups of the bio-adsorbents of vegetal origin. The lower adsorption onto pine bark

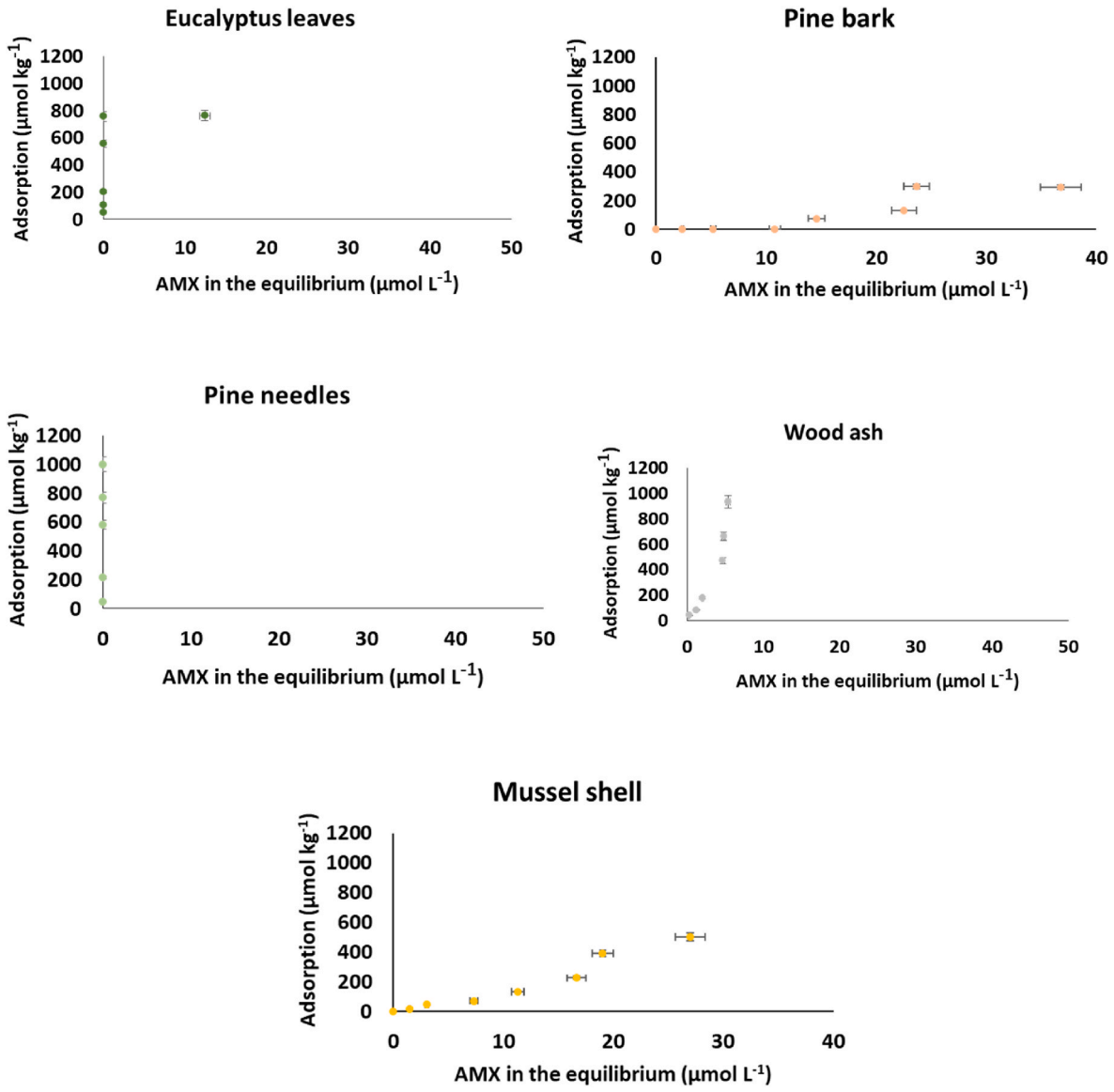

Fig. 1. AMX adsorption curves for the five bio-adsorbents. Average values (n = 3), with error bars showing that coefficients of variation were always <5%.

Table 2
Fitting of the adsorption data to the Freundlich, Langmuir and Linear models. K_F (L^n µmol^{1-n} kg^{-1}); K_L (L µmol^{-1}); q_m (µmol kg^{-1}); K_d (L kg^{-1}); -: error values too high for fitting.

	Freundlich					Langmuir					Linear		
	K_F	Error	n	Error	R^2	K_L	Error	q_m	Error	R^2	K_d	Error	R^2
Eucalyptus leaves	–	–	–	–	–	–	–	–	–	–	–	–	–
Pine bark	–	–	1.37	0.43	0.822	–	–	–	–	–	7.92	1.13	0.789
Pine needles	–	–	–	–	–	–	–	–	–	–	–	–	–
Wood ash	100.88	77.59	1.21	0.49	0.816	–	–	–	–	–	139.61	16.15	0.812
Mussel shell	5.23	2.99	1.39	0.18	0.966	–	–	–	–	–	17.25	1.21	0.933

compared to pine needles and eucalyptus leaf is due to the different chemical composition of their organic compounds and also to the fact that the bark has a higher content in Fe and Al non-crystalline minerals, which would be positively charged at that pH.

In a previous research, Cela-Dablanca et al. (2021) studied the adsorption of the antibiotic cefuroxime onto the same bio-adsorbents used in this work, finding that pine bark and pine needles had the lowest adsorption capacities. However, Conde-Cid et al. (2021) found that pine bark performed as the best bio-adsorbent retaining three sulfonamides (sulfadiazine, sulfamethazine and sulfachloropyridazine), while wood ash and mussel shell presented lower retention potential.

Fig. 1 shows AMX adsorption curves for the five bio-adsorbents, while Table 2 shows the fitting of the experimental data to the Freundlich, Langmuir, and Linear adsorption models.

The adjustment to the Freundlich model is valid just for mussel shell and wood ash (R^2 being 0.966 and 0.816, respectively), and partially valid for pine bark (R^2 = 0.822 for the n parameter), due to the high errors associated to the adjustment for the other adsorbents. The values

of the K_F parameter (related to the adsorption capacity) in the bio-adsorbents that fit the model range between 5.23 and 100.88 L^n μmol^{1-n} kg^{-1}, for mussel shell and wood ash, respectively. The values of the *n* parameter (which are related to the reactivity and heterogeneity of the adsorption sites) range between 1.21 and 1.39. K_F is much higher for wood ash than for mussel shell, but it is much lower than that obtained in previous studies for the adsorption of tetracycline antibiotics onto these same adsorbents (Conde-Cid et al., 2019).

AMX adsorption fitted well the linear model for mussel shell, wood ash and pine bark (R^2 of 0.933, 0.812 and 0.789, respectively). The K_d values vary from 139.61 to 7.92 L kg^{-1}, following the sequence: wood ash ≫ mussel shell > pine bark. Therefore, according to these data, among the materials that fit the linear model, wood ash is the one that adsorbs AMX with the strongest interactions. The K_d values for wood ash and mussel shell are higher than those obtained in most of the soils evaluated in a previous study (Cela-Dablanca et al., 2021). However, these K_d values are lower than those reported by Cela-Dablanca et al. (2021) for the antibiotic cefuroxime in the same bio-adsorbents (mussel shell, woos ash and pine bark).

The high errors associated to the fitting of the experimental data to the Langmuir model make it invalid in this case (Table 2).

Regarding desorption, no release was detected when the maximum AMX concentration (50 μmol L^{-1}) was added, indicating that the adsorption process was not reversible. In fact, the desorption procedure showed no release taking place from any of the five bio-sorbent materials.

Overall, in the current study eucalyptus leaf, pine needle and wood ash performed as good bio-adsorbents for AMX, while pine bark and mussel shell would be less suitable to adsorb this antibiotic. These results are not coincident with those obtained for the antibiotic cefuroxime in a previous study (Cela-Dablanca et al., 2021), in which mussel shell gave good results, while pine needles and eucalyptus leaves could not be recommended for removing cefuroxime. However, in another previous study, Conde-Cid et al. (2019) found that pine bark was a good adsorbent for tetracycline antibiotics, since it had high adsorption and low desorption values, while mussel shell was not suitable for adsorbing these compounds.

4. Conclusions

Among the five bio-adsorbents here studied, eucalyptus leaf, pine needles, and wood ash showed high adsorption for the antibiotic AMX, with non-crystalline Al and Fe, exchange cations and organic carbon contents present in each bio-adsorbent being the parameters with the highest influence on the retention of this antibiotic. Regarding fitting to adsorption models, it was possible for pine bark, mussel shell and wood ash as regards the Linear and Freundlich equations. Referred to desorption, no release was detected when the highest concentration of the antibiotic was added. Therefore, except for pine bark and mussel shell, the remaining three materials would be good bio-adsorbents for AMX, with wood ash showing the best results. The overall results of this research encourage additional studies to elucidate in further detail key additional details on the use of these bio-adsorbents to protect environmental compartments from emerging pollutants such as AMX.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113621.

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Supplementary Material

Low-cost bio-adsorbents to retain amoxicillin in contaminated environments

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Characterization of the sorbent materials

The values corresponding to pH in water and pH in KCl (solid:liquid ratio 1:2.5) were measured with a CRISON-2001 pHmeter (Crison, Barcelona, Spain). Total C and N were determined by means of elemental analysis, using a LECO CHN-100 equipment (Leco Corporation, St. Joseph, MI, USA). The exchangeable cations (Cae, Mge, Nae, Ke and Ale) were displaced using a 1 M NH4Cl solution in a 1:10 sorbent: solution ratio, and were then quantified by means of atomic absorption spectrophotometry, using a Perkin Elemer AAnalyst 200 apparatus (Perkin Elmer, Inc, USA). The effective cation exchange capacity (eCECe) was determined as the sum of these five exchangeable cations. Available phosphorus was extracted using 0.5 M $NaHCO_3$ and quantified using a UV-visible spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). To determine total non-crystalline Fe and Al, a specific extraction was performed using ammonium oxalate buffered at pH 3, stirred for 4 hours in the dark, then adding 5 drops of 0.25% superfloc to the resulting extract, then centrifuging at 2000 rpm for 10 minutes, filtering and diluting

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the supernatant to 1:5. Finally, Fe and Al in non-crystalline form (Feo and Alo) were quantified by atomic absorption spectrophotometry. Total contents of different elements (Na_T, Mg_T, Al_T, K_T, Ca_T, Cr_T, Mn_T, Fe_T, Co_T, Ni_T, Cu_T, Zn_T, As_T and Cd_T) were determined following the EPA 3051 method, performing an acid digestion in a microwave with a 65% HNO3 solution, and subsequent determination with ICP-MS.

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Table S1. Characteristics of the bio-adsorbent materials. Ca_e, Mg_e, Na_e, K_e, Al_e: elements in the exchange complex; Sat. Al: Al-saturation in the exchange complex; eCEC: effective cation exchange capacity; XT: total content of the element (X); Alo, Feo: non-crystalline Al and Fe. Average values (n=3), with coefficients of variation always <5%.

Parameter	Unit	Eucalyptus leaves	Pine bark	Pine needles	Wood ash	Mussel shell
C	%	53.05	48.70	50.31	13.23	11.43
N	%	1.35	0.08	1.08	0.22	0.21
C/N		39.18	608.75	46.76	60.14	55.65
pH_{water}		4.88	3.99	3.68	11.31	9.39
pH_{KCl}		4.81	3.42	3.51	13.48	9.04
Ca_e	$cmol_c\ kg^{-1}$	7.95	5.38	2.13	95.0	24.75
Mg_e	$cmol_c\ kg^{-1}$	8.53	2.70	7.15	3.26	0.72
Na_e	$cmol_c\ kg^{-1}$	1.36	0.46	1.42	12.17	4.37
K_e	$cmol_c\ kg^{-1}$	12.93	4.60	11.09	250.65	0.38
Al_e	$cmol_c\ kg^{-1}$	0.13	1.78	2.15	0.07	0.03
eCEC	$cmol_c\ kg^{-1}$	30.90	14.92	23.94	361.15	30.25
Sat Al	%	0.42	11.93	8.98	0.02	0.10
Available-P	$mg\ kg^{-1}$	262.84	70.45	217.95	462.83	54.17
Na_T	$mg\ kg^{-1}$	242.31	68.92	271.54	2950	5174.00
MgT	mg/kg	840.96	473.55	653.40	26171	980.66
Al_T	$mg\ kg^{-1}$	80.58	561.50	246.95	14966	433.24
K_T	$mg\ kg^{-1}$	4464.10	737.84	4123.44	99515	202.07
Ca_T	$mg\ kg^{-1}$	2262.96	2318.81	538.96	136044	280168
Cr_T	$mg\ kg^{-1}$	0.13	1.88	0.74	36.28	4.51
Mn_T	$mg\ kg^{-1}$	614.92	30.19	356.28	10554	33.75
Fe_T	$mg\ kg^{-1}$	43.13	169.78	47.27	12081	3535
Co_T	$mg\ kg^{-1}$	0.03	0.20	0.38	17.25	1.02
Ni_T	$mg\ kg^{-1}$	2.17	1.86	0.93	69.25	8.16
Cu_T	$mg\ kg^{-1}$	2.80	<LD	3.81	146.33	6.72
Zn_T	$mg\ kg^{-1}$	7.66	6.98	5.78	853.00	7.66
As_T	$mg\ kg^{-1}$	0.02	<LD	0.02	8.36	1.12
Cd_T	$mg\ kg^{-1}$	0.0	0.13	0.05	19.93	0.07
Al_o	$mg\ kg^{-1}$	45.0	315.0	169.0	8323	178.33
Fe_o	$mg\ kg^{-1}$	77.0	74.0	15.0	4233	171.0

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HPLC analyses

AMX was quantified using a HPLC-UV with LPG 3400 SD equipment (Thermo-Fisher, USA). The HPLC-UV was equipped with a Luna C18 column (150 mm long, 4.6 mm internal diameter, 5 μm particle size) provided by Phenomenex (Madrid, Spain), as well as a pre-column (4 mm long, 2 mm in diameter, 5 μm particle size) packed with the same material as the column. The injection volume was 50 μL and the flow rate was 1.5 mL min^{-1}. The mobile phase consisted of acetonitrile (phase A) and 0.01 M phosphoric acid (phase B). A linear gradient was used varying from 5% to 15% of phase A and from 95% to 85% of phase B. The initial conditions were re-established in 2 min and maintained for 2 min. The total analysis time was 10 min, with a retention time of 3.3 min, and the wavelength used for detection was 230 nm. The amounts of antibiotic adsorbed were calculated by the difference between the concentrations initially present in the added solutions and those remaining in the solutions at equilibrium. All determinations were made in triplicate. Figure S1 (Supplementary Material) shows some selected chromatograms corresponding to the quantification of AMX.

Figure S1 shows some selected chromatograms corresponding to the quantification of AMX.

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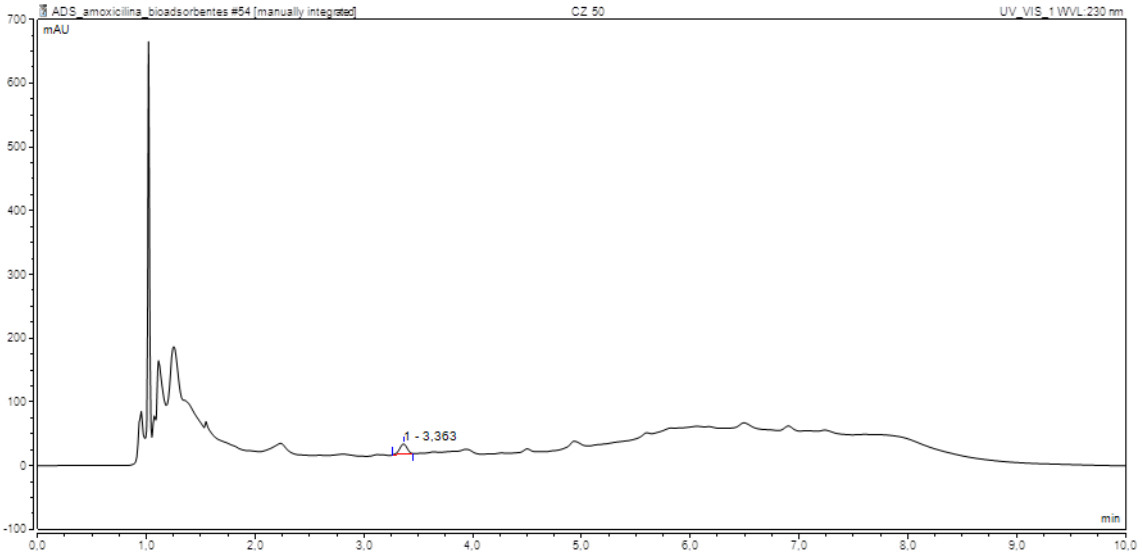
Adsorption on wood ash. Concentration of AMX added 50 µmol/L



Adsorption on pine needles. Concentration of AMX added 40 µmol/L

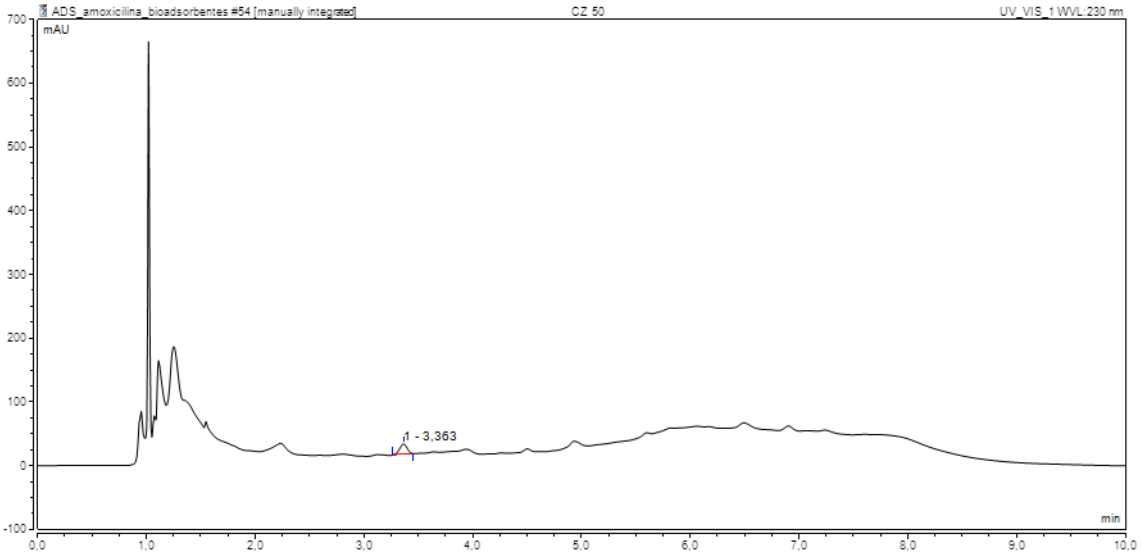


Figure S1: Selected chromatograms corresponding to the detection of AMX after adding various concentrations to the adsorbents used in the study

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PUBLICACIÓN VII

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Amoxicillin Retention/Release in Agricultural Soils Amended with Different Bio-Adsorbent Materials

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Abstract: The antibiotic amoxicillin (AMX) may reach soils and other environmental compartments as a pollutant, with potential to affect human and environmental health. To solve/minimize these hazards, it would be clearly interesting to develop effective and low-cost methods allowing the retention/removal of this compound. With these aspects in mind, this work focuses on studying the adsorption/desorption of AMX in different agricultural soils, with and without the amendment of three bio-adsorbents, specifically, pine bark, wood ash and mussel shell. For performing the research, batch-type experiments were carried out, adding increasing concentrations of the antibiotic to soil samples with and without the amendment of these three bio-adsorbents. The results showed that the amendments increased AMX adsorption, with pine bark being the most effective. Among the adsorption models that were tested, the Freundlich equation was the one showing the best fit to the empirical adsorption results. Regarding the desorption values, there was a decrease affecting the soils to which the bio-adsorbents were added, with overall desorption not exceeding 6% in any case. In general, the results indicate that the bio-adsorbents under study contributed to retaining AMX in the soils in which they were applied, and therefore reduced the risk of contamination by this antibiotic, which can be considered useful and relevant to protect environmental quality and public health.

Keywords: antibiotics; bio-adsorbents; emerging pollutants; soil pollution

check for updates

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1. Introduction

Emerging pollutants include a wide range of chemical compounds, such as various pharmaceutical products, and specifically antibiotics [1,2]. In 2020, in the European Union (EU), the average total consumption of anti-bacteria compounds for systemic use (ATC Group J01) was 16.4 defined daily doses (DDD) per 1000 inhabitants [3]. These compounds are not fully absorbed in the intestine, causing them to be excreted in significant amounts (up to 90%) through urine and feces [4,5], thus passing to wastewater in the case of humans, and to manure pits or manure accumulations in the case of animal farms. These contaminants can pass into various environmental compartments and may directly cause undesirable effects in soils [6], including the promotion of antibiotic resistance [7–9], and/or be absorbed by plants used for human or animal consumption, entering the food chain, as has been shown for lettuce and other vegetables [7,10,11].

One of the most widely used antibiotics in both human and veterinary medicine is amoxicillin (AMX), which is frequently used as a first-choice drug for the treatment of serious infections [12]. Between 80 and 90% of this antibiotic is excreted due to its poor absorption [13], then reaching the environment, and achieving concentrations of 127.49 ng L^{-1} in wastewater [14].

Different authors point out that antibiotics are frequently detected in treated wastewater, because they come from human use, but also from other sources such as agriculture and livestock production [15–18]. In this regard, one of the current strategies to alleviate water scarcity is the reuse of previously treated wastewater, which could result in public health issues due to the presence of different chemical pollutants and microbes [19]. Wastewater treatment tries to decrease nutrient loads [20] and pathogens [21], among other contaminants, but many current treatments are not sufficiently effective in retaining and inactivating pharmaceuticals such as antibiotics [22,23].

Current EU and United States (US) legislations do not include concentration limits for antibiotics in treatment plant effluents [24,25], making it more probable that antibiotics reach soils through WWTP-purified water used in irrigation [26]. In addition, antibiotics may be spread on soils through WWTP sludge used as fertilizer in agricultural crops and silvo-pastoral systems [27], and can subsequently be incorporated into the food chain.

Soils have a relevant buffering capacity and filtering potential due to the colloids present in the clay fraction and in organic matter, which help in preventing environmental pollution [28–30]. The dynamics followed by antibiotics in the soil depends on their physicochemical properties, as well as on those of the soil, and also on the time of application of the residual materials, as well as weather conditions [31–33]. Among the various processes that antibiotics can undergo in the soil environment, it is worth highlighting mineralization, degradation, volatilization, leaching, surface runoff, bioaccumulation, and adsorption.

The specific behavior of the antibiotic AMX in the soil is highly conditioned by the pH of the medium, which affects the ionization of the compound and the surface charge of the soil colloids [34,35]. In this regard, it is highly relevant that AMX has amphoteric properties due to three functional groups present in its structure: -NH_2, -COOH, and -OH [36]. The dissociation constants (pk_a) of a molecule indicate its ionization state as a function of pH [37]. In the case of AMX, pk_{a1} corresponds to carboxyl groups (-COOH), pk_{a2} is represented by amine groups (-NH_2), while pk_{a3} corresponds to phenolic groups (where hydroxyl (-OH) is attached to a C atom integrated in an aromatic ring), so that, at different pH values, AMX may appear as a cation, anion, or zwitterion [38]. Specifically, at pH < pk_{a1} AMX will appear as a cation, at pH > pk_{a3} it will appear as an anion, while at pH values between pk_{a1} and pk_{a3} it will appear as a zwitterion [39].

The presence of antibiotics in soils and water that have received the spreading/disposal of wastewater and/or sewage sludge is a matter of concern [40], so different investigations have focused on the design of a variety of systems intended to its removal [41]. Several technologies have been proposed to achieve antibiotics removal during wastewater treatment, such as the use of ozone [42] or advanced oxidation [39], although these methods tend to generate unwanted toxic side-products. In contrast, adsorption is considered a rather simple and sustainable alternative [43]. In this way, commonly used adsorbents include mineral and biological materials, as well as activated carbon, with this last adsorbent being widely used, although it has a high cost and regeneration issues [44]. In view of this, there is increasing interest in using low-cost adsorbents, such as industrial waste or by-products, for which it is necessary to determine their pollutant adsorption capacity, and in particular their potential to retain/remove antibiotics [45,46].

Among them, certain residues/by-products from the food industry, such as mussel shell, and from the forestry industry, such as pine bark and wood ash, are abundant, easily accessible and low-cost, making it interesting to determine their capacity to adsorb contaminants such as antibiotics that reach different environmental compartments. These three bio-adsorbent materials could be added to the soil or used in modules specifically designed and installed in wastewater treatment plants, to minimize the risk of dispersion of these pollutants in the environment. Mussel shell, pine bark and wood ash have already been studied previously regarding their ability to retain heavy metals, inorganic anions and antibiotics from the group of tetracyclines and sulfonamides, obtaining very promising results [47–56]. There are also previous studies dealing with the adsorption of AMX present in wastewater by means of adsorbents such as wheat grain, almond shell ash, palm bark,

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bentonite or activated carbon [36]. However, there are no previous publications that have focused on evaluating the effects on AMX retention derived from amending crop soils with pine bark, wood ash and mussel shell. In fact, the efficacy of these bio-adsorbents to increase the adsorption of antibiotics in soils has been previously proven for sulfonamides in the case of pine bark [57], while wood ash and mussel shell showed worse results. In addition, mussel shell has been widely studied as a bio-adsorbent in soils contaminated with heavy metals [58], and wood ash has also been investigated for this purpose (for example [59]), but studies on the application of these materials in the retention of pharmaceutical products are very scarce.

In view of the above background, the present study was performed to investigate for the first time AMX adsorption and desorption on/from different agricultural soils with and without the presence of the bio-adsorbents pine bark, wood ash and mussel shell, assessing their potential to decrease the dispersion of this antibiotic, which can be considered of relevance with regard to environmental preservation and public health protection.

2. Materials and Methods

2.1. Soils and Bio-Adsorbents

Four agricultural soils, devoted to maize and vineyard cultivation, located in different areas of Galicia (NW Spain), were selected in function of their pH values and organic matter (OM) contents. All four were characterized as detailed in the Supplementary Materials. Table S1 (Supplementary Materials) shows values corresponding to soil properties., Within them, soil pH_{water} was between 5.01 and 6.04, while organic matter (OM) content was in the range 3.06–4.59%. The texture of two of the soils (soils M1 and M2) was clay loam, while it was sandy clay loam for the other two soils (soils M3 and VO).

In addition, the following materials were used as bio-adsorbents/amendments: (a) two forest by-products: pine bark (commercially distributed by Geolia, Madrid, Spain), and wood ash (from a local boiler at Lugo, Spain); (b) mussel shell (crushed at <1 mm), from Abonomar (Pontevedra, Spain). These bio-adsorbents were characterized as indicated in the Supplementary Materials, with results shown in Table S2. Some additional data regarding characteristics of these materials have been included in previous publications [55,56].

Different soil + amendment mixtures were elaborated adding the bio-adsorbents to soil samples at doses of 48 t ha^{-1}, followed by 72 h of stirring at 50 rpm using a rotatory shaker, and further homogenization by means of a Retsch splitter (Haan, Germany), all this carried out at stable temperature of 25 ± 2 °C. The pH of the different soil + bio-adsorbent mixtures was analysed, with results shown in Table 1.

Table 1. pH values of the different soils and soil + bio-adsorbent mixtures. VO: vineyard soil; M: maize soils; A: wood ash; MS: mussel shell; PB: pine bark. Average values (n = 3), with coefficients of variation always <5%.

Soils and Mixtures	pH	Soils and Mixtures	pH
M1	5.33	M3	5.01
M1 + A	6.93	M3 + A	6.93
M1 + MS	5.29	M3 + MS	5.46
M1 + PB	4.92	M3 + PB	4.79
M2	5.65	VO	6.04
M2 + A	7.04	VO + A	7.81
M2 + MS	5.76	VO + MS	5.92
M2 + PB	5.24	VO + PB	5.35

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2.2. Chemical Reagents

The antibiotic AMX used (with purity ≥ 95%) was from Sigma-Aldrich (Barcelona, Spain), while acetonitrile (purity ≥ 99.9%) and phosphoric acid (85% extra pure) were from Fisher Scientific (Madrid, Spain), and $CaCl_2$ (95% purity) was from Panreac (Barcelona,

Spain). All the solutions needed for HPLC analyses were prepared using milliQ water (from Millipore, Madrid, Spain).

2.3. Adsorption and Desorption Experiments

AMX adsorption and desorption were studied by means of batch experiments, performed on the different soils amended with the bio-adsorbent materials, which were added to the soils in doses of 48 t ha^{-1}. For this, 2 g of the soil + bio-adsorbent mixtures was weighed, then adding a volume of 5 mL of a solution with different concentrations of the antibiotic (2.5, 5, 10, 20, 30, 40, 50 μmol L^{-1}), which also contained 0.005 M $CaCl_2$ as background electrolyte. The resulting suspensions were shaken for 48 h in the dark, using a rotary shaker. Previous kinetic tests indicated that the 48 h period is enough to reach equilibrium (data not shown). This step was followed by centrifuging the suspensions (15 min at 4000 rpm), and by subsequent filtration of the supernatants through 0.45 μm nylon syringe filters. Finally, AMX concentration was quantified using specific HPLC-UV equipment (an LPG 3400 SD device, by Thermo-Fisher Scientific, Madrid, Spain). Details on AMX HPLC determinations are provided in Supplementary Materials. Additionally, example chromatograms are shown in Figure S1 (Supplementary Materials).

Regarding desorption, it was studied adding 5 mL of 0.005 M $CaCl_2$ to the material derived from the adsorption experiments, then repeating the procedure performed for the previous adsorption phase. In all cases, triplicate determinations were carried out.

2.4. Data Treatment

The experimental adsorption data were fitted to the Freundlich (Equation (1)), Langmuir (Equation (2)) and Linear (Equation (3)) models [60].

$$q_e = K_F C_{eq}^n \quad (1)$$

$$q_e = \frac{q_m K_L C_{eq}}{1 + K_L C_{eq}} \quad (2)$$

$$K_d = q_e / C_{eq} \quad (3)$$

with q_e being the amount of AMX retained, which was calculated as the difference between the concentration added and that remaining in the equilibrium; C_{eq} is the AMX concentration in the equilibrium solution; K_F is the Freundlich parameter related to the adsorption capacity; n is a Freundlich parameter related to the degree of heterogeneity in adsorption; K_L is the Langmuir adsorption constant; q_m is the maximum adsorption capacity in the Langmuir model; and K_d is the partition coefficient in the Linear model.

The fitting of the experimental data to the Langmuir, Freundlich and Linear models was studied by means of the SPSS Statistics 21 software (IBM, Armonk, NY, USA).

3. Results

3.1. Adsorption

As shown in Figure 1, as well as in data included in Table S3 (Supplementary Materials), pine bark performed as a very effective material for increasing AMX adsorption in the soils amended with this bio-adsorbent. Table 1 shows that the pH of each soil changes when the different bio-adsorbents are added. Specifically, pine bark (which has pH = 3.99) generally causes an acidification of the amended soil.

Table 2 presents the values of the parameters corresponding to AMX adsorption as per the Freundlich, Langmuir and Linear models.

Considering R^2 values, all the non-amended soils (except M1) presented an overall good fit to all three models, given that $R^2 > 0.90$ for VO and M3, and $R^2 > 0.80$ for M2. Focusing on both the non-amended soils and those amended with bio-adsorbents, the errors in some parameters of the Linear model, and especially in the Langmuir model, were

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too high, invalidating the adjustment in those cases, so the Freundlich's model shows the best results.

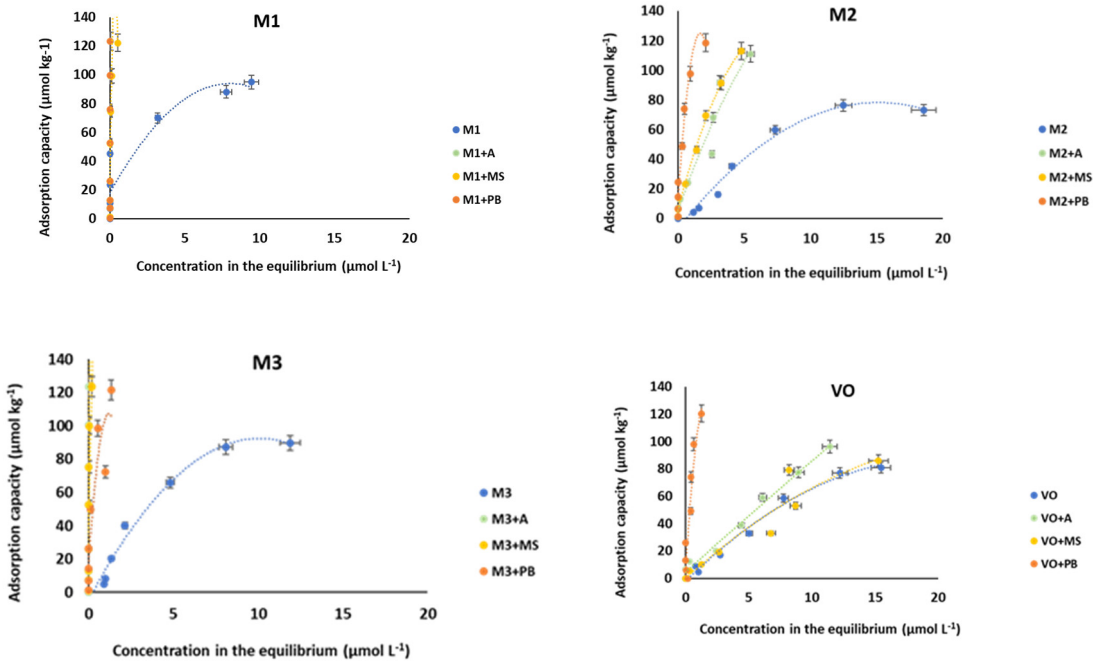

Figure 1. Adsorption curves for AMX in unamended and bio-adsorbent-amended soils. Average values (n = 3), with coefficients of variation always <5%.

Table 2. Values of the adsorption parameters corresponding to the Freundlich (K_F, expressed in L^n $\mu mol^{1-n} kg^{-1}$, and n–dimensionless-), Langmuir (K_L, expressed in L μmol^{-1}, and q_m -$\mu mol\ kg^{-1}$-) and Linear (K_d, expressed in L kg^{-1}) models. M: maize soil; VO: vineyard soil; A: wood ash; MS: mussel shell; PB: pine bark; –: fitting not possible.

	Freundlich					Langmuir					Linear		
Sample	K_F	**Error**	n	**Error**	R^2	K_L	**Error**	q_m	**Error**	R^2	K_d	**Error**	R^2
M1	50.79	34.56	0.274	0.344	0.723	–	–	–	–	–	3.699	0.122	0.983
M1 + A	–	–	–	–	–	0.78	0.209	2066.7	0	0.344	1525.8	358.85	0.344
M1 + MS	139.24	36.56	0.191	0.145	0.745	27.983	29.168	129	30.23	0.746	–	–	–
M1+ PB	–	–	–	–	–	–	–	–	–	–	–	–	–
M2	11.81	4.224	0.676	0.141	0.896	0.074	0.039	140.85	40.68	0.935	5.057	0.568	0.813
M2 + A	31.042	6.881	0.758	0.16	0.932	–	–	–	–	–	22.265	1.671	0.911
M2 + MS	40.022	3.142	0.672	0.062	0.986	0.183	0.064	243.06	53.74	0.984	26.378	1.625	0.939
M2 + PB	91.91	6.984	0.391	0.108	0.923	1.725	0.795	154	27.49	0.934	69.95	11.36	0.633
M3	19.17	4.626	0.67	0.114	0.928	0.124	0.049	161.82	34.48	0.961	3.084	0.113	0.978
M3 + A	–	–	–	–	–	0.05	0	–	–	–	–	–	–
M3 + MS	107.418	7.279	0	0.038	0.978	103.812	287.076	–	–	–	–	–	–
M3 + PB	98.89	11.05	0.282	0.15	0.85	7.342	6.343	112.88	20.25	0.85	94.94	15.65	0.622
VO	9.579	2.155	0.806	0.091	0.974	0.037	0.017	232.67	75.91	0.982	5.934	0.312	0.957
VO + A	10.99	2.246	0.892	0.094	0.982	–	–	–	–	–	8.694	0.313	0.979
VO + MS	10.25	4.764	0.795	0.196	0.893	–	–	–	–	–	6.287	0.571	0.875
VO + PB	109.63	14.06	0.766	0.214	0.815	–	–	–	–	–	112.34	13.83	0.778

Figure 2 shows the AMX adsorption results (expressed as percentage values) for the different soils with or without bio-adsorbents. It is evident that, in general, adsorption is lower in the vineyard soil, and in the four soils studied, the amount of AMX adsorbed increases when amending with the bio-adsorbents, especially for the three highest concentrations of antibiotic added (30, 40 and 50 µmol L^{-1}). In three of the soils (VO, M2 and M3), adsorption increases when amending with the bio-adsorbents, and this takes place for any of the AMX concentrations added; however, in soil M1, this increase occurs just for the

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three highest concentrations of antibiotic, because for lower concentrations the soil adsorbs 100% of the added antibiotic. These graphs show that the greatest increases in adsorption occur in both VO and M2 soils, especially after the addition of pine bark, while in soils M1 and M3, no differences were found regarding adsorption onto the different bio-adsorbents (Figure 2).

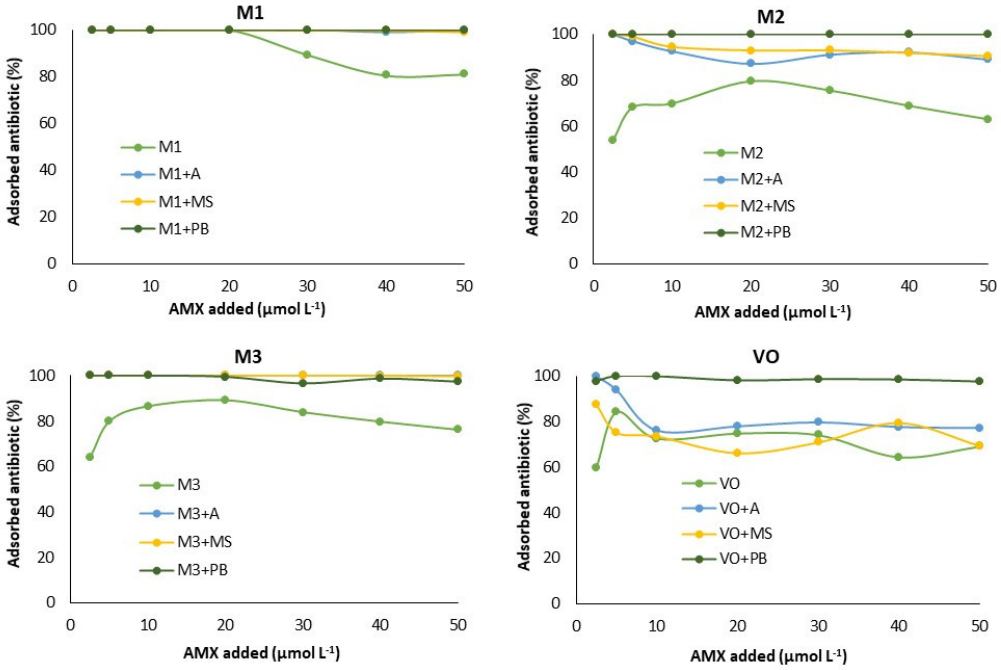

Figure 2. Adsorbed antibiotic (%) for each soil and the mixtures soil + bio-adsorbent in relation to the concentration of AMX added. M: maize soil; VO: vineyard soil; A: wood ash; MS: mussel shell; PB: pine bark; AMX: amoxicillin. Average values (n = 3), with coefficients of variation always <5%.

3.2. Desorption

Table 3 shows the values of AMX desorption from the different soils depending on the concentration of antibiotic added and the bio-adsorbent used. In general, the higher the concentration of antibiotic added, the greater the desorption from soils, both with and without bio-adsorbent amendments. In some soils, this progressive increase is observed up to 40 µmol L^{-1} of AMX added, with further increase being very scarce or null from this concentration up to 50 µmol L^{-1}. In most cases, desorption was lower in soils with one bio-adsorbent than in soils without bio-adsorbents.

Table 3. AMX desorption, in µmol kg^{-1} and in percentage between brackets, from the soils studied, with or without bio-adsorbents, as a function of the concentration of antibiotic added (C_0). M: maize soils; VO: vineyard soil; A: wood ash; MS: mussel shell; PB: pine bark; –: no value. Average values (n = 3), with coefficients of variation always <5%.

	C_0 (µmol L^{-1})						
Sample	**2.5**	**5**	**10**	**20**	**30**	**40**	**50**
M1	0.349 (10.9)	1.181 (12.5)	2.331 (12.8)	2.819 (11.1)	4.781 (7.2)	4.816 (11.6)	6.21 (16.9)
M1 + A	0 (0)	0 (0)	0 (0)	0.414 (0.79)	1.698 (2.26)	2.482 (2.51)	5.062 (4.10)
M1 + MS	0 (0)	0 (0)	0 (0)	0.233 (0.44)	2.259 (3.05)	3.943 (3.98)	6.105 (4.99)
M1 + PB	0 (0)	0 (0)	0 (0)	0 (0)	0.824 (1.09)	0.834 (0.84)	1.851 (1.50)
M2	0.767 (9.25)	1.339 (12.19)	3.029 (16.12)	5.032 (13.17)	5.211 (6.07)	11.504 (8.07)	18.489 (8.36)
M2 + A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.935 (0)
M2 + MS	0 (0)	0.047 (0.34)	0.176 (0.77)	–	0.69 (0.99)	1.061 (1.16)	3.488 (3.09)
M2 + PB	–	0.164 (1.13)	0.329 (1.35)	0.713 (1.46)	1.075 (1.46)	1.788 (1.83)	2.597 (2.19)

Table 3. *Cont.*

	C_0 (μmol L^{-1})						
Sample	**2.5**	**5**	**10**	**20**	**30**	**40**	**50**
M3	0.384 (7.45)	0.828 (8.88)	2.6 (10.40)	4.639 (6.11)	–	6.151 (9.33)	6.107 (9.67)
M3 + A	0.283 (3.82)	0.313 (2.45)	–	0.949 (1.80)	1.055 (1.40)	2.319 (2.31)	–
M3 + MS	–	0.258 (2.01)	–	2.488 (4.74)	2.694 (3.58)	–	4.684 (3.79)
M3 + PB	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.14 (0.14)	1.276 (1.05)
VO	0.357 (7.67)	0.735 (13.41)	2.115 (13.85)	2.446 (8.58)	4.741 (8.15)	8.139 (6.26)	8.682 (7.68)
VO + A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO + MS	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
VO + PB	0 (0)	0 (0)	0 (0)	0 (0)	0.219 (0.30)	0.712 (0.73)	–

4. Discussion

4.1. Adsorption

In the current study pine bark (with pH 3.99) generally causes an acidification of the amended soil. In this regard, it must be noted that greater acidification is associated with more pronounced AMX adsorption increases, as occurs in soils VO and M2. In previous studies, Githinji et al. [61] found a decrease in AMX adsorption as pH increased from 3.5 to 8.5, whereas other researchers also described a decrease in adsorption for pH values > 5, using pistachio shell [62] or activated carbon [63] as adsorbents.

In the current piece of research, the pH of the soils is above 5, and it was expected that lowering it by incorporating acid adsorbents would facilitate AMX adsorption. In this regard, it is worth noting that, depending on the environmental acid-base conditions, most antibiotics can behave as cations, anions or zwitterions [64], and in the case of AMX the electrical charge of the molecule changes depending on the pH, associated with the charge density present in different functional groups. For AMX, when the pH is lower than its pk_{a1} value (2.98), the amine groups are protonated and the molecule acquires a positive charge; when the pH value is between pk_{a1} (2.98) and pk_{a2} (7.4), the molecule behaves like a zwitterion, with deprotonated carboxyl groups (negative charge density) and protonated amine groups (positive charge density); on the other hand, at pH values between pk_{a2} (7.4) and pk_{a3} (9.6), deprotonated carboxyl and amine groups predominate (with negative charge density); and, finally, at pH > pk_{a3} the phenolic groups are also deprotonated, and the charge is even more negative [65]. When soil pH decreases due to amending with acidic bio-adsorbents (such as pine bark), more positive charges appear on the variable-charge components of those soils, which are summed to those present on the bio-adsorbents, thus being able to bind functional groups of AMX with negatively charged sites, due to electrostatic interactions.

In the current study, the wood ash amendment increased the pH of all soils, while the addition of mussel shell clearly increased the pH of only two of them (M2 and M3) (Table 1). However, an increase in AMX adsorption was also observed with these two amendments (wood ash and mussel shell), similarly to what was achieved when pine bark was added. This is due to the fact that the increase in pH derived from the addition of wood ash and mussel shell causes the appearance of a large number of negative charges in organic matter and in the non-crystalline minerals of the soil, which are very abundant in the soils of this study (Table S1, Supplementary Materials), to be summed to the fact that non-crystalline minerals are also very abundant in wood ash (Table S2, Supplementary Materials). Thus, these negative charges present in the soils and in the bio-adsorbents will facilitate bonds with deprotonated carboxyl groups of the AMX molecule, stablished through cationic bridges (in which Ca probably plays an important role, given its abundance both in wood ash and in mussel shell, Table S2, Supplementary Materials). However, it should be noted that, in the VO soil, despite the fact that wood ash and mussel shell increase the pH, the increase in AMX adsorption is clearly lower than that achieved by amending with pine

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bark, because organic matter and non-crystalline mineral contents are much lower in this soil than in the other three.

Regarding the fittings to adsorption models, starting with the Linear model the values of the distribution coefficient (K_d) were in the range between 1 and 1525.76 L kg^{-1} in maize soils, and between 5.93 and 112.34 L kg^{-1} in the vineyard soil (Table 2). These values are lower than those reported in previous studies for tetracycline antibiotics [54], but are higher than for sulfonamides [66]. This would indicate that the interactions with soils that give rise to AMX adsorption are weaker than those taking place with tetracycline antibiotics, but stronger than those affecting sulfonamides. Regarding the Freundlich model, the K_F values (affinity coefficient, related to adsorption capacity) vary between 1 and 139.24 L^n μmol^{1-n} kg^{-1} in maize soils, and between 9.58 and 109.63 L^n μmol^{1-n} kg^{-1} in the vineyard soil. These results are also lower than those obtained by other authors [56] for tetracyclines, but higher than those obtained for sulfonamide [64]. As for the Freundlich's n values, in the case of maize soils they are between 0 and 0.926, while in the vineyard soil they range between 0.298 and 0.892. These n values are lower than 1 in all soils, which would indicate that adsorption is not linear, coinciding with that obtained by other authors [61] for other materials. In fact, values of $n < 1$ indicate the presence of heterogeneous adsorption sites and a non-linear and concave curve, which means that the number of available adsorption sites decreases when the concentration of the added contaminant increases, occupying firstly the high energy adsorption sites [67,68]. Regarding the Langmuir model, the K_L values range between 0.05 and 103.81 L μmol^{-1} in maize soils, and between 0.037 and 0.975 L μmol^{-1} in vineyard soils (Table 2).

As shown in Figure 1 and in Table S3 (Supplementary Materials), both soils M1 and M3 have very high AMX adsorption scores (sometimes close to 100%) for most of the antibiotic concentrations added. For these two soils, the incorporation of bio-adsorbents causes modifications in adsorption that are lower than the results reached in the other two soils. In the soils that adsorb less AMX (VO and M2), the mixtures with wood ash, mussel shell or pine bark generally continue to present high R^2 values (>0.80) for the three models (Table 2), but the high errors associated with the estimation of some parameters invalidate the fittings in several cases (especially in the Langmuir model).

4.2. Desorption

Regarding desorption, focusing on the maximum concentration added (50 $\mu mol\ L^{-1}$), unamended soils desorb between 6% and 17% of the AMX previously adsorbed, while the release of the antibiotic from the mixtures of soil + bio-adsorbent was always lower than 6%. The greatest decrease in desorption occurred in soil M2 when adding pine bark, going from 17% to 2.5%. Similar results were obtained previously for tetracyclines and sulfonamides [56,66], by researchers who added pine bark to different soils, detecting a decrease in desorption of up to 12% for tetracyclines, and up to 17% for sulfonamides. In this regard, a previous study [69] indicated that the presence of tannins in pine bark favors adsorption (and decreases desorption). It should be noted that AMX desorption has been mainly studied in wastewater, while most of the studies on the adsorption of this antibiotic in soils omit desorption processes. In aqueous matrices, the reported AMX desorption values went from 5% when almond shell ashes were added [70] up to 40% in cases where clay materials were used as adsorbents [71].

5. Conclusions

The pH and the abundance of non-crystalline minerals and organic matter are the most determining factors in the adsorption processes of the antibiotic amoxicillin (AMX) in the soils evaluated in this study, both alone and amended with the three tested bio-adsorbents (pine bark, wood ash and mussel shell). It was evidenced that AMX adsorption increased when the crop soils used (devoted to maize and vineyard cultivation) were mixed with the different bio-adsorbents. This increase was higher when pine bark (the bio-adsorbent with the most acidic pH) was added. In addition, AMX desorption decreased when the bio-

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adsorbent materials were incorporated into the soil, reaching values that did not exceed 6%. The overall results obtained in the current research show that, regarding its applicability, the incorporation of the three bio-adsorbents into agricultural soils contaminated by AMX reduced the risk of transport and passage of the antibiotic to surface and groundwater, and therefore to the food chain, which in fact entails important implications for the environment and public health. In future studies, it would be interesting to evaluate the effect of other bio-adsorbents, as well as soils with different characteristics compared to those used here. Furthermore, possible additional studies could delve into the mechanisms that explain the retention and release processes of AMX when it reaches the environment as a pollutant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ma15093200/s1. Table S1. Values corresponding to the basic parameters determined in the various soils studied. M: maize soils; VO: vineyard soils; OC: organic carbon; OM: organic matter; eCEC: effective cation exchange capacity; Cae, Mge, Nae, Ke, Ale: elements in the exchange complex; o subindex: non-crystalline form; pyr subindex: crystalline form. Average values (n = 3), with coefficients of variation always <5%.; Table S2. Characteristics of the bio-adsorbent materials. Cae, Mge, Nae, Ke, Ale: elements in the exchange complex; Sat. Al: Al-saturation in the exchange complex; eCEC: effective cation exchange capacity; XT: total content of the element (X); Alo, Feo: non-crystalline Al and Fe; <LD: below detection level. Average values (n = 3), with coefficients of variation always <5%.; Table S3. AMX adsorption, expressed in μmol kg^{-1} (and in percentage between brackets), for the various soils studied, with or without bio-adsorbents, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils; A: ashes; MS: mussel shell; PB: pine bark. Average values (n = 3), with coefficients of variation always <5%.; Figure S1. Example chromatograms corresponding to AMX adsorption onto soils amended with bio-adsorbents

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Supplementary material

Amoxicillin retention/release in agricultural soils amended with different bio-adsorbent materials

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Characterization of soils and sorbent materials

The values corresponding to pH in water and pH in KCl (solid:liquid ratio 1:2.5) were measured with a CRISON-2001 pHmeter (Crison, Barcelona, Spain). Total C and N were determined by means of elemental analysis, using a LECO CHN-100 equipment (Leco Corporation, St. Joseph, MI, USA). The exchangeable cations (Ca_e, Mg_e, Na_e, K_e and Al_e) were displaced using a 1 M NH_4Cl solution in a 1:10 soil/bio-adsorbent: solution ratio and were then quantified by means of atomic absorption spectrophotometry, using a Perkin Elemer Analyst 200 apparatus (Perkin Elmer, Inc, USA). The effective cation exchange capacity (eCEC) was determined as the sum of these five exchangeable cations. Available phosphorus was extracted using 0.5 M $NaHCO_3$ and quantified using a UV-visible spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). To determine total non-crystalline Fe and Al, a specific extraction was performed using ammonium oxalate buffered at pH 3, stirred for 4 hours in the dark, then adding 5 drops of 0.25% superfloc to the resulting extract, then centrifuging at 2000 rpm for 10 minutes, filtering and diluting the supernatant to 1:5. Finally, Fe and Al in non-crystalline form (Fe_o and Al_o) were quantified by atomic absorption spectrophotometry. In addition, Fe and Al were determined after extraction with sodium pyrophosphate at pH 10. The extraction was performed shaking 1 g of sample with 100 ml of sodium pyrophosphate during 16 h. Finally, Fe and Al were quantified by atomic absorption spectrophotometry. Total contents of different elements (Na_T, Mg_T, Al_T, K_T, Ca_T, Cr_T, Mn_T, Fe_T, Co_T, Ni_T, Cu_T, Zn_T, As_T and Cd_T) were determined following the EPA 3051 method, performing an acid digestion in a microwave with a 65% HNO_3 solution, and subsequent determination with ICP-MS.

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Table S1. Values corresponding to the basic parameters determined in the various soils studied. M: maize soils; VO: vineyard soils; OC: organic carbon; OM: organic matter; eCEC: effective cation exchange capacity; Ca_e, Mg_e, Na_e, K_e, Al_e: elements in the exchange complex; o subindex: non-crystalline form; pyr subindex: crystalline form. Average values (n=3), with coefficients of variation always < 5%

Soil	pH_{water}	OC	OM	N	Sand	Clay	eCEC	Ca_e	Mg_e	Na_e	K_e	Al_e	Fe_o	Fe_{pyr}	Al_o	Al_{pyr}
		%					$cmol_c\ kg^{-1}$						$mg\ kg^{-1}$			
M1	5.33	2.66	4.59	0.27	43.42	24.65	6.88	5.35	0.67	0.02	0.44	0.40	5745	3159	3401	2871
M2	5.65	2.58	4.44	0.25	43.57	22.43	7.48	6.18	0.77	0.00	0.28	0.24	5780	3315	3881	2717
M3	5.01	2.04	3.52	0.21	45.64	22.43	5.94	4.58	0.67	0.10	0.17	0.43	4545	2923	2896	1945
VO	6.04	1.77	3.06	0.10	49.57	24.14	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141

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Table S2. Characteristics of the bio-adsorbent materials. Ca_e, Mg_e, Na_e, K_e, Al_e: elements in the exchange complex; Sat. Al: Al-saturation in the exchange complex; eCEC: effective cation exchange capacity; X_T: total content of the element (X); Al_o, Fe_o: non-crystalline Al and Fe; <LD: below detection level. Average values (n=3), with coefficients of variation always <5%.

Parameter	Unit	Pine bark	Wood ash	Mussel shell
C	%	48.70	13.23	11.43
N	%	0.08	0.22	0.21
C/N		608.75	60.14	55.65
pH_{water}		3.99	11.31	9.39
pH_{KCl}		3.42	13.48	9.04
Ca_e	$cmol_c\,kg^{-1}$	5.38	95.0	24.75
Mg_e	$cmol_c\,kg^{-1}$	2.70	3.26	0.72
Na_e	$cmol_c\,kg^{-1}$	0.46	12.17	4.37
K_e	$cmol_c\,kg^{-1}$	4.60	250.65	0.38
Al_e	$cmol_c\,kg^{-1}$	1.78	0.07	0.03
eCEC	$cmol_c\,kg^{-1}$	14.92	361.15	30.25
Sat Al	%	11.93	0.02	0.10
Available-P	$mg\,kg^{-1}$	70.45	462.83	54.17
Na_T	$mg\,kg^{-1}$	68.92	2950	5174.00
MgT	mg/kg	473.55	26171	980.66
Al_T	$mg\,kg^{-1}$	561.50	14966	433.24
K_T	$mg\,kg^{-1}$	737.84	99515	202.07
Ca_T	$mg\,kg^{-1}$	2318.81	136044	280168
Cr_T	$mg\,kg^{-1}$	1.88	36.28	4.51
Mn_T	$mg\,kg^{-1}$	30.19	10554	33.75
Fe_T	$mg\,kg^{-1}$	169.78	12081	3535
Co_T	$mg\,kg^{-1}$	0.20	17.25	1.02
Ni_T	$mg\,kg^{-1}$	1.86	69.25	8.16
Cu_T	$mg\,kg^{-1}$	<LD	146.33	6.72
Zn_T	$mg\,kg^{-1}$	6.98	853.00	7.66
As_T	$mg\,kg^{-1}$	<LD	8.36	1.12
Cd_T	$mg\,kg^{-1}$	0.13	19.93	0.07
Al_o	$mg\,kg^{-1}$	315.0	8323	178.33
Fe_o	$mg\,kg^{-1}$	74.0	4233	171.0

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Table S3. AMX adsorption, expressed in μmol kg^{-1} (and in percentage between brackets), for the various soils studied, with or without bio-adsorbents, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils; A: ashes; MS: mussel shell; PB: pine bark. Average values (n=3), with coefficients of variation always < 5%

Soil	AMX added (μmol/L)	AMX adsorbed, concentration and %	Soil	AMX added (μmol/L)	AMX adsorbed, concentration and %
M1	0	0 (0)	M2	0	0 (0)
	2.5	7.02 (100)		2.5	4.15 (53.60)
	5	10.73 (100)		5	6.79 (68.07)
	10	23.67 (100)		10	16.12 (69.80)
	20	45.20 (100)		20	35.22 (79.57)
	30	70.05 (89.37)		30	59.63 (75.47)
	40	88.02 (80.57)		40	76.21 (68.76)
	50	94.87 (81.07)		50	73.08 (62.88)
M1+A	0	0 (0)	M2+A	0	0 (0)
	2.5	7.42 (100)		2.5	6.76 (100)
	5	12.71 (100)		5	12.91 (97.03)
	10	25.99 (100)		10	24.03 (92.60)
	20	52.30 (100)		20	43.36 (87.15)
	30	75.01 (100)		30	67.99 (91.11)
	40	98.83 (98.82)		40	91.69 (92.14)
	50	123.33 (99.85)		50	110.99 (89.01)
M1+MS	0	0 (0)	M2+MS	0	0 (0)
	2.5	7.33 (100)		2.5	6.32 (100)
	5	12.71 (100)		5	13.94 (98.98)
	10	26.01 (100)		10	22.99 (94.51)
	20	52.44 (100)		20	46.24 (92.94)
	30	74.14 (99.85)		30	69.37 (92.96)
	40	99.17 (99.67)		40	91.38 (91.84)
	50	122.17 (98.92)		50	113.03 (90.42)
M1+PB	0	0 (0)	M2+PB	0	0 (0)
	2.5	7.34 (100)		2.5	7.34 (100)
	5	12.88 (100)		5	12.88 (100)
	10	26.07 (100)		10	26.07 (100)
	20	52.76 (100)		20	52.76 (100)
	30	75.74 (100)		30	75.74 (100)
	40	99.62 (100)		40	99.62 (100)
	50	123.37 (100)		50	123.37 (100)

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Soil	AMX added (μmol/L)	AMX adsorbed, concentration and %	Soil	AMX added (μmol/L)	AMX adsorbed, concentration and %
M3	0	0 (0)	VO	0	0 (0)
	2.5	4.80 (63.96)		2.5	4.54 (59.82)
	5	8.28 (80)		5	8.81 (84.22)
	10	20.33 (86.63)		10	16.84 (72.64)
	20	40.04 (89.23)		20	32.87 (74.87)
	30	65.98 (83.93)		30	58.64 (74.16)
	40	87.21 (79.77)		40	76.89 (64.45)
	50	89.74 (76.21)		50	80.81 (69.06)
M3+A	0	0 (0)	VO+A	0	0 (0)
	2.5	7.41 (100)		2.5	6.72 (100)
	5	12.78 (100)		5	12.30 (93.94)
	10	26.21 (100)		10	19.94 (76.04)
	20	52.66 (100)		20	38.77 (77.93)
	30	75.50 (100)		30	59.17 (79.68)
	40	100.40 (100)		40	77.24 (77.63)
	50	123.74 (100)		50	96.23 (77.16)
M3+MS	0	0 (0)	VO+MS	0	0 (0)
	2.5	7.40 (100)		2.5	5.30 (87.65)
	5	12.84 (100)		5	9.96 (75.26)
	10	25.96 (100)		10	19.33 (73.60)
	20	52.52 (100)		20	32.95 (66.22)
	30	75.20 (100)		30	52.96 (70.96)
	40	99.92 (99.92)		40	79.10 (79.50)
	50	123.59 (99.57)		50	85.75 (69.46)
M3+PB	0	0 (0)	VO+PB	0	0 (0)
	2.5	7.04 (100)		2.5	5.93 (97.75)
	5	14.16 (100)		5	13.15 (100)
	10	26.26 (100)		10	25.92 (99.97)
	20	49.73 (99.45)		20	49.03 (98.05)
	30	72.48 (96.64)		30	73.92 (98.57)
	40	98.65 (98.65)		40	97.94 (98.43)
	50	121.41 (97.34)		50	120.44 (97.49)

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Quantification of AMX

The HPLC determinations were carried out with a Model LPG 3400 SD equipment (Thermo-Fisher, USA). Some example chromatograms are included in Figure S1.

For HPLC quantifications, a Luna C18 column (150 mm long, 4.6 mm internal diameter, 5 μm particle size) was used, being provided by Phenomenex (Madrid, Spain), as well as a pre-column (4 mm long, 2 mm in diameter, 5 μm particle size) packed with the same material as the column. The injection volume was 50 μL and the flow rate was 1.5 ml min^{-1}. The mobile phase consisted of acetonitrile (phase A) and 0.01 M phosphoric acid (phase B). A linear gradient was used varying from 5% to 15% of phase A and from 95% to 85% of phase B. The initial conditions were re-established in 2 min and maintained for 2 min. The total analysis time was 10 min, with a retention time of 3.3 min, and the wavelength used for detection was 230 nm. The amounts of antibiotic adsorbed were calculated by the difference between the concentrations initially present in the added solutions and those remaining in the solutions at equilibrium. All determinations were made in triplicate.

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M3 soil with pine bark amendment; AMX concentration added 50 μmol L^{-1}.

M2 soil with mussel shell amendment, AMX concentration added 20 μmol L^{-1}

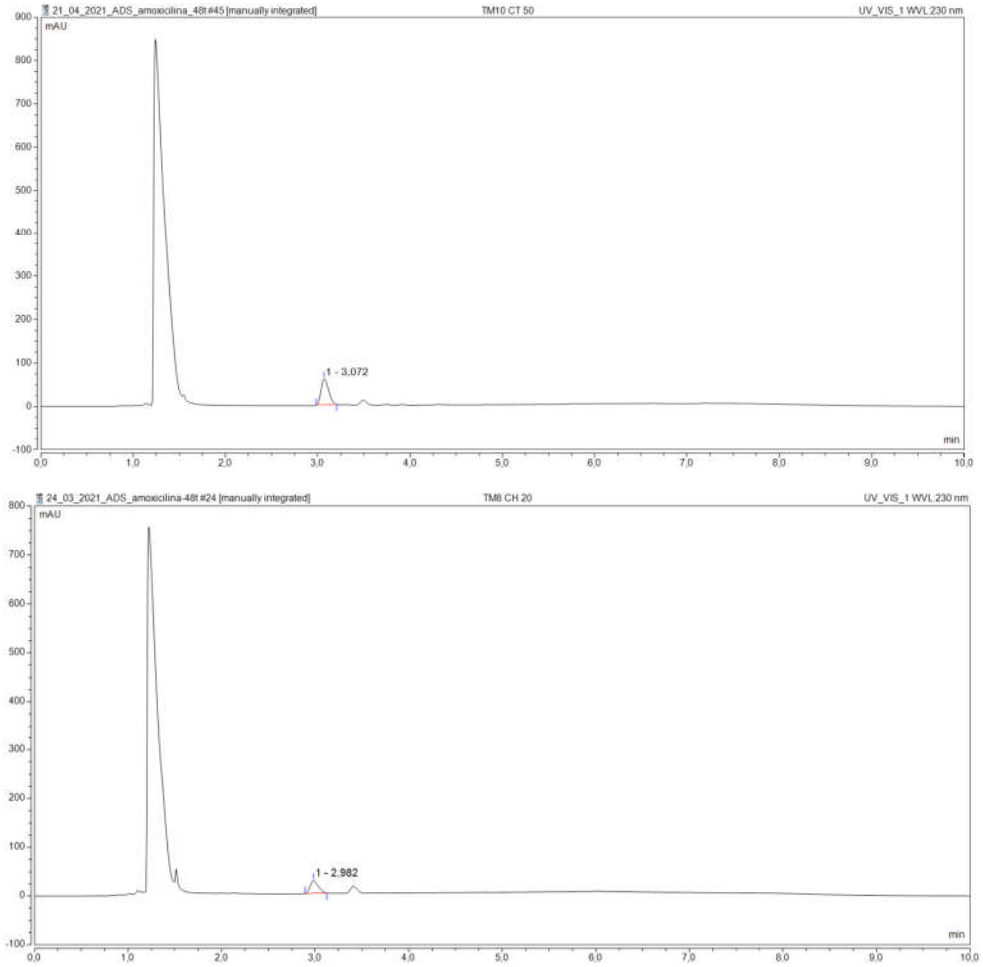

Figure S1. Example chromatograms corresponding to AMX adsorption onto soils amended with bio-adsorbents.

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PUBLICACIÓN VIII

Cela-Dablanca, Raquel[1]., Barreiro, Ana[1]., Rodríguez-López, Lucía[2]., Pérez-Rodríguez, Paula[2]., Arias-Estévez, Manuel[2]., Fernández-Sanjurjo, María J[1]., Álvarez-Rodríguez, Esperanza[1]., & Núñez-Delgado, Avelino[1]. (2022). **Azithromycin adsorption onto different soils**. *Processes*, *10*(12), 2565. https://doi.org/10.3390/pr10122565

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Article

Azithromycin Adsorption onto Different Soils

Raquel Cela-Dablanca [1,*], Ana Barreiro [1], Lucía Rodríguez-López [2], Paula Pérez-Rodríguez [2], Manuel Arias-Estévez [2], María J. Fernández-Sanjurjo [1], Esperanza Álvarez-Rodríguez [1] and Avelino Núñez-Delgado [1]

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Abstract: The antibiotic azithromycin (AZM) is one of the most persistent in the environment, with potential to cause serious health and environmental problems. As some polluting discharges containing this antibiotic can reach the soil, it is clearly relevant determining the ability of soils with different characteristics to retain it. In this research, AZM adsorption and desorption were studied for a variety of soils, using batch-type experiments. The results show that, at low doses of antibiotic added (less than or equal to 50 μmol L^{-1}), the adsorption always reached 100%, while when higher concentrations were added (between 200 and 600 μmol L^{-1}) the highest adsorption corresponded to soils with higher pH values. Adsorption data were fitted to the Linear, Langmuir and Freundlich models, with the latter showing the best fit, in view of the determination coefficient. No desorption was detected, indicating that AZM is strongly adsorbed to the soils evaluated, suggesting that the risks of environmental problems due to this contaminant are minimized for these edaphic media. These results can be considered relevant with respect to risk assessment and possible programming of measures aimed at controlling environmental contamination by emerging contaminants, especially from the group of antibiotics, and in particular from AZM.

Keywords: antibiotic; emerging contaminants; adsorption; desorption; environment

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1. Introduction

Azithromycin (AZM) is a semi-synthetic broad spectrum antibiotic belonging to the subclass of second-generation macrolides [1]. It is used to treat bacterial infections in infants and in people with weaker immune systems, among other diseases [2]. In the US, AZM is among the first-line agents prescribed for infectious diseases [3]. In 30 European countries, this group of antimicrobials together with beta-lactams, lincosamides, streptogramins and tetracyclines accounted for 83.5% of total antibiotic sales in 2013 [4]. This antibiotic is on the DU75 list (among the 75% of the most consumed antibiotics) in 24 of 46 countries in the European area [5]. In addition, during 2020 the use of AZM increased significantly in Spain [6].

When AZM, as well as other antibiotics and other emerging contaminants, reach the environment through polluting discharges, it is considered a cause of concern, especially taking into account the high concentrations detected in aquatic environments [7–10]. In fact, there are several routes for antibiotics to reach the environment as pollutants, but the main one is through wastewater [11]. The cause is that, after their administration as drugs, these antimicrobials are partially metabolized and released through urine and feces [12]. Specifically regarding AZM, 75% of it is excreted after being administered [13], reaching wastewater treatment plants.

The efficacy of these treatment plants is dependent on factors such as the type of treatment, or the nature and properties of the antibiotic to be treated. Specifically, AZM belongs to a group of antibiotics of special relevance in view of its prevalence in the

environment, due to its persistence and resistance to biological degradation [14]. The effectiveness of different treatments applied in wastewater treatment plants to retain this antibiotic was studied by Mirzaei et al. [15], finding that the efficiency of one of the treatments ranged from 0% to 74.9%, while another treatment was not effective in removing AZM. In addition, in a study carried out in twelve wastewater plants in China [16], it was found that AZM was one of the antibiotics that appeared most frequently, and it was affected by the lowest elimination rate efficiency, specifically 6.3%.

Antibiotics that cannot be eliminated after wastewater treatment persist in these waters and/or end up in the sludge generated by this treatment, reaching agricultural soils through the application of irrigation wastewaters and/or biosolids [17]. In a study by Rodríguez-Mozaz et al. [18], the authors found the presence of AZM in a range of 45.2–597.5 ng L^{-1}, with Portugal showing the highest concentrations of this antibiotic, while the minimum values were obtained in Cyprus. In North America, Europe and elsewhere, the use of biosolids as agricultural soil amendment is permitted [19], and specifically, in the EU-27, 53% of the total sludge produced is recycled in agriculture directly or after composting [20]. In some countries such as Denmark, France, the Walloon region of Belgium, Ireland, Spain and the United Kingdom, more than half of the sludge production ends up on agricultural land, while in other countries such as Finland, the Netherlands, Slovakia, Greece and Slovenia, the amounts are less than 5% [21].

The persistence of antibiotics in these sludges can lead to contamination of the soil and other environmental compartments, such as surface water and groundwater by leaching or runoff processes, or even enter the food chain through vegetables grown in contaminated soils [22]. Walters et al. [23] carried out a study on the persistence of antibiotics in mixtures of biosolids with soils and found that AZM had a half-life of 408–3466 days. Of special concern as regards health risks related to the presence of antibiotics in soil are the emergence and spread of antibiotic resistance in pathogenic bacteria [24].

The characterization of the retention and release processes of these antibiotics in soils amended with biosolids is essential to evaluate their transport and the risks associated with their presence [25]. The behavior of these contaminants in the soil depends on edaphic characteristics, such as organic carbon and clay contents, texture and pH [26] and on antibiotics properties, such as hydrophobicity, solubility and molecular structure [27] and their degrees of ionization [28]. The dissociation constant (pK_a) is a parameter to predict the ionization state of a molecule with respect to pH [29]. The antibiotic AZM has two pk_a values, pk_{a1} = 8.74 and pk_{a2} = 9.45, causing that in most soils the molecule behaves as cation [30], however, some authors indicate only one pk_a value, being 7.25 [31] or 8.96 [32]. This antibiotic presents multi-basic amines, having pk_a values that could allow suffering protonation in a rather specific (physiological) pH range [33].

It is also interesting to determine and bear in mind the sensitivity of these molecules to different environmental conditions that could affect their degradation [34].

Taking all of the above into account, the objective of this work is to determine AZM adsorption and desorption on/from soils with different physical-chemical properties. This will be key to evaluating the risk that the presence of this antibiotic may pose in the soil environment, as well as, due to its eventual mobility, the risk of pollution affecting to other different environmental compartments.

2. Materials and Methods

2.1. Soils

A total of 21 soils were selected for the study, all of them previously sampled at different areas of Galicia (NW Spain). Six of the soils correspond to plots planted with corn/maize (designated with codes from M1 to M6), while twelve soils were from vineyards (from VO1 to VO5 -Ourense province-, and from VP1 to VP7 -Pontevedra province), and three were forest soils, of which one was pine forest (FP), another eucalyptus (FE) and another oak (*Q. robur*) (FR). These soils were selected based on their pH values and organic

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matter contents. Table S1 (Supplementary Material) shows some characteristics of the areas where the soil samples were collected.

Each soil sample was made up of 10 subsamples collected in a zig-zag pattern in the surface layer (0–20 cm). Once collected, the samples were dried at 40 °C to constant weight, and then sieved through a 2 mm diameter sieve and stored until analysis. The soils used in this work were previously characterized by Cela-Dablanca et al. [35]. Table S2 (Supplementary Material) shows the main physical-chemical properties of the selected soils.

2.2. Chemical Reagents

The AZM used was supplied by Sigma-Aldrich (Barcelona, Spain). Figure S1 (Supplementary Material) shows the molecular structure of AZM. Potassium phosphate (purity $\geq$ 99.5%) and acetonitrile (purity $\geq$ 99.9%) used for HPLC were supplied by Fisher Scientific (Madrid, Spain) and $CaCl_2$ (95% purity) by Panreac (Barcelona, Spain). To carry out HPLC determinations, all solutions were prepared with milliQ water (Millipore, Madrid, Spain).

2.3. Sorption and Desorption Experiments

Batch-type experiments were carried out to study the adsorption/desorption of AZM on/from the different soils. For this, 2 g of each sample were weighed, then adding 5 mL of a solution with different concentrations of the antibiotic (2.5, 5, 10, 20, 30, 40, 50, 200, 400, and 600 μmol L^{-1}), also containing 0.005 M $CaCl_2$ as background electrolyte. The suspensions were shaken in the dark for 48 h (time enough to reach equilibrium, according to previous kinetic tests) using a rotary shaker. These suspensions were then centrifuged at 4000 rpm for 15 min (G force: 1931.91). The resulting supernatants were filtered through 0.45 μm nylon syringe filters. Finally, the antibiotic concentrations in the equilibrium solution were determined by HPLC-UV with a LPG 3400 SD equipment (Thermo-Fisher, Waltham, MA, USA). The quantification method, as well as further details regarding adsorption and desorption experiments, are shown in Supplementary Material.

2.4. Data Treatment

The experimental data obtained in the adsorption/desorption tests were adjusted to the Freundlich (Equation (1)), Langmuir (Equation (2)) and Linear (Equation (3)) models [36].

$$q_e = K_F C_{eq}^n \quad (1)$$

$$q_e = \frac{K_L\, C_{eq} q_m}{1 + K_L C_{eq}} \quad (2)$$

$$q_e = K_d C_{eq} \quad (3)$$

where q_e (expressed in μmol kg^{-1}) is the amount of antibiotic retained onto the soil (calculated as the difference between the concentration added and that remaining in the equilibrium solution); K_F (Ln μmol^{1-n} kg^{-1}) is the Freundlich constant related to the adsorption capacity; C_{eq} (μmol L^{-1}) is the concentration of antibiotic present in the solution at equilibrium; n (dimensionless) is a parameter of the Freundlich model associated with the degree of heterogeneity of the adsorption; K_L (L μmol^{-1}) is the Langmuir adsorption constant; q_m (μmol kg^{-1}) is the maximum adsorption capacity according to the Langmuir model; and K_d (L kg^{-1}) is the partition coefficient in the linear model.

In addition, soil properties were correlated with the parameters obtained in the adjustments to the adsorption models, determining the Pearson correlation coefficients.

The SPSS Statistics 21 software was used to carry out the adjustment of the data derived from the adsorption experiments to the Langmuir, Freundlich and Linear models, as well as any further statistical analysis.

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3. Results and Discussion

3.1. Adsorption

Figure 1 shows relations among equilibrium concentrations and AZM adsorption for the different soils. The results indicate that the corn/maize soils have a maximum adsorption value of 1256.77 μmol kg^{-1}, similar to that found in vineyard soils, which specifically reach 1229.38 and 1318.21 μmol kg^{-1} in granite soils and in slate/schist soils, respectively. Regarding forest soils, AZM adsorption is much lower than in crop soils, with maximum value (228.84 μmol kg^{-1}) found in the eucalyptus soil sample. Regarding the minimum adsorption scores, for the highest AZM concentration added they were the following: 754.04 μmol kg^{-1} in soils with corn cultivation, 690.96 μmol kg^{-1} in granite vineyard soils, 401.27 μmol kg^{-1} in slate/schist vineyard soils, and 187.29 μmol kg^{-1} in forest soils.

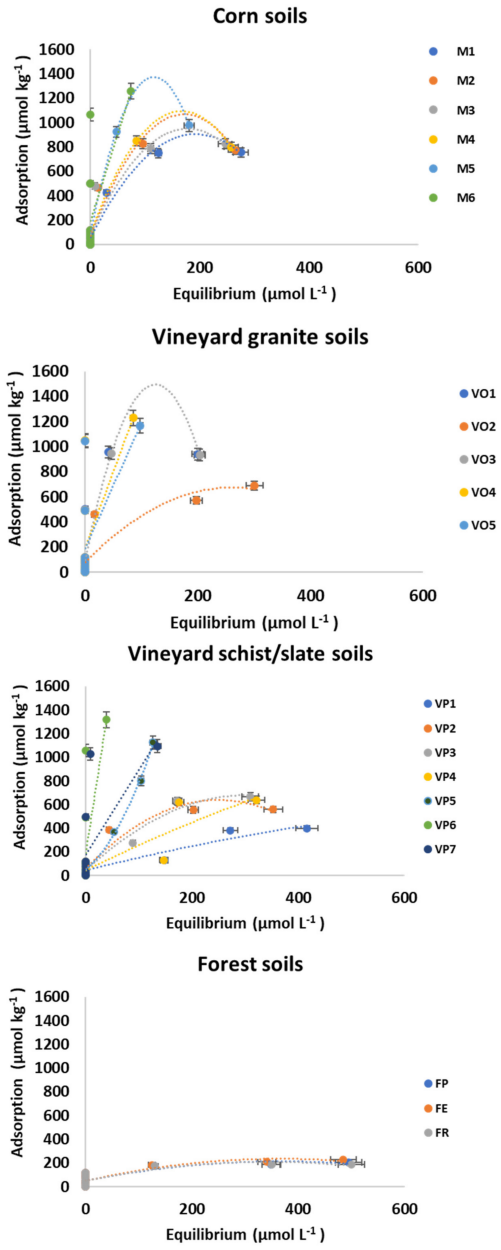

Figure 1. AZM adsorption curves for the different soils studied. M: corn/maize soils, VO: vineyard soils (Ourense Province), VP: vineyard soils (Pontevedra province), FP: forest soil (*P. pinaster*), FE: forest soil (*E. nitens*), FR: forest soil (*Q. robur*), 1–7: different soil samples. Error bars indicate standard deviation.

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Figure 2 shows that the percentage adsorption values were 100% for all the soils when the antibiotic concentrations added were in the range of 2.5–50 μmol L^{-1}, while marked differences are observed among the different soils when the highest AZM concentrations (200–600 μmol L^{-1}) were added. To note that, in general, in this last range of concentrations added the percentage of adsorption decreases as the concentration of antibiotic added increases, probably due to the saturation of the adsorption sites [37]. Considering specific values, most maize soils show high adsorption percentages, ranging between 52.29% and 100%, very close to those obtained in granite vineyard soils, which range between 47.92% and 100%. Adsorption on slate/schists vineyard soils varied over a broader range (from 26.12% to 100%). On the other hand, forest soils were those that presented the lowest adsorption scores (between 13.25% and 37.27%).

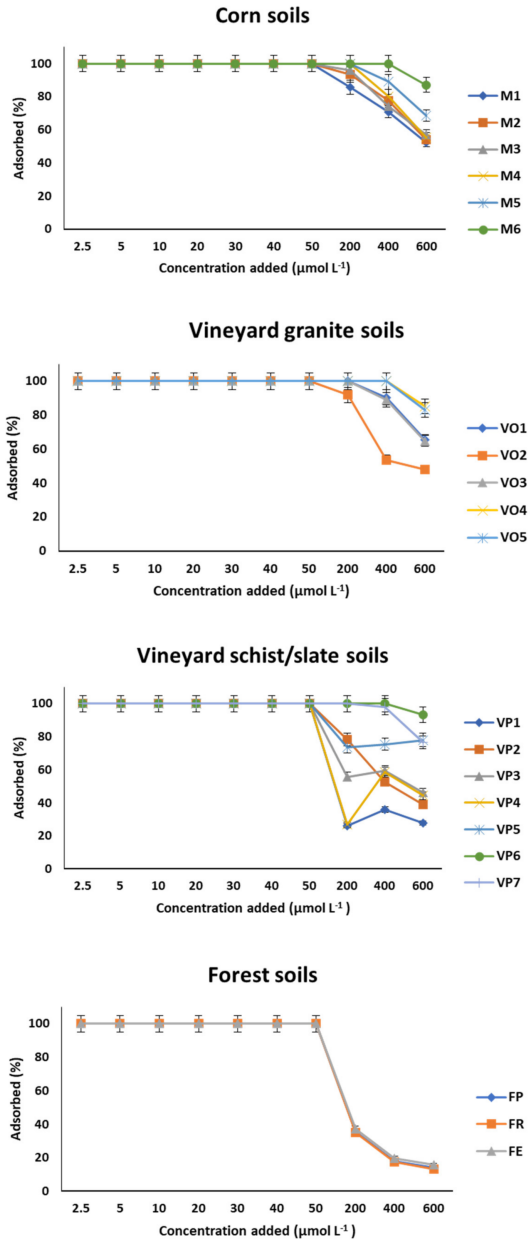

Figure 2. AZM adsorption percentages for the soils studied, as a function of the AZM concentration added. M: corn/maize soils, VO: vineyard soils (Ourense Province), VP: vineyard soils (Pontevedra province), FP: forest soil (*P. pinaster*), FE: forest soil (*E. nitens*), FR: forest soil (*Q. robur*), 1–7: different soil samples. Error bars indicate standard deviation.

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When comparing adsorption data with soil characteristics (Table S2, Supplementary Material) it is clear that the soils showing higher adsorption were those having higher pH values (M5, M6, VP6 and VP7). In fact, adsorption data were correlated with soil properties, indicating that AZM adsorption correlates positively and significantly with soil pH (r = 0.562 and $p < 0.01$) (Table 1). To note that pH is one of the parameters having greater influence on antibiotic-adsorbent interactions, since it simultaneously affects the chemical speciation of the pollutants and adsorbent surfaces [38]. Both antibiotics and soil components have functional groups that may suffer protonation/deprotonation, depending on the pH of the solution. This makes it possible for there to have positive, negative or neutral charges on the reactive surfaces, and therefore allows the formation of different types of bonds [39]. The pK_a of AZM is approximately between 8.6–9.5, so in an acid medium this antibiotic is protonated [40]. In the pH range of the soils included in this study (4.68–8.02), AZM has positively charged functional groups, thus favoring electrostatic interactions with the negatively charged surfaces of minerals and organic matter [41,42].

Table 1. Correlations between AZM adsorption values and soil properties. OM: organic matter; Al_{ox}: Non-crystalline aluminum (extracted with ammonium oxalate).

Soil Property	Correlation Coefficient (r)	Significance Level (p)
pH	0.562	0.01
OM	0.530	0.05
Al_{ox}	−0.43	0.05

Soil organic matter and the non-crystalline Fe and Al components have variable charge, with negative charge increasing as the pH rises, favoring higher AZM adsorption to these charged surfaces, as AZM is mainly present as a divalent cation. For this reason, forest soils, despite the fact that they have a high content of both organic matter and non-crystalline minerals, present low AZM adsorption when the antibiotic is added at concentrations above 200 μmol L^{-1}. This is due to the fact that their pH is very low and there are few negative charges. However, soils having lower organic matter and non-crystalline minerals contents (M5, M6, VO4, VP6, VP7), show higher adsorption than forest soils, because they have a pH >5.8 and higher presence of negative charges. This would explain the significant ($p < 0.05$) and negative correlation obtained between the maximum adsorption of each soil with the organic matter content (r = −0.53) and the total non-crystalline Al extracted with ammonium oxalate (r = −0.43) (Table 1). Other authors also found no positive correlations between non-crystalline Fe components and AZM adsorption [43]. However, in our study we not found any correlations (positive or negative) with this parameter. The high AZM adsorption obtained in the current study for various agricultural soils (Figures 1and 2) is consistent with that found in other researches carried out with high concentrations of this antibiotic in amended crop soils, which on the other hand showed that this drug does not cause toxicity in crops or soil microorganisms [44,45].

The adsorption data were fitted to the Linear, Freundlich and Langmuir models, which are the most commonly used to establish equilibrium relations between an adsorbent and an adsorbate, or between the amount adsorbed to a solid phase and that which remains in solution at a given temperature under equilibrium conditions [30].

Table 2 shows the adsorption parameters obtained from the fit of the experimental data to the Freundlich, Langmuir and Linear adsorption models.

Taking into account the values of the coefficient of determination (R^2), it can be considered that the model showing a better fit was the Freundlich equation, with R^2 greater than 0.85 for the 33% soils, while the Langmuir model obtained a R^2 value > 0.85 for 23% of the soils, and for the Linear model this porcentage decrease until 9%. However, in the Freundlich and in the Langmuir model, very high error values were associated with the estimation of the parameters in many cases, so they would not satisfactorily explain AZM adsorption in these soils. To note that some authors indicate that AZM follows linear

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adsorption models in soils amended with biosolids [46], although the soils in the current study have higher pH values.

Table 2. Fitting of the adsorption data to the Freundlich, Langmuir and Linear models. K_F expressed in L^n μmol^{1-n} kg^{-1}; n is dimensionless; K_L expressed in L μmol^{-1}; q_m expressed in μmol kg^{-1}; K_d expressed in L kg^{-1}; -: error values too high for fitting.

	Freundlich					Langmuir					Linear		
Soil	K_F	**Error**	n	**Error**	R^2	K_L	**Error**	q_m	**Error**	R^2	K_d	**Error**	R^2
M1	95.986	50.522	0.387	0.103	0.89	0.028	0.012	901.315	100.849	0.94	3.403	0.55	0.67
M2	341.346	94.081	0.161	0.057	0.95	0.097	0.037	852.124	60.454	0.96	3.653	0.77	0.52
M3	354.449	72.119	0.159	0.042	0.97	0.167	0.058	837.409	49.227	0.97	4.026	0.72	0.62
M4	-	-	-	-	-	-	-	-	-	-	3.787	0.9	0.44
M5	-	-	-	-	-	-	-	995.768	258.357	0.8	6.309	1.42	0.49
M6	9.471	0	1.136	0.073	0.31	-	-	1600	0	0.31	16.97	5.08	0.31
VO1	-	-	-	-	-	-	-	-	-	-	5.456	1.43	0.39
VO2	314.979	91.637	0.128	0.057	0.94	0.143	0.085	-	-	0.94	2.532	0.41	0.66
VO3	-	-	-	-	-	-	-	-	-	-	5.376	1.35	0.42
VO4	9.219	0	1.101	0.072	0.31	-	-	1600	0	0.31	14.46	4.36	0.31
VO5	8.87	0	1.066	0.073	0.27	-	-	1554.29	0	0.27	11.99	3.78	0.27
VP1	-	-	0.829	0.365	0.79	-	-	-	-	-	1.08	0.13	0.79
VP2	203.771	95.973	0.178	0.089	0.93	0.041	0.026	608.522	66.977	0.93	1.952	0.32	0.65
VP3	-	-	0.552	0.182	0.91	-	-	-	-	-	2.546	0.26	0.86
VP4	-	-	0.872	0.418	0.77	-	-	-	-	-	2.149	0.29	0.77
VP5	-	-	1.326	0.213	0.98	-	-	-	-	-	8.274	0.4	0.97
VP6	10.24	0	1.327	0.082	0.36	-	-	1600	0	0.36	33.87	9.59	0.36
VP7	-	-	-	-	–	-	-	1099.382	190.379	0.84	8.581	2.56	0.3
FP	-	-	-	-	–	-	-	209.883	80.292	0.44	0.493	0.11	0.2
FR	-	-	-	-	–	-	-	192.928	73.297	0.4	0.466	0.11	0.1
FE	-	-	-	-	–	-	-	242.635	87.674	0.54	0.559	0.11	0.33

The values of the distribution coefficient of the linear model (K_d), a parameter related to the adsorption intensity, range between 3.403 and 16.973 L kg^{-1} in maize soils, between 2.532 and 14.463 L kg^{-1} in vineyard granite soils, between 1.080 and 33.867 L kg^{-1} in slate/schists vineyards, and between 0.466 and 0.599 L kg^{-1} in forest soils. The lower values of forest soils indicate greater AZM mobility in them [47]. These values are higher than those obtained in previous studies for sulfonamides [48], but lower than those obtained for tetracyclines [49], indicating that interactions with these soils that give rise to AZM adsorption are stronger than those of sulfonamides and weaker than those of tetracyclines. As for the values of the Freundlich affinity coefficient (K_F), related to the soil adsorption capacity, they indicate that corn and vineyard soils on granite are the ones with the highest affinity for AZM adsorption.

Bearing in mind that the lower the value of n, the more heterogeneous the adsorption surface [50], and also that values of this parameter being between 1 and 10 indicate favorable conditions for adsorption [51], the fact that some of the maize and vineyard soils in this study are the ones with the lowest n values would indicate that they are the ones with a more heterogeneous surface. As comparison, Bao et al. [52] obtained lower n values for tetracycline in forest than in agricultural soils. Regarding the Langmuir model, the lowest values of the q_m parameter (the maximum Langmuir's adsorption capacity) corresponded to some of the forest soils, which would confirm that these soils are the ones with the lowest adsorption capacity.

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3.2. Desorption

The desorbed AZM concentrations were lower than the detection limit in all the soils studied and for all the antibiotic concentrations added. These results indicate that AZM is adsorbed very strongly onto these soils, causing that the retention process could be

considered almost not reversible under the conditions of this study. These results are consistent with previous research indicating strong AZM adsorption in both biosolids [42] and biosolids-amended soils [30].

4. Conclusions

When AZM concentrations of up to 50 μmol L^{-1} are added to the soils used in this research, its adsorption was 100% in all of them, while when the concentration added was equal to or greater than 200 μmol L^{-1}, some differences were observed, with those soils having higher pH showing higher adsorption. Adsorption data fitted better to the Freundlich model, in the sense of presenting higher determination coefficients. Regarding AZM desorption, its concentrations were always lower than the detection limit, indicating that the antibiotic was adsorbed in a very strong manner to the soils studied. These results can be considered relevant in terms of assessment of risks of pollution due to AZM, both in soils and in other environmental compartments to which the antibiotic could migrate. It should be noted that, although AZM is present in the environment at lower concentrations than those found in this research, its consumption has clearly increased in recent years, which makes the current work relevant as regard increasing the knowledge on the factors that influence this fact, and facilitating the future development of solutions to the problem. In addition, taking into account that desorption is practically absent from the soils studied, it is suggested that these edaphic environments could help to prevent AZM leaching/transportation to other environmental compartments such as water bodies, especially in case that the adsorption capacity of these soils could be increased by means of low-cost sorbents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10122565/s1. Table S1: Basic details corresponding to the different soils used in this work. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils; Table S2: Values corresponding to the basic parameters determined in the various soils studied. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils. OC: organic carbon; OM: organic matter; N: nitrogen; eCEC: effective cation exchange capacity; Alox and Feox: Al and Fe extracted with ammonium oxalate; Alpir and Fepir: Al and Fe extracted with sodium pyrophosphate. Average values (n = 3), with coefficients of variation always <5%; Table S3: AZM adsorption expressed in μmol kg^{-1} (and in percentage between brackets), for the soils studied, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils. Average values (n = 3), with coefficients of variation always <5%; Figure S1: Molecular structure of AZM; Figure S2. Molecular structure of AZM with amine groups selected; Figure S3: Selected chromatograms corresponding to AZM adsorption onto soils.

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Supplementary Material

Azithromycin Adsorption onto Different Soils

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Table S1. Basic details corresponding to the different soils used in this work. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils

Code	Crop/Trees	Parent material	Mean temperature (° C)	Annual rainfall (mm)
M1	Corn	Schists, quartzite, pelite, sandstones and granites	12	995
M2 M3 M4 M5 M6	Corn	Schist and amphibolite	13	1270
VO1 VO2 VO3	Vineyard	Schist Slate Slate	11	850-1000
VO4 VO5	Vineyard	Granite and mica-schists	14.5	950
VP1 VP2 VP3	Vineyard	Granite	13.8	1540
VP4 VP5	Vineyard	Granite	14	1000-2000
VP6 VP7	Vineyard	Granite	13.8	1540
FP FE FR	Pine, Eucalyptus, Q. robur	Granite and gneiss	11.2	1389

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Table S2. Values corresponding to the basic parameters determined in the various soils studied. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils. OC: organic carbon; OM: organic matter; N: nitrogen; eCEC: effective cation exchange capacity; Al_{ox} and Fe_{ox}: Al and Fe extracted with ammonium oxalate; Al_{pir} and Fe_{pir}: Al and Fe extracted with sodium pyrophosphate. Average values (n=3), with coefficients of variation always < 5%

Soil	pH_{H20}	OC	OM	N	Sand	clay	eCEC	Ca	Mg	Na	K	Al	Fe_{ox}	Fe_{pir}	Al_{ox}	Al_{pir}
				%			$cmol_c\ kg^{-1}$						$mg\ kg^{-1}$			
M1	8.02	4.98	8.58	0.33	49.57	22.50	42.81	39.44	2.07	0.60	0.69	0.01	4140	1501	5351	2111
M2	5.33	2.66	4.59	0.27	43.42	24.65	6.88	5.35	0.67	0.02	0.44	0.40	5745	3159	3401	2871
M3	5.65	2.58	4.44	0.25	43.57	22.43	7.48	6.18	0.77	0.00	0.28	0.24	5780	3315	3881	2717
M4	5.01	2.04	3.52	0.21	45.64	22.43	5.94	4.58	0.67	0.10	0.17	0.43	4545	2923	2896	1945
M5	6.11	7.67	13.21	0.63	63.35	12.36	20.84	18.81	1.60	0.08	0.33	0.02	6990	3719	11651	6179
M6	6.43	5.56	9.59	0.53	59.28	16.50	24.35	21.65	1.60	0.16	0.93	0.02	6525	3231	4991	4087
VO1	5.6	0.63	1.09	0.06	61.40	13.14	5.96	2.85	0.73	1.39	0.31	0.68	1665	1273	634	1961
VO2	5.7	1.00	1.72	0.11	48.05	22.44	5.43	3.15	0.32	1.48	0.43	<0.06	2515	715	484	173
VO3	5.6	1.61	2.78	0.18	43.50	22.90	6.02	3.38	0.26	1.83	0.5	<0.06	2555	1047	894	1047
VO4	5.87	1.94	3.35	0.11	47.50	18.22	6.41	3.69	0.88	0.15	0.52	1.17	1980	1421	1601	1321
VO5	6.04	1.77	3.06	0.10	49.57	24.14	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141
VP1	5.6	3.69	6.36	0.26	68.60	14.23	19.52	4.74	1.07	1.64	11.91	0.16	3045	2353	5739	4543
VP2	6.1	4.38	7.55	0.37	50.16	20.25	22.58	8.43	1.56	2.46	10.12	<0.05	3220	6309	4024	3897
VP3	6.1	2.83	4.88	0.23	60.62	18.35	37.23	6.33	1.48	18	11.4	<0.05	3460	4889	4294	3005
VP4	5.5	6.58	11.35	0.49	65.54	21.07	10.51	6.55	0.37	2.60	0.27	0.72	3540	6459	6624	14857
VP5	5.7	4.81	8.30	0.40	58.22	21.38	17.36	13.40	1.09	2.32	0.55	<0.10	2765	4519	3679	3965
VP6	7.05	2.43	4.19	0.14	67.42	22.43	9.09	8.11	0.51	0.08	0.37	0.02	1720	1225	3981	1703
VP7	7.27	3.56	6.14	0.22	67.50	12.22	12.32	10.53	1.04	0.44	0.27	0.04	1945	823	4576	1597
FP	4.88	7.70	13.27	0.48	69.35	12.86	6.43	0.07	0.00	0.11	0.09	6.16	5410	9239	8184	10767
FE	4.8	9.78	16.87	0.67	67.28	12.86	6.59	0.05	0.00	0.12	0.12	6.29	5805	8249	10189	11347
FR	4.68	7.15	12.32	0.49	69.35	14.86	7.48	0.18	0.07	0.00	0.22	7.01	3055	6789	5619	9457

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Table S3. AZM adsorption expressed in μmol kg^{-1} (and in percentage between brackets), for the soils studied, as a function of the concentration of antibiotic added. M: maize (corn) soils; VO: vineyard soils (Ourense province); VP: vineyard soils (Pontevedra province); F: forest soils. Average values (n=3), with coefficients of variation always < 5%

Soil			Soil		
M1			VP2		
	AZM added (μmol/L)	AZM adsorbed. concentration and %		AZM added (μmol/L)	AZM adsorbed. concentration and %
M1 M2	0	0 (0)	VP2 VP2	0	0 (0)
	2.5	0 (0)		2.5	6.25 (100)
	5	12.44 (100)		5	12.44 (100)
	10	24.88 (100)		10	25.00 (100)
	20	50.00 (100)		20	49.75 (100)
	30	74.63 (100)		30	75.00 (100)
	40	100 (100)		40	100.00 (100)
	50	115.77 (100)		50	116.35 (100)
	200	425.85 (85.60)		200	130.60 (26.12)
	400	750.40 (70.88)		400	383.40 (36.03)
	600	754.04 (52.30)		600	401.27 (27.83)
M2 M3	0	0 (0)	VP2 VP3	0	0 (0)
	2.5	6.25 (100)		2.5	6.22 (100)
	5	12.44 (100)		5	12.44 (100)
	10	24.88 (100)		10	25.00 (100)
	20	50.00 (100)		20	49.75 (100)
	30	75.00 (100)		30	75.00 (100)
	40	99.50 (100)		40	99.50 (100)
	50	115.77 (100)		50	116.35 (100)
	200	464.62 (93.39)		200	388.81 (78.15)
	400	824.86 (77.53)		400	555.55 (52.48)
	600	775.53 (54.05)		600	559.44 (38.80)
M3	0	0 (0)	VP3	0	0 (0)
	2.5	6.25 (100)		2.5	6.22 (100)
	5	12.44 (100)		5	12.44 (100)
	10	25.00 (100)		10	25.00 (100)
	20	49.75 (100)		20	49.75 (100)
	30	75.00 (100)		30	74.63 (100)
	40	99.50 (100)		40	99.50 (100)
	50	115.77 (100)		50	115.77 (100)
	200	479.98 (96)		200	277.23 (55.72)
	400	785.36 (74.18)		400	630.94 (59.60)
	600	825.44 (57.25)		600	667.08 (46.26)

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	0	0 (0)		0	0 (0)
	2.5	6.22 (100)	VP4	2.5	6.25 (100)
	5	12.44 (100)	VP4 VP5	5	12.50 (100)
M4	10	25.00 (100)	VP4	10	24.88 (100)
	20	49.75 (100)		20	50.00 (100)
	30	74.63 (100)		30	75.00 (100)
	40	100.00 (100)		40	99.50 (100)
	50	116.35 (100)		50	116.35 (100)
	200	497.51 (100)	VP4	200	133.22 (26.78)
M4 M5	400	849.73 (80.26)	VP5 VP5	400	622.20 (58.77)
	600	799.38 (55.44)	VP6	600	638.92 (44.31)
	0	0 (0)		0	0 (0)
	2.5	6.25 (100)		2.5	6.25 (100)
	5	12.50 (100)		5	12.44 (100)
	10	25.00 (100)		10	25.00 (100)
	20	50.00 (100)		20	50.00 (100)
	30	75.00 (100)		30	75.00 (100)
	40	99.50 (100)		40	99.50 (100)
	50	115.77 (100)		50	116.35 (100)
	200	497.51 (100)	VP5	200	368.40 (73.68)
M5 M6	400	923.49 (88.97)	VP6 VP6	400	802.96 (75.47)
	600	975.90 (68.70)	VP7	600	1125.46(78.05)
	0	0 (0)		0	0 (0)
	2.5	6.25 (100)		2.5	6.25 (100)
	5	12.44 (100)		5	12.50 (100)
	10	24.88 (100)		10	25.00 (100)
	20	49.75 (100)		20	49.75 (100)
	30	74.63 (100)		30	74.63 (100)
	40	100.00 (100)		40	100.00 (100)
	50	115.77 (100)		50	115.77 (100)
	200	500.00 (100)	VP6	200	490.20 (100)
M6 VO1	400	1063.98 (100)	VP7 VP7	400	1058.69 (100)
	600	1256.77 (87.16)		600	1318.21(93.25)
	0	0 (0)		0	0 (0)
	2.5	6.22 (100)		2.5	6.25 (100)
	5	12.44 (100)		5	12.44 (100)
	10	25.00 (100)		10	25.00 (100)
	20	49.75 (100)		20	50.00 (100)
	30	75.00 (100)		30	75.00 (100)
	40	100.00 (100)		40	100.00 (100)
VO1	50	116.35 (100)	VP7 FP	50	116.35 (100)
	200	500.00 (100)		200	497.51 (100)
	400	957.74 (90.47)		400	1027.40 (98.01)
	600	941.88 (65.32)		600	1094.55 (76.67)

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	0	0 (0)		0	0 (0)
	2.5	6.25 (100)		2.5	6.25 (100)
	5	12.50 (100)		5	12.44 (100)
	10	24.88 (100)		10	24.88 (100)
			FP		
			FE		
			FE		
			FR		
	20	49.75 (100)		20	49.75 (100)
VO2			FP		
	30	75.00 (100)		30	75.00 (100)
	40	99.50 (100)		40	99.50 (100)
	50	116.35(100)		50	116.35 (100)
	200	459.23 (91.85)		200	174.23 (35.54)
	400	570.73 (53.64)	FP	400	186.81 (17.99)
	600	690.96 (47.92)	FE	600	205.02 (14.22)
VO2			FE		
VO3	0	0 (0)	FR	0	0 (0)
			FE		
	2.5	6.25 (100)	FR	2.5	6.25 (100)
	5	12.50 (100)	FR	5	12.50 (100)
	10	25.00 (100)		10	24.88 (100)
	20	50.00 (100)		20	49.75 (100)
	30	75.00 (100)		30	75.00 (100)
	40	99.50 (100)		40	100.00 (100)
	50	116.35 (100)		50	116.35 (100)
	200	497.51 (100)		200	175.00 (37.26)
	400	947.65 (89.07)		400	186.73 (19.79)
	600	933.52 (64.73)	FE	600	187.29 (15.95)
VO3			FR		
VO4	0	0 (0)	FR	0	0 (0)
			FR		
	2.5	6.22 (100)		2.5	6.22 (100)
	5	12.50 (100)		5	12.50 (100)
	10	24.88 (100)		10	24.88 (100)
	20	50.00 (100)		20	49.75 (100)
	30	74.63 (100)		30	74.63 (100)
	40	100.00 (100)		40	99.50 (100)
	50	116.35 (100)		50	116.35 (100)
	200	492.61 (100)		200	183.57 (35)
	400	1048.26 (100)		400	209.51 (17.73)
	600	1229.38 (85.26)		600	228.84 (13.25)
VO4					
VO5	0	0 (0)	FR		
	2.5	6.25 (100)			
	5	12.50 (100)			
	10	25.00 (100)			
	20	50.00 (100)			
	30	75.00 (100)			
	40	100.00 (100)			
	50	116.35 (100)			
VO5	200	490.20 (100)			
	400	1043.12 (100)			

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Quantification of AZM and further details on adsorption and desorption experiments

The amounts of antibiotic adsorbed were calculated by the difference between the concentrations initially present in the added solutions and those remaining in the solutions at equilibrium. All determinations were made in triplicate. Figure S3 (Supplementary Material) shows some of the chromatograms corresponding to the quantification of AZM.

Once the adsorption experiments were carried out, desorption was studied, allowing to evaluate the reversibility of the process. For this, a volume of 5 mL of 0.005 M CaCl2 (without antibiotic) was added to the material resulting from the adsorption process and then the subsequent procedure carried out for adsorption was repeated. All determinations were made in triplicate.

The HPLC determinations were carried out with a Model LPG 3400 SD equipment (Thermo-Fisher, USA). A Luna C18 column (150 mm long, 4.6 mm internal diameter, 5 μm particle size) and a pre-column (4 mm long, 2 mm diameter; 5 μm particle size) packed with the same material as the column were also used, both provided by Phenomenex (Madrid, Spain). During the HPLC analysis, the injection volume was 200 μL and the flow rate was 1 ml min^{-1}, with the mobile phase being acetonitrile (phase A) and 0.025 M potassium monophosphate (phase B). A linear gradient was used, varying from 5% to 70% of phase A, and from 95% to 30% of phase B during 18 minutes. Then, the initial conditions (5% of phase A and 95% of phase B) were re-established in 2 minutes and maintained during 3 minutes. The total analysis time was 22 minutes, with a retention time of 9.8 minutes. The wavelength used for detection was 210 nm.

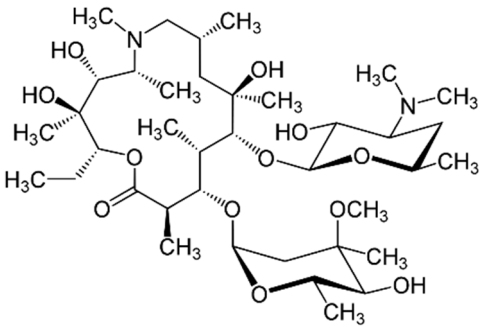

Figure S1. Molecular structure of AZM

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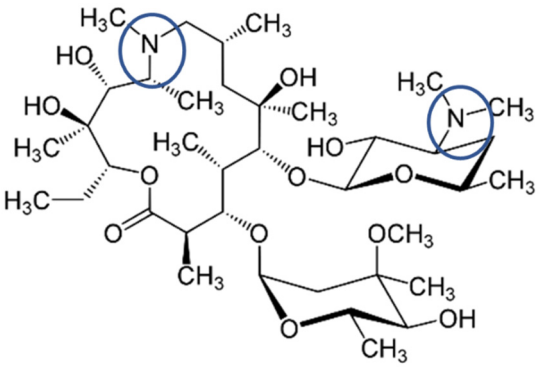

Figure S2. Molecular structure of AZM with amine groups remarked

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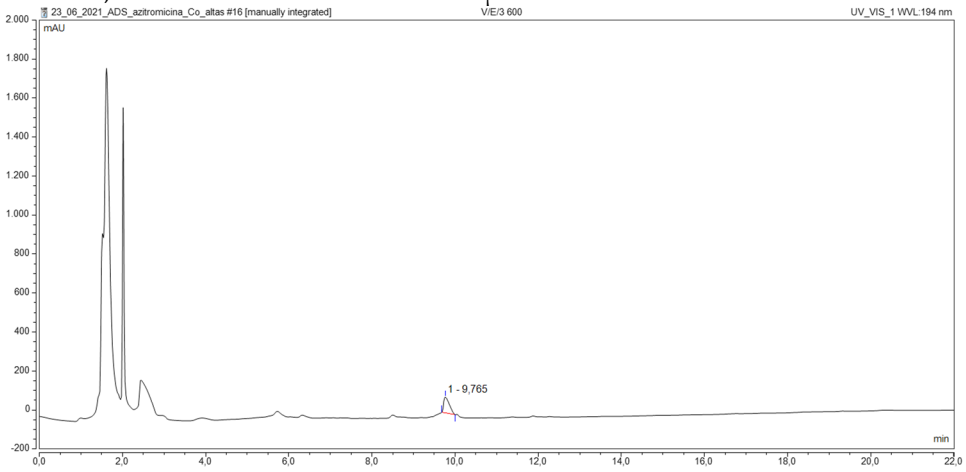
Soil VP1; AZM concentration added 600 µM



Soil M1; AZM concentration added 600 µM

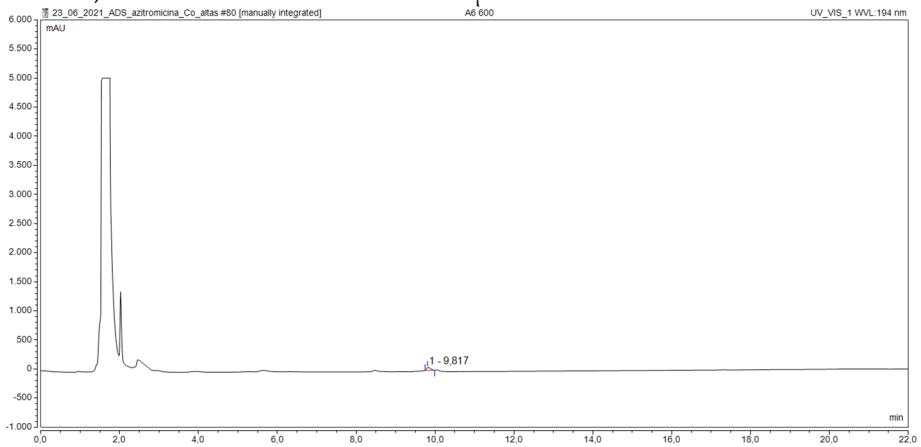


Figure S3. Selected chromatograms corresponding to AZM adsorption onto soils

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PUBLICACIÓN IX

Cela-Dablanca, Raquel[a]., Barreiro, Ana[a]., Rodríguez-López, Lucía[b]., Arias-Estévez, Manuel[b]., Fernández-Sanjurjo, María J[a]., Álvarez-Rodríguez, Esperanza[a]., & Núñez-Delgado, Avelino[a]. (2024). **Azithromycin removal using pine bark, oak ash and mussel shell.** *Environmental Research*, *252*, 119048. https://doi.org/10.1016/j.envres.2024.119048

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Azithromycin removal using pine bark, oak ash and mussel shell

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ABSTRACT

Adsorption is considered an interesting option for removing antibiotics from the environment because of its simple design, low cost, and potential efficiency. In this work we evaluated three by-products (pine bark, oak ash, and mussel shell) as bio-adsorbents for the antibiotic azithromycin (AZM). Furthermore, they were added at doses of 48 t ha^{-1} to four different soils, then comparing AZM removal for soils with and without bio-adsorbents. Batch-type experiments were used, adding AZM concentrations between 2.5 and 600 μmol L^{-1} to the different bio-adsorbents and soil + bio-adsorbent mixtures. Regarding the bio-adsorbents, oak ash showed the best adsorption scores (9600 μmol kg^{-1}, meaning >80% retention), followed by pine bark (8280 μmol kg^{-1}, 69%) and mussel shell (between 3000 and 6000 μmol kg^{-1}, 25–50% retention). Adsorption data were adjusted to different models (Linear, Freundlich and Langmuir), showing that just mussel shell presented an acceptable fitting to the Freundlich equation, while pine bark and oak ash did not present a good adjustment to any of the three models. Regarding desorption, the values were always below the detection limit, indicating a rather irreversible adsorption of AZM onto these three by-products. Furthermore, the results showed that when the lowest concentrations of AZM were added to the not amended soils they adsorbed 100% of the antibiotic, whereas when the highest concentrations of AZM were spread, the adsorption decreased to 55%. However, when any of the three bio-adsorbents was added to the soils, AZM adsorption reached 100% for all the antibiotic concentrations used. Desorption was null in all cases for both soils with and without bio-adsorbents. These results, corresponding to an investigation carried out for the first time for the antibiotic AZM, can be seen as relevant in the search of low-cost alternative treatments to face environmental pollution caused by this emerging contaminant.

1. Introduction

Emerging contaminants (including substances such as pharmaceuticals, perfluorinated compounds, fire retardants, and many others) are causing an increasing concern as regards environmental and human health (Kumar et al., 2023; Núñez-Delgado et al., 2023; Puri et al., 2023; Ramírez-Malule et al., 2020; Wells et al., 2010).

In the last twenty years, pharmaceutical products have been found as micro contaminants in soils and waters, due to the improvement of the capacity to detected them by using advanced chemical analysis (Boxall, 2004; Carvalho and Santos, 2016; Puri et al., 2023). Within this group, antibiotics stand out (Fick et al., 2009; Cela-Dablanca et al., 2024; Conde-Cid et al., 2020; Rodríguez-López et al., 2022; Santás-Miguel et al., 2023). For example, in Beijing (China), in soils fertilized for greenhouse vegetable production different antibiotics were detected, within a wide range when expressed in μg kg^{-1} (Li et al., 2015; Wei et al., 2019).

The excessive use of antibiotics causes their environmental accumulation and may induce a negative impact in human health and degrade the environment (Cherian et al., 2023; Verlicchi and Zambello, 2015), since these compounds can reduce the microbial populations in soils and waters, delaying or blocking contaminant bioremediation, and being toxic to microorganisms (Rodríguez-González et al., 2023; Selvam et al., 2012). Furthermore, antibiotics in the environment are related to a significant increase of resistant genes (ARGs) and resistant bacteria (ARB) (Han et al., 2016; Hu et al., 2016; Koch et al., 2021; Li et al., 2023; Zhu et al., 2017). Several studies have indicated that, in soils, there is a correlation between residual antibiotic levels and ARGs (Awad et al., 2015; Lu et al., 2023; Ma et al., 2020; Tang et al., 2015; Zhang et al., 2023), although it is not always clear (Sun et al., 2022). This problem

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got worse recently with the appearance of COVID-19, which produced an increase in the antibiotic consumption to treat and prevent this disease (Núñez-Delgado et al., 2022; Race et al., 2020; Rawson et al., 2020).

Among antibiotics, AZM was used as a therapy for the treatment of SARS-CoV-2 pneumonia given its antiviral and immunomodulatory activity with well-known safety profile (Parnham et al., 2014; Gautret et al., 2020). In addition, it is considered as one of the relevant broad-spectrum therapeutic molecules (Firth and Prathapan, 2020), and overall is indicated for respiratory, urogenital, dermal and other bacterial infections, and exerts immunomodulatory effects in chronic inflammatory disorders (Parnham et al., 2014).

ARGs spreading in soils is mainly related to agricultural practices, such as intensive livestock farming (Forsberg et al., 2012; Gao et al., 2023). On the other hand, antibiotics of human consumption, which are excreted by feces and urine, enter the environment through effluents and sludge from wastewater treatment plants (WWTPs) (Carlesi-Jara et al., 2007; Hazra et al., 2022). Thereby, Hernández et al. (2019) detected the presence of eight different antibiotics in treated wastewater, with higher concentrations of two quinolones, ciprofloxacin and norfloxacin (at 0.89 and 0.75 μg L^{-1} respectively), and three macrolides, AZM and clarithromycin (with concentrations averaging 0.4 μg L^{-1}), and erythromycin (at 0.003 μg L^{-1}).

The presence of antibiotics like AZM and ciprofloxacin, transferred by biosolids, can introduce ARG transcriptional activities and mobilize genetic elements in biosolids and soils previously amended, in environmentally relevant concentrations (Sidhu et al., 2021). For this reason, there is a great concern regarding the entry of antibiotics into the environment through irrigation with reclaimed water or through the spreading of manure and sewage sludge used as fertilizers (Christou et al., 2017; Jia et al., 2023; Martínez, 2009; Sultana et al., 2023). The interest for the use of sewage sludge as fertilizer is owing to its characteristics such as elevated nutrient content, optimum properties to restore overexploited agricultural soils, and capacity to improve the organic matter content and water retention on sandy or eroded soils (Clarke and Smith, 2011; Inglezakis et al., 2014; Seleiman et al., 2020). Most WWTPs are expected to run well for organic matter and some macro-elements, such as C, N and P, but these treatment plants are not performant enough to effectively remove microcontaminants, like antibiotics (de Kreuk et al., 2010; Novo et al., 2013; Obaideen et al., 2022; Pal et al., 2015; Pronk et al., 2015). Therefore, WWTPs are one of the main sources of antibiotics that enter the environment (Gulkowska et al., 2008; Osińska et al., 2020). For example, in a WWTP in Beijing 22 different antibiotics were detected (eight quinolones, nine sulfonamides and five macrolides) with concentrations between 365 and 4916 ng L^{-1} (Li et al., 2013). Rodriguez-Mozaz et al. (2020) studied the presence of 53 antibiotics in WWTPs in seven different European countries and detected 17 antibiotics, among which ciprofloxacin, AZM, clarithromycin and trimethoprim stood out. Despite this, in Europe there is no legislation to regulate antibiotics concentration released into the environment from WWTPs (Carvalho and Santos, 2016; Marutescu et al., 2023). The 2008/105/EC Directive includes a list with 33 priority substances or groups of substances and their respective environmental quality standards, and in 2015 three antibiotics of the macrolides group (AZM, clarithromycin and erythromycin) were added (Barbosa et al., 2016). The UE included these three antibiotics in the list "Watch list of contaminants of emerging concern" of contaminants that can be controlled according to the Decision 2015/495 (European Union, 2015).

The maximum acceptable concentration for AZM, according to the Directive 2008/105/EC, is 90 mg L^{-1}. This antibiotic presents a low elimination rate from wastewater when using conventional treatments (Barbosa et al., 2016). In recent years, several studies have found the presence of AZM in wastewater effluents under conventional treatment. Specifically, Mirzaei et al. (2019) observed that the elimination of the macrolides AZM and erythromycin was not successful in a WWTP in Teheran (Iran). In previous studies, azithromycin was detected in influents of three WWTP and the antibiotic concentrations were 145 ng L^{-1}, 110 ng L^{-1} and 896 ng L^{-1} (Mirzaei et al., 2022). Furthermore, in previous decades, Mowery and Loganathan (2007) detected AZM in all the WWTPs analyzed in their research in Kentucky (USA), in concentrations up to 23.4 ng L^{-1}. On the other hand, this antibiotic was detected in treated wastewater recycled for agricultural use (Panthi et al., 2019). This demonstrates the convenience of developing new alternative treatments in order to achieve the complete (or much higher) removal of AZM and minimize the adverse effects caused by the presence of this antibiotic in wastewater and environmental compartments (Cano et al., 2020).

There are several techniques to remove or degrade antibiotics, such us hydrolysis, photodegradation, oxidization and biodegradation (Yang et al., 2021). Specifically, adsorption has been widely used for the removal of a variety of pollutants (Ahmed et al., 2021; Peña-Rodríguez et al., 2013), including antibiotics and ARGs, obtaining promising results (Fathy et al., 2017; Pan, 2020), because of its simple design and performance, low cost, and high efficiency removing antibiotics and not generating toxic subproducts (Ahmadi et al., 2017). The study of adsorption methods allows to analyze the retention/mobility of antibiotics in soils and their possible transport to groundwater (lixiviation), superficial water (run-off) and crops (plant uptake) (Pikkemaat et al., 2016; Toakoak-Asho and Cho, 2016). The adsorption process not only can determine the bioavailability of these compounds (Botero-Coy et al., 2018), but also would influence degradation reactions (Xiang et al., 2019). This technique has several advantages over other techniques used to remove antibiotics, so it would be interesting introduce an integrated system into existing treatment plants for a complete remediation (Mangla et al., 2022). The efficiency of this process depends on the type of adsorbent, adsorbate properties and the waste stream composition (Acevedo-García et al., 2020; Aksu and Tunç, 2005). There are multiple studies that analyze the use of adsorbents to remove antibiotics, including clay compounds and biochar to remove tetracycline (Premarathna et al., 2019); magnetic ion exchange resins to remove sulfamethoxazole, tetracycline and amoxicillin (Wang et al., 2017); magnetic imprinted polymers for the selective removal of erythromycin (Ou et al., 2015) or powdered zeolites to remove AZM, ofloxacin and sulfamethoxazole (de Sousa et al., 2018). Paredes-Laverde et al. (2018) also studied the elimination of norfloxacin by using natural adsorbents such as rice husk and coffee. Furthermore, various vegetable residues turned out promising as natural adsorbents for the treatment of pollutants, including grapefruit peel (Zou et al., 2012), papaya seed (Pavan et al., 2014), consumed tea leaves (Hameed, 2009), peanut hull (Ali et al., 2016), shell of wheat and lentil (Aydin et al., 2008), and banana and mango peel (Bhatnagar et al., 2015).

With the previous background and constituting the first steps in a more complex project (Núñez-Delgado, 2024), the main objective of the current research is to assess the capacity of three different by-products to adsorb AZM and their influence in AZM adsorption on agricultural soils with different physicochemical properties. Two of these bio-adsorbents derive from the forestry industry (pine bark and oak ash) and the other one from the food industry (mussel shell). Promoting the recycling of by-products while favoring the retention/removal of antibiotics that have reached the environment as pollutants can be considered of relevance at the level of research and social implications.

2. Material and methods

2.1. Soils and bio-adsorbent materials

Four soils were selected for this study, three of them under corn crops (C soils) and the fourth one being a vineyard soil (V soil), all of them situated in Galicia (NW Spain). The main characteristics of these soils are detailed in Table 1. It could be highlighted that these soils show pH values between 5.01 and 6.04, organic matter contents in the range 3.06–4.59%, and cation exchange capacity scores between 5.94 and

Table 1
Values corresponding to the basic physicochemical properties determined in the soils used in this research. Average values (n = 3), with coefficients of variation always <5%.

Soil	pH H_2O	OC	OM	N	Sand	clay	eCEC	Ca	Mg	Na	K	Al	Feo	Fepir	Alo	Alpir
		%					$cmol_c\ kg^{-1}$						$mg\ kg^{-1}$			
C1	5.33	2.66	4.59	0.27	43.42	24.65	6.88	5.35	0.67	0.02	0.44	0.40	5745	3159	3401	2871
C2	5.65	2.58	4.44	0.25	43.57	22.43	7.48	6.18	0.77	0.00	0.28	0.24	5780	3315	3881	2717
C3	5.01	2.04	3.52	0.21	45.64	22.43	5.94	4.58	0.67	0.10	0.17	0.43	4545	2923	2896	1945
V	6.04	1.77	3.06	0.10	49.57	24.14	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141

C: corn soils; V: vineyard soils. OC: organic carbon; OM: organic matter; N: nitrogen; eCEC: effective cation exchange capacity; Al_o, Fe_o: non-crystalline Al and Fe (extracted with ammonium oxalate); Al_{pir}, Fe_{pir}: Al and Fe extracted with sodium pyrophosphate.

7.48 $cmol_c\ kg^{-1}$.

Three bio-adsorbents were used to carry out this work, with pine bark being provided by Geolia (Madrid, Spain), oak ash being from a combustion boiler in Lugo (Spain), and mussel shell provided by the factory of Abonomar S.L, Pontevedra (Spain). The main characteristics of these bio-adsorbents are detailed in Supplementary Material (Table S1). To highlight that pine bark presents an acid pH (3.99), while mussel shell and oak ash have alkaline pH values (9.39 and 11.31, respectively).

For specific experiments, these bio-adsorbents were added as amendments to the soils, in a dose of 48 t ha^{-1}. Table S2 (Supplementary Material) shows the pH values of the soils and the soil + bio-adsorbent mixtures.

2.2. Chemical reagents

AZM was provided by Sigma-Aldrich (Barcelona, Spain). The reagents used for HPLC measurements, potassium phosphate (purity ≥99.5%), and acetonitrile (purity ≥99.9%), were supplied by Fisher Scientific (Madrid, Spain). $CaCl_2$ (95% purity) was provided by Panreac (Barcelona, Spain).

All the solutions used were prepared with milliQ water (Millipore, Madrid, Spain).

2.3. Adsorption and desorption experiments

Batch-type experiments were carried out to study the adsorption/desorption of AZM on/from the different bio-adsorbents and mixtures soil + bio-adsorbent. For this purpose, solutions with different antibiotic concentrations were prepared (in the range 2.5–600 μmol L^{-1}), using 0.005 M $CaCl_2$ as background electrolyte. These solutions were added to 0.5 g of each bio-adsorbent sample and to 2 g of each mixture soil + bio-adsorbent sample. The suspensions were shaken in the dark during 48 h (time needed to reach the equilibrium, as determined in previous experiments), using a rotary shaker. Then, the suspensions were centrifuged for 15 min at 4000 rpm by means of an Eppendorf centrifuge 5810 R (Eppendorf, Germany) and filtered through 0.45 μm nylon syringe filters. Finally, the antibiotic concentrations in the equilibrium solution were determined by means of an HPLC-UV LPG 3400 SD equipment (Thermo-Fisher, USA), with additional details included in Supplementary Material. AZM adsorbed was determined as the difference between the concentrations in the added solutions and concentrations present in the equilibrium. Triplicate determinations were performed in all cases.

Desorption experiments were carried out adding 10 mL of 0.005 M $CaCl_2$ to the material derived from the adsorption phase, then applying the same procedure as for the adsorption process. Fig. S1 (Supplementary Material) shows example chromatograms corresponding to the quantification of AZM.

2.4. Data treatment

The adsorption experimental data were adjusted to the Freundlich (Eq. (1)), Langmuir (Eq. (2)) and Linear (Eq. (3)) models, as indicated in Conde-Cid et al. (2021), and Cela-Dablanca et al. (2022a).

For this, the following equations were used:

$$qe = K_F Ceq^n \quad \text{(Eq. 1)}$$

$$qe = \frac{q_m K_L Ceq}{1 + K_L Ceq} \quad \text{(Eq. 2)}$$

$$Kd = qe/Ceq \quad \text{(Eq. 3)}$$

where qe is the amount of antibiotic retained by the adsorbent material (calculated as the difference between the concentration added and that remaining in the equilibrium solution, referred to the amount of adsorbent used); Ceq is the antibiotic concentration present in the solution at equilibrium; K_F is the Freundlich parameter related to the adsorption capacity; n is a parameter of the Freundlich model associated with the degree of heterogeneity of the adsorption; K_L is the Langmuir adsorption constant; q_m is the maximum adsorption capacity according to the Langmuir model; and K_d is the partition coefficient in the Linear model.

The SPSS statistics software (version 21) was used to perform the adjustments, as well as any additional statistical treatment and calculations needed.

3. Results and discussion

3.1. AZM adsorption on the three bio-adsorbent materials

Fig. S2 (Supplementary Material) shows a rise in AZM adsorption (expressed in μmol kg^{-1}) when the concentration added increased, especially for oak ash and pine bark, while the behavior was rather erratic when expressed as percentage. In addition, when analyzing the AZM adsorption values (expressed in percentage) by the different bio-adsorbents, it was observed that oak ash was the material showing the highest adsorption, with values greater than 80% for all the concentrations added (except for the lowest, 50 μmol L^{-1}). In the case of pine bark, the maximum adsorption was 69%, while mussel shell adsorption decreased from 50% (reached for the lowest AZM concentration added) to 25% (for the highest AZM concentration applied). Similar results were obtained by Kabir et al. (2022) in a study where AZM adsorption (studied for different bio-adsorbents) significantly decreased when the highest concentration was added, probably due to the progressive saturation of the adsorption sites (Wu et al., 2020). Conde-Cid et al. (2019) studied the adsorption of three tetracyclines on the same three bio-adsorbents used in the current research, in a ternary system, obtaining the highest adsorption scores for oak ash and pine bark.

Before performing the current investigation focused on by-products, we have previously studied AZM adsorption onto Galician soils (Cela-Dablanca et al., 2022b), while other authors have shown results corresponding to AZM retention/removal onto other different soils (Bustos-López et al., 2023) or on sorbent materials different to those used in the current research (Ameen et al., 2023; Balarak et al., 2021; Upoma et al., 2022), all of them needed to provide different but complementary results helping to increase the overall knowledge of this

specific matter. In fact, previous studies have shown the applicability of low-cost sorbent materials to remove antibiotics from environmental compartments (Anthony et al., 2021; Anuar et al., 2023; Cela-Dablanca et al., 2022a), thus encouraging further research in this field.

Regarding adsorption curves (Fig. 1), it is shown that, for the highest concentration of antibiotic added, oak ash was the bio-adsorbent that presented the highest adsorption, reaching 9300 μmol kg^{-1}, followed by pine bark that reached 8054 μmol kg^{-1}. However, mussel shell showed a much smaller adsorption, with maximum of 2035 μmol kg^{-1}. These values are much higher than the ones obtained for the same antibiotic in a previous study with different soils (Cela-Dablanca et al., 2022b). Similarly, other authors have described high adsorption results for this antibiotic in other materials such as biosolids (Sidhu et al., 2019), and a high affinity for soils has been reported (Vermillion Maeir and Tjeerdema, 2018).

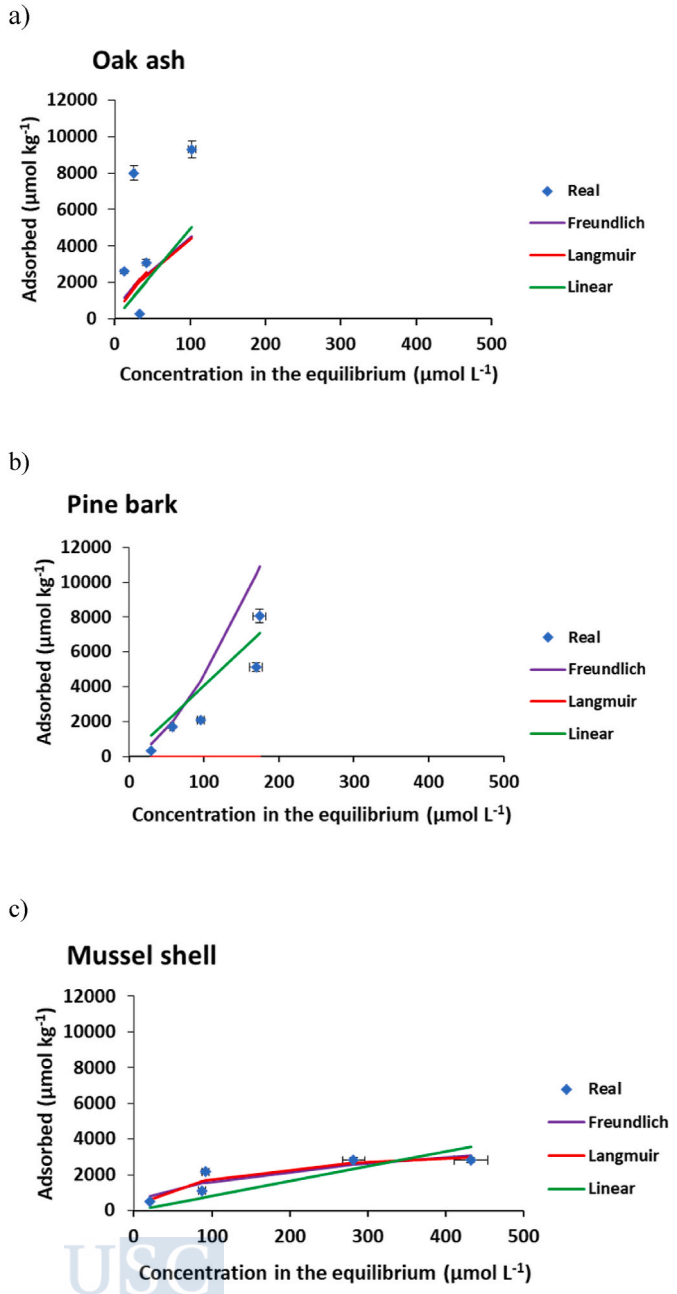

Fig. 1. Real and estimated adsorption curves for AZM corresponding to each of the three bio-adsorbents [a), b), and c)]. Average values (n = 3) with coefficients of variation always lower than 5%.

The results showed that the two bio-adsorbents having alkaline pH (oak ash and mussel shell) had adsorption capacities clearly different between them. Furthermore, comparing two of the bio-adsorbents having very different pH values, such as oak ash and pine bark, it was observed that both materials had high adsorption capacities. These facts suggest that the pH of these bio-adsorbents had not marked influence on AZM adsorption. However, in a previous work Cela-Dablanca et al. (2022b) found clearly increased AZM adsorption for the soils with higher pH within their study.

This controversial behavior may be related to the very different pH values characterizing the bio-adsorbents used in the current research. In fact, oak ash had a very alkaline pH (11.31), as well as non-crystalline Fe and Al concentrations much higher than the other two bio-adsorbents (Table S1; Supplementary Material), relevant due to being variable charge components negatively charged at these pH values. To note that Gravesen and Judy (2020) used multiple linear regression analyses showing relations between AZM adsorption and total Fe content, due to the propensity of AZM to react with Fe species. Furthermore, we must bear in mind that the pH value of the oak ash used in the current research (pH = 9.39) was higher than the pK_a of AZM (8.74) (Davoodi et al., 2019), therefore, at this pH AZM is deprotonated and negatively charged. Specifically, these authors reported that, due to that reason, AZM adsorption onto raw diatomite increased at pH > 9 and was highest at pH 11. Owing to this, the union between AZM and oak ash would take place by means of electrostatic interactions (Anastopoulos et al., 2020). However, mussel shell had fewer non-crystalline minerals and organic matter contents than oak ash and hence less negatively charges surface available for AZM adsorption. Related to that, Conde-Cid et al. (2019) also obtained higher adsorption for three tetracyclines (oxytetracycline, chlortetracycline and tetracycline) using oak ash than with mussel shell. On the contrary, pine bark pH was very acidic (3.99), although having a high organic matter content (>47%) (Table S1, Supplementary Material), and in these conditions there would be deprotonated carboxyl groups that acquire negative charge to adsorb this antibiotic. In this case, the union between AZM and the adsorbent would be by hydrogen bonds, since the acidic pH would generate an electrostatic repulsion between positive charges of the pine bark surface and the AZM cationic form (Zhao et al., 2016).

Data obtained from the AZM adsorption experiments were adjusted to the Freundlich, Langmuir and Linear models. As shown in Table S3 (Supplementary Material), for mussel shell real data adjusted well to the Freundlich model, while the fitting was clearly poorer for the Langmuir and Linear models. For pine bark, all three models were affected by high errors, invalidating the fittings. For oak ash, the best adjustment (although poor) corresponded to the Linear model. In a previous publication, Upoma et al. (2022) found the best fitting for the Freundlich model when studying AZM adsorption on graphene oxide.

In the current study, the values corresponding to the Freundlich *n* parameter were <1 (Table S3), indicating the presence of heterogeneous adsorption sites, where those being of high energy were first occupied (Foo and Hameed, 2010; Behnajady and Bimeghdar, 2014). As indicated above, for both oak ash and pine bark the fitting to the models was poorer or inexistent (Table S3). However, in previous research focused on a different antibiotic (amoxicillin), Cela-Dablanca et al. (2022a) found good fitting for the Freundlich and Linear models using the same three bio-adsorbents.

3.2. AZM desorption from the three bio-adsorbents

The results indicated that the three bio-adsorbents used adsorbed the AZM added in a highly irreversible way, as no antibiotic was detected in the equilibrium solution. Similar results were obtained in previous studies using soils (instead of bio-adsorbent materials) as adsorbent (Cela-Dablanca et al., 2022b), as well as for the antibiotic amoxicillin using the same bio-adsorbents (Cela-Dablanca et al., 2022a). However, the results were different for cefuroxime, where pine bark presented

desorption percentages between 29.1% and 51.9% (Cela-Dablanca et al., 2021). Sidhu et al. (2019) also reported a high retention for this antibiotic on biosolids, with desorption no exceeding 1% in any case, while Vermillion Maier and Tjeerdema (2018) obtained a value of 9.2% for maximum desorption from soils. In addition, in the case of alluvial sediments Senta et al. (2021) described the recalcitrant character of AZM. Overall, it should be noted that the persistence of these emerging pollutants for long periods of time is considered as a factor able to favor the emergence of gene antibiotic resistance, for example in agricultural soils (Lau et al., 2020), especially when high doses are spread.

3.3. AZM adsorption on soils with and without bio-adsorbents

Fig. 2 shows the adsorption curves for each of the four soils used in this research, both without amendments and amended with each of the three bio-adsorbents at a dose of 48 t ha^{-1}.

The layouts clearly indicate that the amendments caused that all the AZM added was adsorbed in most cases, although it was very high even for not amended soils, especially when the lowest concentrations of the antibiotic were added.

The adsorption data corresponding to these experiments cannot be fitted to adsorption models due to the fact that no antibiotic remains in solution in most cases.

Considering the soils without amendment, AZM adsorption was 100% up to doses of 400 and 200 μmol L^{-1} added, for soils V and C3, respectively, while for the C1 and C2 soils, adsorption was 100% when the concentration added was 50 μmol L^{-1} or less. However, for the highest concentration added (600 μmol L^{-1}) adsorption oscillated between 50% and 85%, being the vineyard soil (V) the one showing the highest score. This could indicate a saturation of the adsorbent surfaces when the highest antibiotic concentration was added.

Fig. 3 shows AZM adsorption (expressed in μmol kg^{-1} and in percentage) onto the four agricultural soils with and without each of the three bio-adsorbents, as a function of increasing concentrations of the antibiotic added. The results indicate that adsorption increased in all cases after amending with the bio-adsorbents, and the difference was more marked for the highest concentrations of AZM added (400 and 600 μmol L^{-1}).

After the amendment, when any of the bio-adsorbents was added to the soils, AZM retention increased for all soils when the highest concentrations of the antibiotic were spread, reaching 100% adsorption in most cases (with the exception of the C3 soil mixed with mussel shell, where the maximum adsorption was limited to 87% when a concentration of 600 μmol L^{-1} of the antibiotic was added).

It must be noted that oak ash and mussel shell have a high pH value, increasing the pH of the soils amended with these materials (clearly in all cases, for oak ash, or slightly and not so clear, for mussel shell) (Table S2, Supplementary Material), raising the negative charge of the variable charge components (organic matter and non-crystalline substances), and therefore increasing the adsorption of the AZM molecules that are in cationic form over a wide range of pH (4–9) (Sidhu et al., 2019). These same authors found an increase in AZM adsorption when soils were amended with biosolids in comparison with soils without biosolids, due to the increase in pH caused by the addition of these materials (Sidhu et al., 2019). In addition, Davoodi et al. (2019) studied the capacity of raw and saponin-modified nano diatomite to absorb AZM from aqueous solutions, obtaining that adsorption was similar both at pH 5 and 7 (<60%), but when pH increased to 9 and 11 adsorption rose to 79% and 92%, respectively. However, in the case of raw nano diatomite, pH had no influence on adsorption. Upoma et al. (2022) studied the adsorption of AZM using wastes derived from graphene oxide and obtained a slight increase in adsorption at high pH values. At these pHs, adsorption can be explained due to the deprotonation taking place and generation of negative charge, which favor the electrostatic attraction between the negative charge of the adsorbents and the positively charged groups of AZM (Davoodi et al., 2019).

On the other hand, the amendment with pine bark (pH = 3.99) slightly decreased the pH of soils (Table S2, Supplementary Material), and also caused an increase in the organic matter content of the mixture. This bio-adsorbent can acquire negative charge in carboxyl groups in the range of pH 4.2–4.5, where AZM presents amino groups in protonated form (Vajdle et al., 2017). Thereby, in this case the adsorption

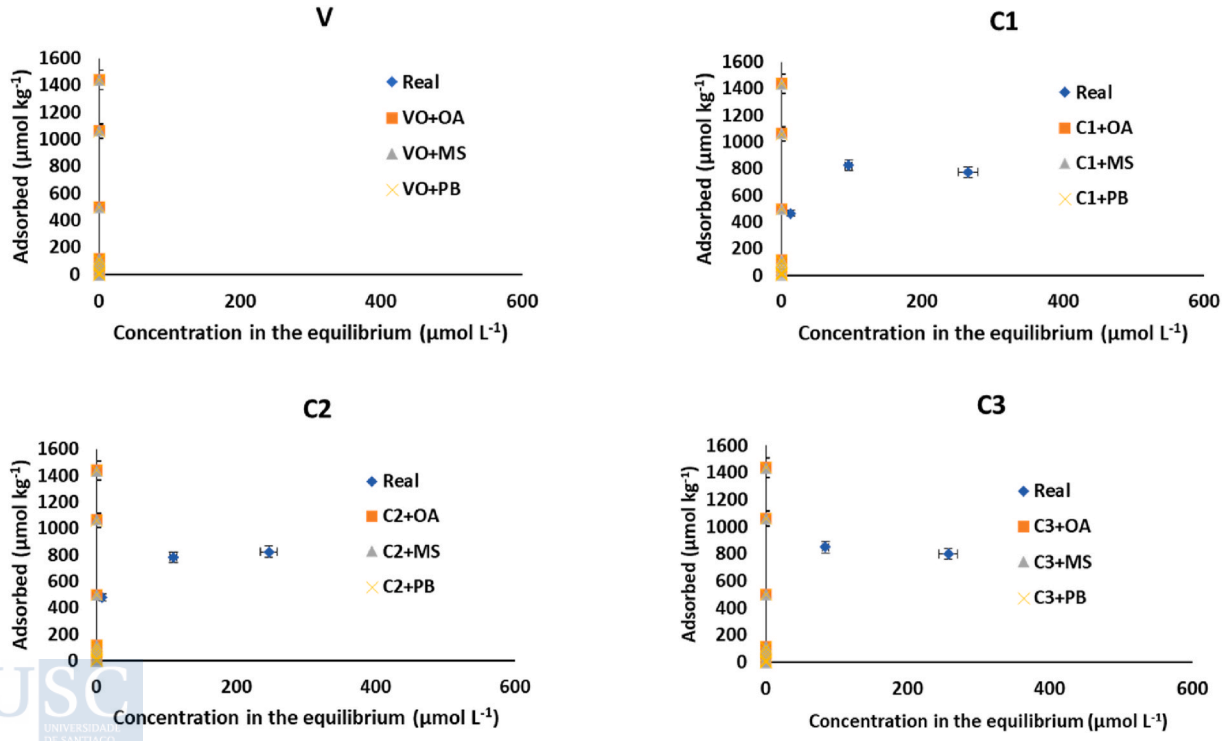

Fig. 2. AZM adsorption curves corresponding to four agricultural soils (V, C1, C2, and C3), with and without bio-adsorbents amendment. Real: unamended soil. V and VO: vineyard soil; C: corn soils; AO: oak ash; MS: mussel shell; PB: pine bark. Average values (n = 3) with coefficients of variation always lower than 5%.

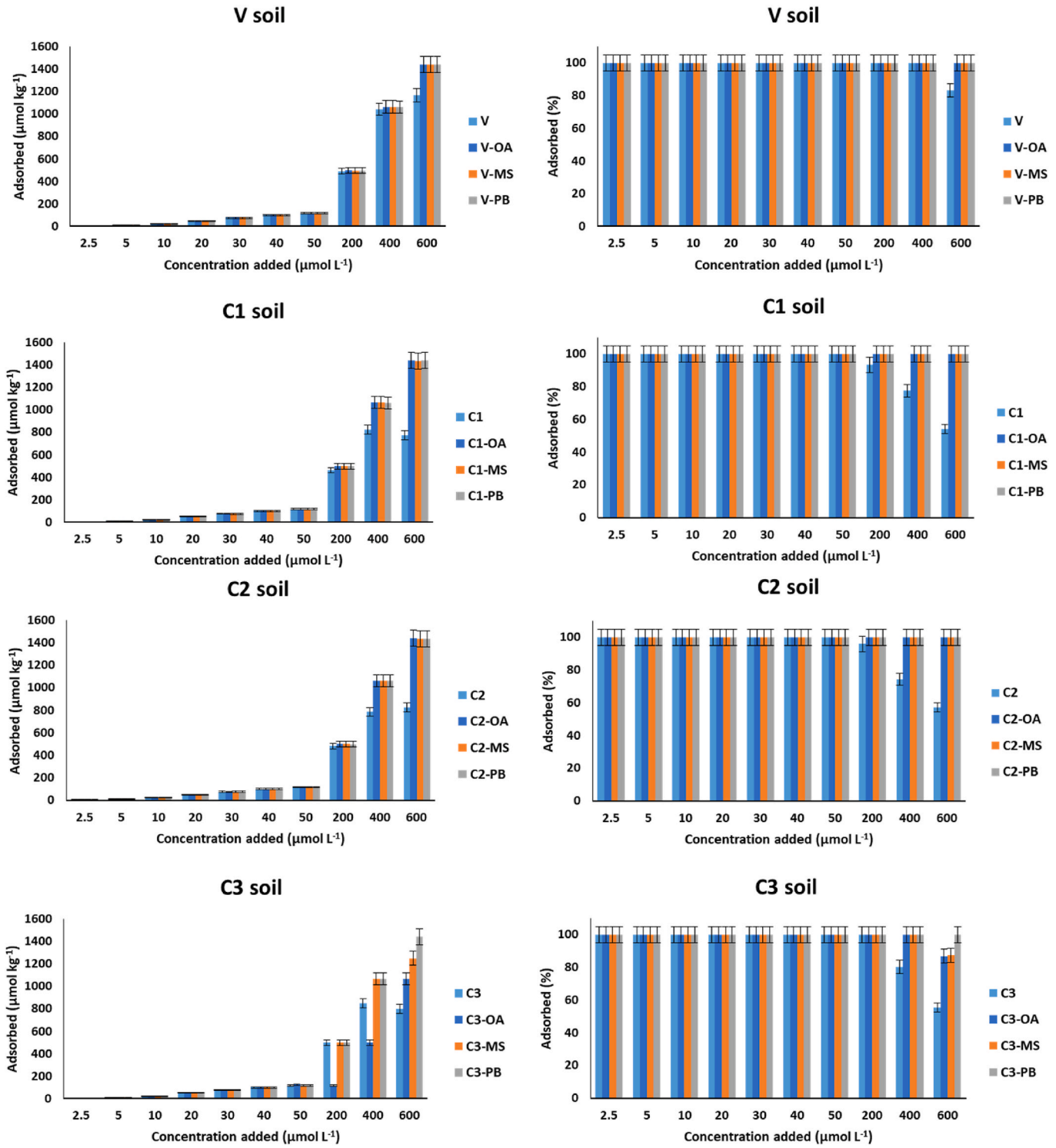

Fig. 3. AZM adsorption (expressed in μmol kg^{-1} and in percentage) for increasing concentrations of the antibiotic added to four agricultural soils, with and without bio-adsorbents amendment. V: vineyard soil; C: corn soils; OA: oak ash; MS: mussel shell; PB: pine bark. Average values (n = 3) with coefficients of variation always lower than 5%.

mechanism must be attributed to H-bonding and electrostatic interaction between the negative charges of the bio-adsorbent and the positive charges of AZM (de Sousa et al., 2018).

3.4. AZM desorption from soils with and without bio-adsorbents amendment

There was no AZM desorption from soils with or without bio-adsorbents amendment. These results are in line of those previously found for the three bio-adsorbents. In addition, Cela-Dablanca et al. (2022b) reported a strong retention of AZM in studies focused on soils not amended with bio-adsorbents. High AZM retention was also found for biosolids (Gravesen and Judy, 2020), for soils amended with bio-solids (Sidhu et al., 2019), and for biochar (Mutera et al., 2019). Senta et al. (2021) reported a high AZM adsorption on sediments, while Vermillion Maier and Tjeerdema (2018) found desorption between 0.1

and 9% from riverbank soils. In a previous study carried out for the antibiotic amoxicillin using the same soil + bio-adsorbent mixtures as in the current research, desorption clearly decreased after the amendments (Cela-Dablanca et al., 2022c).

4. Conclusions

Among the three bio-adsorbents studied, oak ash was the one showing the highest adsorption capacity for the antibiotic AZM, being above the other sorbent with also alkaline pH (mussel shell). The third bio-adsorbent (pine bark) also showed high AZM adsorption capacity, in spite of having acidic pH, suggesting that the influence of pH in the adsorption of AZM on these materials was low. Regarding desorption, it was inexistent or very low, indicating that AZM presents a strong and rather irreversible adsorption onto the three bio-adsorbents. At the highest AZM concentrations added, the soils used were not able to retain 100% of the antibiotic they received, but the retention increased up to 100% when amending with any of the bio-adsorbents used (pine bark, oak ash or mussel shell). In addition, in the conditions tested there was no desorption, indicating a strong and rather irreversible retention of AZM after the amendments. These results show a rather low risk of AZM pollution for the soils studied and under most of the experimental conditions considered, although with potential hazards increasing as a function of the concentration of the antibiotic present in the environmental compartment being investigated. In these cases, the amendment with an appropriated bio-adsorbent (as the three included in this research) would be a good option to increase the overall adsorption capacity, preventing that this or similar emerging pollutants end up into other environmental compartments such as surface water and groundwater. In addition, it is relevant that the use of low-cost materials as effective bio-adsorbents promote recycling, sustainability, and the circular economy.

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CRediT authorship contribution statement

Raquel Cela-Dablanca: Writing – original draft, Visualization, Software, Investigation, Data curation. **Ana Barreiro:** Writing – original draft, Visualization, Software, Investigation, Data curation. **Lucía Rodríguez-López:** Visualization, Investigation. **Manuel Arias-Estévez:** Visualization, Validation, Supervision, Methodology, Conceptualization. **María Fernández-Sanjurjo:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Conceptualization. **Esperanza Álvarez-Rodríguez:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Data curation, Conceptualization. **Avelino Núñez-Delgado:** Writing – review & editing, Visualization, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119048.

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Supplementary Material

Azithromycin removal using pine bark, oak ash and mussel shell

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1 Methods and results corresponding to the analyses of soils and bio-adsorbents

The methods used to analyse the soils and bio-adsorbents were the following.

The values corresponding to pH in water and pH in KCl (solid:liquid ratio 1:2.5) were measured with a CRISON-2001 pHmeter (Crison, Barcelona, Spain) (Guitián and Carballás, 1976). Total C and N were determined by means of elemental analysis, using a LECO CHN-100 equipment (Leco Corporation, St. Joseph, MI, USA). Sand and clay contents were determined using the International Method of the Robinson Pipette (Guitián and Carballás, 1976). The exchangeable cations (Ca_e, Mg_e, Na_e, K_e and Al_e) were displaced using a 1 M NH_4Cl solution (Peech et al., 1947) in a 1:10 sorbent:solution ratio, and were then quantified by means of atomic absorption spectrophotometry, using a Perkin Elemer AAnalyst 200 apparatus (Perkin Elmer, Inc, USA). The effective cation exchange capacity (eCEC) was determined as the sum of these five exchangeable cations (Kamprath, 1970). Available phosphorus was extracted using 0.5 M $NaHCO_3$ and quantified using a UV-visible spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan) (Olsen and Sommers, 1982). Total contents of different elements (Na_T, Mg_T, Al_T, K_T, Ca_T, Cr_T, Mn_T, Fe_T, Co_T, Ni_T, Cu_T, Zn_T, As_T and Cd_T) were determined following the EPA 3051 method, performing an acid digestion in a microwave with a 65% HNO_3 solution, and subsequent determination with ICP-MS. To determine total non-crystalline Fe and Al, a specific extraction was performed using ammonium oxalate buffered at pH 3, stirred for 4 hours in the dark, then adding 5 drops of 0.25% superfloc to the resulting extract, then centrifuging at 2000 rpm for 10 minutes, filtering and diluting the supernatant to 1:5. To determine the total non-crystalline Fe and Al contents (Fe_o and Al_o), an extraction was carried out with ammonium oxalate buffered at pH 3 (Blakemore, 1978), stirring for 4 hours in the dark; five drops of 0.25% superfloc were added to the resulting extract, then centrifuged at 2000 rpm for 10 minutes; it was then filtered, and the supernatant is diluted 1:5; Fe and Al in non-crystalline form were measured by atomic absorption spectrophotometry. The

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determination of Fe and Al bound to organic matter (Fe_{pir} and Al_{pir}) was carried out by extraction with sodium pyrophosphate at pH 10 (Bascomb, 1968); the extraction was carried out by shaking 1 g of sample with 100 mL of sodium pyrophosphate for 16 hours; the samples were diluted in half with distilled water and finally Fe and Al were measured in by atomic absorption spectrophotometry.

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Table S1. Main characteristics of the bio-adsorbent materials used in this research. Average values (n=3), with coefficients of variation always <5%

Parameter	Unit	Pine bark	Oak ash	Mussel shell
C	%	48.70	13.23	11.43
N	%	0.08	0.22	0.21
C/N		608.75	60.14	55.65
pH_{water}		3.99	11.31	9.39
pH_{KCl}		3.42	13.48	9.04
Ca_e	$cmol_c\ kg^{-1}$	5.38	95.0	24.75
Mg_e	$cmol_c\ kg^{-1}$	2.70	3.26	0.72
Na_e	$cmol_c\ kg^{-1}$	0.46	12.17	4.37
K_e	$cmol_c\ kg^{-1}$	4.60	250.65	0.38
Al_e	$cmol_c\ kg^{-1}$	1.78	0.07	0.03
eCEC	$cmol_c\ kg^{-1}$	14.92	361.15	30.25
Sat Al	%	11.93	0.02	0.10
Available-P	$mg\ kg^{-1}$	70.45	462.83	54.17
Na_T	$mg\ kg^{-1}$	68.92	2950	5174.00
Mg_T	mg/kg	473.55	26171	980.66
Al_T	$mg\ kg^{-1}$	561.50	14966	433.24
K_T	$mg\ kg^{-1}$	737.84	99515	202.07
Ca_T	$mg\ kg^{-1}$	2318.81	136044	280168
Cr_T	$mg\ kg^{-1}$	1.88	36.28	4.51
Mn_T	$mg\ kg^{-1}$	30.19	10554	33.75
Fe_T	$mg\ kg^{-1}$	169.78	12081	3535
Co_T	$mg\ kg^{-1}$	0.20	17.25	1.02
Ni_T	$mg\ kg^{-1}$	1.86	69.25	8.16
Cu_T	$mg\ kg^{-1}$	<LD	146.33	6.72
Zn_T	$mg\ kg^{-1}$	6.98	853.00	7.66
As_T	$mg\ kg^{-1}$	<LD	8.36	1.12
Cd_T	$mg\ kg^{-1}$	0.13	19.93	0.07
Al_o	$mg\ kg^{-1}$	315.0	8323	178.33
Fe_o	$mg\ kg^{-1}$	74.0	4233	171.0

Ca_e, Mg_e, Na_e, K_e, Al_e: elements in the exchange complex; Sat. Al: Al-saturation in the exchange complex; eCEC: effective cation exchange capacity; X_T: total content of the element (X); Al_o, Fe_o: non-crystalline Al and Fe.

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2 Quantification of AZM using HPLC equipment

AZM concentrations were determined by means of HPLC-UV LPG 3400 SD equipment (Thermo-Fisher, USA), with a Luna C18 column (150 mm length x 4.6 mm internal diameter x 5 µm particle size) provided by Phenomenex (Madrid, Spain), and a precolumn (4 mm length x 2 mm in diameter x 5 µm particle size). The injection volume was 200 µL and the flow rate was 1 mL min^{-1}. The mobile phase consisted of acetonitrile (phase A) and 0.025 M monopotassium phosphate (phase B). A linear gradient was used varying from 5% to 70% of phase A and from 95% to 30% of phase B for 18 minutes. Then, the initial conditions were re-established in 2 min and maintained for 3 min. The total analysis time was 22 min, with a retention time of 9.8 min, and the wavelength used for detection was 210 nm.

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Table S2. pH values of the different soils and soil + bio-adsorbent mixtures. Average values (n=3), with coefficients of variation always <5%

Soils and Mixtures	**pH**	**Soils and Mixtures**	**pH**
V	6.04	C2	5.65
V+OA	7.81	C2+OA	7.04
V+MS	5.92	C2+MS	5.76
V+PB	5.35	C2+PB	5.24
C1	5.33	C3	5.01
C1+OA	6.93	C3+OA	6.93
C1+MS	5.29	C3+MS	5.46
C1+PB	4.92	C3+PB	4.79

V: vineyard soil; C: corn soils; OA: oak ash; MS: mussel shell; PB: pine bark

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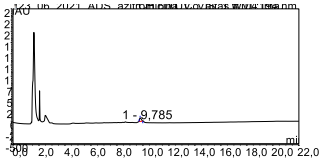
Mussel shell. AZM concentration added 600 µmol L^{-1}



C3 soil amended with pine bark. AZM concentration added 50 µmol L^{-1}

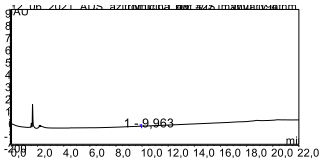


Figure S1. Selected chromatograms corresponding to the detection and quantification of AZM

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3 Complementary data about AZM adsorption

Further details regarding data obtained during AZM adsorption experiments are provided in Table S4 and in Figure S2.

Table S3. Fitting of adsorption data to the Freundlich, Langmuir and Linear models. K_F ($L^n\mu mol^{1-n}\,kg^{-1}$); K_L ($L\,\mu mol^{-1}$); q_m ($\mu mol\,kg^{-1}$); Kd ($L\,kg^{-1}$); -: error values too high for fitting

	Freundlich					Langmuir					Linear		
	K_F	Error	N	Error	R^2	K_L	Error	q_m	Error	R^2	Kd	Error	R^2
Oak ash	-	-	0.65	0.41	0.47	-	-	-	-	-	49.18	12.77	0.37
Mussel shell	90.47	48.067	0.48	0.1	0.93	-	-	2078.79	224.76	0.97	4.503	0.739	0.51
Pine bark	-	-	-	-	-	-	-	-	-	-	-	-	-

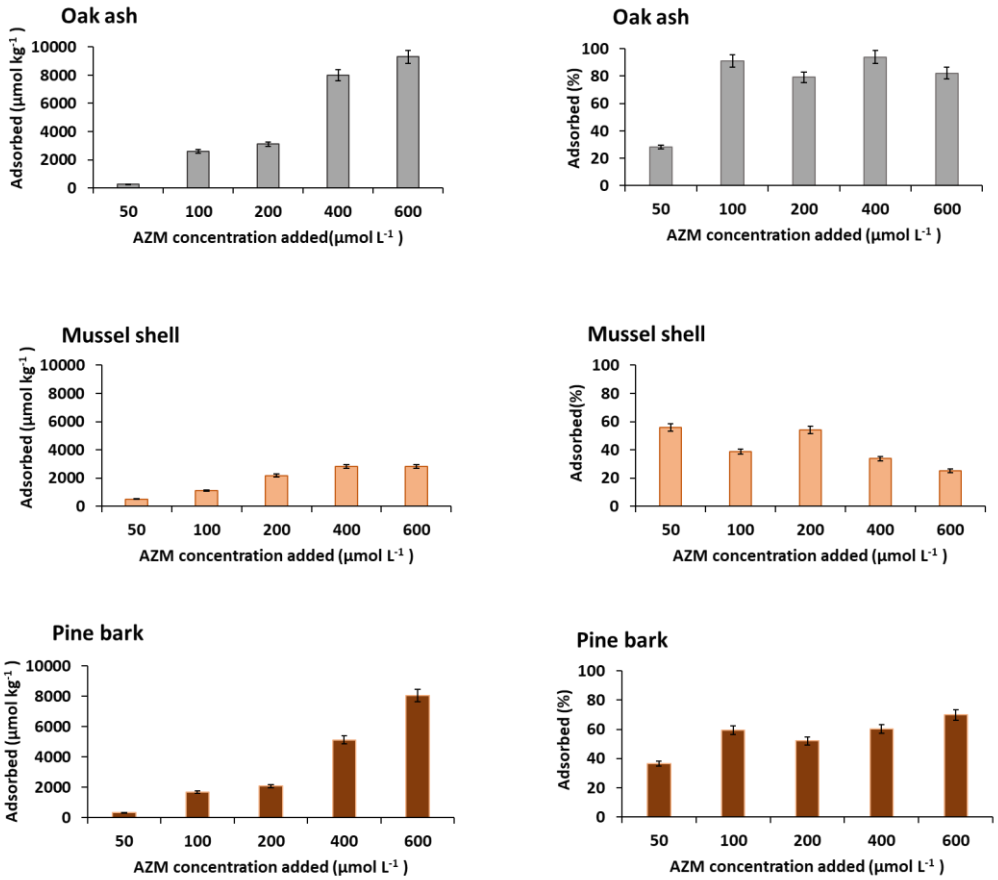

Figure S2. AZM adsorption (in $\mu mol\,kg^{-1}$ and percentage) on the three bio-adsorbents as a function of the concentration of antibiotic added. Average values, with error bars related to coefficients of variation always lower than 5%

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PUBLICACIÓN X

Cela-Dablanca, Raquel[a]., Míguez-González, Ainoa[a]., Barreiro, Ana[a]., Rodríguez-López, Lucía[b]., Arias-Estévez, Manuel[b]., Núñez-Delgado, Avelino[a]., Fernández-Sanjurjo, María J[a]., Castillo-Ramos, Ventura[c]., & Álvarez-Rodríguez, E[a]. (2024). **Simultaneous adsorption of amoxicillin and ciprofloxacin on agricultural soils and by-products used as bio-adsorbents: Unraveling the interactions in complex systems**. *Environmental Research*, *240*, 117535. https://doi.org/10.1016/j.envres.2023.117535

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Simultaneous adsorption of amoxicillin and ciprofloxacin on agricultural soils and by-products used as bio-adsorbents: Unraveling the interactions in complex systems

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ABSTRACT

The presence of pharmaceuticals in agricultural soils, like amoxicillin (AMX) and ciprofloxacin (CIP), poses a significant environmental challenge with potential implications for ecosystems and human well-being. This study explores the simultaneous adsorption of AMX and CIP on crop soils and bio-adsorbents, focusing on competitive adsorption dynamics. Tests were conducted with varying pharmaceutical concentrations in six soils and three bio-adsorbents. CIP consistently exhibited higher adsorption than AMX, particularly at higher concentrations. In the binary system, AMX's adsorption exceeded the individual system at higher concentrations, implying a synergistic effect. Bio-adsorbents, especially pine bark and oak ash, displayed superior adsorption capacities compared to soils. Some soils exhibited enhanced adsorption and retention of both antibiotics simultaneously, aligning with the cooperative adsorption model. Freundlich's adsorption model described the competitive adsorption systems well. These findings have implications for addressing antibiotic contamination in agricultural ecosystems, offering insights into complex interactions in soil environments amid rising pharmaceutical concerns.

1. Introduction

The extensive utilization of antibiotics in recent years has resulted in their dissemination and subsequent detection in water bodies and cultivated soils, posing a significant threat to both human health and ecosystems (Zhang et al., 2017). Given that up to 90% of these biocides are excreted from the body through feces and urine (due to not complete absorption in the intestine), they accumulate in farm solid excreta and effluents or get released into wastewaters and sludge generated from wastewater treatment plants (WWTP) after their use in human medicine (Russell and Yost, 2021)– (Rodriguez-Mozaz et al., 2020). Regrettably, most WWTPs are not adequately equipped to inactivate or remove antibiotics effectively, leading to their substantial release in effluents and accumulation in sludge (Gogoi et al., 2018), (Michael et al., 2013).

The incorporation of WWTP sludge and/or wastewater effluents into soil to enhance fertility can inadvertently introduce these pollutants into crop soils. This may result in the emergence of resistant bacteria, ecotoxicity, and the potential entry of antibiotics into the food chain through water and crops, classifying these compounds as emerging pollutants with adverse effects on human and animal health (Pan and Chu, 2016)– (Rahbar Shahrouzi et al., 2019).

Adsorption processes stand out as simple, cost-effective, highly efficient, eco-friendly, and renewable approaches to improve the retention of antibiotics in soils (Singh et al., 2020)– (Karoui et al., 2020). Employing adsorption to retain antibiotics in soils could present a viable and affordable solution to control the dispersion of these pollutants into the food chain. Other methods like advanced oxidation, hydrolysis, photodegradation and biodegradation have been explored as alternatives to adsorption. However, most of these processes generate toxic by-products, and lack sufficient reliability for antibiotic removal (Du and Liu, 2012), (Cheng et al., 2018).

The concurrent presence of amoxicillin and ciprofloxacin in crop

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soils holds considerable scientific importance due to its potential environmental ramifications and impact on agricultural systems. Once introduced into the soil, these antibiotics may exhibit prolonged persistence and interact with soil components, thereby exerting long-term effects on soil quality, microbial communities, and nutrient cycling processes (Grenni et al., 2018), (Rodríguez-González et al., 2022). Moreover, the coexistence of both antibiotics could give rise to synergistic or antagonistic effects, altering their adsorption behavior and mobility, and consequently influencing their bioavailability to plants and potential uptake by crops (Roose-Amsaleg et al., 2021). Understanding the simultaneous adsorption of amoxicillin and ciprofloxacin in crop soils is pivotal in comprehending their environmental fate and the associated risks of entering the food chain. This underscores the significance of employing sustainable agricultural practices to mitigate potential adverse effects on human and ecosystem health.

Drawing from this context and our prior research works (Míguez--González et al., 2023)– (Cela-Dablanca et al., 2022b), the primary aim of this study is to investigate the retention capacity of six crop soils, namely VO5, M1, FC, VP1, VP6, and VP7, along with three distinct bio-adsorbents materials (pine bark, mussel shell, and oak ash), concerning amoxicillin and ciprofloxacin adsorption. Additionally, we seek to explore the simultaneous adsorption behavior of both antibiotics within these systems. This comprehensive approach ensures a realistic representation of real-world scenarios, enabling an in-depth analysis of the intricate interactions among the pharmaceuticals, soils, and bio-adsorbents. Through elucidating the underlying mechanisms, this study significantly could contribute to the understanding of the functioning of such real systems and their potential implications.

2. Materials and methods

2.1. Screening and selection of soils samples and bio-adsorbents

The soil sampling procedure encompassed the collection of 60 distinct soil samples distributed across Galicia, a geographic area in northwestern Spain. Among these, 27 samples were derived from corn crops, and 33 samples originated from vineyard soils. Additionally, three samples were acquired from forest soils due to their differential organic matter content and pH values compared to crop soils, both of which play pivotal roles in adsorption/desorption processes (as depicted in Table S1, Supplementary Material).

The sampling process involved obtaining ten sub-samples from each plot within the topsoil layer (0–20 cm) using an Edelman probe in a zig-zag pattern. These ten sub-samples were combined to form a comprehensive and representative sample. Subsequently, the collected samples were transported to the laboratory and subjected to oven drying (at a specific temperature) until a constant weight was achieved. Following this, the samples were sieved to a 2-mm size and properly stored.

From the initial pool of 60 soil samples, a subset of six soils was selected based on their respective pH and organic matter contents. These soils were designated as M1, VO5, VP1, VP6, VP7, and FC.

The study also employed biosorbents sourced from the forestry and agro-food industry. Specifically, phyto mass ash was obtained from oak log combustion at a combustion boiler in Lugo, Spain. The uncalcined mussel shell, with a particle size smaller than 1 mm, was provided by Abonomar S.L. on Isla de Arousa, Pontevedra, Spain. Lastly, the Geolia trademark pine bark from Madrid, Spain, was crushed and sieved to a particle size of 0.63 mm.

2.2. Chemicals and reagents

The antibiotic ciprofloxacin (CIP) was of 98% purity while amoxicillin trihydrate (AMX) was of >95% purity, all of them supplied by Sigma-Aldrich (Madrid, Spain). All the reagents needed for the quantification of the antibiotics were of HPLC grade, provided by Sigma-Aldrich (Madrid, Spain). A Millipore system (Madrid, Spain) was used to provide ultrapure water.

2.3. Characterizations of soils and bio-adsorbents

The granulometry of the soils was determined following the Robinson International Pipette Method (Guitian Ojea and Carballas, 1976), which allowed for the separation of sand, silt, and clay fractions. The soil textures were then classified using the Soil Taxonomy approach (Baillie, 2001).

The pH measurement was conducted in water and KCl solutions using a soil-to-liquid ratio of 1:2.5 (Guitian Ojea and Carballas, 1976). The pH readings were performed using a CRISON model 2001 pH-meter (Spain), with a contact time of 10 min for water and 2 h for KCl. To analyze organic matter (OM) and nitrogen contents, elemental analysis was employed, utilizing a TRUSPEC CHNS equipment from LECO model (USA). For the determination of exchange cations, a 1M NH_4Cl extract was utilized, with a soil-to-solution ratio of 1:10, and the extraction process was conducted over 12 h (Peech, 1947). Atomic adsorption/emission spectrophotometry was employed, with the addition of 1% $LaCl_3$ to prevent any potential interferences. The elements Al, Ca, Mg, Na, and K were quantified through this method. To assess the effective cation exchange capacity (eECE), the sum of the exchange cations was calculated (Fox and Kamprath, 1970). For the non-crystalline iron (Fe_{ox}) and aluminum (Al_{ox}) fractions, an extraction procedure was carried out using an ammonium oxalate buffered solution at pH = 3 (v Blackmore, 1978). Additionally, the fractions bound to organic matter (Fe_{pir} and Al_{pir}) were obtained using a sodium pyrophosphate solution at pH = 10 (Bascomb, 1968). The quantification of these fractions was performed using atomic absorption spectrophotometry. Tables S1 and S2 (Supplementary Material) provide an overview of the characteristic physicochemical parameters of the six soils and three bio-adsorbents studied.

2.4. Simple and binary adsorption/desorption tests and antibiotics quantification

Batch experiments were conducted in simple and binary systems, each containing either one of the antibiotics (amoxicillin/ciprofloxacin) or both and were exposed to five different concentrations of the respective antibiotics (ranging from 0 to 400 μmol L^{-1}). To initiate the tests, 4 g of the soils (0.5 g in the case of bio-adsorbents) were mixed with 10 mL of a 0.005 M $CaCl_2$ solution, which served as the background electrolyte to maintain a constant ionic strength. The antibiotic concentrations were varied independently for each individual antibiotic. The mixture was stirred for 48 h, a duration determined based on prior kinetics experiments, to ensure that equilibrium was reached (Rodríguez-López et al., 2022), (Cela-Dablanca et al., 2022b). Following the adsorption phase, the samples were subjected to centrifugation at 4000 rpm for 15 min, and the resulting supernatants were filtered through 0.45 μm nylon-type syringe filters.

The quantification of antibiotic concentrations was performed using High-Performance Liquid Chromatography (HPLC) with a Thermo-Fisher Model LPG 3400 SD equipment (USA) coupled with a HPG-3400 quaternary pump (USA), a WPS3000 autosampler (USA), a thermostated compartment for the TM-120 column, and an ultraviolet–visible detector of the UltiMate 3000 series (USA). Data processing was facilitated using the Chromeleon software, and a Luna C18 column (150 mm long; 4.6 mm internal diameter; 5 μm particle size) provided by Phenomenenex (Madrid, Spain) was used, along with a pre-column (4 mm long; 2 mm internal diameter; 5 μm particle size) packed with the same material as the column. The injection volume was set at 50 μL, and the flow rate was maintained at 1.5 mL min^{-1}. Regarding the mobile phase, acetonitrile (phase A) and 0.01 M phosphoric acid at pH = 2 (phase B) were used for amoxicillin, while for ciprofloxacin, the phases remained the same, but with a different linear gradient, varying from 5% to 32% of phase A and from 95% to 68% of phase B over 10.5 min. The wavelengths used for quantification were

212 nm for CIP and 230 nm for AMX.

After the adsorption step, desorption tests were conducted. The remaining material post-adsorption was weighed, and 10 mL of 0.005 M $CaCl_2$ was added. The samples were stirred for 48 h, followed by centrifugation and filtration using the same conditions as in the adsorption process. The concentration of the corresponding antibiotic in the equilibrium solution was then determined using a similar procedure as that used in the adsorption tests.

2.5. Freundlich's adsorption modelling for simple and binary systems

To describe the individual adsorption process of each antibiotic, the Freundlich model was used (eq. (1)).

$$q_e = K_f C_e^n \quad (1)$$

where q_e (μmol kg^{-1}) is the amount of antibiotic adsorbed per unit mass of adsorbent (difference between the added amount and the amount remaining at equilibrium); C_e (μmol L^{-1}) is the equilibrium concentration of the antibiotic in the solution, K_f (L^n kg^{-1} μmol^{1-n}) is the Freundlich constant related to the adsorption capacity, and n (dimensionless) is a Freundlich parameter related to the adsorption intensity (Sukul et al., 2008).

Furthermore, to study a possible competition between antibiotics for adsorption sites, the Freundlich equation was adapted (eq. (2)) following the literature (Russell and Yost, 2021), focusing on the total amount adsorbed for both antibiotics (eq. (2)).

$$(Q_{aCIP} + Q_{aAMX}) = K_F \left(C_{eqCIP} + C_{eqAMX}\right)^n \quad (2)$$

where Q_a is the individual amount adsorbed for each antibiotic; C_{eq} is the equilibrium concentration of each antibiotic; K_F and n are the parameters mentioned above.

3. Results and discussion

3.1. Adsorption of amoxicillin and ciprofloxacin in a binary system by different soils and bio-adsorbents

Fig. S1 (Supplementary Material) depicts the adsorption capacity (in μmol kg^{-1}) of AMX and CIP in individual and binary systems of the six soils under investigation, while Fig. S2 (Supplementary Material) represents the adsorbed amount as a percentage.

The addition of the two or even all three lower concentrations (25, 50, and 100 μmol L^{-1}) generally does not show significant differences in the adsorbed amount, neither between the two antibiotics (AMX and CIP) nor between the two systems (individual and binary) for each antibiotic (Fig. S1, Supplementary Material). This behavior changes for the two higher concentrations added (200 and 400 μmol L^{-1}), where generally a higher adsorption of CIP than AMX is observed in both systems. Furthermore, in the case of AMX, there is a higher retention in the binary compared to the individual system (Fig. S1, Supplementary Material).

By expressing the data as a percentage of adsorption relative to the amount added, it is generally observed that the adsorption rate of CIP is higher than that of AMX in both systems, especially when the two higher concentrations are added. In the case of AMX, the adsorption rate is higher in the binary than in the individual system, particularly at the two higher concentrations. However, this rate decreases as the added concentrations increase, particularly in the individual system. As for CIP, the behavior is less defined. Some soils, such as M1, adsorb almost all of the added concentrations in both systems, while others, as VO5, show an increasing adsorption percentage with the added concentration. Soils such as FC and VP6 exhibit higher adsorption rates in the binary system (between 80% and 100%) than in the individual system (between 40% and 100%), with the percentage increasing as the added concentration increases. Finally, soils like VP1 and VP7 show higher adsorption in the individual than in the binary system, with the former adsorbing 100% of the added concentrations, while the latter shows an increasing retention with concentration, reaching 100% for the two highest doses (Fig. S2, Supplementary Material).

The higher adsorption of CIP compared to AMX in both the individual and binary systems (Fig. S1, Supplementary Material) can be related to the fact that CIP has various functional groups (hydroxyl, carboxyl, and fluoride) that play an important role in the adsorption process (Cukierman et al., 2019). These groups are found in cationic or zwitterionic form within the pH range of the studied soils (Genç et al., 2013), electrostatically interacting with negatively charged soil components. The non-crystalline components, which have a high presence in the studied soils, contribute to the strong affinity of these substances. In contrast, AMX exists in zwitterionic or anionic form (Homsirikamol et al., 2016), resulting in less favorable electrostatic interactions with soil components. Several authors have also reported higher adsorption of CIP compared to AMX (Githinji et al., 2011), (Chandrasekaran et al., 2020).

The fact that AMX is generally more adsorbed in the presence of CIP becomes more noticeable at higher concentrations, especially at 400 μmol L^{-1}, where AMX adsorption in the binary system can increase up to 2.7 times in soils M1 and VP7, and between 50% and 75% in the rest of the soils, except for VP1. This synergistic relation in the binary system, favoring AMX adsorption in the presence of CIP, is more significant in soils M1 and VP7, which have higher pH values (8.02 and 7.27, respectively). At these pH values, AMX acts as an anionic species with deprotonated amino and carboxyl groups, while CIP exists as a zwitterionic species, allowing for electrostatic attractions between the positively charged groups of CIP and the anionic AMX. This suggests that the presence of CIP adsorbed in the soils enhances the incorporation of AMX onto the adsorbent. Due to the high amount of organic matter in both soils (8.58% for M1 and 6.14% for VP7), which carries a negative charge at basic pH values, electrostatic repulsions between AMX and the soils are expected in the individual system, leading to the adsorption of a certain amount of the antibiotic possibly through cationic bridges due to the high amounts of exchangeable calcium in these soils (De Oliveira et al., 2018) (39.44 μmol kg^{-1} for M1 and 10.53 μmol kg^{-1} for VP7). The high affinity of soil M1 for adsorbing CIP at all concentrations promotes the incorporation of AMX in the binary system, resulting in AMX adsorptions also approaching 100%. Thus, a ternary complex of soil-CIP-AMX is formed, where CIP provides positive charges for the binding of the anionic AMX. This synergistic phenomenon between antibiotics has been described by other authors in the binary adsorption of tetracycline and sulfadiazine (Cela-Dablanca et al., 2022c).

Regarding CIP, the adsorption in the binary system shows a different behavior compared to the individual system, depending on the concentrations added. For the two highest concentrations, no differences are observed between the two systems (Fig. S2, Supplementary Material), indicating that AMX has no influence on the adsorption of CIP. On the other hand, at the two lower concentrations, two different behaviors are observed. In soils FC and VP6, there is higher adsorption of CIP in the binary system compared to the individual system, and it is also higher than that of AMX. However, in soils VP1 and VP7, the adsorption is higher in the individual system, and in the binary system, it is lower than that of AMX (Fig. S2, Supplementary Material). Therefore, at low concentrations, AMX sometimes has a synergistic effect on CIP adsorption, while in other cases, it has an antagonistic effect. Some authors have found that at low concentrations, CIP is more adsorbed in individual systems than in binary systems, indicating that it cannot efficiently compete with AMX. Conversely, at high concentrations, greater adsorption in the binary system than in the individual system has been observed, which is interpreted as a decrease in mass transfer resistance for the adsorption of CIP species in this binary system (Manjunath et al., 2020).

Fig. S3 (Supplementary Material) shows the adsorption of CIP and AMX by the bio-adsorbents in both the individual and binary systems, in

terms of μmol kg^{-1} and as a percentage. It is worth noting that all three bio-adsorbents used exhibit much higher adsorption capacities than the soils in the present study (Figs. S1 and S3, Supplementary Material). It can be observed that, generally, both in the individual and binary systems, mussel shell and especially pine bark adsorb more CIP than AMX, while oak ash shows the opposite trend (Fig. S3, Supplementary Material). Therefore, oak ash is an excellent adsorbent for AMX, and pine bark is efficient for CIP in both systems. Expressing the data as percentage values, it can be observed that the adsorption of CIP, both in binary and single systems, is 100% onto pine bark. In mussel shell and oak ash, similar adsorptions of CIP are obtained, while AMX is adsorbed more than CIP only onto oak ash.

Comparing the single and binary systems, different behaviors are observed depending on the antibiotic and bio-adsorbent used. In the case of AMX, it is found that in oak ash, at low concentrations added, the adsorption is higher in the single than in the binary system, indicating that CIP competes favorably with AMX under these conditions. However, at higher concentrations, the adsorption is similar in both systems. In contrast, in pine bark and especially in mussel shell, the effect of CIP on AMX is synergistic (Fig. S3, Supplementary Material).

Chandrasekaran et al. (2020) found that the adsorption of AMX by *Prosopis juliflora*, in a binary system AMX + CIP, was lower than in the single system, indicating that CIP has antagonistic/competitive effects on the adsorption of AMX, as observed in the case of the oak ash used in the current research. This may be due to both antibiotics being negatively charged at the pH of oak ash, leading to the most probable mechanism being cationic bridges established by the abundant exchangeable calcium with the soil components, as mentioned before. This suggests that CIP has a higher affinity than AMX for forming cationic bridges.

Regarding the synergistic effect of CIP on AMX observed in pine bark and especially in mussel shell, this can be related to the cooperative adsorption model proposed by several authors (Ringot et al., 2007), (Foo and Hameed, 2010). According to this model, when a solute is retained by a site on a homogeneous adsorbent surface, it can influence the consecutive active sites of that surface, promoting new adsorptions and stronger retentions.

As for CIP, the presence of AMX generally affects its adsorption very little in oak ash and pine bark, while in mussel shell, it decreases the adsorption in the binary system (at high concentrations), indicating that in this case, AMX competes with CIP (Fig. S3, Supplementary Material).

3.2. Adsorption isotherms in the amoxicillin-ciprofloxacin binary system for soils and bio-adsorbents

Table 1 presents the values obtained after applying the Freundlich model for the binary system of AMX with CIP for each soil.

Fig. 1 shows the relation between equilibrium concentration (μmol L^{-1}) and the adsorbed amount of AMX and CIP (μmol kg^{-1}) by the different soils used. The data is fitted to the Freundlich model.

As can be observed in Table 1, the Freundlich model for the binary system AMX + CIP fits well, with high R^2 values. Therefore, as mentioned earlier, the Freundlich model can be satisfactorily adapted to describe these competitive adsorption systems.

The obtained K_F values for the binary system AMX + CIP ranged from 1.12 to 129.80 Ln kg^{-1} μmol^{1-n} (Table 1), which are lower than those found in a previous study for the binary adsorption of tetracycline + sulfonamide (ranging from 34.30 to 1130.45 Ln kg^{-1} μmol^{1-n}) in six cultivation soils (Cela-Dablanca et al., 2020). In the binary system of AMX + CIP, the K_F values were higher than for the individual systems of each antibiotic in FC, VP6, and M1 soils, indicating that the simultaneous presence of both antibiotics favors their adsorption onto these three soils, probably due to the previously described synergistic effects.

Regarding the parameter n for the binary system, the VP7 soil presents a value of 1.79 (the highest among all the studied soils), which indicates chemical adsorption between the simultaneously present antibiotics and the soil. If we compare the value of n in the AMX + CIP system for this soil with the respective values of the individual systems, it can be appreciated that the presence of both antibiotics may cause modifications in the adsorption mechanisms with the soil surface (Cela-Dablanca et al., 2020). Other authors found values of $n > 1$ for binary adsorption systems of chlortetracycline + sulfonamide, suggesting strong interactions between the antibiotics and the adsorption sites of the adsorbents (Jiang et al., 2020). For the rest of the soils of the current research, the n values were close to 1 (linear adsorption, Fig. 1) or less than 1, indicating the presence of heterogeneous adsorption surfaces, with the highest energy sites being occupied first.

Table 1
Freundlich's model adsorption parameters for the binary system CIP + AMX and simple systems in soils (K_F, in L^n kg^{-1} μ mol^{1-n}, and n, dimensionless); -: error value too high for fitting.

Soil	Antibiotic	Freundlich				
		K_F	Error	n	Error	R2
VO5	CIP	–	–	–	–	–
	AMX	–	–	0.629	0.199	0.868
	CIP + AMX	**8.777**	**5.046**	**0.658**	**0.112**	**0.975**
FC	CIP	0.00004	0	5.798	1.360	0.854
	AMX	9.339	5.516	0.739	0.108	0.969
	CIP + AMX	**29.431**	**13.411**	**0.77**	**0.093**	**0.982**
VP6	CIP	–	–	–	–	–
	AMX	0.000004	0	3.274	1.869	0.862
	CIP + AMX	**4.564**	**3.335**	**1.042**	**0.138**	**0.979**
M1	CIP	–	–	–	–	–
	AMX	–	–	1.072	0.541	0.783
	CIP + AMX	**129.796**	**33.558**	**0.684**	**0.071**	**0.979**
VP1	CIP	–	–	–	–	–
	AMX	–	–	0.758	0.219	0.872
	CIP + AMX	**6.731**	**2.209**	**1.03**	**0.065**	**0.994**
VP7	CIP	–	–	–	–	–
	AMX	12.222	17.619	0.795	0.282	0.872
	CIP + AMX	**1.12**	**0.826**	**1.79**	**0.183**	**0.981**

In Fig. 2, the relation between equilibrium concentration (μmol L^{-1}) and the adsorbed amount of AMX and CIP (μmol kg^{-1}) by the different bio-adsorbents used is depicted, both real and according to the Freundlich model.

As can be seen in Table 2, the R^2 values in all cases exceed 0.97, indicating a good fit of the AMX and CIP adsorption in the binary system to the Freundlich model for all three bio-adsorbents.

The values of the Freundlich affinity coefficient (K_F), related to the adsorption capacity of the adsorbent, range from 56.746 to 83.643 L^n μmol^{1-n} kg^{-1} for the binary adsorptions. The significantly higher values are obtained in oak ash and pine bark, which align with their high adsorption capacity. Other authors found K_F values for the binary adsorption of tetracycline + sulfonamide on these same bio-adsorbents between 35.15 and 123.29 L^n μmol^{1-n} (Cela-Dablanca et al., 2020), higher than those in the present study.

Regarding the adsorption intensity (n), it takes values below 1 for pine bark, indicating the presence of heterogeneous adsorption surfaces and high-energy adsorption sites, which are occupied first (Sukul et al., 2008). In the case of mussel shell and oak ash, n takes values of 1.657 and 1.128, respectively, suggesting a favorable intensity model for adsorption as the added concentration of antibiotics increases (Khanifari et al., 2023). Studies conducted by Cela-Dablanca et al. (2020) regarding the adsorption of tetracycline with sulfadiazine by the bio-adsorbents used in this study also reported values of n greater than 1 for oak ash.

3.3. Binary desorption of amoxicillin and ciprofloxacin by different soils and bio-adsorbents

Fig. 3 displays the desorption, in μmol kg^{-1}, in both the single and

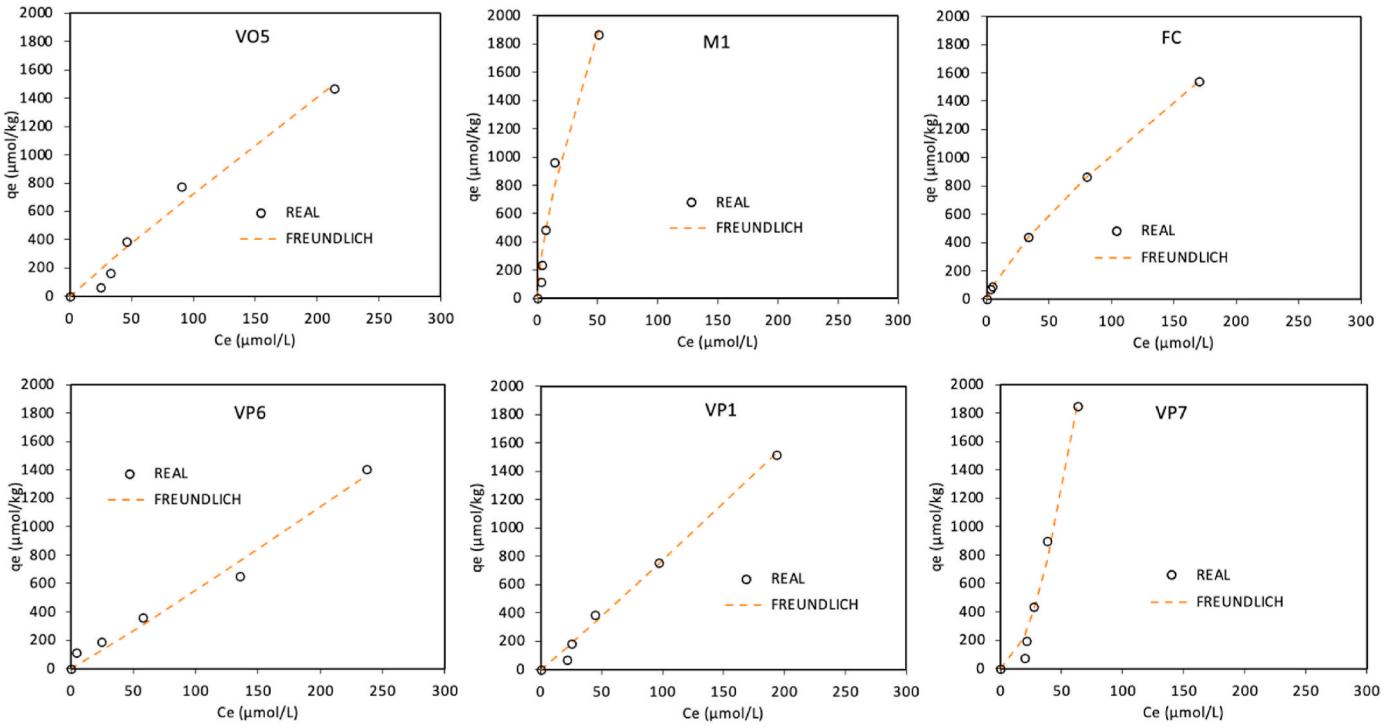

Fig. 1. Adsorption curves for AMX and CIP in the binary system onto the studied soils, both real and according to the Freundlich model.

Table 2
Freundlich's model adsorption parameters for the binary system CIP + AMX and simple systems in bio-adsorbents (K_F, in $L^n\ kg^{-1}\ \mu\ mol^{1-n}$, and n, dimensionless); -: error values too high for fitting.

Bio-adsorbent	Antibiotic	Freundlich				
		K_F	Error	n	Error	R2
Ash	CIP	–		0.81	0.36	0.73
	AMX	1824.031	685.92	0.58	0.14	0.94
	CIP + AMX	**56.746**	**31.953**	**1.128**	**0.129**	**0.979**
Shell	CIP	–	–	1.47	0.42	0.81
	AMX	2.767 10^{-6}	0	3.22	2.00	0.69
	CIP + AMX	**-**	**-**	**1.657**	**0.284**	**0.971**
Pine bark	CIP	–	–	–	–	–
	AMX	–	–	0.62	0.38	0.58
	CIP + AMX	**83.643**	**25.958**	**0.843**	**0.057**	**0.992**

binary systems of AMX and CIP by the soils used in this study. In Fig. 4, these results are expressed as percentage.

In general, it can be observed that the desorption rate of CIP and, especially, AMX is higher in the individual system than in the binary system, particularly at lower added concentrations (Fig. 4). CIP desorbs more than AMX at lower concentrations, while the opposite occurs at higher concentrations. Previously, Chandrasekaran et al. (2020) found higher desorption of CIP than AMX in *Prosopis juliflora*.

Looking at the binary system, it can be noted that the desorption percentage for AMX is always below 10% in all soils, being particularly low from M1, FC, and VP6 soils. These soils also retain CIP very strongly, as its desorption in the binary system is very low (<5%) for all added concentrations, while in the other three soils (VO5, VP1, and VP7), there is significant desorption when the two lower concentrations are added (up to 100% of the adsorbed), being slightly higher than in the single system (Fig. 4). Therefore, particularly in M1, FC, and VP6 soils, the simultaneous presence of both antibiotics not only has a synergistic effect on increasing the adsorption of both pollutants, but it also enhances their retention strength, resulting in less release into the environment. This can be explained by the cooperative adsorption model proposed by several authors (Ringot et al., 2007), (Foo and Hameed, 2010) as

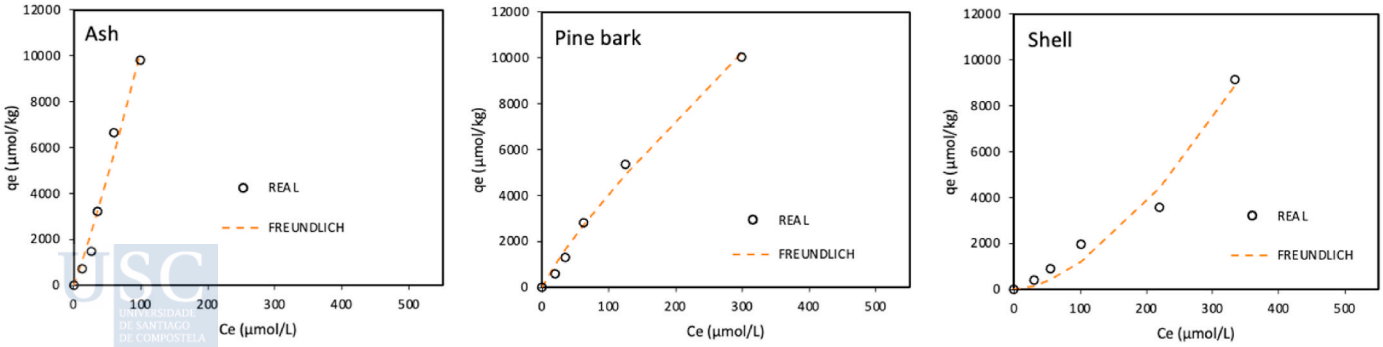

Fig. 2. Adsorption of AMX and CIP in the binary system in bio-adsorbents, real and according to the Freundlich model.

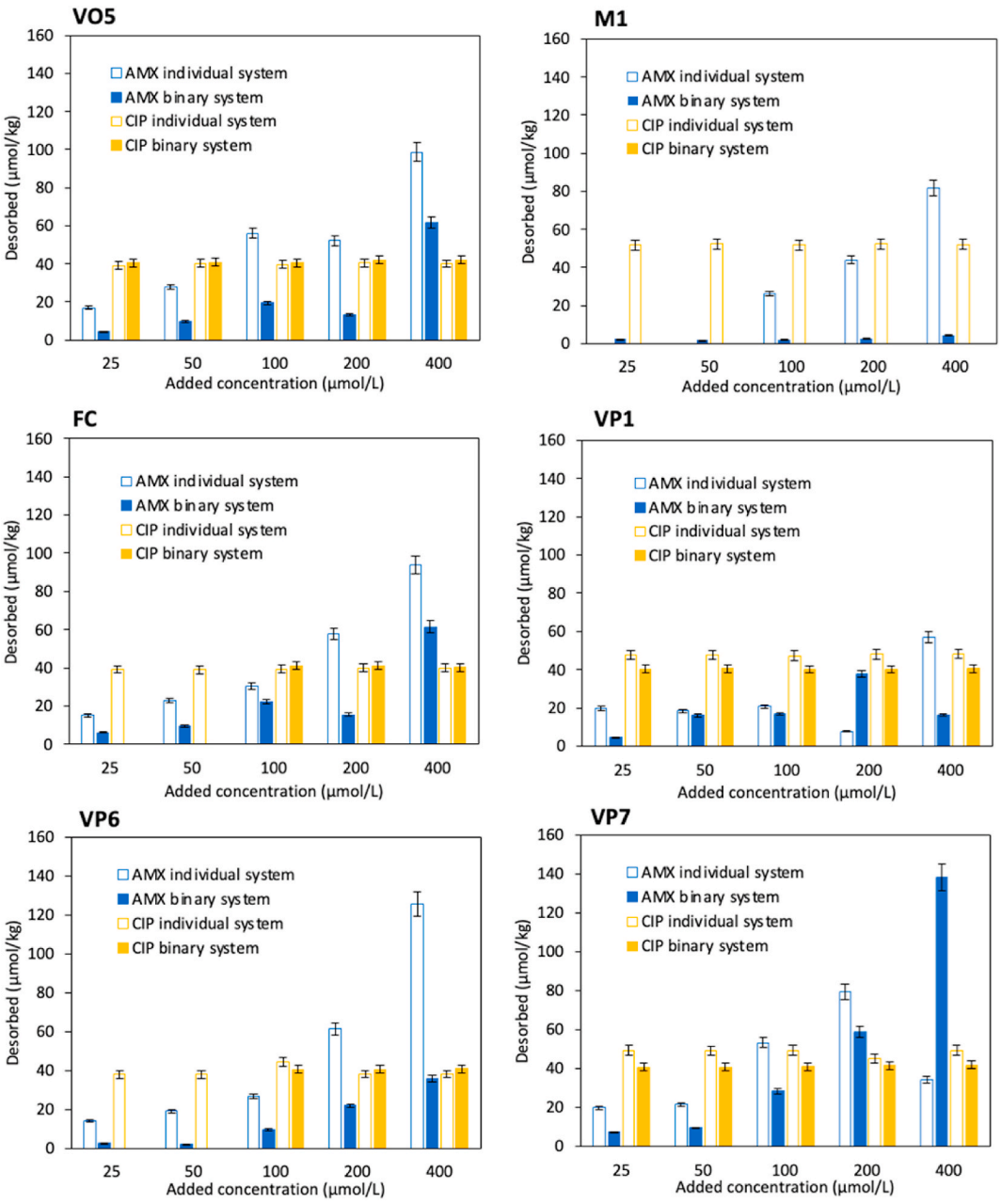

Fig. 3. Simple/binary desorption of amoxicillin and ciprofloxacin as µ mol/kg from the six soils of study.

mentioned before, which assumes that when a solute is retained by a site on an adsorbent surface, it can influence consecutive active sites on that surface, promoting new adsorptions and stronger retentions.

Regarding the bio-adsorbents, the desorption of AMX and CIP in the single and binary systems is shown in Fig. 5, expressed in µmol kg^{-1} and as a desorption percentage.

It can be observed that the highest desorption rate generally occurs for the lowest added concentrations, both in the individual and binary systems (Fig. 5). The behavior is different for each antibiotic and each bio-adsorbent, similar to what happens in adsorption. For instance, in the case of AMX, oak ash, which had the highest retention capacity, exhibits very low desorption, negligible at high concentrations. Thus, this by-product is capable of irreversibly retaining a significant amount of AMX. The other two bio-adsorbents have low retention in both systems (less than 60% in mussel shell and less than 30% in pine bark), and the release at low concentrations can reach 70% in mussel shell in the individual system, and close to 100% in pine bark in the binary system.

Regarding CIP, when low concentrations are added, the desorption is very high in the single system for all three bio-adsorbents (up to 100% of the adsorbed), and it is also high in the binary system for oak ash and mussel shell (Fig. 5). Pine bark, which showed 100% retention in both systems and for any concentration, retains this antibiotic strongly in binary systems, while in the individual system, desorption is high, especially at the lowest added concentrations (Fig. 5). Once again, the cooperative adsorption model can explain the stronger adsorptions both in the binary system and when increasing the antibiotic concentrations (Ringot et al., 2007), (Foo and Hameed, 2010).

In studies conducted by Cela-Dablanca (Cela-Dablanca et al., 2020) on the same bio-adsorbents with tetracycline and sulfadiazine, it was found that pine bark did not desorb tetracycline but did desorb sulfadiazine in the binary system, similar to what occurs in this case where it does not desorb CIP but does desorb AMX. The high retention of CIP by pine bark could be attributed to its high organic matter content, with an abundance of adsorption sites with a high affinity for CIP compounds. As

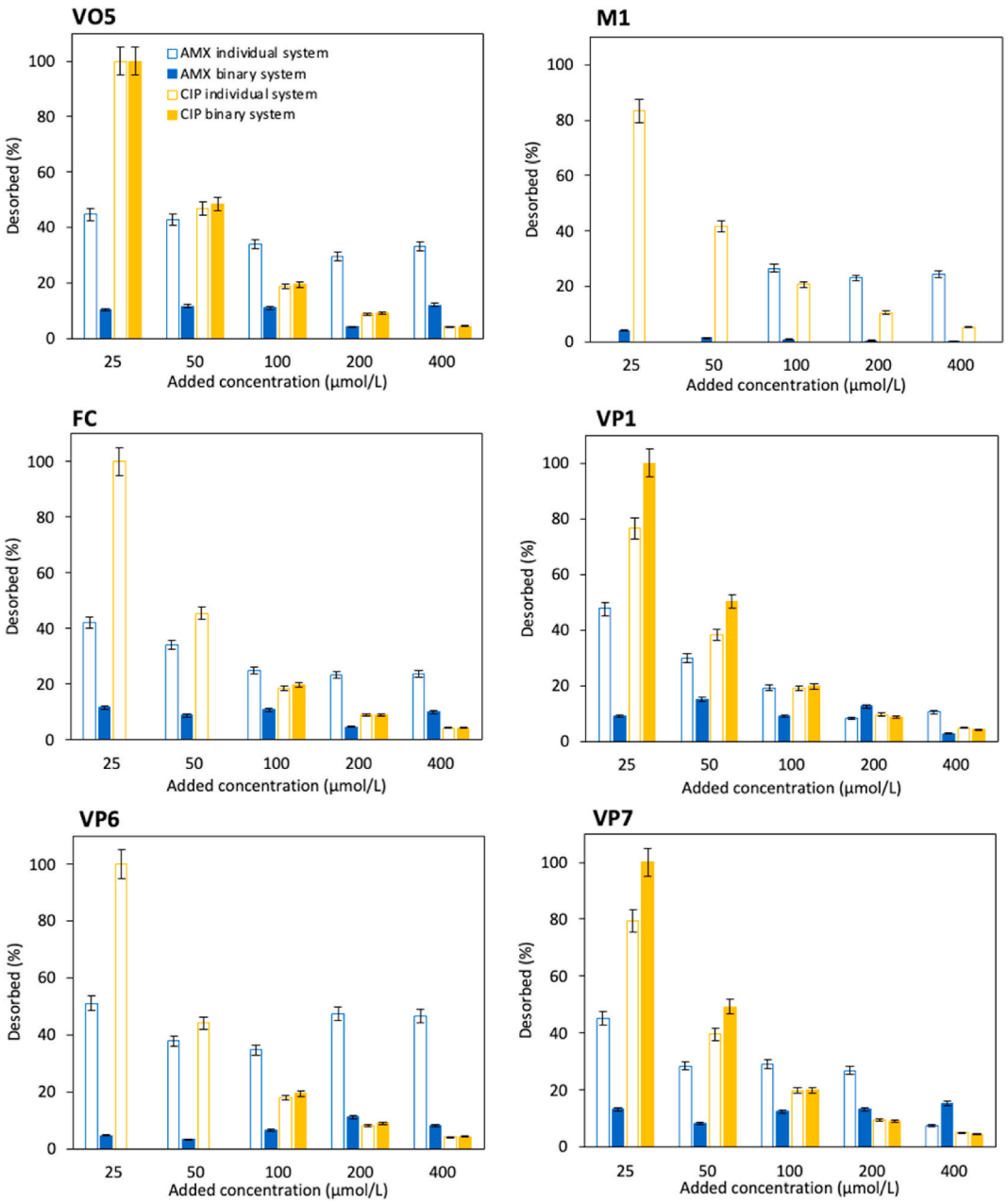

Fig. 4. Simple/binary desorption of amoxicillin and ciprofloxacin as percentages from the six soils of study.

for mussel shell, it can be seen that it is the bio-adsorbent that exhibits the highest CIP desorption and some AMX desorption, besides being the bio-adsorbent with the lowest adsorptions of these antibiotics. These results are comparable to those obtained by Conde-Cid et al. (2019) in a binary system of chlortetracycline + tetracycline and chlortetracycline + oxytetracycline. These authors indicate that mussel shell is the by-product that presented the highest desorption percentages in the binary system, being also the bio-adsorbent with the lowest adsorption capacity for these antibiotics. In the same study, Conde Cid et al. (Conde-Cid et al., 2019) also mention that oak ash has a very good retention capacity for the three aforementioned tetracyclines, as it barely showed desorption for these antibiotics, similar to what happens in this case with AMX.

4. Conclusions

The adsorption capacity of the antibiotics AMX and CIP in individual and binary systems exhibits variations based on the concentration of antibiotics and the specific soil or bio-adsorbent being examined. Notably, CIP demonstrates consistently higher adsorption than AMX in both individual and binary systems, particularly at elevated concentrations of the pollutant. In the binary system, AMX's adsorption surpasses that of the individual system at higher concentrations, especially in soils with higher pH levels, implying a synergistic effect for AMX and CIP that enhances AMX adsorption. The presence of CIP in the binary system shows either synergistic or antagonistic behavior on AMX adsorption, depending on the soil type and antibiotic concentrations. Among the bio-adsorbents, oak ash and pine bark stand out for their superior adsorption capacities for CIP compared to AMX, both in individual and binary systems. Conversely, AMX and CIP desorption tends to be higher in the individual than in the binary system, particularly at lower antibiotic concentrations. The bio-adsorbents also exhibit varying degrees of desorption for AMX and CIP in both individual and binary systems, with pine bark displaying the highest retention capability and mussel shell

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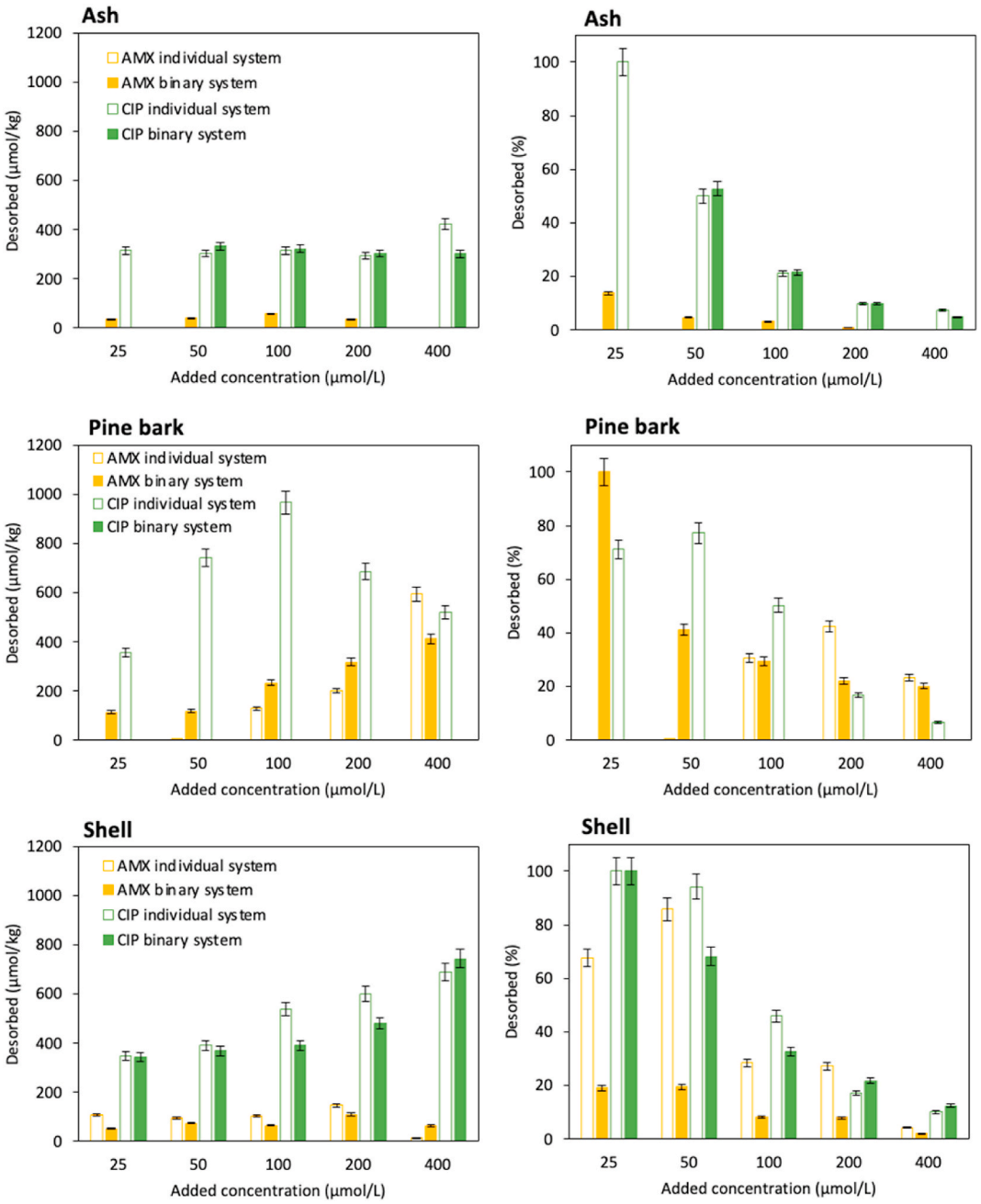

Fig. 5. Simple/binary desorption of amoxicillin and ciprofloxacin in µ mol/kg (left) and as percentages (right) from the three bio-adsorbents used.

exhibiting the highest desorption. The application of the Freundlich model provided satisfactory descriptions for the competitive adsorption systems of AMX and CIP in the investigated soils and bio-adsorbents. The findings highlight the potential influence of the simultaneous presence of multiple antibiotics on their adsorption mechanisms and retention strength in the examined environmental contexts. Consequently, considering the coexistence of multiple antibiotics in environmental systems becomes essential for a comprehensive understanding of their fate and potential impact on the environment.

Author contribution statement

Raquel Cela-Dablanca: Data curation, Formal análisis, Investigation, Methodology, Software. Ainoa Míguez-González: Conceptualization, Data curation, Investigation, Methodology, Software, Roles/Writing – original draft, Writing – review & editing. Ana Barreiro: Formal análisis, Investigation, Methodology, Validation, Visualization. Lucia Rodríguez-López: Formal análisis, Investigation, Resources, Validation, Visualization. Manuel Arias-Estévez: Conceptualization, Project administration, Resources, Supervision, Validation. Avelino Núñez-Delgado: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. María J. Fernández-Sanjurjo: Formal análisis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing. Ventura Castillo-Ramos: Conceptualization, Data curation, Formal analysis, Methodology, Roles/Writing – original draft, Writing – review & editing. Esperanza Álvarez-Rodríguez: Conceptualization, Data curation, Formal análisis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.117535.

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Supplementary Material

Simultaneous Adsorption of Amoxicillin and Ciprofloxacin on Agricultural Soils and By-products Used as Bio-adsorbents: Unraveling the Interactions in Complex Systems

Raquel Cela-Dablanca[1], Ainoa Míguez-González[1], Ana Barreiro[1], Lucia Rodríguez-López[2], Manuel Arias-Estévez[2], Avelino Núñez-Delgado[1], María J. Fernández-Sanjurjo[1], Ventura Castillo-Ramos[3,*], Esperanza Álvarez-Rodríguez[1]

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Table S1. Physicochemical properties of the crop soils

Parameter	Unit	Soils					
		M1	VO5	VP1	VP6	VP7	FC
Sand	%	49.57	49.57	68.60	67.42	67.50	69.35
Silt	%	27.93	26.29	17.17	10.14	20.29	15.78
Clay	%	22.50	24.14	14.23	22.43	12.22	14.86
OM	%	8.58	3.06	6.36	4.19	6.14	12.32
N	%	0.330	0.097	0.260	0.138	0.218	0.495
C	%	4.98	1.77	3.69	2.43	3.56	7.15
C/N		15.09	18.25	14.19	17.61	16.33	14.44
pH $_{(H2O)}$		8.0	6.0	5.6	7.1	7.3	4.7
pH $_{(KCl)}$		7.8	4.8	4.9	6.5	6.5	4.1
eCEC	cmolc kg^{-1}	42.81	7.42	19.52	9.09	12.32	7.48
Ca_e	cmolc kg^{-1}	39.44	4.94	4.74	8.11	10.53	0.18
Mg_e	cmolc kg^{-1}	2.07	0.86	1.07	0.51	1.04	0.07
Na_e	cmolc kg^{-1}	0.60	0.16	1,64	0.08	0.44	0
K_e	cmolc kg^{-1}	0.69	0.69	11.91	0.37	0.27	0.22
Al_e	cmolc kg^{-1}	0.01	0.77	0.16	0.02	0.14	7.01
Fe_{ox}	mg kg^{-1}	4140	1790	3045	1720	1945	3055
Fe_{pir}	mg kg^{-1}	1501	1171	2353	1225	823	6789
Al_{ox}	mg kg^{-1}	5351	1556	5739	3981	4576	5619
Al_{pir}	mg kg^{-1}	2111	1141	4543	1703	1597	9457

OM: Organic Matter; N: total nitrogen; C: total carbon; C/N: carbon to nitrogen ratio; eCEC: effective cation exchange capacity; Ca_e, Mg_e, Na_e, K_e, Al_e: Exchangeable cations; Al_{ox}, Fe_{ox}: Al and Fe extracted with ammonium oxalate; Al_{pir}, Fe_{pir}: Al and Fe extracted with sodium pyrophosphate

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Table S2. Physicochemical properties of the bio-adsorbents

Parameter	Unit	Bio-adsorbents		
		Oak ash	Mussel shell	Pine bark
OM	%	22.76	19.66	83.76
N	%	0.22	0.21	0.08
C	%	13.23	11.43	48.70
C/N		60.14	55.65	608.75
pH $_{(H2O)}$		11.31	9.39	3.99
pH $_{(KCl)}$		13.48	9.04	3.42
eCEC	cmolc kg^{-1}	361.15	30.25	14.92
Ca_e	cmolc kg^{-1}	95.00	24.75	5.38
Mg_e	cmolc kg^{-1}	3.26	0.72	2.70
Na_e	cmolc kg^{-1}	12.17	4.37	0.46
K_e	cmolc kg^{-1}	250.65	0.38	4.60
Al_e	cmolc kg^{-1}	0.07	0.03	1.78
Fe_{ox}	mg kg^{-1}	4233	171	74
Al_{ox}	mg kg^{-1}	8328	178	315

OM: Organic Matter; N: total nitrogen; C: total carbon; C/N: carbon to nitrogen ratio; eCEC: effective cation exchange capacity; Ca_e, Mg_e, Na_e, K_e, Al_e: Exchangeable cations; Al_{ox}, Fe_{ox}: Al and Fe extracted with ammonium oxalate

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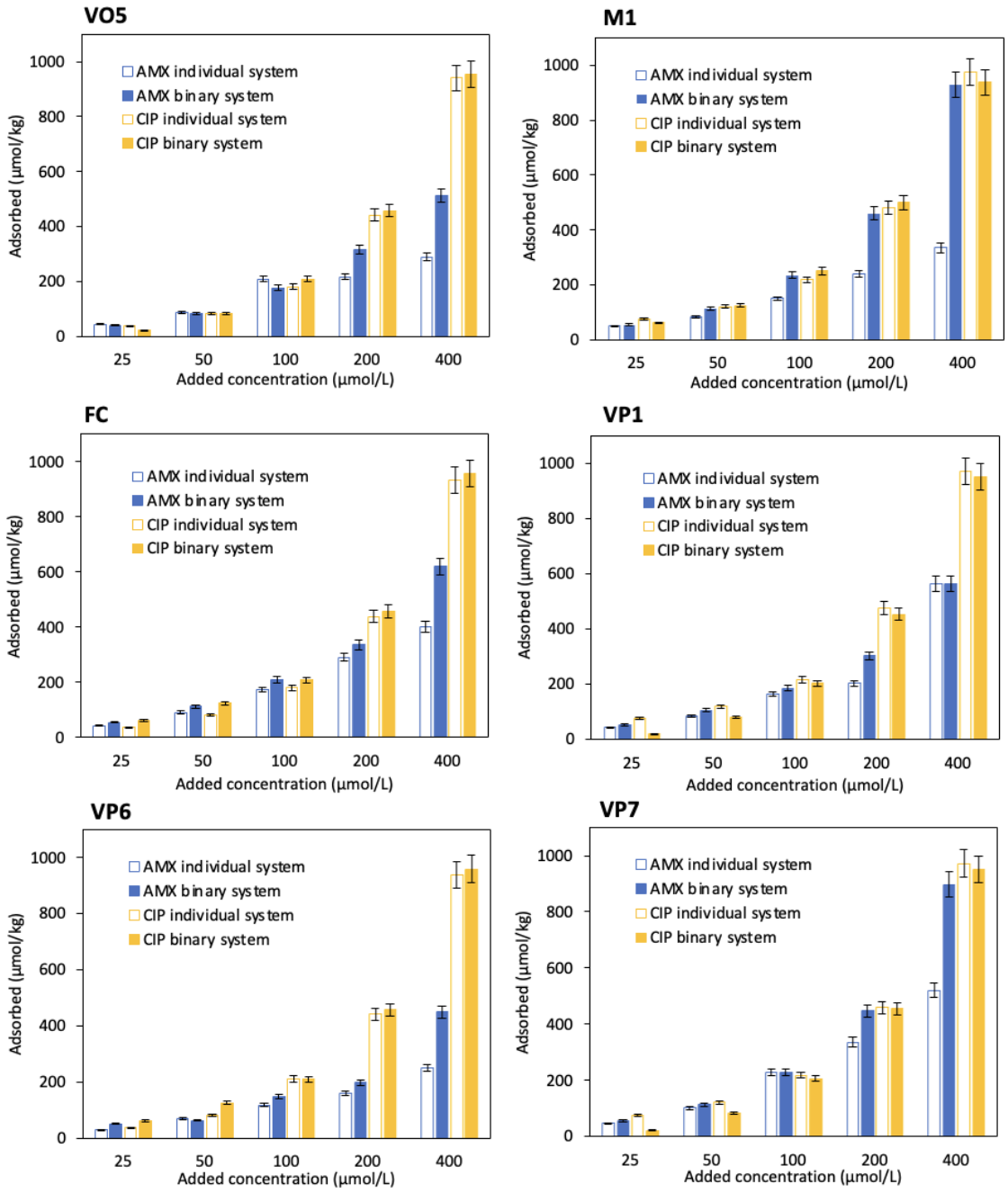

Fig. S1. Simple/binary adsorption of amoxicillin and ciprofloxacin (in µmol/kg) onto the six soils studied

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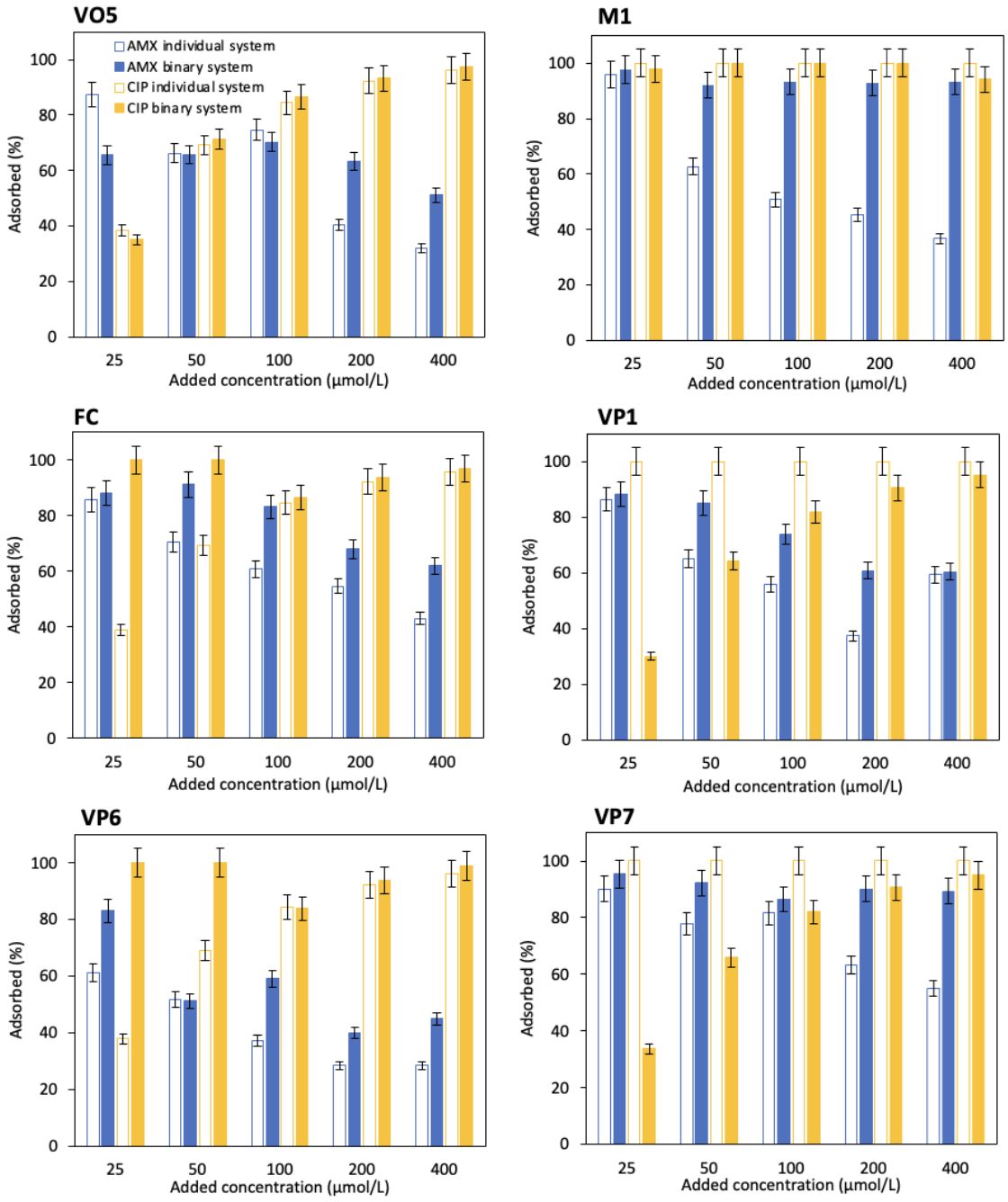

Fig. S2. Simple/binary adsorption (as percentages) of amoxicillin and ciprofloxacin onto the six soils studied

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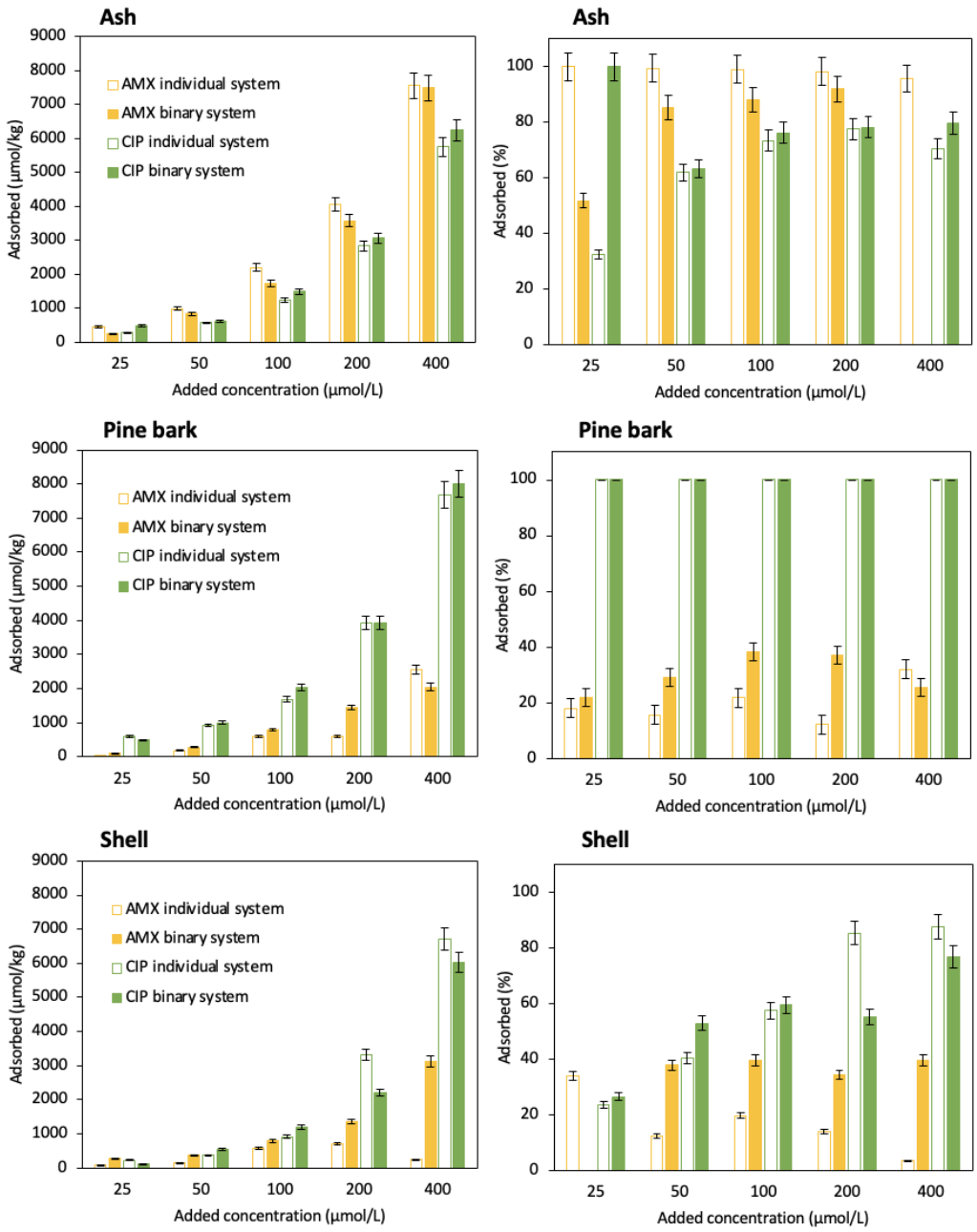

Fig. S3. Simple/binary adsorption of amoxicillin and ciprofloxacin (in µmol/kg, on the left) and as percentages (on the right) onto the three bio-adsorbents used

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PUBLICACIÓN XI

Cela-Dablanca, Raquel[a]., Míguez-González, Ainoa[a]., Barreiro, Ana[a]., Rodríguez-López, Lucía[b]., Arias-Estévez, Manuel[b]., Núñez-Delgado, Avelino[a]., Álvarez-Rodríguez, Esperanza[a]., Fernández-Sanjurjo, María J[a]. (2025). **Role of soils and bio-adsorbents in the adsorption dynamics of amoxicillin, cefuroxime and azitrhomycin in single and ternary systems**. Journal of Environmental Management (Enviado).

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ROLE OF SOILS AND BIO-ADSORBENTS IN THE ADSORPTION DYNAMICS OF AMOXICILLIN, CEFUROXIME AND AZITRHOMYCIN IN SINGLE AND TERNARY SYSTEMS.

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ABSTRACT

Antibiotics are poorly absorbed and largely excreted through feces and urine, entering the environment. Although previous research focused on the adsorption of cefuroxime (CFX), amoxicillin (AMX) and azithromycin (AZM) onto soils and bio-adsorbents, the effect of the simultaneous presence of these antibiotics was not investigated, although being common in the environment. Hence, this work studied the adsorption of these antibiotics when added together to six soils and to three bio-adsorbents (oak ash, pine bark and mussel shell), and compared the results with those obtained for each antibiotic individually. AZM exhibited the highest adsorption on soil. AMX adsorption by soils increased in the presence of CFX and AZM (from 76% to 88%). However, the adsorption of the other two antibiotics decreased in ternary systems: CFX dropped from 99% to 96%, and AZM from 100% to 42%. Regarding bio-adsorbents, oak ash demonstrated the highest adsorption efficiency for the three antibiotics, exceeding 90% in the ternary system. Pine bark and mussel shell showed lower adsorption efficiencies. The Freundlich model best described adsorption in soils, while only mussel shell fits well this model among the bio-adsorbents. Desorption from soils increased when antibiotics were applied simultaneously, with AMX showing the highest desorption. For bio-adsorbents, desorption was higher in the single-compound systems. Overall, AMX adsorption was enhanced by the presence of CFX and AZM, while both CFX and AZM adsorption were negatively affected by the presence of other antibiotics, suggesting competitive interactions. All these results can be seen as relevant for the environment and public health.

Keywords: antibiotics, bio-adsorbents, retention, soils

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1. INTRODUCTION

Emerging pollutants include pharmaceuticals and their transformation products, personal care products, pesticides, certain biological materials, and others (Cukierman et al., 2019). In recent years, low concentrations of these contaminants have been detected in wastewater, surface water, groundwater and drinking water, which can generate great risks to human health and natural ecosystems due to short- and long-term toxicity (Fischer et al., 2017; Richardson e Kimura, 2017; Zhao et al., 2018).

Regarding antibiotics, in 2021 the population weighed average consumption in the EU/EEA, measured in mg of active substance per kg of estimated biomass, was 125.0 mg kg^{-1} in humans and 92.6 mg kg^{-1} in food-producing animals (ECDC, 2024), representing a 53% reduction in the latter between 2011 and 2022 (EMA, 2023). Regarding humans, the average total consumption of systemic antibacterial agents used in Europe in 2023 was estimated to be 20 daily doses per 1000 inhabitants per day, considering both hospital and no-hospital use, while non-hospital consumption, was 18.3 daily doses per 1000 inhabitants per day (ECDC, 2023).

Approximately, 60-85% of antibiotics consumed are excreted in feces and urine as parent compounds or transformation products (Wang and Wang, 2015; Wei et al., 2017; Duan et al., 2021). After excretion, these antimicrobials may end up retained in sludge and/or eliminated by biotic and abiotic degradation in wastewater disposal systems. However, molecular breakdown is often incomplete, and antibiotics and their metabolites end up entering the environment (Danner et al., 2019). Golchin et al. (2021) detected amoxicillin in concentrations between 4.7-16.25 g L^{-1} in treated effluents. Moreover, sewage sludge that contains antibiotics is used as organic amendment in agricultural soils, with repeated applications contributing to the entry of antimicrobials to terrestrial ecosystems (Ternes et al., 2004; Yudong et al., 2010; Du and Liu, 2012; Buta et al., 2021).

Soil is often the main sink for pollutants and can potentially reduce environmental risks related to antibiotics, as physical and chemical processes could immobilize these molecules. The retention of antibiotics onto soils depends on soil characteristics (pH, organic matter, clays, metal oxides, etc.), as well as on characteristics of the antibiotics (photo-stability, biodegradation, solubility in water, electrical charge, etc.) (Conde-Cid et al., 2020b). However, soil can also become a source of emissions when its capacity to retain the affected pollutants is exceeded (Pan et al., 2019). Biel-Maeso et al. (2018) detected antibiotics in concentrations up to 5.45 ng g^{-1} in soil samples collected in Spain. In the same geographic area of the current study, Conde-Cid (2018) reported the presence of various tetracyclines and sulfonamides in soils at concentrations of up to 0.6 mg kg^{-1}. In addition, Barreiro et al. (2022) detected amoxicillin (AMX) and cefuroxime (CFX) in soil samples, with concentrations reaching up to 57 ng g^{-1} and 276 ng g^{-1}, respectively.

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Due to its simple design and operation, along with the advantage of not introducing undesirable byproducts into the system, adsorption has been deemed a promising method for removing pharmaceuticals in the fields of pollution control and waste management (Tong et al., 2010; Grassi et al., 2012). Additionally, it is interesting to study the capacity of different materials to adsorb antibiotics, as good results could make them a viable option for incorporation into WWTP or soils with low adsorption capacity to increase pollutants removal. Several studies have documented the interaction and adsorption mechanisms of antibiotic pollutants on various adsorbent materials. Examples of these adsorbents include activated carbon, graphene oxide, bamboo biochar (Balarak et al., 2017; Wang et al., 2015; Zhang Hiew et al., 2019), and hybrid magnetic composite sorbents (Wu et al., 2018).

Among the most widely used antibiotic types, β-lactams represent the largest proportion of consumption in human and veterinary medicine, being the most common antibiotics found in wastewater and surface waters (Stavbar et al., 2017). Quinolones and their subgroup fluoroquinolones, sulfonamides and macrolides are also emerging groups of antibiotics in the environment due to their slow and difficult biodegradation (Seifrtová et al., 2009).

Previous studies have investigated the individual adsorption of two β-lactam antibiotics, cefuroxime (CFX) and amoxicillin (AMX), as well as one macrolide antibiotic, azithromycin (AZM), onto various soils and bio-adsorbents (Cela-Dablanca et al., 2021a, b; 2022a, b, c; 2024b). However, usual environmental conditions are more complex, often involving the simultaneous presence of multiple contaminants, which may result in competitive interactions (Li and Werth, 2001; Bonin and Simpson, 2007). Such interactions can involve competition for sorption sites, leading to sorption inhibition, physical displacement of one compound by another, decreased adsorption efficiency, and increased mobility or release (Conkle et al., 2010).

To date, no studies have evaluated the competitive adsorption and desorption behavior of amoxicillin, cefuroxime, and azithromycin when present simultaneously in a polluted environment. Therefore, the objective of the present work is to investigate the competitive adsorption of these three antibiotics (AMX, CFX, and AZM) onto six soils with varying physicochemical characteristics, as well as onto three low-cost adsorbents (mussel shell, pine bark, and oak ash) which could be used to enhance soil adsorption capacity and in water decontamination processes, which could be of relevance for human and environmental health.

2. MATERIAL AND METHODS

2.1 Soil and bio-adsorbents

2.1.1 Soils

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Six soils with different physicochemical properties were selected. Table S1 (Supplementary Material) shows the detailed physicochemical properties of the

soils. The pH values ranged between 4.68 and 8.02, whereas organic matter content oscillated from 3.06% to 12.32%, and cationic exchange capacity (CECe) varied between 7.42 $cmol_c\ kg^{-1}$ and 42.81 $cmol_c\ kg^{-1}$.

2.1.2 Bio-adsorbents

Three bio-adsorbents were selected, two of them from the forestry industry (pine bark and oak ash), and the third one from the food industry (mussel shell). All of them were previously characterized (Cela-Dablanca et al., 2021b), showing very different physicochemical properties. Pine bark has an acid pH (3.99), while mussel shell and oak ash present an alkaline pH (9.39 and 11.31). Organic carbon ranged between 11.43% (mussel shell) and 48.70% (pine bark), while eCEC values oscillated from 14.92 $cmol_c\ kg^{-1}$ (pine bark) to 361.15 $cmol_c\ kg^{-1}$ (oak ash). Table S2 (Supplementary Material) shows the detailed physicochemical properties of the bio-adsorbents.

2.2 Chemical reagents

Cefuroxime (purity ≥ 95%), amoxicillin (with ≥ 95% of purity) and azithromycin (neat) were supplied by Sigma-Aldrich (Barcelona, Spain). Phosphoric acid (being 85% extra pure) were from Acros Organics (Barcelona, Spain), potassium phosphate (purity ≥ 99.5%), acetonitrile (with ≥99.9% of purity) was from Fisher Scientific (Madrid, Spain) and $CaCl_2$ (with purity of 95%) was provided by Panreac (Barcelona, Spain). MilliQ water (Millipore, Madrid, Spain) was used for preparing all solutions needed for HPLC quantification procedures.

2.3 Adsorption and desorption experiments

Batch-type experiments were carried out to study the adsorption/desorption of CFX, AMX and AZM by different materials in ternary systems. Two g of each soil were weighed and then 5 mL of a solution with different concentrations of each antibiotic (0; 2.5; 5; 10; 20; 30; 40; 50 $\mu mol\ L^{-1}$) were added, using 0.005 M $CaCl_2$ as background electrolyte. In the case of the bio-adsorbents, the relation adsorbent to solution used was 0.5:10. The suspensions were shaken in the dark for 48 h (time enough to reach equilibrium, according to previous kinetic tests) using a rotary shaker. These suspensions were then centrifuged at 4000 rpm for 15 min (G force: 1931.91). The resulting supernatants were filtered through 0.2 µm nylon syringe filters. Finally, the antibiotic concentrations in the equilibrium solution were determined by HPLC-UV with a LPG 3400 SD equipment (Thermo-Fisher, Waltham, MA, USA).

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2.4 Data treatment

The experimental data obtained in the adsorption/desorption tests were adjusted to the Freundlich (Eq.1) and Linear (Eq. 2) models (Ayawei et al., 2017).

$$q_e = K_F C_{eq}{}^n \quad \text{Eq. 1}$$

$$Kd = q_e / C_{eq} \quad \text{Eq. 2}$$

where q_e (expressed in μmol kg^{-1}) is the amount of antibiotic retained in the adsorbent (calculated as the difference between the concentration added and that remaining in the equilibrium solution); K_F (L^n μmol^{1-n} kg^{-1}) is the Freundlich constant related to the adsorption capacity; C_{eq} (μmol L^{-1}) is the concentration of antibiotic present in the solution at equilibrium; n (dimensionless) is a parameter of the Freundlich model associated with the degree of heterogeneity of the adsorption, and K_d (L kg^{-1}) is the partition coefficient in the linear model.

Freundlich and Linear models were adapted for competitive systems (Eq. 3 and Eq. 4, respectively).

$$(q_{e\,AMX} + q_{e\,CFX} + q_{e\,AZM}) = KF(C_{eq\,AMX} + C_{eq\,CFX} + C_{eq\,AZM})^n \quad \text{Eq. 3}$$

$$(q_{e\,AMX} + q_{e\,CFX} + q_{e\,AZM}) = Kd(C_{eq\,AMX} + C_{eq\,CFX} + C_{eq\,AZM}) \quad \text{Eq. 4}$$

In addition, soil properties were correlated with the parameters obtained in the adjustments to the adsorption models, determining the Pearson correlation coefficients.

The SPSS Statistics 21 software was used to carry out the adjustment of the data derived from the adsorption experiments to the Langmuir, Freundlich and Linear models, as well as any further statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Soil adsorption

Figure 1 shows adsorption curves for AMX, AZM and CFX in the six soils studied, for both the simple system (when one antibiotic was added alone) and the ternary system (when three antibiotics were added simultaneously). Figure 2 shows the percentage of adsorption for all antibiotics in simple and ternary systems.

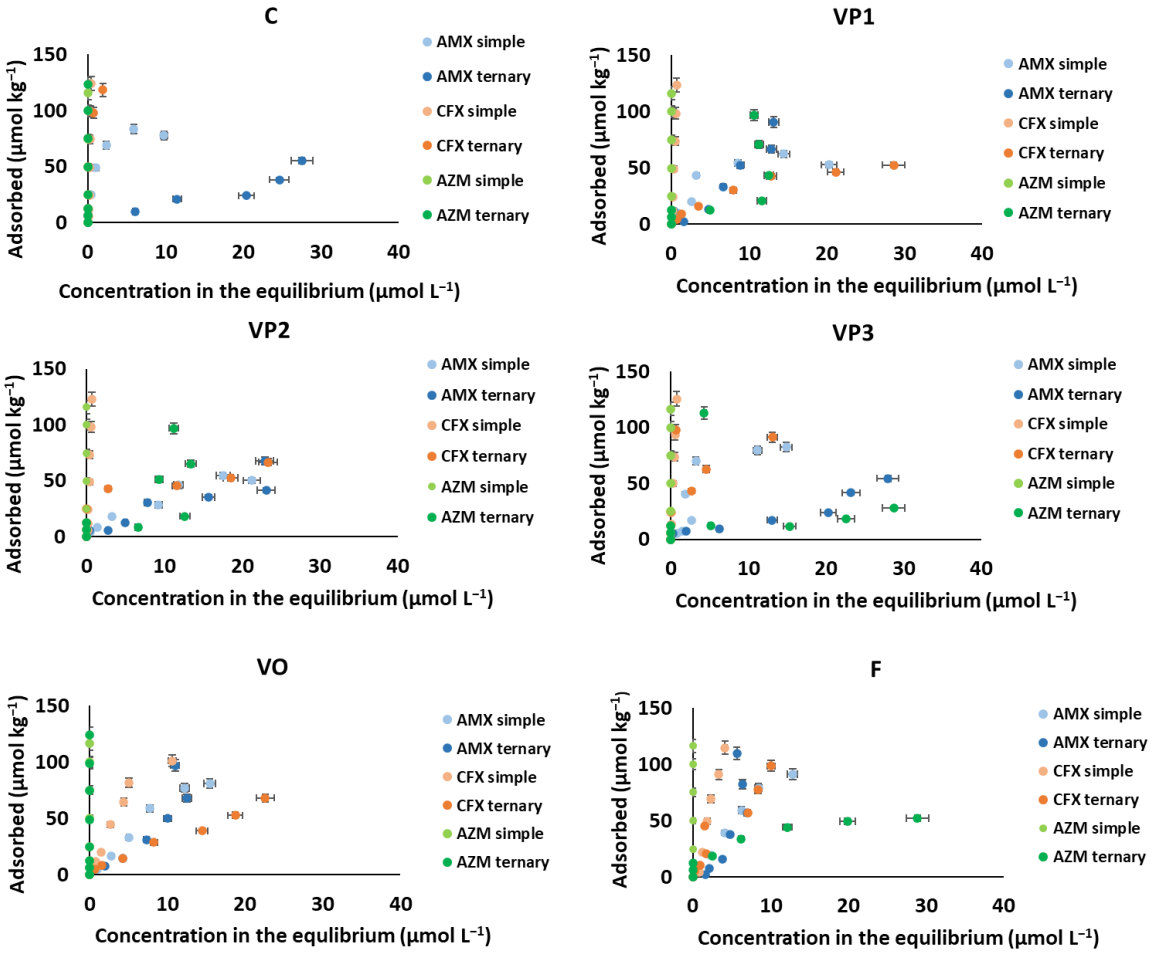

Figure 1. Adsorption curves for each antibiotic obtained from simple and ternary systems. AMX: amoxicillin; AZM: azithromycin; CFX: cefuroxime; C: corn soil; VO: vineyard soil (Ourense province); VP: vineyard soil (Pontevedra province); F: forest soil

The figures show that AZM was the antibiotic that generally presented the highest adsorption percentages in the simple system, followed by CFX and finally AMX. Comparing both systems, AZM and CFX exhibited higher adsorption percentages when they are alone (simple systems) than in the ternary system, especially at high antibiotic doses added. An exception is soil C, which consistently adsorbs over 100% of both antibiotics, and VO soil, which adsorbs 100% of AZM. Regarding AMX, the results are more variable: there is usually greater adsorption in the single system than in the ternary one, especially at low antibiotic doses. These differences tend to decrease as the dose increases, and in some cases, even higher adsorption of AMX is observed in the ternary system (soils VP1 and F) (Fig. 2). When the highest concentration of antibiotic (50 µmol L^{-1}) was added, AZM adsorption percentages were 100% in all soils in simple systems. However, in the ternary system these values ranged between 42% (F soil) and 100% (C and VO soils). In the case of CFX, adsorption percentages oscillated between 79% (VO soil) and 99% (the rest of soils) in simple systems, and between 43% (VP1 soil) and 96% (C soil) in the ternary

system. For AMX, these values ranged from 49% (VP2 soil) to 76% (C soil) in simple systems and from 44% (VP3 soil) to 88% (F soil) in the ternary system. In a previous study on the adsorption competition between AMX and ciprofloxacin (CIP), a synergistic effect between the two antibiotics was observed, with the adsorption of AMX being enhanced by the presence of CIP (Cela-Dablanca et al., 2024a). This was attributed to a cooperative adsorption, pointed out by several authors (Zhang et al., 2007; Zhong et al., 2013; Schmidtová et al., 2020). According to this cooperative model, when a solute is retained by a site on a homogeneous adsorbent surface, it can influence the consecutive active sites of that surface, promoting new adsorptions and stronger retentions.

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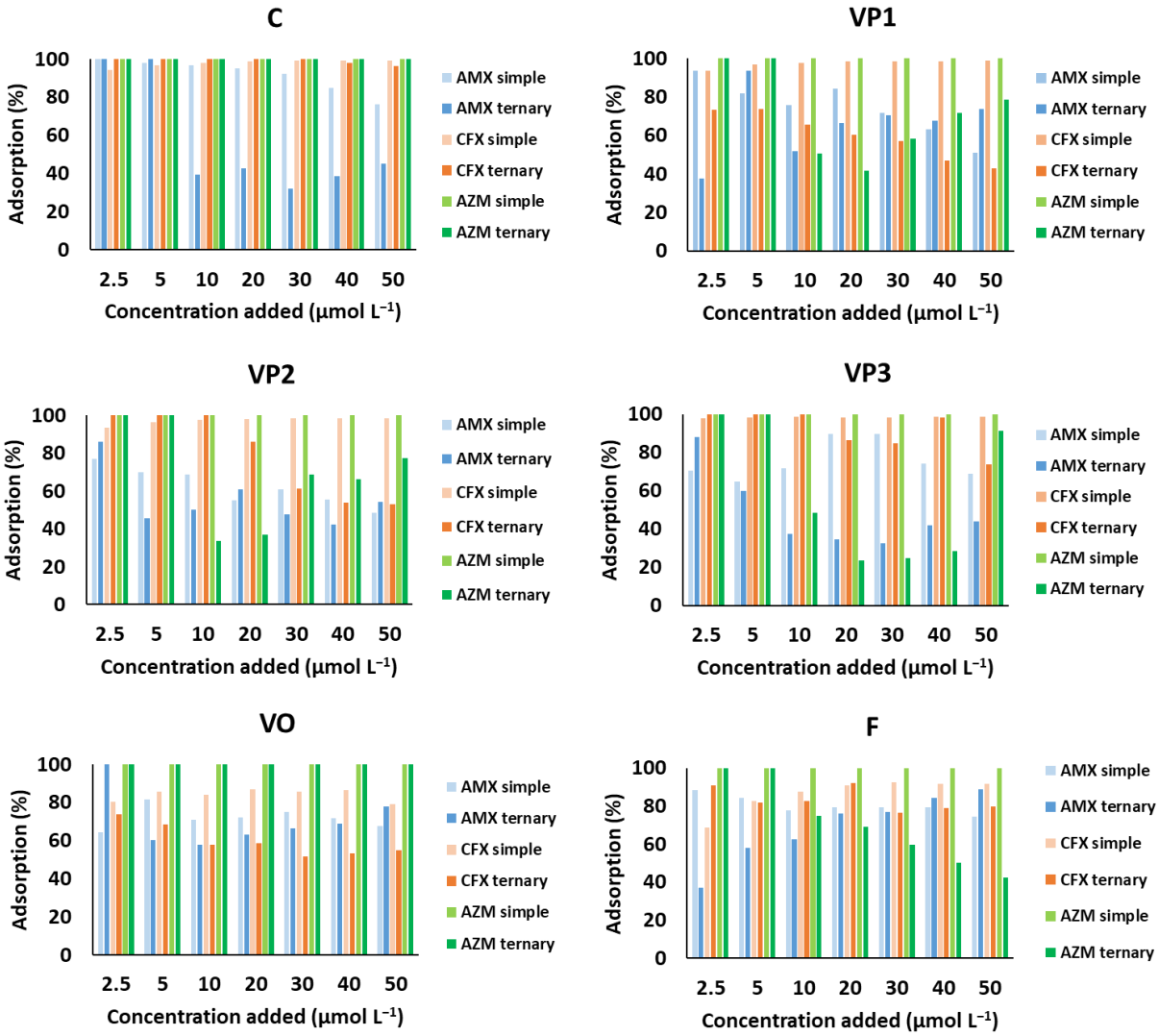

Figure 2. Adsorption (in percentages) for each antibiotic (amoxicillin, azithromycin and cefuroxime) obtained from simple and ternary systems, as a function of the antibiotic concentrations added. AMX: amoxicillin; AZM: azithromycin; CFX: cefuroxime; C: corn soils; VO: vineyard soil (Ourense province); VP: vineyard soil (Pontevedra province); F: forest soil

Comparing the adsorption values obtained for the six soils, several differences have been observed. Both, antibiotics and soil components, contain functional groups that can undergo protonation or deprotonation depending on the pH of the solution. In the case of azithromycin, this antibiotic has one pK_a approximately between 8.6-9.5 (Balarak et al., 2021), so in the range of the soils included in this study (4.68-8.02), AZM has positively charged functional groups, favoring the electrostatic interactions with the negative charges of minerals and organic matter. In the simple system no significant differences were observed between the studied soils, with AZM adsorption approaching 100% in all of them. In the ternary system, notable differences were observed among the various soils regarding their adsorption

behavior. In higher-pH soils (C and VO), azithromycin (AZM) adsorption remained unaffected by the presence of the other two antibiotics. In contrast, the remaining soils exhibited a pronounced reduction in AZM adsorption relative to the simple system, indicating potential competitive interactions for adsorption sites. In the ternary system, azithromycin adsorption showed a negative and significant correlation with exchangeable Al (r=-0.867, p<0.05), Fe_{pir} (r=-0.916, p<0.05) and Al_{pir} (r=-0.927, p<0.01). Among the soils evaluated, F exhibited the lowest AZM adsorption percentages in the ternary system (42% when 50 µmol L^{-1} of AZM were added). This soil has the lowest pH value and the highest Al and Fe oxalate contents, which estimate the poor crystalline fractions. These non-crystalline compounds have variable charge, with more positive charges at low pH values. At the pH levels of soil F (pH=4.68) the non-crystalline components, abundant in these soils, will be positively charged, as well as azithromycin. Therefore, under these conditions, the low-crystallinity components would not contribute to the retention of this antibiotic, but the –COOH groups of the organic matter would begin to deprotonate, facilitating electrostatic interactions with the positively charged groups of AZM. However, the overall negative charge at this pH value is still limited, resulting in lower adsorption in more acidic soil compared to those with higher pH, so the other two antibiotics may compete with AZM for adsorption sites, leading to a reduction in its adsorption compared to the simple system. In the other soils (with adsorption values exceeding 80% in the ternary system), the content of non-crystalline minerals is lower than in soil F. However, due to their higher pH range (6.04–8.02, except for VP1), these compounds exhibit negative charges. This, combined with the increased negative charge of organic matter at elevated pH levels, reduced competition for adsorption sites among antibiotics, resulting in a minimal decrease in AZM adsorption compared to the single-component system. Additional mechanisms may also contribute to AZM adsorption in the studied soils, including hydrogen bonding with organic matter and Van der Waals forces. Balarak et al. (2021) also observed a gradual increase in AZM adsorption as pH increased, using activated porous carbon derived from *Azolla filiculoides*, with maximum adsorption at pH values between 9 and 11.

Regarding CFX, minimal differences were observed among the different soils when this antibiotic was added individually. However, in the ternary system (AMX+CFX+AZM), differences in adsorption among the different soils were observed. CFX has two dissociation constants (pk_{a1}=3.15 and pk_{a2}= 10.97) and can carry a positive or negative charge or exist as a zwitterion, depending on the pH of the medium (Ribeiro and Smith., 2017). Within the pH range of the studied soils, CFX will exist as a zwitterion, with both negative and positive charges. Soils with high pH values (soil C has pH 8.02, being 7.27 for soil VP3) presented the highest adsorption scores for CFX (between 73 and 100%) for all the concentrations added, especially in the ternary system. At these pH values, CFX exists in zwitterionic form, meaning that the positively charged amino groups of the antibiotic interact electrostatically with the negatively charged adsorbent surfaces of the soils, while the anionic groups

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(COO^-) of CFX bind to these negatively charged surfaces via a cation bridge (Legnoverde et al., 2014). In the case of soil F, it showed a rather high adsorption percentage (80%), despite having a pH of 4.68. This elevated value for adsorption could be related to the high content of organic matter in this soil (12.33 %) and non-crystalline compounds (Table S1), because at pH<5, same -COOH groups of the organic matter can dissociate (Yu et al., 2011) and could interact with the positive charges of CFX by electrostatic interactions. It is also possible that protonated amino groups in the soil organic matter and positively charged non-crystalline components interact with anionic groups of the antibiotic. In contrast, the VO soil presents a relatively low adsorption considering its pH (6.04), which is probably justified by being the soil with the lowest organic matter content (3.05%) and also with the lowest concentrations of non-crystalline compounds (Table S1).

Regarding AMX, in the single system it was generally the least adsorbed compared to the other two antibiotics, particularly at the highest concentration tested (50 μmol L^{-1}), where adsorption values ranged between 50% and 80% (Fig. 2). In the ternary system, AMX also exhibited the lowest adsorption among the three antibiotics, with values ranging from 40% to 90% (Fig. 2). When comparing the single-compound system to the ternary one, synergistic or antagonistic effects of the other antibiotics on AMX adsorption were observed, depending on the soil. In the two soils with the highest pH values (C and VP3), the presence of the other antibiotics had an antagonistic effect, reducing AMX adsorption, possibly due to competition for adsorption sites, an effect that becomes more pronounced at higher concentrations. In contrast, in the remaining four soils, the effect was synergistic, with CFX and AZM enhancing AMX adsorption. Statistical analyses were conducted, revealing a highly significant negative correlation (r=-0.953; p<0.01) between pH and AMX adsorption in the ternary system. This antibiotic has three dissociation constants, due to the presence of different functional groups such as carboxyl (with pKa1 = 2.68), amino (pKa2 = 7.49) and phenolic (pKa3 = 9.63) (Homsirikamol et al., 2016), causing that in most of the studied soils it would be found as a zwitterion. In the forest soil (pH<5), deprotonated carboxyl groups of AMX can bind to the positive charges in the soil (non-crystalline minerals and amino groups of the organic matter) through electrostatic interactions, and also $-COO^-$ groups of the organic matter could interact with the positive charges of AMX. Soils with pH between 5 and 6.5 (VO and VP1) will present more negative charge than soil F, which can interact with protonated groups of AMX. In soils with pH>7 (C, VP2 and VP3), negative charges will predominate, especially in soil C (pH=8.02) and AMX will have more deprotonated groups, favoring adsorption through a cationic bridge, and under these conditions there is competition between AMX and the other two antibiotics for the adsorption sites.

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3.2 Adjustment of AMX, CFX and AZM adsorption onto soils to adsorption models

Adsorption results were adjusted to the Linear and Freundlich models. Table 1 shows the values obtained for the different parameters of these models.

Table 1. Parameters of the Linear and Freundlich models for amoxicillin, cefuroxime and azithromycin adsorption onto soils in simple and ternary systems.

Soil	Antibiotic	Freundlich					Linear		
		KF	Error	n	Error	R^2	Kd	Error	R^2
C	AMX	44.17	4.64	0.306	0.06	0.94	10.72	2.30	0.37
	CFX	478.87	152.34	1.58	0.29	0.893	247.34	29.38	0.797
	AZM	95.986	50.522	0.387	0.103	0.89	3.403	0.55	0.67
	AMX+CFX+AZM	**8.628**	**4.418**	**1.032**	**0.16**	**0.969**	**9.554**	**0.418**	**0.969**
VP1	AMX	99.53	20.66	1.09	0.28	0.84	93.63	0.67	0.48
	CFX	228.8	25.58	1.59	0.18	0.971	157.031	11.182	0.923
	AZM	--	--	--	--	--	1.08	0.13	0.79
	AMX+CFX+AZM	**0.529**	**0.432**	**1.544**	**0.215**	**0.968**	**4.069**	**0.285**	**0.927**
VP2	AMX	--	--	--	--	--	--	--	--
	CFX	207.294	19.52	1.451	0.15	0.975	157.181	11.182	0.923
	AZM	10.24	0	1.327	0.082	0.36	33.87	9.59	0.36
	AMX+CFX+AZM	**--**	**--**	**1.251**	**0.29**	**0.926**	**3.375**	**0.248**	**0.917**
VP3	AMX	116.05	9.89	0.93	0.13	0.97	120.34	5.63	0.97
	CFX	164.23	5.17	1.099	0.05	0.996	156.031	3.176	0.993
	AZM	--	--	--	--	--	8.581	2.56	0.3
	AMX+CFX+AZM	**--**	**--**	**0.839**	**0.78**	**0.553**	**2.99**	**0.611**	**0.551**
VO	AMX	19.29	5.08	0.66	0.14	0.90	9.68	1.15	0.79
	CFX	21.68	4.085	0.685	0.1	0.948	11.48	1.085	0.863
	AZM	8.87	0	1.066	0.073	0.27	11.99	3.78	0.27
	AMX+CFX+AZM	**3.638**	**1.932**	**1.218**	**0.158**	**0.977**	**7.531**	**0.332**	**0.97**
F	AMX	13.44	1.89	0.79	0.07	0.99	8.49	0.38	0.97
	CFX	20.15	3.37	1.251	0.14	0.971	26.738	1.548	0.949
	AZM	--	--	--	--	--	0.466	0.11	0.1
	AMX+CFX+AZM	**12.543**	**2.805**	**0.795**	**0.064**	**0.988**	**6.079**	**0.259**	**0.971**

KF expressed in $L^n\ kg^{-1}\ \mu mol^{1-n}$; n = dimensionless; Kd expressed in $L\ kg^{-1}$; -- = error value too high for fitting. C = corn soil; VO and VP = vineyard soils; AMX: amoxicillin; CFX: cefuroxime; AZM: azithromycin

As shown in table 1, the adsorption of AMX, CFX and AZM in the ternary system presented a rather acceptable adjustment to the Linear and Freundlich models in most cases, with R^2 values higher than 0.9, except for soil VP3.

Regarding the Freundlich model, the KF parameter, which is related to the adsorption capacity, ranged between 0.526 and 12.543 $L^n\ kg^{-1}\ \mu mol^{1-n}$, values lower than those obtained for simple systems corresponding to each antibiotic. This

difference is especially evident for CFX, which shows the most significant decrease in adsorption within the ternary system. KF values also were lower than those obtained by Conde-Cid et al. (2019a) for tetracyclines in a ternary system, and by Cela-Dablanca et al. (2024a) in a binary system with AMX and CIP. However, these values were similar to those obtained in a ternary system for three sulfonamides (Conde-Cid et al., 2020c). KF was significantly and positively correlated ($p<0.05$) with different properties of the soils: with C ($r=0.881$), N ($r=0.897$) and Fe_{pir} ($r=0.828$). The observation of strong correlations with non-crystalline Fe contents in the soils may indicate high adsorption energy for certain antibiotics when bound to these non-crystalline minerals (Conde-Cid et al., 2019b). On the other hand, Conde-Cid et al. (2020a) found that soils with higher organic matter content adsorbed all the added antibiotic, whereas soils with low organic matter did not perform equally. This may be because soils with less organic matter have fewer adsorption sites, which become saturated. Considering the Freundlich n parameter, two of the soils (VP3 and F) had $n<1$, indicating a heterogeneous adsorption surface, where the highest energy sites are occupied first, while the rest exhibited $n>1$. When n values are higher than 1, adsorption would be promoted as the concentration of added antibiotics increases (Khanifari et al., 2023).

Regarding the Linear model, in the ternary system the kd value oscillated between 2.99 and 9.554 L kg^{-1}. These values are lower than those obtained for these antibiotics in simple systems, and similarly to what was observed for the KF parameter, with this difference being more evident in the case of CFX.

Figure S1 (Supplementary material) shows the adsorption curves for the three antibiotics added simultaneously and their adjustments to the Freundlich and Linear models.

3.3 Bio-adsorbents adsorption

Figure 3 shows adsorption curves for AMX, CFX and AZM, both in simple and ternary systems, corresponding to oak ash, pine bark and mussel shell, while figure 4 shows the adsorption data expressed in percentages.

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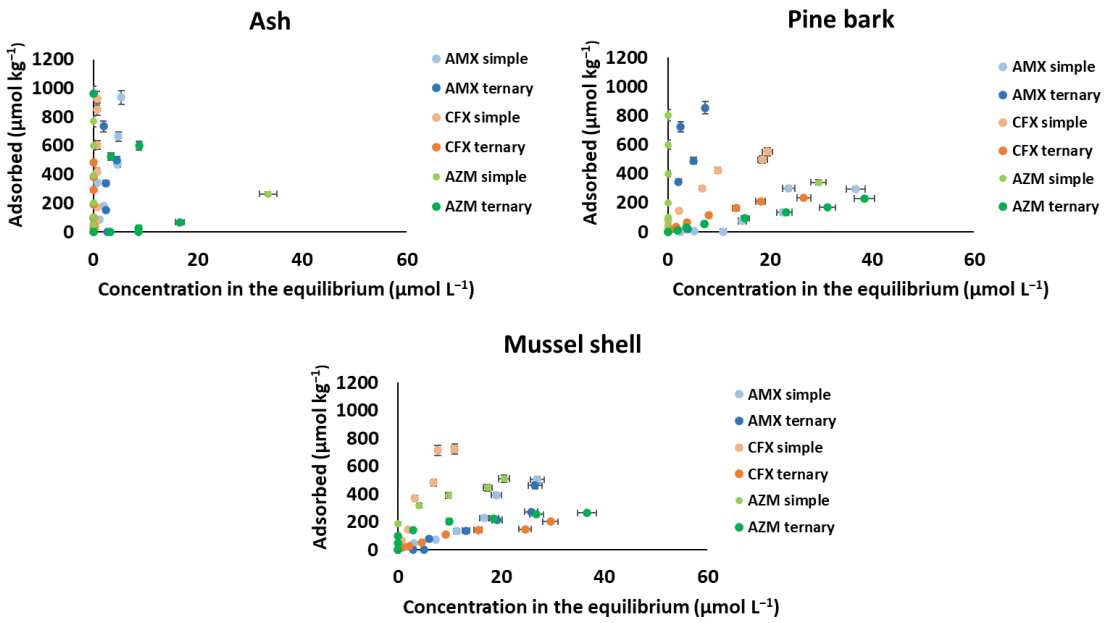

Figure 3. Adsorption curves for each antibiotic obtained from simple and ternary systems. AMX: amoxicillin; AZM: azithromycin; CFX: cefuroxime

Oak ash was the bio-adsorbent that presented the highest adsorption for all the antibiotics in both systems, more pronounced in the case of CFX and AMX. For pine bark and mussel shell, the results are very variable, with a tendency towards higher adsorption using pine bark, especially at higher doses of antibiotic. In a previous study for three tetracyclines in a ternary system, pine bark and oak ash showed high adsorption scores, while mussel shell presented lower adsorption, suggesting that, for the first two materials, the adsorption surfaces were not saturated at the antibiotic concentrations used, and no competition among antibiotics was observed (Conde-Cid et al., 2019b).

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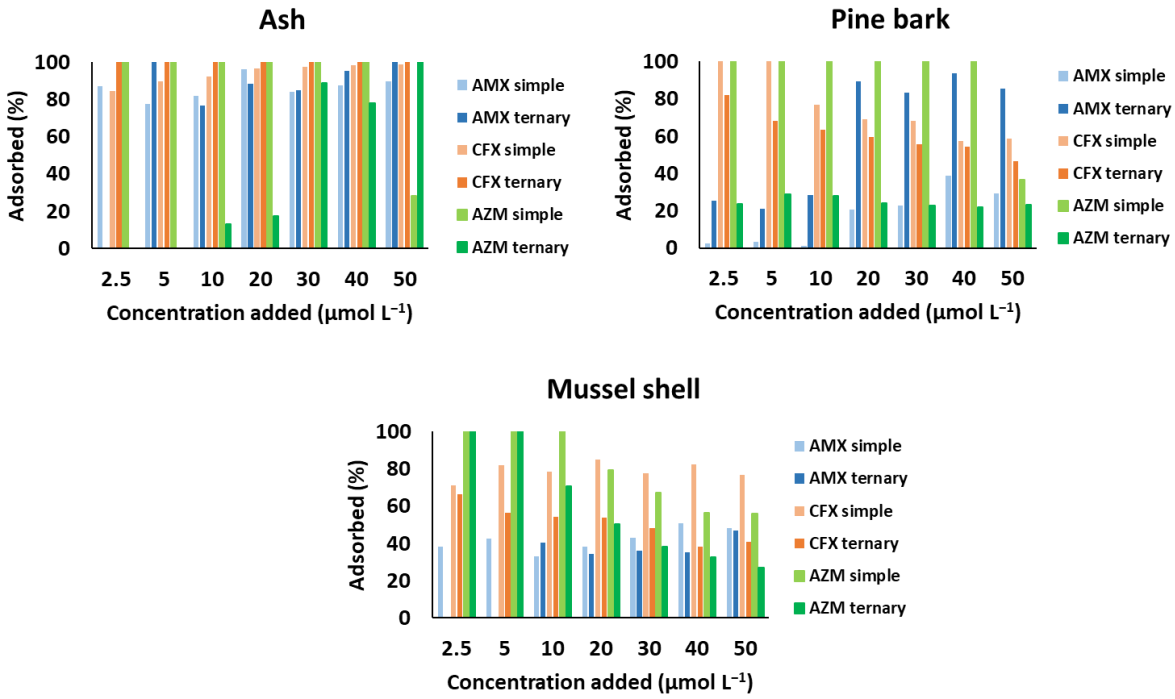

Figure 4. Adsorption by the bio-adsorbents (in percentages) for each antibiotic (amoxicillin=AMX, azithromycin=AZM, cefuroxime=CFX) obtained from simple and ternary systems, as a function of the antibiotic concentrations added.

Comparing both systems, although oak ash adsorbs generally high percentages in both systems, reaching 100% in many cases, there is a tendency for higher adsorption in the ternary system, especially at higher doses added. These results indicate that there is a synergistic effect between all antibiotics, that can be related to the cooperative adsorption model described by several authors (Ringot et al., 2007; Foo and Hameed, 2010). The alkaline nature of this material and its abundance of variable charge components, specifically non-crystalline Fe and Al (Fe_{ox}, Al_{ox}) would not favor the retention of antibiotics, as this alkaline pH would result in a high negative charge density for both the antibiotics and the variable charge components present in the adsorbent, preventing binding through electrostatic attractions (Conde-Cid et al., 2019b). However, other mechanisms, such as adsorption through cation bridging, may still play a role (Wang and Wang, 2015), probably using Ca as a binding element.

Nevertheless, in the case of pine bark (except for AMX) and mussel shell, the opposite effect occurs, with adsorption being lower when the three antibiotics were added together, indicating competition for adsorption sites. The lower adsorption of AZM and CFX by pine bark is attributed to its acidic pH, as these antibiotics generally exhibit higher adsorption at higher pH levels. Under strongly acidic conditions, pine bark shows low negative surface charge for the adsorption of positively charged AZM and the cationic groups of CFX. As a result, when present in a single-solute system at low concentrations, the adsorption percentage of these antibiotics is high. However, in a multicomponent system, the adsorbent surface becomes saturated,

and the antibiotics compete for the available adsorption sites. In contrast, AMX shows the opposite behavior, with greater adsorption occurring under more acidic conditions and a synergistic effect of the other antibiotics on its adsorption onto pine bark. Mussel shell exhibits an alkaline pH, as oak ash; however, its non-crystalline elements and Ca contents are significantly lower than that of oak ash. Therefore, the adsorption capacity is lower for mussel shell, especially at higher doses of antibiotic, where adsorption values generally do not exceed 47% in the ternary system.

In a previous study investigating the competition between AMX and CIP for the same bio-adsorbents, it was also found that CIP had a synergistic effect on AMX adsorption in both pine bark and mussel shell. However, CIP was affected by the presence of AMX, decreasing its adsorption, and, again, this competition was more evident for mussel shell (Cela-Dablanca et al., 2024a).

3.4 Adjustment of AMX, CFX and AZM adsorption by bio-adsorbents to adsorption models

The obtained adsorption data were fitted to the Linear and Freundlich models. Table 2 shows the results of parameters obtained in the adjustments.

Table 2. Parameters of the Linear and Freundlich models for amoxicillin, cefuroxime and azithromycin adsorption by different bio-adsorbents in simple and ternary systems. KF expressed in $L^n\ kg^{-1}\ \mu mol^{1-n}$; n = dimensionless; Kd expressed in $L\ kg^{-1}$; -- = error value too high for fitting. AMX: amoxicillin; CFX: cefuroxime; AZM: azithromycin

		Freundlich					Linear		
Adsorbent	Antibiotic	KF	Error	n	Error	R^2	Kd	Error	R^2
Oak ash	AMX	100.826	77.588	1.213	0.49	0.816	139.604	16.152	0.812
	CFX	1177.352	772.661	2.17	1.896	0.562	766.994	157.816	0.496
	AZM	11.79	0	0.885	0.476	--	--	--	--
	AMX+CFX+AZM	**--**	**--**	**--**	**--**	**--**	**--**	**--**	**--**
Pine bark	AMX	**--**	**--**	1.37	0.434	0.822	7.919	1.131	0.789
	CFX	110.851	27.973	0.535	0.094	0.965	30.413	2.701	0.867
	AZM	11.452	0	1.002	0.39	--	--	--	--
	AMX+CFX+AZM	**--**	**--**	**0.517**	**0.507**	**0.317**	**14.887**	**5.236**	**0.214**
Mussel shell	AMX	5.3	2.995	1.391	0.183	0.966	17.251	1.213	0.933
	CFX	119.306	33.638	0.782	0.134	0.948	75.246	5.209	0.927
	AZM	208.193	106.681	0.282	0.194	0.82	27.826	4.256	0.592
	AMX+CFX+AZM	**37.496**	**11.458**	**0.691**	**0.072**	**0.978**	**10.015**	**0.613**	**0.934**

The data in table 2 show that the fittings are worse in the ternary than in simple systems. Only mussel shell presented a rather good adjustment to the Freundlich and Linear models, wiht R^2>0.9, while oak ash and pine bark did not fit well to any

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model. Figure S2 (Supplementary Material) shows adsorption curves, both real and according to the Linear and Freundlich models

The Freundlich affinity coefficient (KF), related to the adsorbent adsorption capacity, in the ternary system reached 111.004 $L^n\ kg^{-1}\ \mu mol^{1-n}$ for pine bark and 37.496 $L^n\ kg^{-1}\ \mu mol^{1-n}$ for mussel shell. This value was higher than that obtained in the simple systems of the three antibiotics for pine bark (Table 2). In the case of mussel shell, the score was significantly lower than that obtained in the simple system for CFX and AZM; however, it was much higher than that obtained for AMX in the simple system (Table 2). The KF values obtained in the ternary system were lower than those previously found for three tetracyclines in a ternary system using the same bio-adsorbents (Conde-Cid et al., 2019b), and similar to the obtained for ciprofloxacin and amoxicillin in a binary system by the same bio-adsorbents (Cela-Dablanca et al., 2024a). The n parameter reached a value of 0.517 for pine bark and 0.691 for mussel shell, indicating a heterogeneous adsorption surface. Different results were obtained by Cela-Dablanca et al. (2024a) for a binary system of amoxicillin and ciprofloxacin, where n was higher than 1 for oak ash and mussel shell. Conde-Cid et al. (2019b), in a study dealing with the simultaneous adsorption of three tetracyclines, reported a value of $n<1$ for pine bark; however, in the case of mussel shell it was $n>1$.

Regarding the Linear model, the distribution constant (Kd) reached 14.887 $L\ kg^{-1}$ for pine bark and 10.015 $L\ kg^{-1}$ for mussel shell. These values were generally lower than the obtained for simple system (table 2), indicating lower adsorption capacities when the three antibiotics were added together.

3.5 Soil desorption

Figure 5 shows the desorption percentages of AMX, CFX and AZM in simple and ternary systems by the six soils studied.

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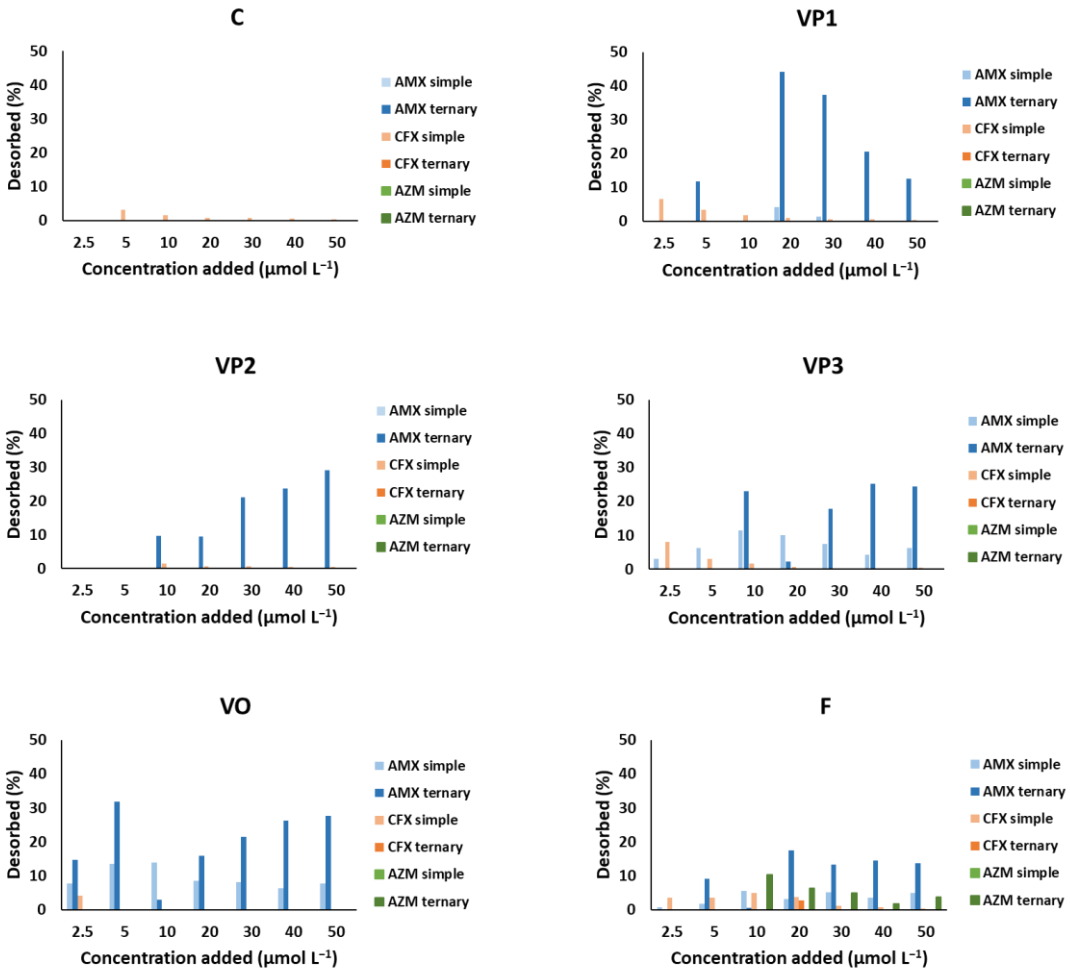

Figure 5. Soil desorption percentages for each antibiotic (amoxicillin, azithromycin and cefuroxime) obtained from simple and ternary systems. AMX: amoxicillin; AZM: azithromycin; CFX: cefuroxime; C: corn soil; VO: vineyard soil (Ourense province); VP: vineyard soil (Pontevedra province); F: forest soil

Figure 5 shows that AMX was the antibiotic with the highest desorption both in simple and ternary systems. AMX desorption was higher in the ternary system, ranging from 0% to 37%, compared to the range 0% to 14% in the simple system. AZM desorption was null in all cases, except for soil F in the ternary system, where it reached a maximum desorption of 10%. This coincides with what was obtained by other authors for three tetracyclines (Conde-Cid et al.,2019a) and three sulfonamides (Conde-Cid et al., 2020c), showing higher desorption from soils when the antibiotics were added simultaneously. However, for CFX the opposite occurs, with desorption being slightly higher in the simple system, although the desorbed values were very low (always lower than 8%). Comparing soils, the one with the lowest pH (F soil) presented higher desorption percentages for CFX and AZM, while the soil with highest pH (C) presented the lowest desorption values. This behavior is opposite to that observed in the adsorption process, where the highest retention occurred in soils with the highest pH, suggesting that the bindings are highly stable and rather irreversible. These results also indicate that adsorption was stronger for

CFX and AZM than for AMX. It would be because AMX in presence of the other two antibiotics occupies low-energy sites, while AZM and CFX bind to the higher-energy sites, this also agrees with the generally higher values of the Freundlich KF parameter obtained for AZM and CFX compared to AMX (Table 1). Chen et al. (2020) found that two sulfonamides (sulfamethizole and sulfamethazine) occupied adsorption sites with different energy, and they concluded that adsorption energy is a decisive factor determining the competition strength.

3.6 Bio-adsorbents desorption

Figure 6 shows the desorption in percentage for the three antibiotics studied both in simple and ternary systems, from mussel shell, pine bark and oak ash.

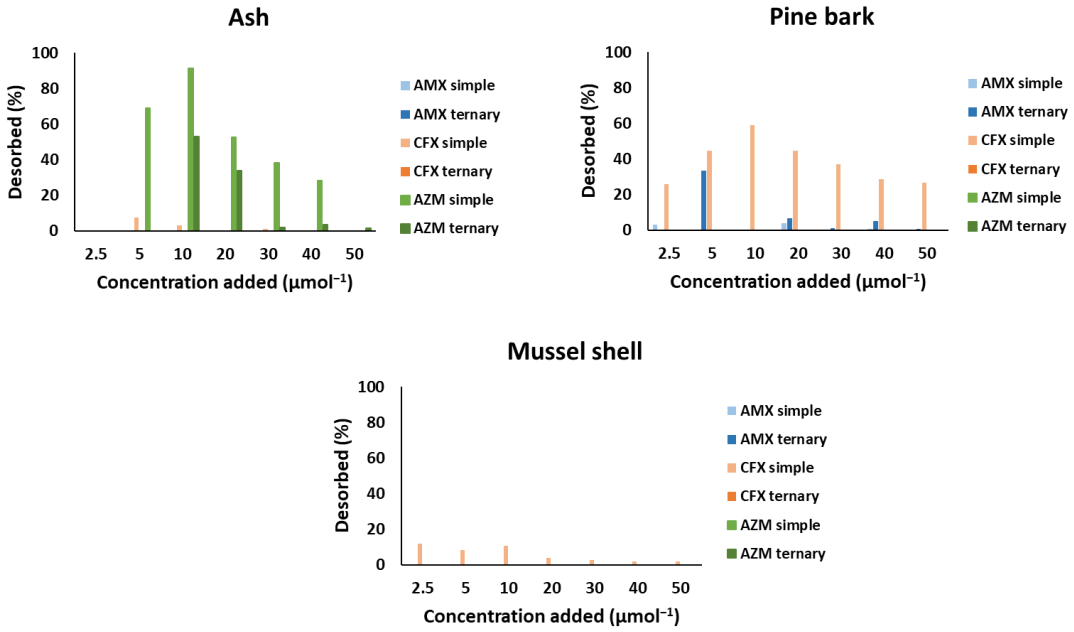

Figure 6. Desorption percentages from the bio-adsorbents for each antibiotic (amoxicillin, azithromycin and cefuroxime), considering both simple and ternary systems. AMX: amoxicillin; AZM: azithromycin; CFX: cefuroxime

Mussel shell was the bio-adsorbent with the lowest desorption values, showing null desorption for all antibiotics in both systems, except for CFX in the simple system, where it reached values of 12%. Regarding pine bark, AZM desorption was null for all concentrations and in both systems, while for AMX the desorption values reached 4% in the simple system and 33% in the ternary system. CFX was the antibiotic which presented the highest desorption percentages for pine bark in the simple system, with values ranging from 59% to 27%, whereas in the ternary system desorption is always null. Oak ash was the bio-adsorbent that presented the highest desorption values for AZM, reaching 91% and 53% in simple and ternary systems, respectively, when 10 µmol L^{-1} were added, whereas, as the antibiotic concentration increased, the desorption percentage decreased to below 2% for both systems. As

for CFX and AMX, desorption levels are practically null for both systems, except for CFX in the simple system, with values not exceeding 8% at low doses.

These findings indicate that oak ash emerged as the most effective bio-adsorbent for AMX and CFX, both in simple and combined (ternary) systems, demonstrating the highest adsorption efficiencies and low desorption. However, it is not suitable for AZM, as although adsorption was high (particularly in the single system and at the highest doses), desorption percentages were significantly elevated at intermediate concentrations, limiting its practical applicability. Pine bark showed good retention capacity for AZM in the single system (up to 40 μmol L^{-1}), but its efficiency declined in the ternary system. It was also ineffective for CFX at higher doses, with adsorption values below 60% and desorption rates reaching approximately 20%. Nevertheless, pine bark displayed its best adsorption capacity for AMX in the ternary system, where high and stable adsorption was observed. Mussel shell, on the other hand, consistently showed poor adsorption capacity for all three antibiotics, particularly at elevated concentrations, with efficiencies generally below 60%, indicating limited suitability as a bio-adsorbent under the tested conditions.

4. CONCLUSIONS

The adsorption of AMX by soils was enhanced in the presence of AZM and CFX, whereas the adsorption of AZM and CFX was reduced when all three antibiotics co-occurred. Despite its increased adsorption, AMX exhibited significantly higher desorption percentages compared to CFX and AZM, suggesting that AMX preferentially binds to lower-energy adsorption sites. The retention of antibiotics by these soils is primarily governed by factors such as pH, organic matter content, non-crystalline minerals and the pKa values of the compounds. Among the tested bio-adsorbents, oak ash demonstrated the highest efficacy for retaining all three antibiotics in the ternary system. Its high pH, abundance of non-crystalline minerals, and high levels of exchangeable calcium contribute to achieve strong and stable adsorption of the pollutants, resulting in minimal desorption. In contrast, mussel shell and pine bark generally exhibited poor efficiency in retaining the antibiotics under the same conditions. These findings highlight that the presence of multiple antibiotics in soil can increase the risk of environmental contamination. Therefore, the use of oak ash may enhance the soil's retention capacity and serve as a potential barrier to antibiotic mobility, offering a promising strategy for mitigating contamination risks in both terrestrial and aquatic environments.

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Declaration of generative AI and AI-assisted technologies in the writing process.

The authors declare that they have not used generative AI and AI-assisted technologies in the development of this work

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SUPPLEMENTARY MATERIAL

ROLE OF SOILS AND BIOADSORBENTS IN THE ADSORPTION DYNAMICS OF AMOXICILLIN, CEFUROXIME AND AZITRHOMYCIN IN SINGLE AND TERNARY SYSTEMS.

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Characterization of soils and sorbent materials

The pH values in water and in KCl (using a 1:2.5 solid-to-liquid ratio) were measured with a CRISON-2001 pH meter (Crison, Barcelona, Spain). Total carbon and nitrogen contents were analyzed via elemental analysis using a LECO CHN-100 analyzer (Leco Corporation, St. Joseph, MI, USA). Exchangeable cations (Ca_e, Mg_e, Na_e, K_e, and Al_e) were extracted by treating the samples with 1 M NH_4Cl at a 1:10 soil or bio-adsorbent to solution ratio and subsequently quantified through atomic absorption/emission spectrophotometry using a Perkin Elmer Analyst 200 instrument (Perkin Elmer Inc., USA). The effective cation exchange capacity (eCEC) was obtained by summing the concentrations of these five cations.

Available phosphorus was extracted using a 0.5 M $NaHCO_3$ solution and its concentration measured using a UV-Vis spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). To quantify non-crystalline forms of Fe and Al (Fe_{ox} and Al_{ox}), samples were treated with ammonium oxalate buffered at pH 3, stirred in the dark for 4 hours; then 0.25% superfloc (5 drops) were added and centrifuged at 2000 rpm for 10 minutes, and finally the supernatant was filtered and diluted (1:5) before analysis by atomic absorption spectrophotometry.

For the determination of Fe and Al extracted with pyrophosphate, 1 g of sample was shaken with 100 mL of sodium pyrophosphate solution (pH 10) for 16 hours. The extract was then analyzed using atomic absorption spectrophotometry.

Finally, total concentrations of various elements (Na_T, Mg_T, Al_T, K_T, Ca_T, Cr_T, Mn_T, Fe_T, Co_T, Ni_T, Cu_T, Zn_T, As_T, and Cd_T) were determined following the EPA 3051 protocol. Samples were digested with 65% HNO_3 in a microwave system, and the resulting solutions analyzed using an ICP-MS device.

Table S1. Values corresponding to the physicochemical properties determined in the soils studied.

Soil	pH_{H2O}	OC	N	Sand	Clay	eCEC	Ca	Mg	Na	K	Al	Fe_{ox}	Fe_{pir}	Al_{ox}	Al_{pir}
		%				$cmol_c\ kg^{-1}$						$mg\ kg^{-1}$			
C	8.02	4.98	0.33	50	23	42.81	39.44	2.07	0.6	0.69	0.01	4140	1501	5351	2111
VP1	5.6	3.69	0.26	69	14	19.52	4.74	1.07	1.64	11.91	0.16	3045	2353	5739	4543
VP2	7.05	2.43	0.14	67	22	9.09	8.11	0.51	0.08	0.37	0.02	1720	1225	3981	1703
VP3	7.27	3.56	0.22	67	12	12.32	10.53	1.04	0.44	0.27	0.04	1945	823	4576	1597
VO	6.04	1.77	0.1	50	24	7.42	4.94	0.86	0.16	0.69	0.77	1790	1171	1556	1141
F	4.68	7.15	0.49	69	15	7.48	0.18	0.07	0	0.22	7.01	3055	6789	5619	9457

C: corn soil; VO: vineyard soil (Ourense province); VP1-VP3: vineyard soils (Pontevedra province); F: forest soil. OC: organic carbon; OM: organic matter; N: nitrogen; eCEC: effective cation exchange capacity; Alox and Feox: Al and Fe extracted with ammonium oxalate; Alpir and Fepir: Al and Fe extracted with sodium pyrophosphate. Average values (n=3), with coefficients of variation always < 5%.

Table S2. Characteristics of the bio-adsorbent materials.

Parameter	Pine bark	Oak ash	Mussel shell
C	48.70	13.23	11.43
N	0.08	0.22	0.21
C/N	608.75	60.14	55.65
pH_{water}	3.99	11.31	9.39
pH_{KCl}	3.42	13.48	9.04
Ca_e	5.38	95.0	24.75
Mg_e	2.70	3.26	0.72
Na_e	0.46	12.17	4.37
K_e	4.60	250.65	0.38
Al_e	1.78	0.07	0.03
eCEC	14.92	361.15	30.25
Sat Al	11.93	0.02	0.10
Available-P	70.45	462.83	54.17
Na_T	68.92	2950	5174.00
Mg_T	473.55	26171	980.66
Al_T	561.50	14966	433.24
K_T	737.84	99515	202.07
Ca_T	2318.81	136044	280168
Cr_T	1.88	36.28	4.51
Mn_T	30.19	10554	33.75
Fe_T	169.78	12081	3535
Co_T	0.20	17.25	1.02
Ni_T	1.86	69.25	8.16
Cu_T	<LD	146.33	6.72
Zn_T	6.98	853.00	7.66
As_T	<LD	8.36	1.12
Cd_T	0.13	19.93	0.07
Al_{ox}	315.0	8323	178.33
Fe_{ox}	74.0	4233	171.0

C: carbon; N: nitrogen; Ca_e, Mg_e, Na_e, K_e, Al_e: elements in the exchange complex; Sat. Al: Al-saturation in the exchange complex; eCEC: effective cation exchange capacity; XT: total content of the element (X); Alox, Feox: non-crystalline Al and Fe. Average values (n=3), with coefficients of variation always <5%. Exchangeable cations and eCEC: $cmol_c\ kg^{-1}$; Available-P and Total content elements: $mg\ kg^{-1}$; C, N and Sat. Al: %.

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Adjustment of AMX, CFX, and AZM Adsorption Data to Linear and Freundlich Models in a Ternary System

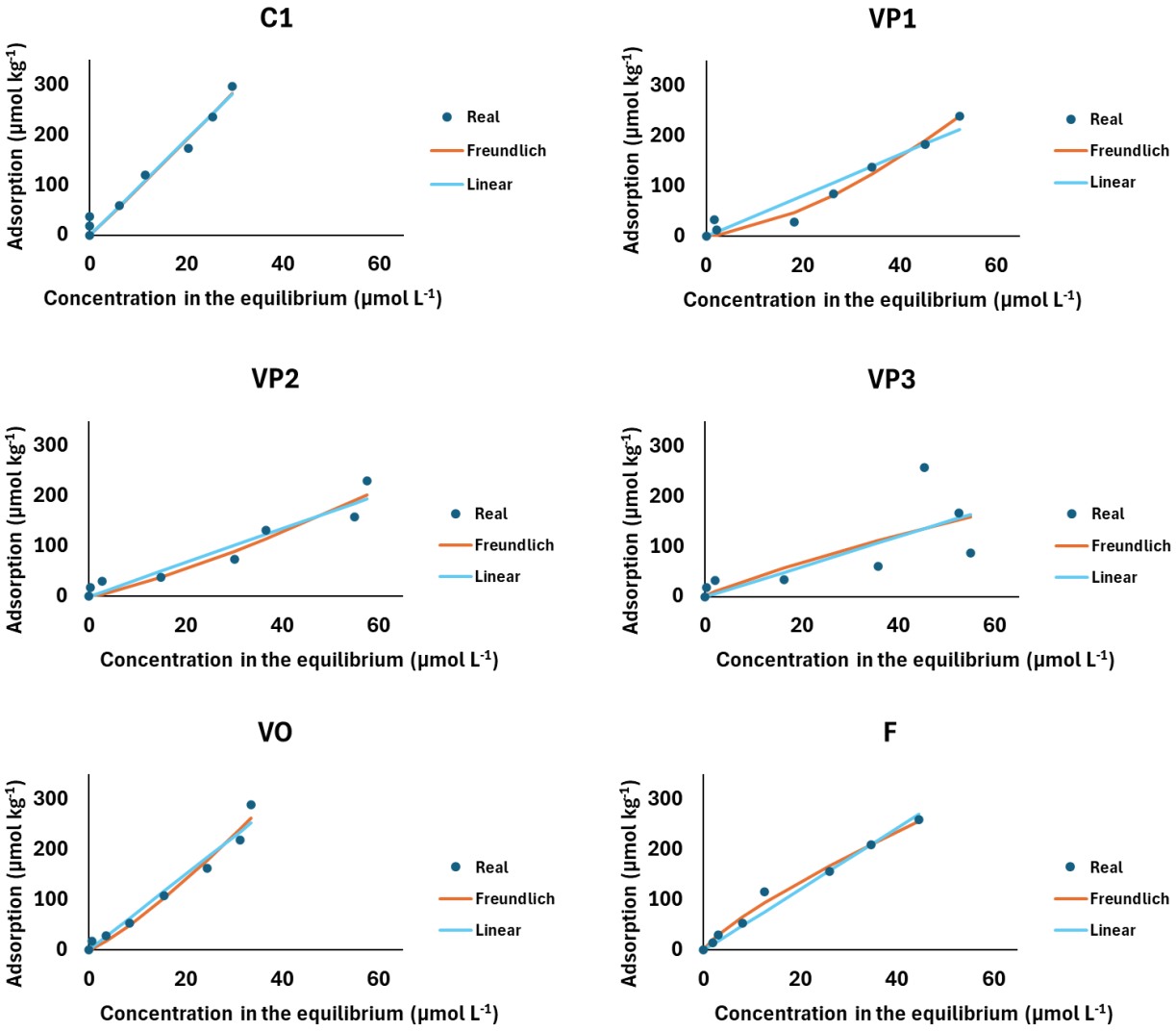

Figure S1. Adsorption curves for ternary system of AMX, CFX and AZM, onto the studied soils, both real and according to the Linear and Freundlich models. C: corn soil; VO: vineyard soil (Ourense province); VP: vineyard soil (Pontevedra province); F: forest soil

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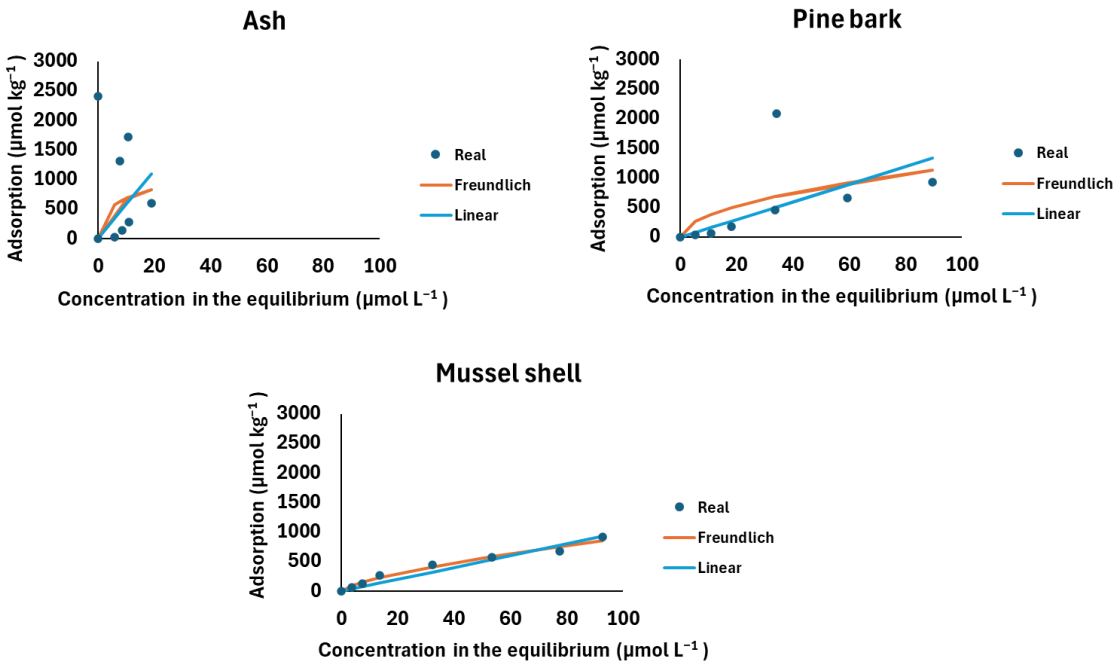

Figure S2. Adsorption curves for AMX, CFX and AZM in the studied bio-adsorbents corresponding to ternary systems, both real and according to the Linear and Freundlich models.

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5. DISCUSIÓN

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5. DISCUSIÓN

5.1. PRESENCIA DE ANTIBIÓTICOS EN LODOS, LODOS GESTORES, SUELOS Y VEGETACIÓN

El primer objetivo de esta Tesis Doctoral consistió en hacer un diagnóstico del estado en que se encuentran los suelos agrícolas y cultivos de Galicia en cuanto a la presencia de los antibióticos más utilizados en medicina humana. Una de las vías de entrada de estos fármacos en el medio ambiente es mediante el uso como fertilizante de lodos de depuradora de aguas residuales (lodos EDAR). En Galicia, estos lodos tienen que ser tratados por gestores autorizados antes de ser incorporados al suelo, por lo que se determinaron los antibióticos antes y después de ser tratados por los gestores, y también en los suelos receptores y en los cultivos establecidos en ellos.

Se realizaron dos muestreos, en primavera y otoño de 2019, recogiendo un total de 50 lodos EDAR (22 en el primer muestreo y 28 en el segundo) en estaciones de depuración de aguas residuales que usan distintos tratamientos, repartidas por toda Galicia. Antes de su incorporación al suelo, estos lodos son tratados por gestores, concretamente en nuestra región son cuatro los que tratan la mayor parte, analizando en este trabajo un total de 13 lodos (6 en el primer muestreo y 7 en el segundo) tras ser procesados por ellos. Finalmente, se analizaron 59 suelos enmendados previamente con estos lodos, así como la vegetación que creció en estos suelos (27 muestras de maíz y 32 de viñedo, separando raíz, tallo, hoja y fruto), recogiendo las muestras en distintas zonas de Galicia. Concretamente, en la provincia de Lugo se muestreó en Castro de Rei y Cospeito, en la de A Coruña en el concello de Arzúa, en la de Ourense en las comarcas del Ribeiro y de Valdeorras y en la de Pontevedra en la comarca do Barbanza, en el concello de Cambados y en otras zonas de las Rías Baixas.

5.1.1 Presencia de antibióticos en lodos EDAR.

En todos los lodos se analizó la presencia de 8 antibióticos distintos, encontrando presencia de 7 de ellos en el primer muestreo y de 5 en el segundo muestreo. En el primero, realizado en primavera, 15 de las 22 muestras de lodos analizadas presentaban al menos un antibiótico de los 8 analizados lo que supone que un 68% de las muestras presentaban estos fármacos. En cuanto al segundo muestreo, este porcentaje decreció hasta el 32%, detectando presencia de antibióticos en 9 de 28 muestras. Esta diferencia en la presencia de antibióticos entre el muestreo de primavera y el de otoño se puede deber a que, en el hemisferio norte, el pico de consumo de antibióticos se encuentra entre los meses de enero y marzo (Van Boeckel et al., 2014). El número de antibióticos detectados en el presente estudio fue generalmente más bajo que el obtenido por otros autores en estudios previos. Concretamente, Aydin et al. (2022) encontraron diez antibióticos diferentes en lodos EDAR en Turquía, con concentraciones máximas detectadas mayores a las de este estudio, mientras que Huang et al. (2020) encontraron 24 antibióticos diferentes en lodos EDAR de China con concentraciones máximas de 11000 ng g^{-1}, frente a valores máximos de 1573 ng g^{-1} obtenidos en el presente estudio. En la misma área geográfica de nuestro estudio, Conde-Cid et al. (2018) encontraron 8 antibióticos diferentes (usados en veterinaria) en un 42% de muestras de purín, pero con un máximo de concentración 60 veces más elevado que los encontrados en el presente estudio.

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En el primer muestreo, el antibiótico que se detectó con más frecuencia en los lodos EDAR fue el ciprofloxacino (CIP), mientras que en el segundo muestreo este fue el segundo antibiótico más detectado, ocupando la primera posición el levofloxacino,

tratándose de antibióticos que pertenecen al mismo grupo, el de las quinolonas. Concretamente, en el primer muestreo el CIP se detectó en el 45% de los lodos muestreados en una concentración máxima de 623 ng g^{-1}, mientras que en el segundo muestreo se detectó en 4 de las 28 muestras analizadas (14%) con un máximo de 1573 ng g^{-1}. Además de ser el antibiótico más frecuente, el CIP fue también el antibiótico que se encontró en concentraciones más altas.

Estudios previos también obtuvieron que el CIP fue uno de los contaminantes emergentes dominantes en todos los compartimentos ambientales, siendo detectado en concentraciones de hasta 1040 ng g^{-1} en lodos (Huang et al., 2020) y valores máximos de 264 ng L^{-1} en efluentes de plantas EDAR (Loos et al., 2013).

5.1.2 Presencia de antibióticos en lodos tratados por gestores de residuos

En los lodos tratados recogidos en el muestreo de primavera se detectó presencia de algún antibiótico en 5 de las 6 muestras analizadas, mientras que en el muestreo llevado a cabo en otoño se encontró algún antibiótico en 4 de las 7 muestras analizadas. El número de antibióticos detectados disminuyó en relación con los encontrados en los mismos lodos sin tratar, ya que en este caso se detectaron 3 de los 8 antibióticos analizados en los lodos del primer muestreo, mientras que en el segundo solo se detectaron 2 antibióticos. Nguyen et al. (2021) y Jelic et al. (2011) en estudios previos indicaron que los métodos de tratamiento aplicados actualmente en las EDAR no son eficaces para eliminar los antibióticos, por lo que se necesita realizar un tratamiento posterior por parte de los gestores de residuos. Generalmente, la gestión que se realizó a las muestras analizadas en este trabajo consistió en la adición de otros residuos orgánicos (animales o vegetales) obteniendo una mezcla final, en algunos casos sometida a compostaje. Como se comentó, los resultados obtenidos en este trabajo mostraron que los lodos tratados por alguno de estos gestores presentaron un menor número de antibióticos y generalmente en menor concentración, que los encontrados en los lodos que no fueron tratados. Esto indica la efectividad de los métodos de tratamiento a los que fueron sometidos por parte de los gestores de residuos, indicando la necesidad de realizar este tratamiento antes de aplicarlos a los suelos, si bien no consiguieron eliminarlos totalmente. Los antibióticos detectados en estos lodos fueron ciprofloxacino, levofloxacino, norfloxacino y lincomicina, por lo que sería necesario aplicar tratamientos adicionales para su total eliminación.

5.1.3 Presencia de antibióticos en suelos previamente enmendados con lodos y los cultivos que crecieron en ellos.

De las 59 muestras de suelo analizadas, solo 7 presentaron niveles detectables de antibióticos, 5 de ellas fueron suelos bajo cultivos de maíz y dos bajo viñedo, lo que supuso un 12% de las muestras. Además, en cada uno de los suelos se detectó un solo antibiótico, con concentraciones que oscilaron entre 41 ng g^{-1} hasta 276 ng g^{-1}. Conde-Cid et al. (2018) encontraron en dos áreas de Galicia con actividad agropecuaria intensiva, antibióticos en el 17% de suelos fertilizados con purines, con concentraciones máximas de 600 ng g^{-1}, duplicando el máximo detectado en los suelos enmendados con los lodos gestionados en el presente estudio. La presencia de antibióticos en estas muestras de suelo podría deberse a su dispersión a través de efluentes contaminados de EDAR (Zuccato et al., 2010) o a la persistencia de antibióticos en el suelo tras varios años de aplicación de lodos (Rutgersson et al., 2020), siendo esta la situación más probable en los suelos del presente trabajo. Un estudio llevado a cabo por Gros et al. (2019) demostró que la aplicación continua de purines y estiércol, especialmente purines porcinos, contribuye a

la acumulación de antibióticos persistentes en las capas superiores del suelo, coincidiendo también con lo señalado por Conde et al. (2018) en suelos de Galicia.

En todas las muestras de suelo recogidas se analizaron los 7 antibióticos más ampliamente utilizados en medicina humana (amoxicilina, cefuroxima, azitromicina, claritromicina, ciprofloxacino, levofloxacino y trimetoprim), obteniendo que 3 de ellos están presentes en estos suelos (amoxicilina, cefuroxima y claritromicina). En cuanto a los antibióticos que se han utilizado en esta Tesis para conocer su dinámica en el suelo (cefuroxima, amoxicilina y azitromicina), se encontró presencia de CFX en 3 suelos, todos ellos con cultivo de maíz y en otros 3 suelos, 2 de viñedo y 1 de maíz, se encontró presencia de AMX. En la figura 11 se representa el número de muestras de suelo en el que se detectaron dos de los antibióticos objeto de estudio y en qué concentración.

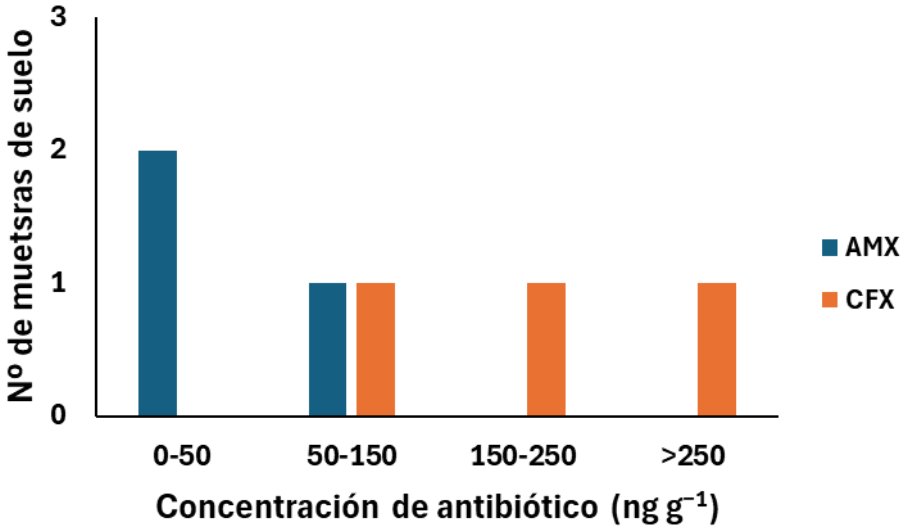

Figura 11. Número de muestras en las que se detectaron los antibióticos amoxicilina (AMX) y cefuroxima (CFX) y el rango de concentración en el que se encuentran.

En cuanto a las muestras vegetales analizadas, no se detectó ningún antibiótico en ninguna de las muestras de raíz, tallo, hojas, o frutos (grano de maíz o uvas). En investigaciones previas, se mostró que diferentes cultivos que crecen en suelos fertilizados con estiércol podían absorber antibióticos (Bassil et al., 2013), pero en algunos casos los valores estaban por debajo del límite de cuantificación (Kang et al., 2013). En Galicia, estudios previos mostraron que el 44% de los cultivos enmendados con purines tenían concentraciones detectables de antibióticos veterinarios (Conde-Cid et al., 2018), porcentajes claramente más elevados que cuando se trata de los antibióticos de uso humano investigados en este trabajo.

5.2 DINÁMICA DE LOS TRES ANTIBIÓTICOS EN LOS SUELOS: ADSORCIÓN/DESORCIÓN

5.2.1 Adsorción

Comparando las curvas de adsorción (adsorción en función de la concentración en la disolución en equilibrio) representadas en las figuras 12, 13 y 14 se puede observar que, de los tres antibióticos estudiados (AMX, CFX y AZM), la AZM es el que presenta una

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mayor adsorción por los suelos, siendo hasta 10 veces superior a la de los otros dos antibióticos (AMX y CFX).

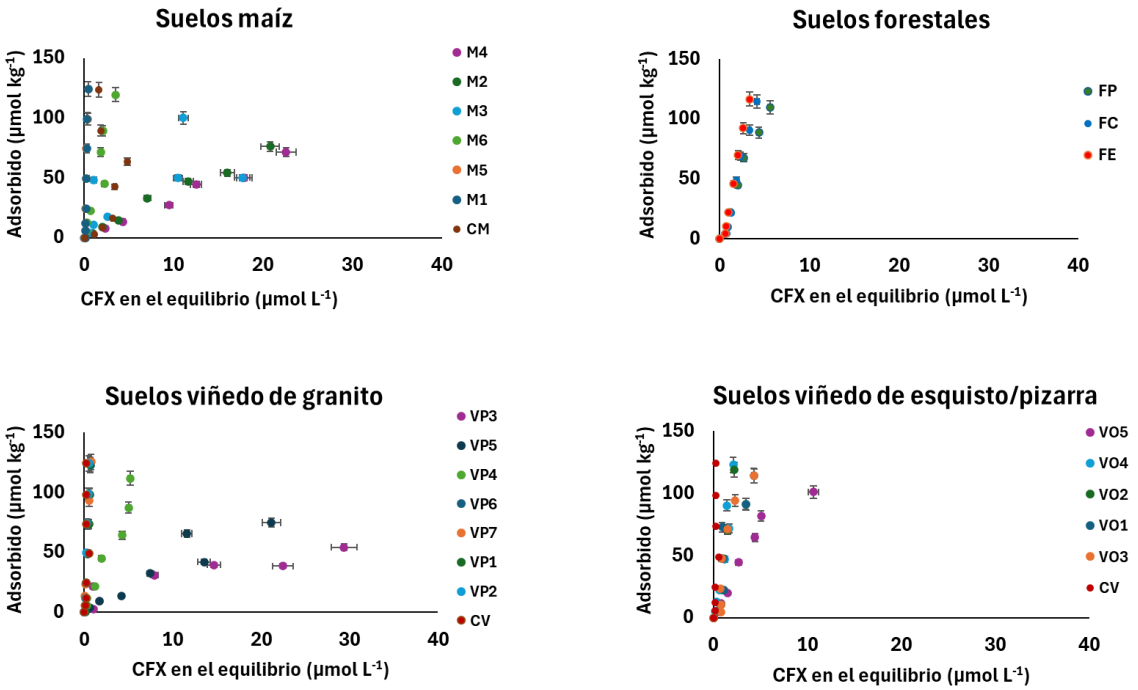

Figura 12. Curvas de adsorción para la cefuroxima (CFX) en los suelos estudiados. M: suelos maíz; VP: suelos viñedo de la provincia de Pontevedra; VO: suelos viñedo de la provincia de Ourense; F: suelos forestales.

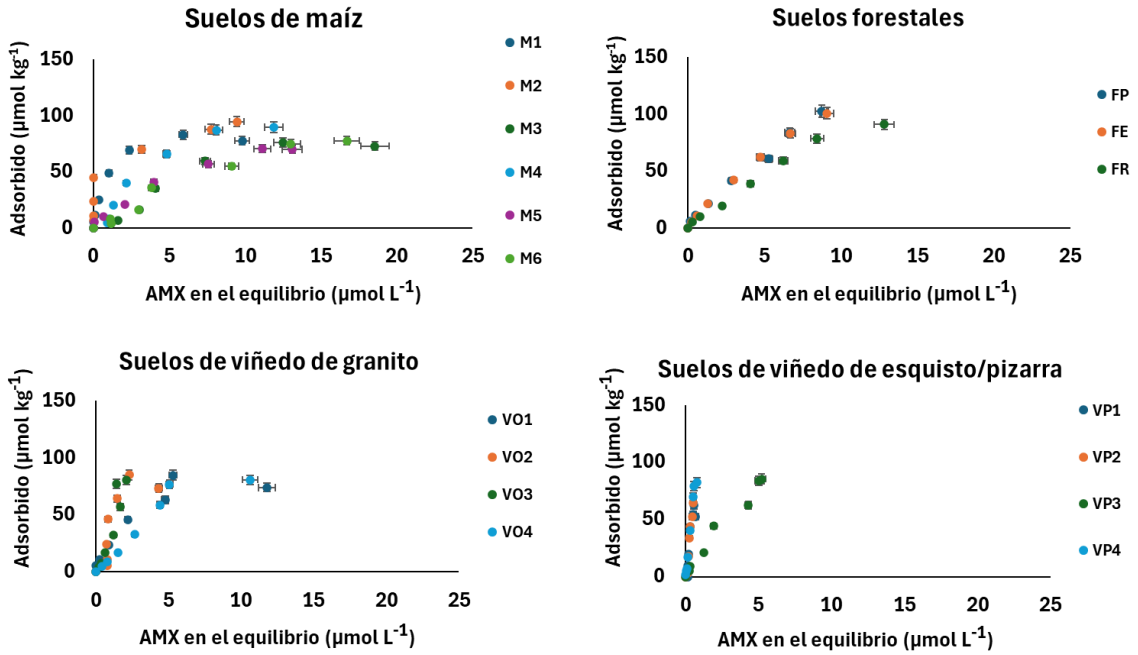

Figura 13. Curvas de adsorción para la amoxicilina (AMX) en los suelos estudiados. M: suelos maíz; VP: suelos viñedo de la provincia de Pontevedra; VO: suelos viñedo de la provincia de Ourense; F: suelos forestales.

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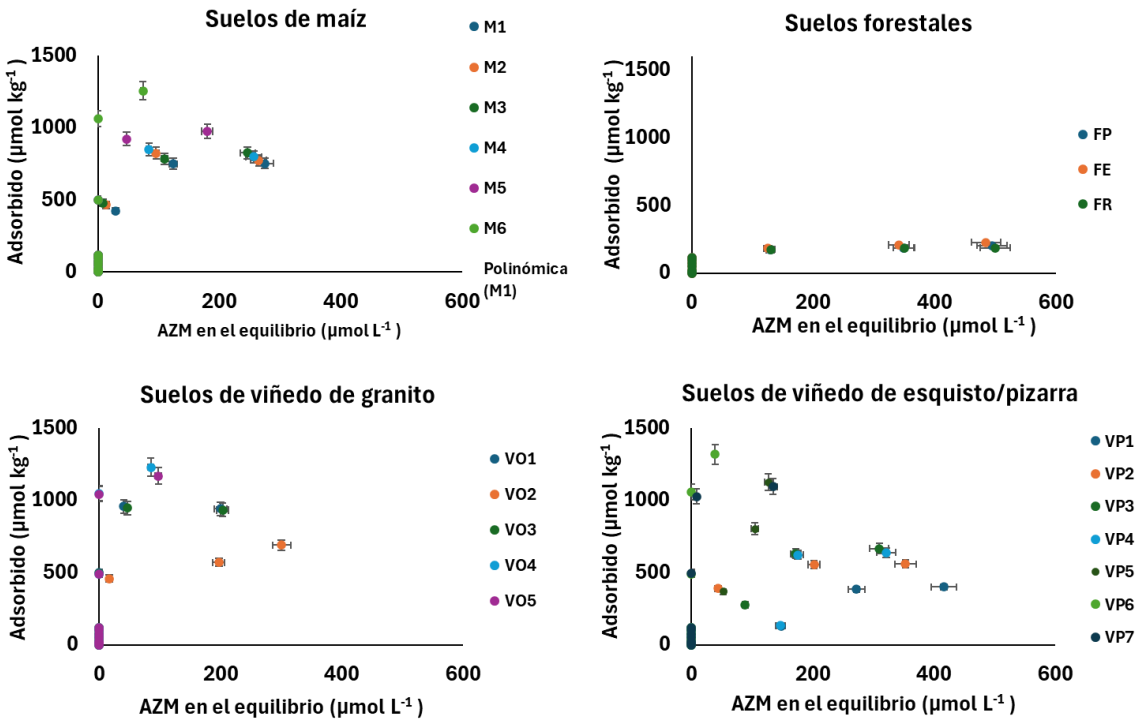

Figura 14. Curvas de adsorción para la azitromicina (AZM) en los suelos estudiados. M: suelos maíz; VP: suelos viñedo de la provincia de Pontevedra; VO: suelos viñedo de la provincia de Ourense; F: suelos forestales

La adsorción de estos tres antibióticos por los suelos fue ajustada a los modelos de adsorción de Freundlich y Langmuir y al modelo Lineal (Tabla 10). Los mejores ajustes para los tres antibióticos se obtuvieron con el modelo de Freundlich (Tabla 10), obteniéndose un R^2>0,9 para la mayoría de los suelos. En el modelo de Langmuir, aunque para AMX y CFX los R^2 son elevados (Tabla 10), también lo es el error, lo que invalida el ajuste a este modelo. En cuanto al modelo Lineal, ninguno de los tres antibióticos presentó un buen ajuste en la mayoría de los suelos.

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Tabla 10. Valores mínimos, máximos, media y mediana de los parámetros obtenidos en los ajustes de adsorción a los modelos de Freundlich, Langmuir y Lineal.

	CFX							
	Freundlich			Langmuir			Lineal	
	KF	n	R^2	KL	qm	R^2	Kd	R^2
Mínimo	3,234	0,41	0,57	0,013	58,46	0,876	2,017	0,328
Máximo	478,866	1,655	0,996	0,15	341,167	0,989	247,337	0,993
Media	105,803	1,054	0,897	0,107	162,880	0,925	69,261	0,836
Mediana	32,17	0,983	0,943	0,1315	125,9455	0,9175	27,873	0,888
	AMX							
Mínimo	9,819	0,306	0,472	0,041	89,525	0,723	3,494	0,365
Máximo	150,337	1,326	0,999	1,242	385,901	0,998	124,74	0,982
Media	41,372	0,777	0,901	0,198	187,919	0,938	27,706	0,803
Mediana	20,155	0,747	0,933	0,075	156,0085	0,9775	10,719	0,886
	AZM							
Mínimo	8,87	0,128	0,269	0,028	192,928	0,269	0,466	0,103
Máximo	354,449	1,327	0,975	0,167	1600	0,968	33,867	0,967
Media	149,815	0,709	0,721	0,079	917,978	0,649	6,568	0,496
Mediana	95,986	0,829	0,892	0,049	852,124	0,734	3,787	0,44

Teniendo en cuenta los parámetros obtenidos en los ajustes a los modelos, la CFX obtuvo valores de la constante de afinidad de Freundlich (KF), relacionada con la capacidad de adsorción, que oscilaron entre 3,234 y 478,86 $L^n\,\mu mol^{1-n}\,kg^{-1}$, mientras que los valores de KF para la AZM variaron entre 8,87 y 354, 44 $L^n\,\mu mol^{1-n}\,kg^{-1}$; la AMX fue la que presentó los valores más bajos, variando entre 9,82 y 150,34 $L^n\,\mu mol^{1-n}\,kg^{-1}$ (tabla 10). Estos datos coinciden con lo observado en las curvas de adsorción en las que claramente la AMX es el antibiótico que presenta la menor adsorción (Figs 12, 13 y 14). Estos valores de KF fueron mucho más elevados que los obtenidos en otros estudios para las sulfonamidas (Conde-Cid et al., 2020c), pero más bajos que los obtenidos para la tetraciclina (Conde-Cid et al., 2019a).

Los análisis de correlación realizados entre el parámetro KF y las variables edáficas indicaron que, en el caso de la CFX, existe una correlación significativa (p<0,05) y positiva de este parámetro con pH el agua (r = 0,56), pH en KCl (r = 0,56), contenido de Ca cambiable (r = 0,71), Mg cambiable (r = 0,45), y con CICe (r = 0,49). Autores como Fakhri y Adami, (2014) y Figueroa-Diva et al. (2010) indicaron que el pH es uno de los parámetros que mayor influencia tiene en las interacciones entre los antibióticos y los adsorbentes, ya que afecta simultáneamente a la especiación química de los antibióticos y a las superficies del adsorbente, pues tanto los antibióticos como los componentes del suelo tienen grupos funcionales que pueden sufrir protonación o deprotonación, dependiendo del pH de la solución. En el caso de la AMX, el parámetro KF se correlacionó significativa y positivamente con el contenido en K (r = 0,712, p < 0,01), mientras que, en el caso de la AZM, este parámetro no se correlacionó con ninguna propiedad de los suelos, posiblemente por el peor ajuste de este antibiótico al modelo de Freundlich en relación con los otros dos (Tabla 10).

En cuanto al parámetro n de Freundlich, relacionado con la reactividad y heterogeneidad de los sitios activos del adsorbente (Proctor y Toro-Vázquez, 1996; Foo y Hameed, 2010), presentó una gran variabilidad entre los distintos suelos para los tres antibióticos. Este parámetro osciló entre 0,41 y 1,655 en el caso de la CFX, entre 0,306 y

1,33 en la AMX y entre 0,128 y 1,327 en la AZM. En el caso de la CFX, se correlacionó positiva y significativamente con el contenido de materia orgánica del suelo (r=0,44; p<0,05), indicando que la adsorción de la CFX sería más fuerte en suelos con más MO. Shafqat y Pierzynski, (2014) también obtuvieron valores de n muy variables para la adsorción de fósforo, lo que atribuyeron a la presencia de cantidades variables de C orgánico, así como de C orgánico lábil. Cuánto más cercano es n a 0, mayor será el grado de heterogeneidad en la superficie de adsorción (Foo y Hameed, 2010; Kong et al., 2012). Además, si los valores de este parámetro se encuentran entre 0 y 1, indican que la adsorción tiene lugar en una matriz heterogénea, en la cual los sitios de mayor energía son ocupados preferentemente, mientras que si n>1, se asocian con desviaciones del modelo de Freundlich, lo cual puede atribuirse a la ocurrencia de procesos irreversibles como la precipitación (Tamer et al., 2018), o-a un tipo de adsorción cooperativa (Foo y Hameed, 2010).

El parámetro Kd del modelo Lineal, relacionado con la intensidad de adsorción, presentó los valores más bajos en la AZM, entre 0,466 y 16,97 L kg^{-1}, mientras que, en el caso de la CFX oscilaron entre 2,017 y 247,33 L kg^{-1} y en la AMX variaron entre 3,49 y 120,34 L kg^{-1}. En un estudio previo se obtuvieron valores de Kd para varias sulfonamidas más altos que los obtenidos en este estudio para la AZM, pero más bajos que los obtenidos para los otros dos antibióticos (CFX y AMX) (Conde-Cid et al., 2020c), sin embargo, las tetraciclinas obtuvieron valores de Kd significativamente más elevados que los antibióticos de este estudio (AMX, CFX y AZM) (Conde-Cid et al., 2019a). Además, el parámetro Kd obtenido en el ajuste de la CFX se correlacionó significativa (p<0,05) y positivamente con el pH en agua (r=0,50), con las concentraciones de Ca y Mg cambiables (r=0,58 y r=0,43, respectivamente) y con CICe (r=0,57). Estos parámetros son los mismos con los que se correlacionó el parámetro KF de Freundlich para la CFX, lo que podría indicar que la capacidad y la intensidad de adsorción de los suelos son mayores cuanto mayor sea el pH, pues a medida que aumenta este parámetro aumentarían las cargas negativas de los coloides del suelo con carga variable, aumentando la capacidad de adsorción.

En cuanto al modelo de Langmuir, el parámetro KL osciló entre 0,013-0,015 L μmol^{-1} en el caso de la CFX, entre 0,028 y 0,167 L μmol^{-1} en el ajuste de la AZM y entre 0,05 y 1,24 L μmol^{-1} en la AMX. El parámetro q_m osciló entre 58,46 y 341 $\mu mol\ kg^{-1}$ en la CFX y entre 89,53 y 293,44 $\mu mol\ kg^{-1}$ en la AMX. La AZM fue la que presentó los valores más altos de q_m, oscilando entre 608,522 y 1554,29 $\mu mol\ kg^{-1}$, lo que coincide con la mayor adsorción de este antibiótico por los coloides del suelo.

5.2.1.1. Influencia de las propiedades fisicoquímicas del suelo en la adsorción de los tres antibióticos

La adsorción de los antibióticos por los suelos depende tanto de las características del antibiótico como de las propiedades fisicoquímicas de los suelos. Los suelos de este trabajo presentan propiedades muy diferentes entre ellos. Como ya se comentó el pH es uno de los parámetros que más influye en las interacciones suelo-antibiótico y el amplio rango de valores de pH (4,68-8,02) de los suelos de este estudio podría explicar las diferencias de adsorción que existen entre los distintos suelos. Tanto los antibióticos como los componentes de los suelos tienen grupos funcionales que pueden sufrir reacciones de protonación/deprotonación dependiendo del pH de la disolución, lo que hace posible que existan cargas negativas o positivas. Además, en el caso de los antibióticos también se pueden comportar como zwitterion con grupos cargados positiva y negativamente a la vez (carga cero) en un determinado rango de pH (Sun et al., 2010).

En la figura 15 se muestran las diferencias en los porcentajes de adsorción de los tres antibióticos en dos suelos con valores de pH muy diferentes entre sí.

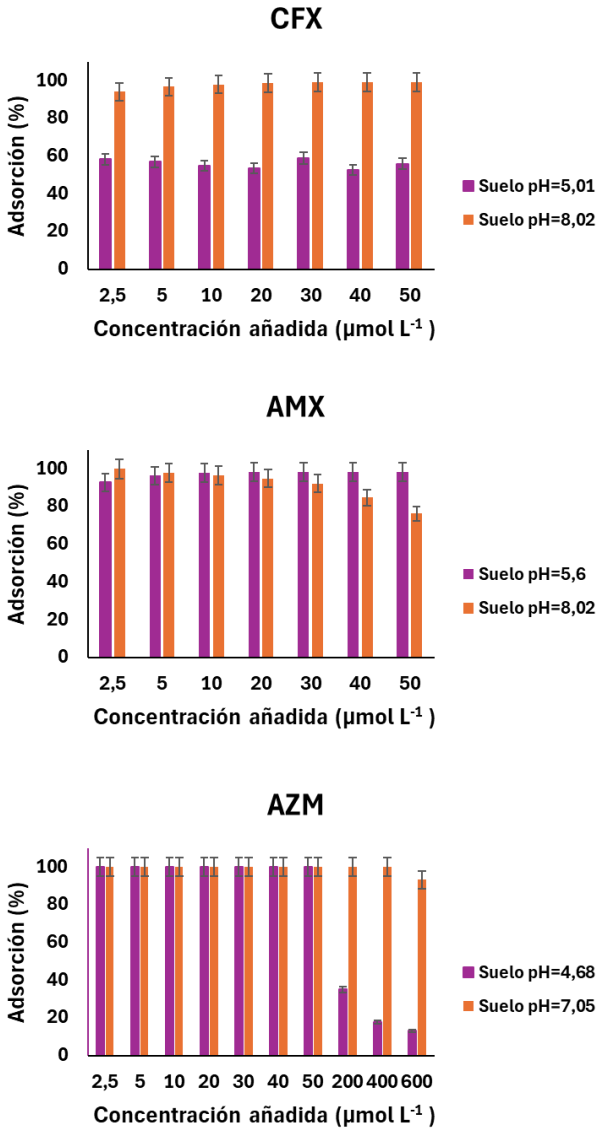

Figura 15. Porcentajes de adsorción de amoxicilina (AMX), cefuroxima (CFX) y azitromicina (AZM) por dos suelos con diferentes valores de pH.

En el caso de la cefuroxima, los suelos que tienen valores de pH más altos son los que presentan los mayores porcentajes de adsorción (Fig.15). Este antibiótico presenta dos valores de pKa, 3,15 y 10,97 (Ribeiro y Schmidt, 2017), por lo que a pH superior a 10,97 está en forma aniónica, mientras que por debajo de 3,15 está como catión y entre ambos valores de pH está como zwitterion. Los suelos, a pH ácido (específicamente a pH<5) pueden tener carga negativa en las arcillas de carga permanente y carga positiva en los componentes de carga variable, mientras que a pH superior a 5 la mayoría de los componentes con carga variable del suelo estarán cargados negativamente. La mayoría de los suelos de este trabajo presentan pH>5,5, por lo que estarían cargados negativamente, mientras que la cefuroxima estaría como zwitterion, de modo que los grupos cargados positivamente (NH_2^+) del antibiótico interaccionarían

electrostáticamente con las superficies adsorbentes de los suelos cargadas negativamente, mientras que los grupos aniónicos (COO^-) de la cefuroxima necesitarían de un puente catiónico para unirse a dichas superficies (Legnoverde et al., 2014). A valores de pH del suelo más altos, habrá mayor cantidad de cargas negativas en el suelo, lo que explicaría la influencia del pH en el proceso de adsorción. Sin embargo, los suelos forestales que hemos estudiado también presentan valores de adsorción altos a pesar de que tienen valores de pH bajos (4,68-4,88) (ver figura 2 de la publicación II). Esto puede estar relacionado con el contenido de MO de estos suelos, que oscila entre 12,32 y 16,86%, valores mucho más elevados que los de los suelos agrícolas (1,09-13,21%); a pH<5, los grupos -COOH de la materia orgánica empiezan a disociarse (Yu et al., 2011) y podrían interactuar electrostáticamente con los grupos cargados positivamente de la CFX, confiriendo a estos suelos un elevado poder de adsorción pese a su fuerte acidez.

La AMX, contrariamente a la CFX, se adsorbe más en los suelos de pH más ácido (en torno a 5), sobre todo en las dosis más altas añadidas (Fig. 15). Esto coincide con lo obtenido para las tetraciclinas en un estudio realizado por Conde-Cid et al. (2019a) en suelos de Galicia. Githinji et al. (2011) también indicaron una disminución de adsorción de AMX a medida que aumentaba el pH del adsorbente de 3,5 a 8,5. En la misma línea, Mohammed et al. (2020) utilizando casca de pistacho como adsorbente, obtuvieron que el máximo de adsorción va disminuyendo para valores de pH>5, resultados similares a los encontrados por Shahrouzi et al. (2019). Esta diferencia en el pH al que se adsorben preferentemente los distintos antibióticos puede relacionarse con sus propiedades fisicoquímicas, principalmente con los valores de pKa, ya que poseen distintos grupos funcionales que generan distintas cargas dependiendo del pH del suelo. La AMX tiene tres valores de pKa y su carga molecular sufre variaciones dependiendo del pH, debido a la presencia de grupos funcionales, tales como carboxilo (pka1=2,68), amina (pka2=7,47) y fenólicos (pka3= 9,63) (Homsirikamol et al., 2016). Por lo tanto, en los suelos con un pH<5, pueden ocurrir interacciones electrostáticas entre los componentes de carga variable positivamente cargados del suelo (MO y componentes no cristalinos muy abundantes en estos suelos) con los grupos carboxilo desprotonados de la AMX, así como interacciones entre los componentes del suelo con carga negativa (arcillas 2:1; algunos grupos carboxilo desprotonados) con los grupos amina protonados del antibiótico. Los suelos con valores de pH entre 5,0-6,5, que son la mayoría de los utilizados en el presente estudio, tienen una carga preferentemente negativa, que puede interactuar con los grupos amina protonados de la AMX. Sin embargo, en los suelos con pH>6 predominarán las cargas negativas, al igual que ocurre en la AMX a este valor de pH, por lo que el mecanismo principal de unión sería mediante un puente catiónico. Además, la adsorción disminuiría con el aumento del pH debido al incremento de iones OH^- en la disolución, que pueden competir con los grupos COO^- del antibiótico para la formación de un puente catiónico con los coloides del suelo.

Finalmente, en el caso de la AZM, se repite el mismo patrón que sucedía en la CFX, ya que a pesar de que a concentraciones añadidas más bajas (hasta 50 μmol L^{-1}), todos los suelos adsorben el 100% de AZM, a concentraciones más altas se observa que los suelos con valores de pH más altos son los que presentan los valores de adsorción más altos (Fig. 15). De hecho, la adsorción de la AZM se correlacionó positiva y significativamente con el pH del suelo (r=0,562; p<0,01). Este antibiótico presenta un valor de pKa que se encuentra entre 8,6 y 9,5, por lo que en todos los suelos de este estudio (valores de pH entre 4,68-8,02) este antibiótico tendrá grupos funcionales con carga positiva, lo que favorecerá la unión con las superficies cargadas negativamente de los minerales y materia orgánica mediante interacciones electrostáticas (Peterson et al., 2009; Chen et al., 2013). Sin embargo, los suelos forestales, a pesar de tener un alto

contenido tanto de minerales no cristalinos como de materia orgánica (MO), presentan una baja adsorción cuando las concentraciones añadidas superan los 200 µmol L^{-1}. Eso es debido a que estos componentes a estos valores de pH tan ácidos desarrollan carga positiva al igual que la que presenta la AZM, de ahí la correlación significativa y negativa obtenida entre la adsorción y el contenido de MO (r=-0,530; (p<0,05) y el Al no cristalino total extraído con oxalato amónico (r=-0,43).

La AZM presentó valores de adsorción significativamente más altos que la CFX y AMX, por ello se añadieron concentraciones más altas de antibiótico (hasta 600 µmol L^{-1}), ya que hasta 50 µmol L^{-1} (la concentración máxima añadida para CFX y AMX) todos los suelos adsorbían el 100% de AZM, independientemente de sus propiedades fisicoquímicas. La elevada adsorción encontrada para la AZM en este estudio coincide con la obtenida en otras investigaciones realizadas con altas concentraciones de este antibiótico en suelos agrícolas enmendados, y debido a esa alta retención se interpreta que este fármaco no causa toxicidad en los cultivos ni en los microorganismos del suelo (Sidhu et al., 2019 a,b).

5.2.2. Suelos: desorción

Los tres antibióticos (CFX, AMX y AZM) presentaron, en general, bajos porcentajes de desorción, indicando que el proceso de adsorción de ellos por los suelos es prácticamente irreversible. En el caso de la AZM esta fue nula en todos los suelos y para todas las concentraciones de antibiótico añadida, mientras que en el caso de la CFX esta nunca superó el 8% (siendo menor del 1% en la mayoría de los casos) (Fig.16). El análisis de correlaciones bivariadas de Pearson entre los valores de desorción de la CFX y las propiedades fisicoquímicas de los suelos indicó que existe una correlación significativa y positiva con el contenido de arena (r=0,45, p<0,05) y negativa con el contenido de arcilla (r=-0,43; p<0,05). Esto indica que las arcillas tendrían un papel importante en la intensidad de la adsorción, reduciendo la reversibilidad del proceso y, contrariamente, la presencia de una fracción más gruesa y menos reactiva como la arena favorecería la desorción.

En el caso de la AMX, también presentó valores bajos de desorción (generalmente inferior al 15%) (Fig. 17), aunque, de los tres antibióticos, fue el que presentó los valores de desorción más altos, existiendo diferencia entre los suelos agrícolas y los forestales. Estos últimos presentaron valores de desorción inferiores al 7% en todos los casos, mientras que los suelos agrícolas alcanzaron un máximo de desorción del 17%. Esto indicaría que los suelos con un pH más bajo y un mayor contenido de MO y minerales no cristalinos (como sucede en los suelos forestales) adsorben más AMX y la retienen con una mayor intensidad.

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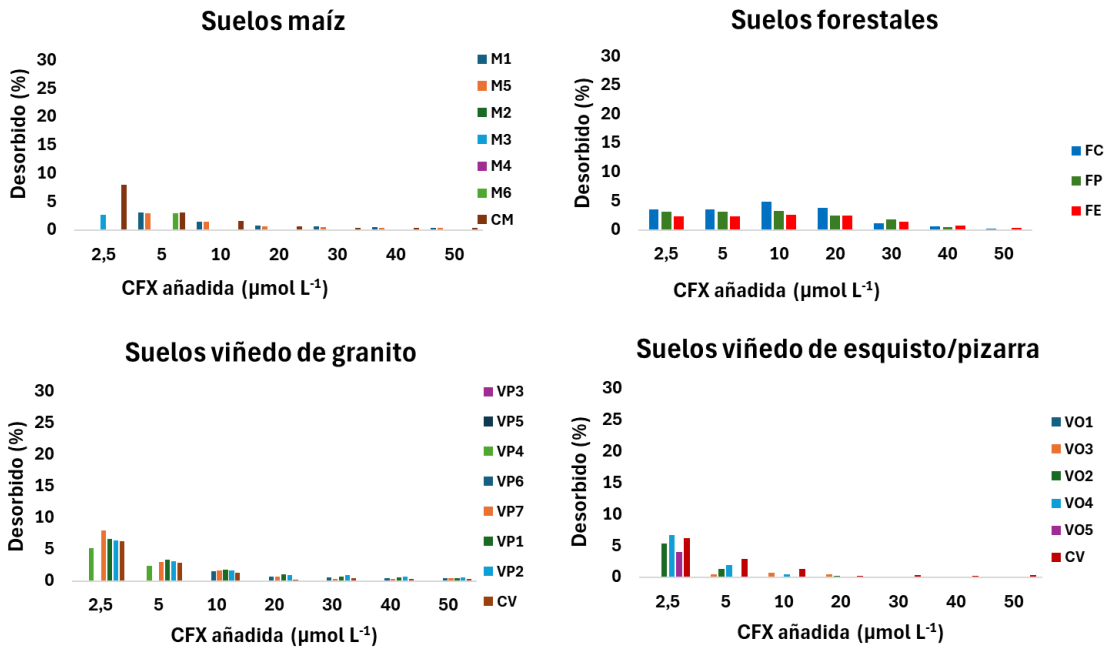

Figura 16. Porcentajes de desorción de CFX en función de la concentración añadida. M: suelos maíz; VP: suelos viñedo de la provincia de Pontevedra; VO: suelos viñedo de la provincia de Ourense; F: suelos forestales

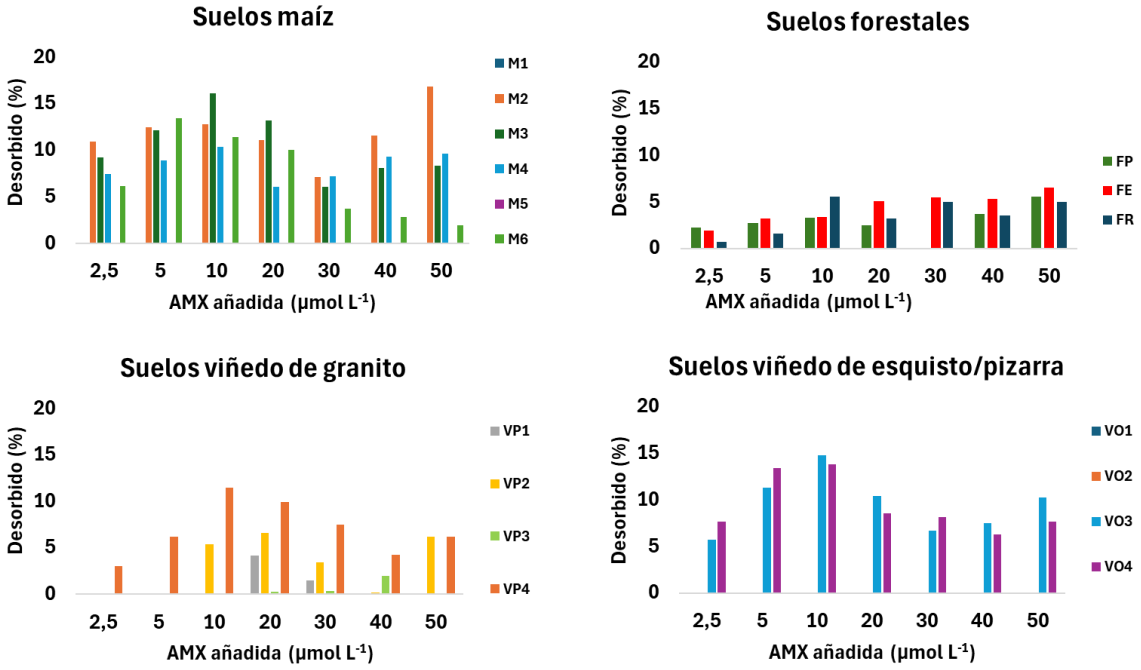

Figura 17. Porcentajes de desorción de AMX en función de la concentración añadida. M: suelos maíz; VP: suelos viñedo de la provincia de Pontevedra; VO: suelos viñedo de la provincia de Ourense; F: suelos forestales

5.3. MATERIALES RESIDUALES/SUBPRODUCTOS: ADSORCIÓN/DESORCIÓN DE LOS TRES ANTIBIÓTICOS

El uso de materiales residuales/subproductos (procedentes de distintas actividades) abundantes en Galicia como bioadsorbentes para retener antibióticos puede ser una

medida eficaz para minimizar el efecto indeseable de estos fármacos en el medio. Además, representa una alternativa más sostenible, ya que su empleo no solo reduce el impacto ambiental asociado a los contaminantes, sino que también permite su revalorización dentro de una economía circular. En este trabajo se utilizaron 5 residuos/subproductos provenientes de la industria forestal y alimentaria (ceniza de roble, corteza y acícula de pino, hoja de eucalipto y concha de mejillón) para estudiar su poder de adsorber los tres antibióticos objeto de estudio. Al igual que en los suelos, se realizaron ensayos tipo batch, donde se estudió la capacidad de estos materiales para adsorber antibióticos.

5.3.1. Materiales residuales/subproductos: adsorción

En la figura 18 se representan las curvas de adsorción de la CFX, AMX y AZM por los distintos materiales residuales. En el caso de los dos primeros antibióticos (CFX y AMX) se utilizaron los 5 materiales citados anteriormente, mientras que en la AZM solo se utilizaron la ceniza de roble, corteza de pino y concha de mejillón.

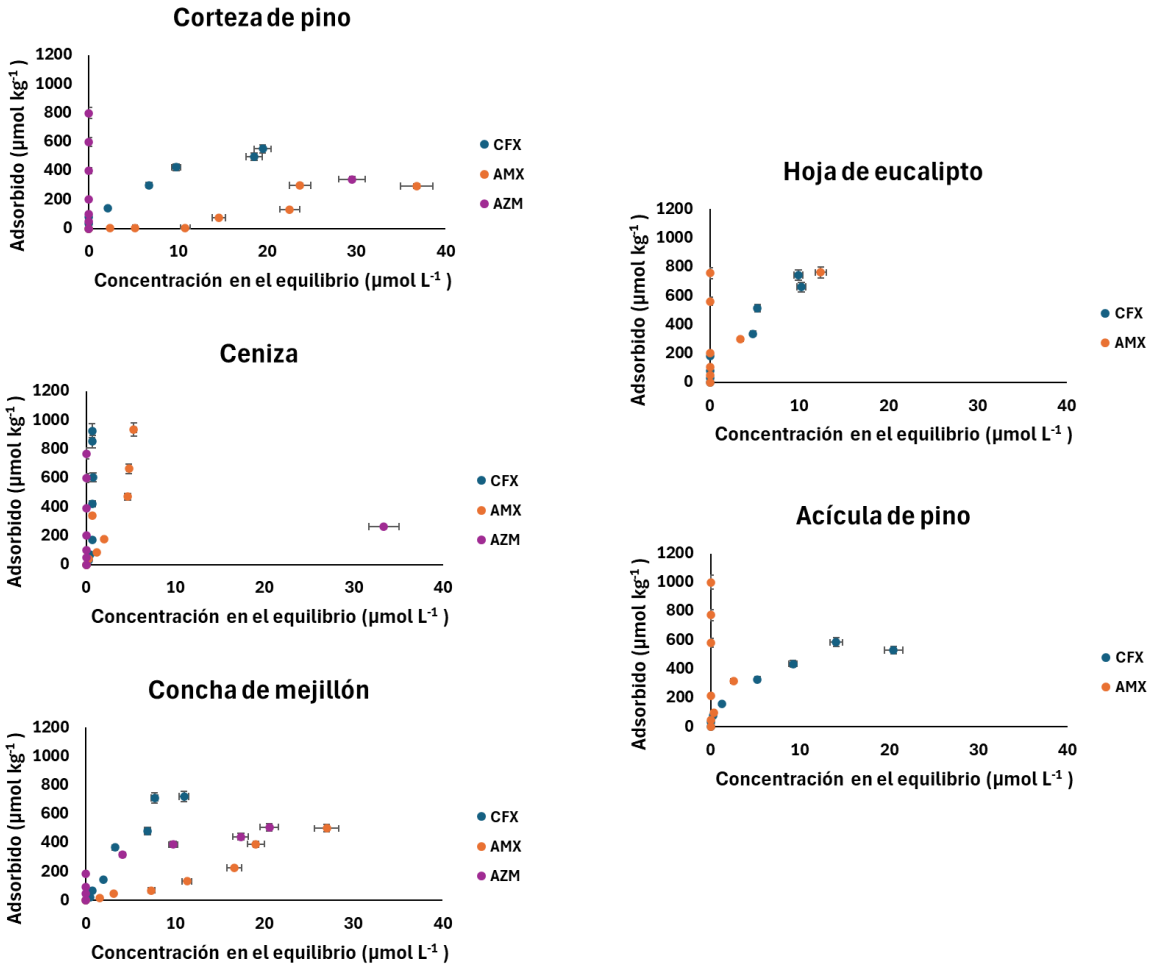

Figura 18. Curvas de adsorción de cefuroxima (CFX), amoxicilina (AMX) y azitromicina (AZM) por los diferentes bioadsorbentes utilizados.

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Al igual que se comentó para los suelos, la AZM es generalmente el antibiótico que presenta los valores de adsorción más altos en la corteza de pino y en la ceniza, ya que en la mayoría de los casos se adsorbió la totalidad de la concentración añadida (no así en la concha de mejillón). Por ello, para este antibiótico se realizaron ensayos con concentraciones añadidas más altas, hasta 600 µmol L^{-1}, que se representan en la figura 19. Como se puede ver en dicha figura, la adsorción es mucho más elevada en la ceniza y en la corteza, en relación con la concha.

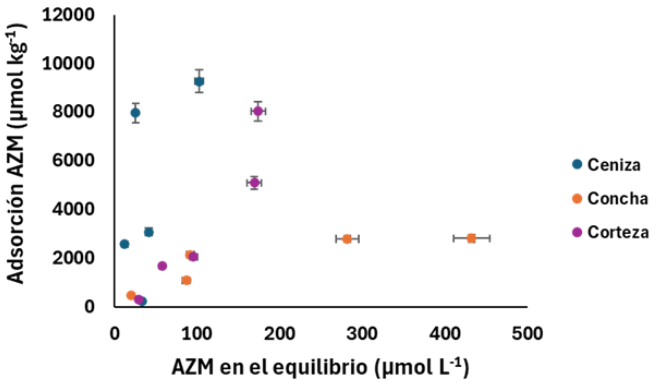

Figura 19. Curvas de adsorción de la azitromicina (AZM) por tres bioadsorbentes cuando se añaden altas concentraciones de antibiótico

Las curvas de adsorción de los tres antibióticos se ajustaron a los modelos más utilizados (Freundlich, Langmuir y lineal). En el caso de la CFX, cuatro de los cinco bioadsorbentes (concha de mejillón, corteza de pino, hoja de eucalipto y acícula de pino) se ajustaron bien (R^2>0,9) a los modelos de Freundlich y Langmuir. En cuanto al modelo Lineal, presentó un buen ajuste con la hoja de eucalipto (R^2=0,929) y con un R^2 menor con la concha de mejillón (R^2=0,884) y la corteza de pino (R^2=0,867). En cuanto a la AMX, la concha de mejillón, la ceniza de biomasa y la hoja de eucalipto presentaron un buen ajuste (R^2>0,8) a los modelos Lineal y de Freundlich, mientras que ninguno de los bioadsorbentes se ajustó bien al modelo de Langmuir. La adsorción de AZM en la concha de mejillón presentó un buen ajuste al modelo de Freundlich (R^2=0,93) y de Langmuir (R^2=0,97). Sin embargo, la adsorción de este antibiótico en la ceniza de roble y la corteza de pino no presentó un buen ajuste a ninguno de los modelos estudiados.

Los valores del parámetro n de Freundlich fueron menores a 1 en todos los bioadsorbentes para los antibióticos CFX y AZM, lo que indicó la presencia de una superficie heterogénea de adsorción, donde los sitios de mayor energía fueron ocupados primero, lo que significa que la energía de adsorción disminuye exponencialmente a medida que la superficie disponible es menor (Foo y Hameed, 2010; Behnajady et al., 2014). Sin embargo, en el caso de la AMX, el parámetro n fue mayor que 1, oscilando entre 1,21 y 1,39, que como ya se comentó para el caso de los suelos, estos valores se asocian con desviaciones del modelo de Freundlich y procesos de adsorción cooperativos.

Teniendo en cuenta los valores del parámetro Kd del modelo lineal, la CFX es el antibiótico que presenta los valores más altos (30,41-597,15 L kg^{-1}), seguida de la AMX (7,92-139,61 L kg^{-1}) y AZM (4,503-49,18 L kg^{-1}). En los tres antibióticos la ceniza fue el bioadsorbente donde se obtuvo el valor más alto, mientras que la corteza de pino presentó el valor más bajo (excepto en el caso de la AZM que no se ajustó a este modelo), indicando

que la ceniza es el material que forma interacciones más fuertes con los antibióticos, mientras que las interacciones antibiótico-corteza son las más débiles.

5.3.1.1. Influencia de las propiedades fisicoquímicas de los bioadsorbentes en la adsorción de los tres antibióticos

En la figura 20 se representa la adsorción en porcentaje de los tres antibióticos (CFX, AMX y AZM) por los diferentes bioadsorbentes en función de la concentración de antibiótico añadido.

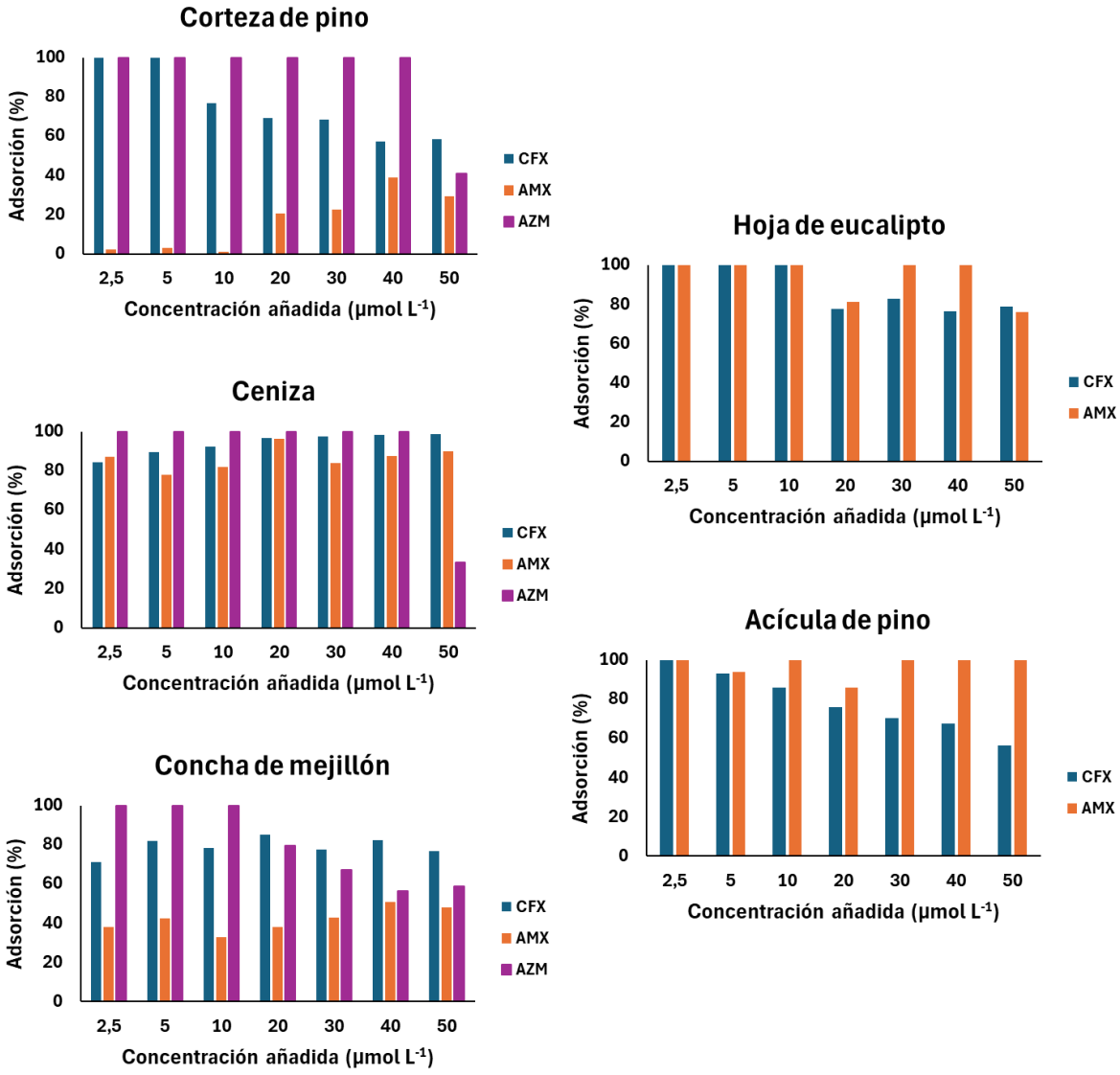

Figura 20. Adsorción en porcentaje de la CFX, AMX y AZM en función de la concentración de antibiótico añadida por los diferentes bioadsorbentes.

Analizando los datos de adsorción de la CFX por los diferentes bioadsorbentes se observa que la ceniza (pH=11,31) es el bioadsorbente con mayor capacidad para adsorber estos antibióticos, con porcentajes de adsorción de aproximadamente el 100% para todas las concentraciones, mientras que la corteza (pH=3,99) y la acícula de pino (pH=3,68)

son los materiales que presentan los porcentajes de adsorción más bajos, disminuyendo hasta el 55% cuándo se añade la máxima concentración de CFX (50µmol L^{-1}). Estos datos confirman la influencia del pH en la adsorción de CFX ya comentada para los suelos, obteniendo la siguiente secuencia: ceniza de roble (pH=11,31)> concha de mejillón (pH=9,39) > hoja de eucalipto (pH=4,88)> corteza de pino (pH=3,99) = acícula de pino (pH=3,68). Fakhri y Adami, (2014) estudiaron la adsorción de CFX en nanopartículas de óxido de magnesio y nanotubos de carbono y obtuvieron los valores de adsorción más elevados a pH>8, indicando que el pH es el parámetro que más influye en la adsorción de los antibióticos del grupo de las cefalosporinas. La CFX tiene dos constantes de disociación, pKa1=3,15 y pKa 2=10,97 y todos los bioadsorbentes, presentan un pH comprendidos entre ambos valores de pKa, excepto la ceniza que tiene un pH mayor a pKa 2. Por ello, este antibiótico va a estar presente como zwitterion en todos los materiales, excepto en la ceniza que estará presente como anión. Cuando la CFX está como zwitterion, los grupos positivos (NH_4^+) del antibiótico interactuarán electrostáticamente con las superficies negativas del bioadsorbente, que serán más abundantes cuanto mayor sea el pH, mientras que los grupos aniónicos (COO^-) de la CFX se unirán a esas superficies mediante un puente catiónico (Legnoverde et al., 2014). Por otro lado, cuando la CFX esté presente como anión (el caso de la ceniza), estará cargada negativamente al igual que ciertos componentes de la ceniza (minerales no cristalinos de Fe y Al), por lo que la adsorción se producirá mediante un puente catiónico, unión que se verá favorecida por la alta concentración de Ca^{2+} que tiene la ceniza. El análisis de correlaciones bivariadas de Pearson demostró que existe una correlación significativa (p<0,05) con el Ca (r=0,918), Na (r=0,903) y K (r=0,927), lo que evidencia su contribución en los enlaces que se forman mediante puentes catiónicos.

En cuanto a la AMX, al contrario de lo que sucede en la CFX, no se ve una influencia clara del pH en la adsorción de la AMX, ya que bioadsorbentes con características ácido-base muy diferentes entre sí presentan adsorciones de AMX muy similares. Concretamente, la acícula de pino (pH=3,68) fue el material que presentó los valores más altos de adsorción (100% en la mayoría de los casos), mientras que la corteza de pino (pH=3,99) presentó los valores más bajos (entre 2,29 y 38,73%). Así, la secuencia de adsorción sería la siguiente: acícula de pino (pH=3,68) > ceniza de biomasa (pH=11,31) > hoja de eucalipto (4,88) > concha de mejillón (pH=9,39) > corteza de pino (pH=3,99). La AMX tiene tres constantes de disociación, pka1=2,68; pka2=7,49 y pka3=9,63, por lo que el pH de la acícula (pH=3,68), de la corteza de pino (pH=3,99) y de la hoja de eucalipto (pH=4,88) se encuentran entre los valores de pka1 y pka2, a los cuales la molécula de AMX se comportará como un zwitterion, con grupos amino protonados y grupos carboxilo deprotonados. La alta adsorción de AMX por la acícula de pino y la hoja de eucalipto puede estar relacionada con el alto contenido de carbono orgánico (50 y 53%, respectivamente), lo que implica la presencia de un amplio número de grupos carboxilo que se pueden disociar y formar carga negativa a pH>3 (Edwards et al., 1996), mientras que la AMX tendrá grupos amino protonados que se unirán mediante interacciones electrostáticas a los grupos carboxilo de los bioadsorbentes de origen vegetal. En cuanto a la baja adsorción de la corteza de pino, comparada con estos materiales de pH similar, puede estar relacionada con la diferente composición química de sus compuestos orgánicos y también al hecho de que la corteza tiene un mayor contenido en minerales no cristalinos de Fe y Al, que estarían cargados positivamente a ese pH. En cuanto a los bioadsorbentes de pH alcalino, ceniza de biomasa (pH=11,31) y concha de mejillón (pH=9,39), también existen diferencias en la adsorción de AMX. Mientras que la ceniza adsorbió entre el 77% y 89% del antibiótico añadida, la concha de mejillón solo adsorbió entre el 32% y 50%. En ambos casos la adsorción se produce mediante un puente

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catiónico entre la superficie cargada negativamente de los materiales adsorbentes y los grupos con carga negativa de la AMX. Sin embargo, la menor adsorción observada en la concha, en comparación con la ceniza, puede relacionarse con su menor contenido de minerales no cristalinos y de calcio intercambiable. Además, el pH de la concha es más bajo y se encuentra por debajo del pKa_3 de la AMX, lo que reduce la densidad de carga negativa del antibiótico.

Finalmente, en el caso de la AZM, la ceniza y la corteza adsorbieron el 100% en todas las concentraciones excepto en la más alta que bajó hasta el 20%; en la concha la adsorción fue del 100% en las concentraciones bajas y descendió hasta el 57% cuándo se añadió la concentración más alta de antibiótico (50 µmol L^{-1}) (Fig.20). Como los suelos presentaron un elevado poder de adsorción de este antibiótico y para poder comparar con ellos, se decidió aumentar la concentración de AZM añadida hasta 600 µmol L^{-1} y los resultados de la adsorción se representan en la figura 21.

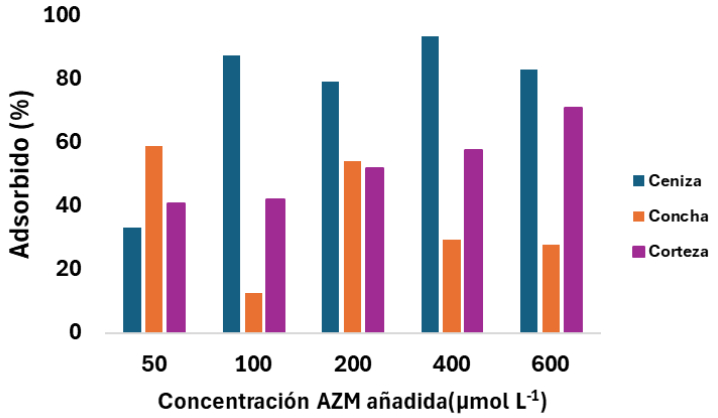

Figura 21. Adsorción en porcentaje de la AZM, al añadir las concentraciones más altas de antibiótico, por los diferentes bioadsorbentes.

Analizando los datos se confirma que la ceniza es el bioadsorbente que más AZM adsorbe, siendo el porcentaje de adsorción generalmente superior al 80%, para dosis superiores a 50 µmol L^{-1}. La corteza de pino presentó un máximo de adsorción del 69% y la concha de mejillón adsorbió el 50%, al añadir la dosis más baja de antibiótico, y decreció hasta el 25% al añadirse la máxima dosis de antibiótico. Un descenso de la adsorción a medida que aumenta la concentración añadida fue obtenido también por Kabir et al. (2022), lo que atribuyen a la progresiva saturación de los sitios de adsorción (Wu et al., 2020). Al comparar la adsorción de la AZM por los tres materiales residuales se obtiene que, al igual que sucedía en la AMX, los de pH alcalino (ceniza de biomasa y concha de mejillón) presentaron capacidades de adsorción muy diferentes entre ellos. Sin embargo, al comparar la ceniza con la corteza de pino, que tienen valores de pH muy diferentes entre sí, se observó que ambos tienen capacidades de adsorción de AZM relativamente similares entre sí. A los valores de pH de la concha de mejillón y la ceniza la AZM estaría presente como anión, ya que este antibiótico tiene un solo pKa, que, según los autores lo sitúan a 8,5 o a 9,6, por lo que el mecanismo de unión sería mediante un puente catiónico entre la superficie cargada negativamente de los materiales y los grupos con carga negativa de la AZM. Por lo tanto, al igual que sucedía con la AMX, la menor adsorción de la concha de mejillón se debe a su menor contenido de MO y de minerales de Fe y Al no cristalinos en comparación con la ceniza de biomasa. En cuanto a la corteza

de pino, esta tiene un pH muy ácido (3,99) y un contenido elevado de MO, por lo que en estas condiciones habrá grupos carboxilo desprotonados que adquieren carga negativa y pueden adsorber este antibiótico mediante atracciones electrostáticas con los grupos catiónico de la AZM; además, a este valor de pH tan ácido se generan cargas positivas en la superficie de la corteza de pino, lo que provocaría una repulsión electrostática con la forma catiónica de la AZM, por lo que en este caso podrían unirse mediante enlaces de H (Zhao et al., 2016).

5.3.2. Materiales residuales/subproductos: desorción

Analizando los datos obtenidos de desorción de los tres antibióticos por los distintos bioadsorbentes se pueden apreciar diferencias entre ellos. La desorción de AMX y AZM fue nula para todos los bioadsorbentes y para todas las concentraciones de antibiótico añadidas, lo que indicó que el proceso de adsorción de ambos antibióticos por los materiales estudiados fue irreversible. Sin embargo, en el caso de la CFX la situación fue diferente (Fig. 22), obteniendo porcentajes de desorción entre 25,9% y 59,1% para la corteza de pino y entre 19,5% y 28,6% para la hoja de eucalipto. Para el resto de materiales residuales, los porcentajes de desorción fueron ligeramente inferiores, situándose el máximo de desorción en 20,6% para la acícula de pino, 12,3% para la concha de mejillón y 0% para la ceniza (Fig. 22).

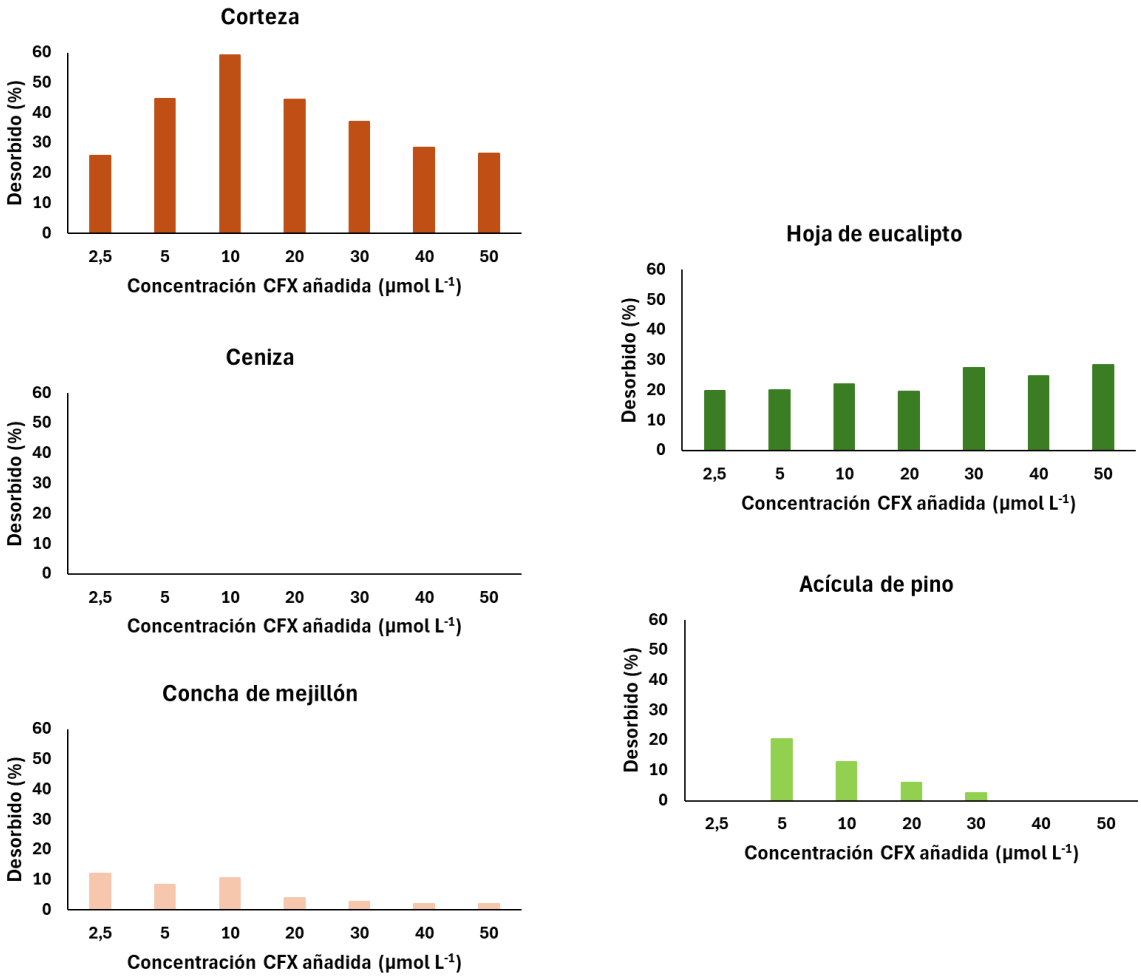

Figura 22. Porcentajes de desorción de CFX en función de la concentración añadida en los bioadsorbentes estudiados

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Los materiales residuales, con pH alcalino (ceniza y concha de mejillón) resultaron ser los mejores para adsorber CFX de forma más estable, pudiendo considerarse unos buenos bioadsorbentes, mientras que el resto de los materiales estudiados (corteza y acícula de pino y hoja de eucalipto) no serían recomendables para retener este antibiótico, ya que presentaron baja adsorción y alta desorción. Sin embargo, en el caso de la AMX los materiales que proporcionaron los mejores resultados fueron la hoja de eucalipto, la acícula de pino y la ceniza, siendo los tres eficaces para la eliminación de este antibiótico, ya que, además de tener una elevada adsorción, el proceso es prácticamente irreversible. Finalmente, en el caso de la AZM, los tres materiales residuales estudiados (ceniza, concha de mejillón y corteza de pino) resultaron ser eficaces a la hora de eliminar este antibiótico, con altos valores de adsorción y una nula desorción, siendo, de nuevo, la ceniza la que proporcionó los mejores resultados, siendo todos ellos materiales recomendables como bioadsorbentes de este antibiótico.

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5. 4. SUELOS ENMENDADOS CON BIOADSORBENTES: ADSORCIÓN/DESORCIÓN DE LOS TRES ANTIBIÓTICOS

5.4.1. Suelos enmendados con bioadsorbentes: adsorción

En las figuras 23, 24 y 25 se representan las curvas de adsorción de la CFX, AMX y AZM en distintos suelos, tanto sin tratamiento como enmendados con ceniza, concha de mejillón y corteza de pino. Los bioadsorbentes fueron añadidos a los suelos en una proporción de 48 t ha^{-1}. Los suelos utilizados son los mismos para los tres antibióticos (para la CFX hay dos suelos a mayores), sin embargo, la nomenclatura utilizada en los distintos artículos publicados no es la misma, siendo la equivalencia: V=VO; C1=M1; C2=M2; C3=M3.

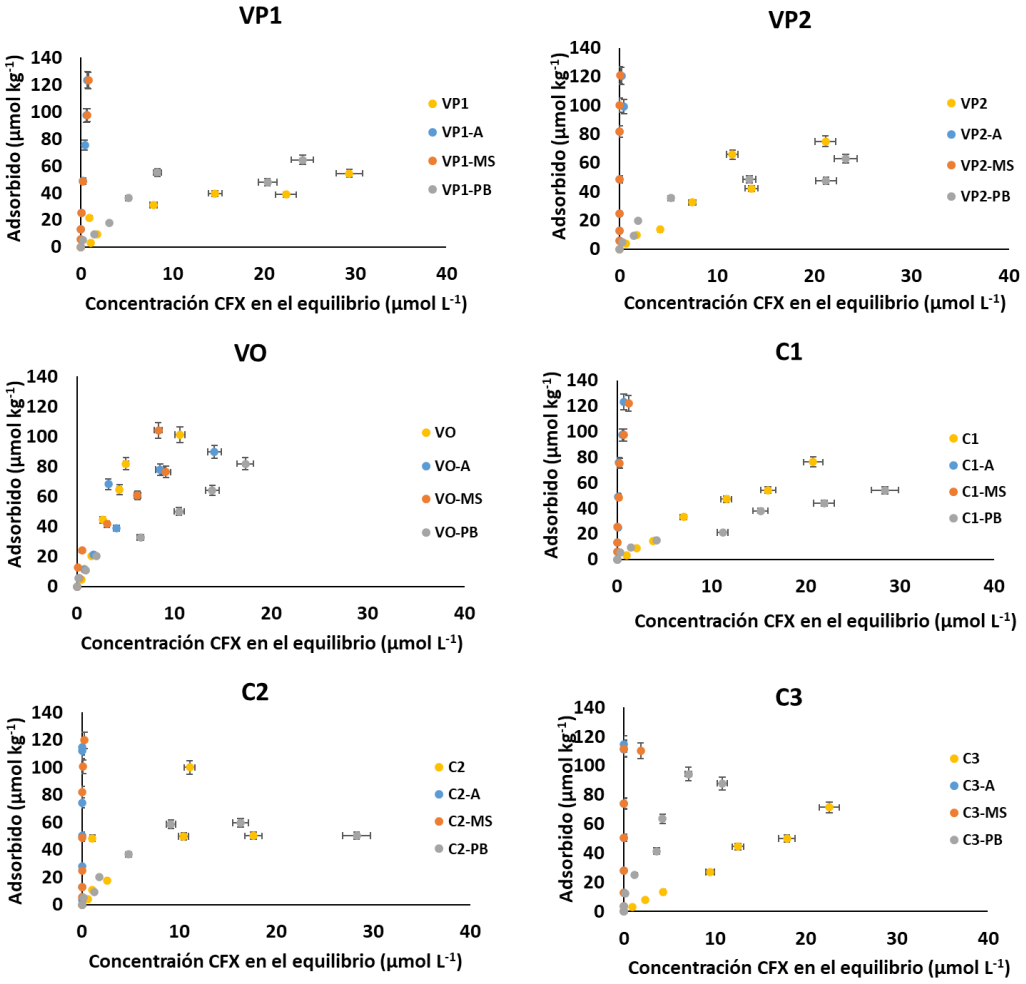

Figura 23. Curvas de adsorción de CFX correspondientes a los suelos sin bioadsorbentes y enmendados con bioadsorbentes en una dosis de 48 t ha^{-1}. C: suelos maíz; VO: suelos de viñedo de la provincia de Ourense; VP: suelos de viñedo de la provincia de Pontevedra; A: ceniza; MS: concha de mejillón; PB: corteza de pino.

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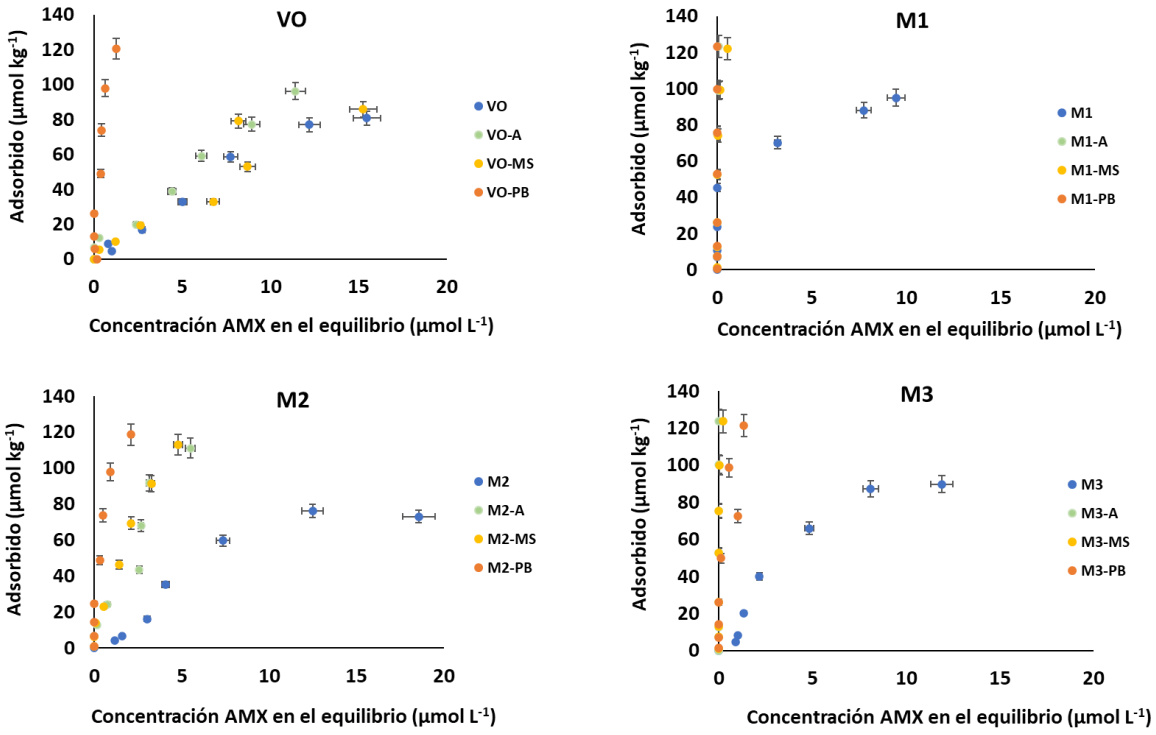

Figura 24. Curvas de adsorción de AMX correspondientes a los suelos sin bioadsorbentes y enmendados con bioadsorbentes en una dosis de 48 t ha^{-1}. M: suelos maíz; VO: suelos de viñedo de la provincia de Ourense; A: ceniza; MS: concha de mejillón; PB: corteza de pino.

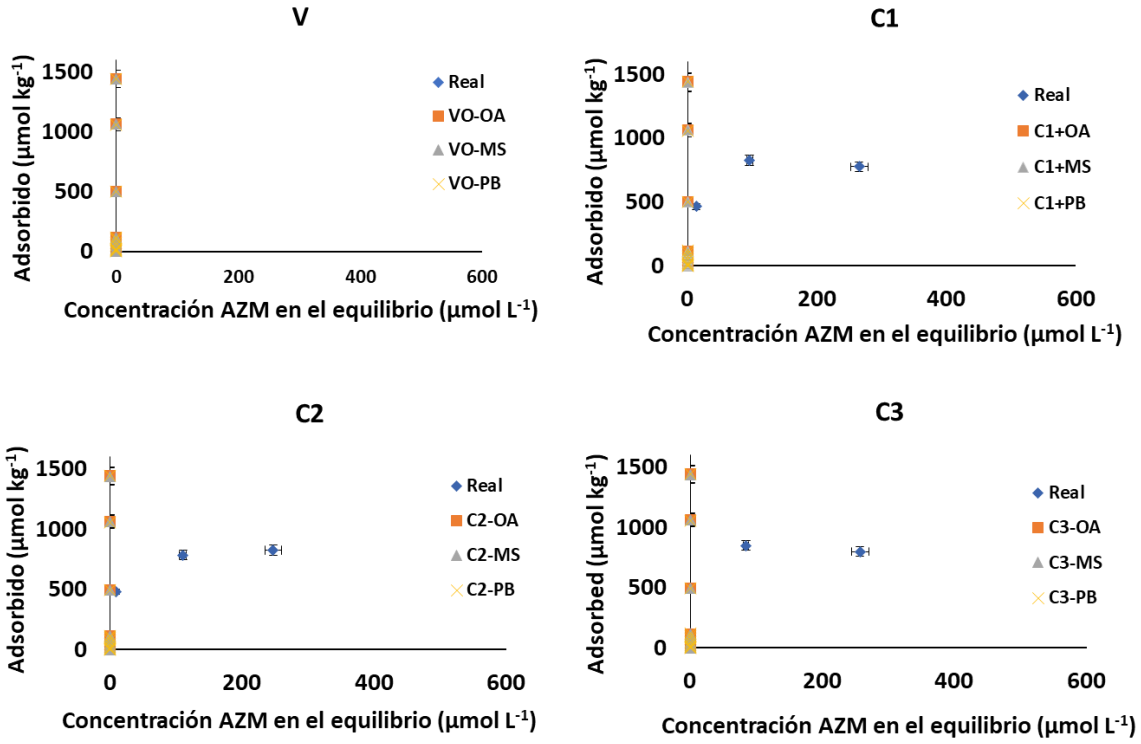

Figura 25. Curvas de adsorción de AZM correspondientes a los suelos sin bioadsorbentes y enmendados con bioadsorbentes en una dosis de 48 t ha^{-1}. C: suelos maíz; V: suelos de viñedo; OA: ceniza; MS: concha de mejillón; PB: corteza de pino.

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Como se puede observar en las gráficas 23, 24 y 25, en la mayoría de los casos, los bioadsorbentes utilizados como enmendantes generan un incremento de la capacidad de adsorción de los suelos para los tres antibióticos.

En el caso de la CFX, los incrementos más notables se produjeron al ser enmendados con ceniza de roble (pH=11,31) o concha de mejillón (pH=9,39) alcanzando valores del 100% de adsorción en la mayoría de los casos (Fig. 26), mientras que al añadirles corteza de pino el aumento de la capacidad de adsorción de los suelos fue mucho menor, e incluso en algunos casos la adsorción disminuyó. Se confirma así la influencia del pH en la adsorción de CFX, al igual que sucedía en los suelos y bioadsorbentes por separado. Se llevaron a cabo análisis estadísticos que confirmaron la existencia de una correlación significativa y positiva entre la adsorción de CFX y el pH (r=0,440; p<0,05). Además del aumento del pH del suelo, que a su vez provoca un incremento de la capacidad de intercambio catiónico del suelo, otras propiedades de los materiales bioadsorbentes, como el elevado contenido de Ca intercambiable de la concha de mejillón y la ceniza de roble o el alto contenido de Fe y Al no cristalinos en esta última, contribuirían a explicar el mayor incremento en la adsorción de CFX provocado por ceniza y concha de mejillón en comparación con la corteza de pino.

En el caso de la AMX, al contrario de lo sucedido para la CFX, fue la corteza de pino (pH=3,99) la que generó los mayores incrementos en la adsorción. La ceniza de roble (pH=11,31) y concha de mejillón (pH=9,39) también provocaron incrementos a pesar de tener un pH muy diferente al de la corteza de pino (Fig. 27). En la adsorción de AZM se observó que tras la enmienda de cualquiera de los bioadsorbentes a los suelos, la retención de este antibiótico aumentó en todos los casos al aplicar las concentraciones más altas, alcanzándose una adsorción del 100 % en la mayoría de los casos (con la excepción del suelo C3 mezclado con concha de mejillón, donde la adsorción máxima se limitó al 87 % al añadirse una concentración de 600 µmol L^{-1} del antibiótico) (Fig 28).

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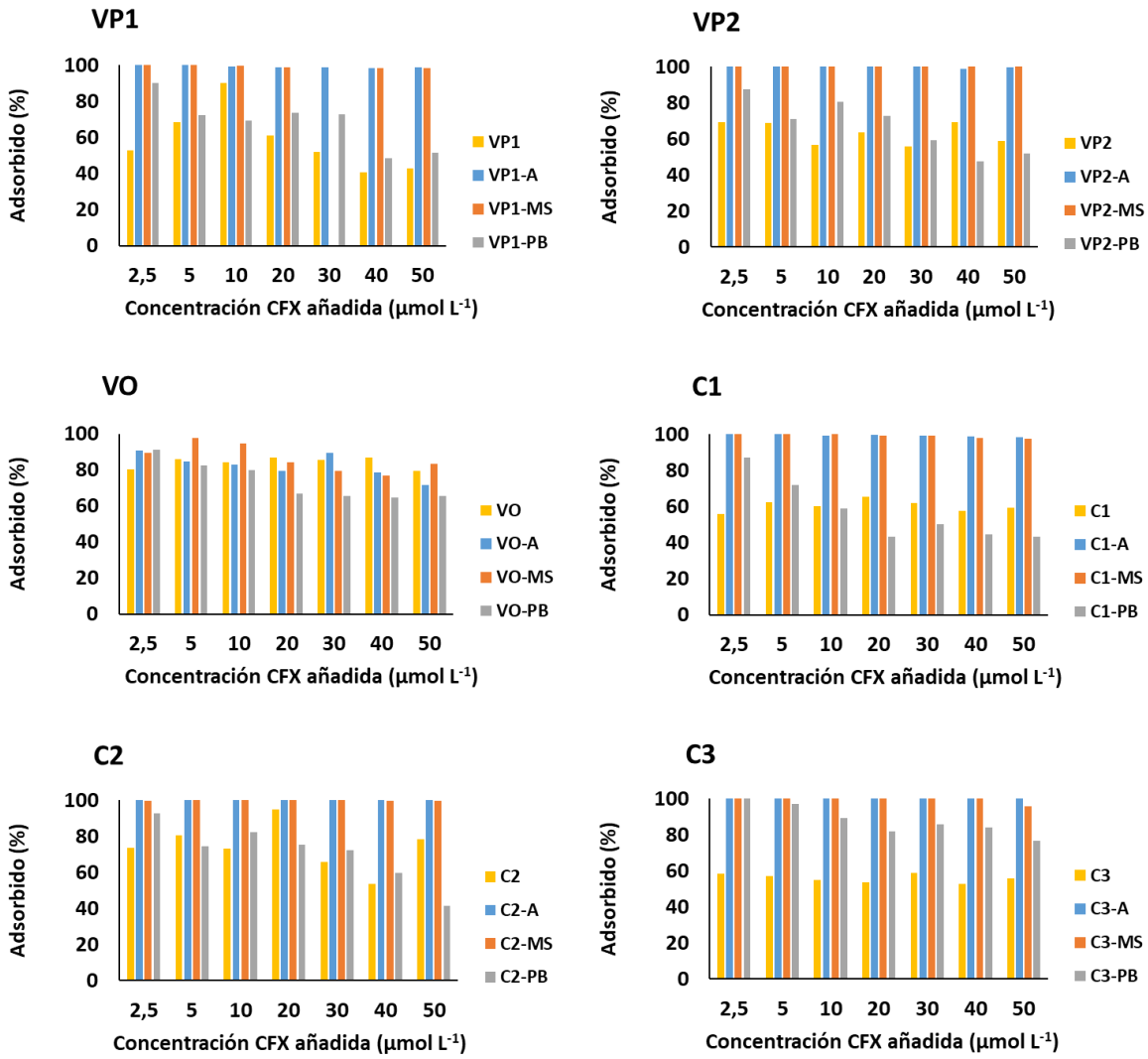

Figura 26. Porcentaje de adsorción de CFX en los suelos sin bioadsorbentes y enmendados con bioadsorbentes en una dosis de 48 t ha^{-1}. C: suelos maíz; VO: suelos de viñedo de la provincia de Ourense; VP: suelos de viñedo de la provincia de Pontevedra; A: ceniza; MS: concha de mejillón; PB: corteza de pino.

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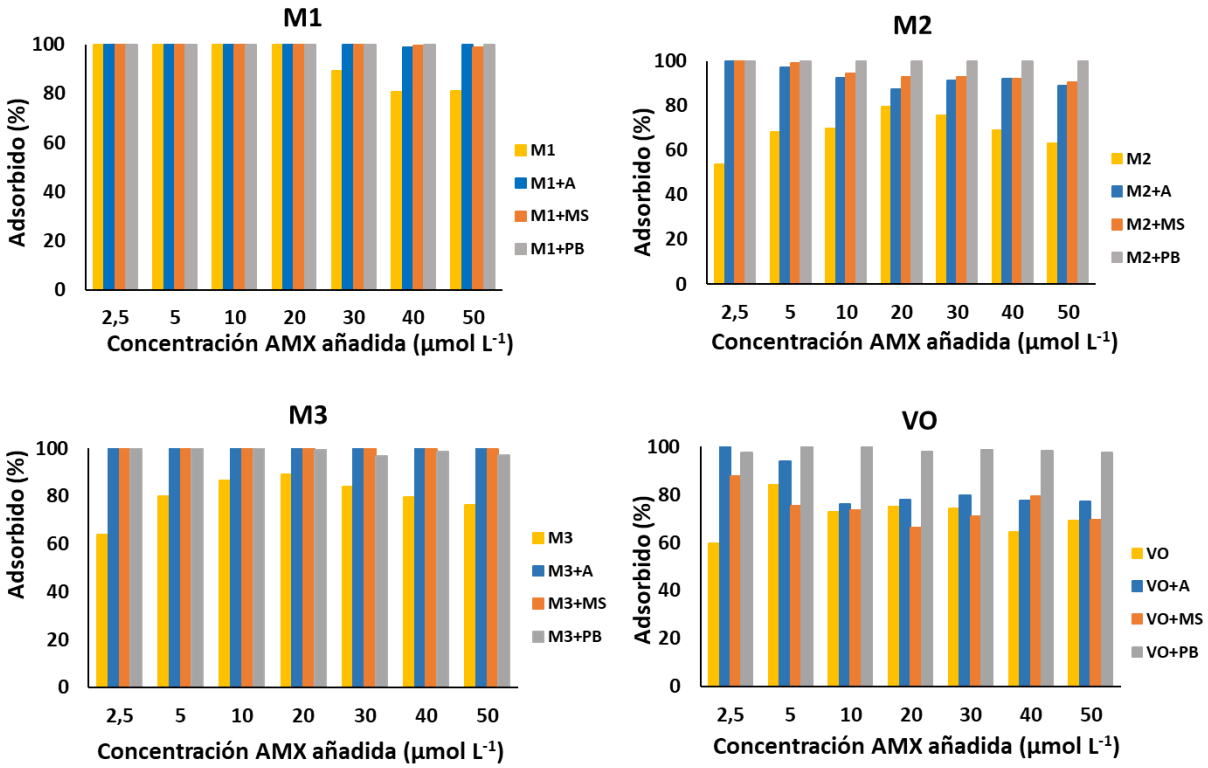

Figura 27. Porcentaje de adsorción de AMX en los suelos sin bioadsorbentes y enmendados con bioadsorbentes en una dosis de 48 t ha^{-1}. M: suelos maíz; VO: suelos de viñedo de la provincia de Ourense; A: ceniza; MS: concha de mejillón; PB: corteza de pino.

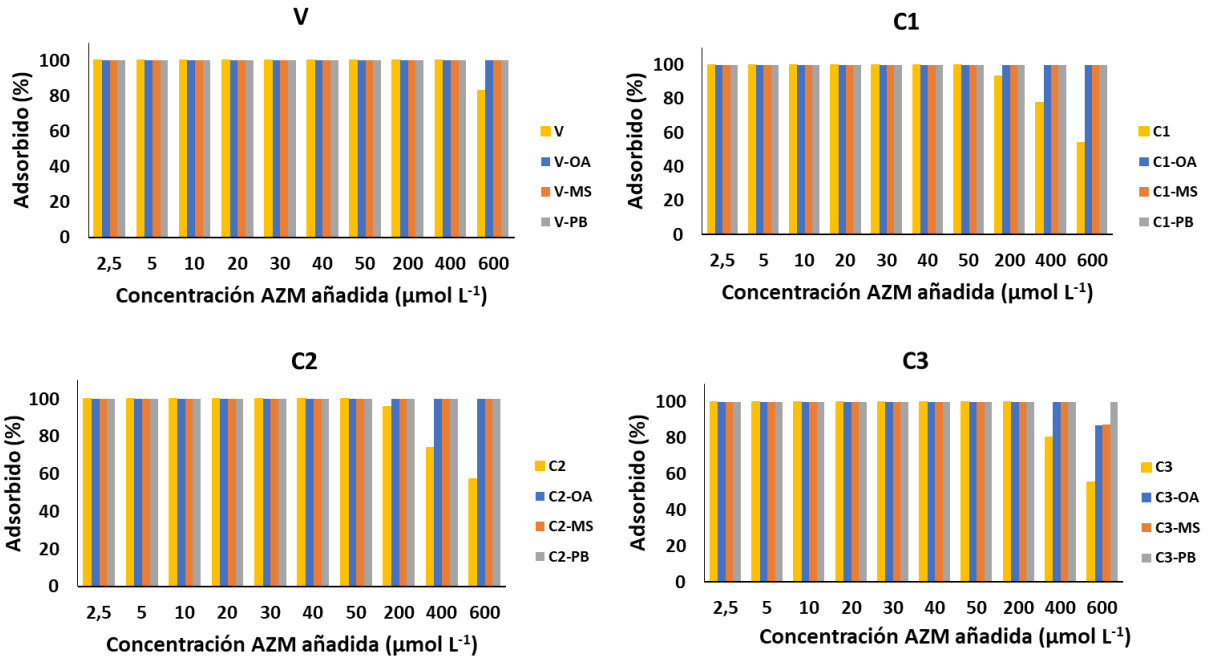

Figura 28. Porcentaje de adsorción de AZM en los suelos sin bioadsorbentes y enmendados con bioadsorbentes en una dosis de 48 t ha^{-1}. C: suelos maíz; V: suelos de viñedo de la provincia de Ourense; OA: ceniza; MS: concha de mejillón; PB: corteza de pino.

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El incremento de la adsorción de la AMX y AZM de los suelos debido a las enmiendas con ceniza de roble y concha de mejillón se debe a que la adición de estos materiales a los suelos aumenta el pH de estos, generando un incremento significativo de cargas negativas, tanto en la materia orgánica como en los minerales no cristalinos del suelo, los cuales son especialmente abundantes en los suelos analizados. Además, a esto se suma que la ceniza de roble también contiene una alta proporción de minerales no cristalinos que a ese pH estarán cargados negativamente. En cuanto a los mecanismos de unión, difieren entre antibióticos y están muy relacionados con sus valores de pKa. Al añadir concha de mejillón y ceniza los grupos carboxilos desprotonados de la AMX se unirán mediante puente catiónico con las cargas negativas presentes en los suelos y bioadsorbentes, comentadas anteriormente, mientras que los grupos cargados positivamente de la AZM se unirán mediante interacciones electrostáticas a estas cargas negativas (Davoodi et al., 2019). Por otro lado, el incremento de adsorción de AMX tras la adición de corteza de pino se debe a que este material acidificó el pH de los suelos, que, como se comentó anteriormente, es más favorable para la adsorción de este antibiótico. En el caso la AZM a valores de pH ácidos presenta grupos amino protonados, mientras que los grupos carboxilo de la corteza de pino pueden adquirir carga negativa según algunos autores a partir de pH 3 (Edwards et al., 1996) y según otros a partir de pH 4,2-4,5 (Vajdle et al., 2017). El mecanismo de adsorción de este antibiótico puede ser también mediante puentes de hidrógeno (de Sousa et al., 2018).

Las diferencias entre el mecanismo de adsorción de la AZM y AMX se debe a que esta última puede estar como catión, zwitterion o anión dependiendo del pH del suelo (Pezoti et al., 2016), estando favorecida la adsorción de este antibiótico a pH ácido, que es cuando se encuentra en forma catiónica o zwiterónica. Por el contrario, la AZM se encuentra únicamente como catión en el rango de pH de estos suelos y la adsorción es más favorable conforme aumenta el pH debido al aumento de cargas negativas en los componentes de carga variable del suelo (Sidhu et al., 2019c).

5.4.2. Mezclas suelo+bioadsorbentes: desorción

La adición de cualquiera de los materiales a los suelos provocó un descenso del porcentaje de desorción en todos los casos. Los suelos sin enmendar presentaron un máximo de desorción de CFX del 10%, mientras que este porcentaje disminuyó al 0% cuando se les añadió algún bioadsorbente, es decir, la adición de estos materiales a los suelos provocó una retención más fuerte de los antibióticos. Los resultados obtenidos para la AMX fueron similares, obteniéndose porcentajes de desorción entre 6% y 17%, cuando se añadió la máxima concentración de antibiótico (50 μmol L^{-1}) en los suelos sin enmendar. En cambio, al añadir algún bioadsorbente a estos suelos, el porcentaje de desorción nunca fue superior al 6%. Resultados similares fueron obtenidos en estudios previos para las tetraciclinas y sulfonamidas, donde se encontró que la adición de corteza de pino a los suelos produjo un descenso en el porcentaje de desorción de un 12% en las tetraciclinas y de un 17% en las sulfonamidas (Conde-Cid et al., 2019b, 2020b). Finalmente, la AZM presentó una nula desorción tanto en los suelos sin enmendar como en los suelos enmendados con algún bioadsorbente. Una alta adsorción de AZM también fue encontrada en estudios previos en biosólidos (Gravesen y Judy, 2020), suelos enmendados con biosólidos (Sidhu et al., 2019c) y en biochars (Mutera et al., 2019).

En conclusión, la utilización de tres materiales residuales (ceniza de roble, corteza de pino y concha de mejillón) como enmendantes de los suelos de Galicia, favorece la adsorción de tres antibióticos ampliamente consumidos a nivel humano (AMX, AZM y CFX), aumentando además la intensidad de las interacciones, lo que hace que, tras su incorporación, el proceso de adsorción sea prácticamente irreversible.

5.5. ESTUDIOS DE COMPETENCIA ENTRE DISTINTOS ANTIBIÓTICOS EN SUELOS Y BIOADSORBENTES

Una vez estudiado el comportamiento individual de los tres antibióticos objeto de estudio en los suelos y los materiales residuales con potencial bioadsorbente, la siguiente fase de la investigación consistió en conocer la posible competencia por los sitios de adsorción cuando estos antibióticos coexisten, que es la situación más habitual. Se estudió la posible competencia por los sitios de adsorción de los tres antibióticos estudiados (AMX, CFX, AZM), así como las interacciones entre AMX y ciprofloxacino (CIP), un antibiótico este último también ampliamente utilizado en medicina humana.

5.5.1 Estudios de competencia en suelos

En la figura 29 se representan las curvas de adsorción de los tres antibióticos (CFX, AMX y AZM) tanto cuando se añaden de forma individual (sistema simple) como cuándo se añaden simultáneamente (sistema ternario) y en la figura 30 se muestran las curvas de adsorción de la AMX y CIP, tanto en el sistema simple como en el sistema binario AMX+CIP.

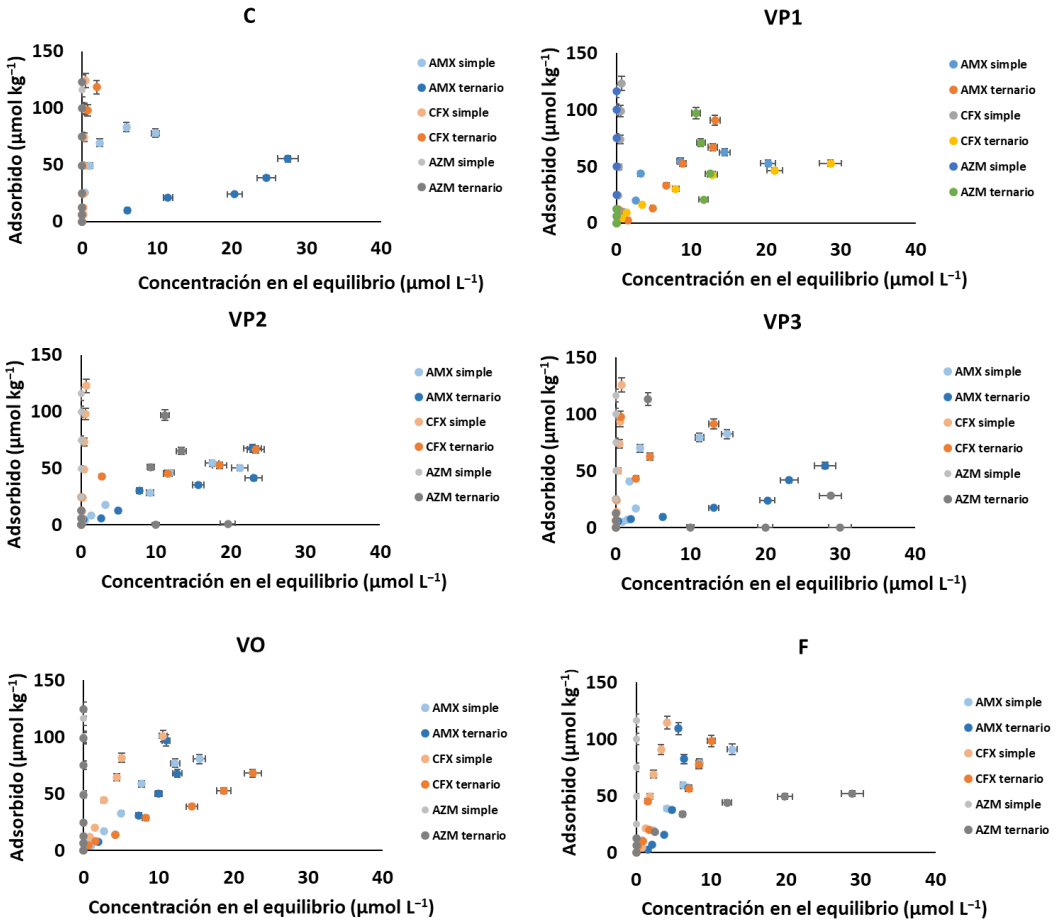

Figura 29. Curvas de adsorción obtenidas para cada antibiótico en el sistema simple y en el ternario por los suelos. AMX: amoxicilina; CFX: cefuroxima; AZM: azitromicina; C: suelo de maíz; VO: suelos de viñedo de la provincia de Ourense; VP: suelos de viñedo de la provincia de Pontevedra; F: suelo forestal.

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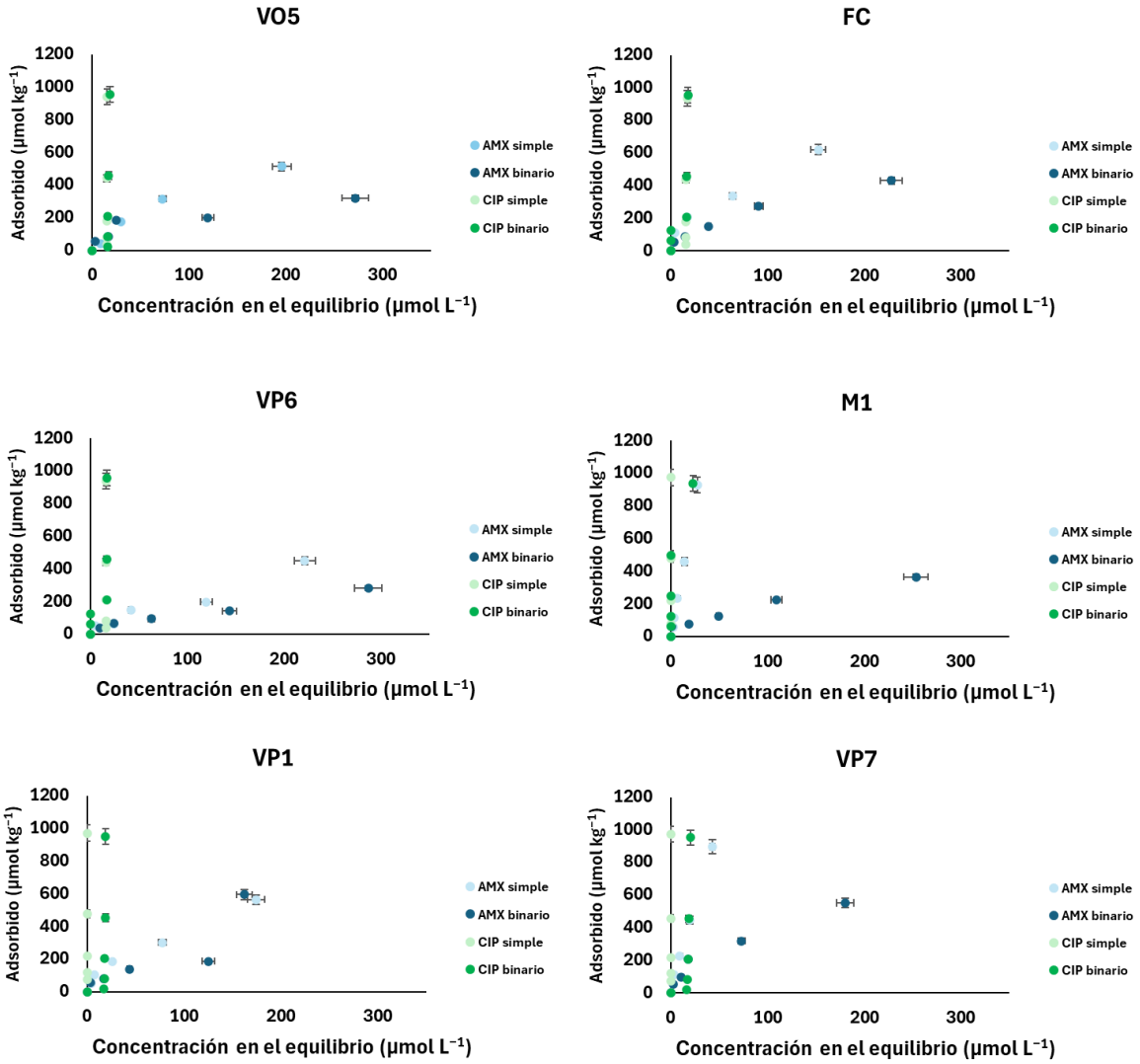

Figura 30. Curvas de adsorción obtenidas para cada antibiótico en el sistema simple y en el binario por los suelos. AMX: amoxicilina; CIP: ciprofloxacino; M: suelo de maíz; VO: suelos de viñedo de la provincia de Ourense; VP: suelos de viñedo de la provincia de Pontevedra; FC: suelo forestal.

Las curvas de adsorción del sistema ternario CFX+AMX+AZM (Fig. 29), así como la del sistema binario AMX+CIP (Fig 30) presentaron un buen ajuste al modelo de Freundlich, obteniendo valores R^2>0,9 para todos los suelos, excepto para el suelo VP3 en el sistema ternario. Los valores de los parámetros obtenidos en los ajustes al modelo de Freundlich de la adsorción de los sistemas binario y ternario se muestran en las tablas 11 y 12. Mientras que los valores de los ajustes obtenidos en los sistemas simples aparecen en la tabla 10 del apartado 5.2.1.

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Tabla 11. Parámetros de los modelos Lineal y de Freundlich para el sistema ternario CFX+AMX+AZM por los suelos. KF expresado en L^n kg^{-1} µ mol^{1-n}; n= sin unidades; Kd expresado en L kg^{-1}

Suelo	Freundlich					Lineal		
	KF	Error	n	Error	R^2	Kd	Error	R^2
C1	8,628	4,418	1,032	0,16	0,969	9,554	0,418	0,969
VP1	0,529	0,432	1,544	0,215	0,968	4,069	0,285	0,927
VP2	1,273	1,445	1,251	0,29	0,926	3,375	0,248	0,917
VP3	5,555	16,555	0,839	0,78	0,553	2,990	0,611	0,551
VO	3,638	1,932	1,218	0,158	0,977	7,531	0,332	0,97
FR	12,543	2,805	0,795	0,064	0,988	6,079	0,259	0,971

Tabla 12. Parámetros de los modelos Lineal y de Freundlich para el sistema binario AMX+CIP por los suelos. KF expresado en L^n kg^{-1} µ mol^{1-n}; n= sin unidades

Suelo	Freundlich				
	KF	Error	n	Error	R^2
VO5	8,777	5	0,958	0,112	0,975
FC	29,431	13,41	0,77	0,093	0,982
VP6	4,564	3,335	1,042	0,138	0,979
M1	129,796	33,558	0,684	0,071	0,979
VP1	6,731	2,209	1,03	0,065	0,994
VP7	1,120	0,826	1,79	0,183	0,981

Los valores del parámetro KF del modelo de Freundlich oscilaron entre 0,526 y 12,543 L^n kg^{-1} µmol^{1-n} en el sistema ternario, mientras que en el sistema binario estos valores fueron significativamente más altos, oscilando entre 34,30 y 1130,45 L^n kg^{-1} µmol^{1-n}. En el sistema ternario estos valores fueron más bajos que los obtenidos en el sistema simple de los tres antibióticos, especialmente en el caso de la CFX, que fue el antibiótico que presentó una mayor disminución de la adsorción en el sistema ternario respecto al simple. Sin embargo, en el sistema binario AMX+CIP, tres de los seis suelos estudiados obtuvieron valores de KF más altos para AMX en el sistema binario que en el simple, lo que indicó que la presencia simultánea de ambos antibióticos favoreció su adsorción en estos suelos, probablemente debido a que se produjo un efecto sinérgico entre ambos fármacos.

El parámetro KF, en el caso del sistema ternario, se correlacionó significativa (p<0,05) y positivamente con algunas propiedades fisicoquímicas de los suelos, como el C (r=0,881), N (r=0,897) y Fe_{pir} (0,828). Conde-Cid et al. (2020c) estudiando la adsorción de tres sulfonamidas por distintos suelos, destacan que los suelos con alto contenido de MO adsorbieron todo el antibiótico añadido, en contraposición a los que presentaban menos compuestos orgánicos, dado que estos últimos tienen menos sitios de adsorción, por lo que se saturan antes. La correlación con el Fe no cristalino presente en los suelos puede indicar una alta energía de adsorción para ciertos antibióticos cuando se unen a estos minerales no cristalinos a través de un puente catiónico (Conde-Cid et al., 2019a). En cuanto al parámetro n, todos los suelos en el sistema ternario, excepto dos, presentaron un n>1, lo que indica un modelo de intensidad de adsorción favorable a medida que aumenta la concentración de antibióticos añadida (Khanifari et al., 2023). En cambio, en el sistema binario AMX+CIP la mayoría de los suelos presentaron un valor de n cercano a 1 o incluso menor, lo que indicó la presencia de una superficie heterogénea de adsorción,

en la que los sitios de mayor energía son ocupados primero. Una excepción es el suelo VP7, el cual presentó un valor de n=1,79 en este sistema binario. Otros autores también encontraron valores de n>1 para el sistema binario de clortetraciclina+sulfonamida, lo que sugiere la existencia de fuertes interacciones entre los antibióticos y los sitios de adsorción de los adsorbentes (Jiang et al., 2020) o adsorciones de tipo cooperativo (Foo y Hameed, 2010).

Por otro lado, la adsorción del sistema ternario CFX+AMX+AZM también fue ajustada al modelo lineal, obteniéndose un buen ajuste con un R^2>0,9, excepto en el suelo VP3, y unos valores del parámetro Kd que oscilaron entre 2,99 y 9,554 L kg^{-1}. Estos datos son similares a los obtenidos por Conde-Cid et al. (2020c) para tres sulfonamidas, sin embargo, son claramente inferiores que los obtenidos en un estudio previo para las tetraciclinas (Teixidó et al., 2012). Esto indica que la intensidad de las interacciones con los sitios de unión del suelo de la CFX, AMX y AZM es similar a la de las sulfonamidas, pero mucho más débil que las de las tetraciclinas.

5.5.2 Influencia de las propiedades fisicoquímicas de los suelos

Al añadir los tres antibióticos (CFX, AMX y AZM) de forma simultánea a los suelos se observó que la AZM fue el antibiótico que presentó los valores más altos de adsorción en la mayoría de los casos, seguido de la CFX y finalmente la AMX. En el caso del sistema binario AMX+CIP, se observó que el CIP presentó para todos los suelos valores de adsorción más elevados que la AMX.

Al comparar los resultados obtenidos en el sistema simple y en el ternario, se observa que la AZM y CFX mostraron porcentajes de adsorción mayores en el sistema simple que en el ternario en la mayoría de los suelos, especialmente en las altas dosis de antibiótico añadido, excepto en el caso del suelo C, que adsorbió sobre el 100% de ambos antibióticos y el suelo VO, el cual adsorbió el 100% de AZM en ambos sistemas. Sin embargo, en la AMX se observaron resultades muy variables: normalmente la adsorción es mayor en el sistema simple que en el ternario, especialmente a dosis bajas, sin embargo, estas diferencias tienden a disminuir a medida que aumenta la dosis y, en algunos casos, se observa incluso una mayor adsorción de AMX en el sistema ternario (suelos VP1 y F). Un comportamiento similar se observó en el sistema binario AMX+CIP, donde la adsorción de la AMX se vio favorecida por la presencia de CIP, con lo cual, parece que la adsorción de la AMX tiende a verse favorecida por la presencia de otros antibióticos.

Por otro lado, al comparar los distintos suelos entre sí se observan diferencias en la adsorción de los distintos antibióticos. En el caso de la AZM, en el sistema simple la adsorción fue del 100% en la mayoría de los casos, sin embargo, en el sistema ternario se observaron diferencias entre los distintos suelos. En los suelos de pH más elevado (C y VO), la adsorción de AZM no se vio afectada por la presencia de los otros dos antibióticos (CFX y AMX), sin embargo, en el resto de suelos sí se apreció una reducción de la adsorción de AZM en el sistema ternario en comparación con el sistema simple, indicando la existencia de una competencia por los sitios de adsorción. Concretamente, el suelo F fue el que mostró el porcentaje de adsorción de AZM más bajo en el sistema ternario (42% cuando se añadió una concentración de 50 µmol L^{-1} de AZM). Este es el suelo que presenta el valor de pH más bajo y los contenidos más altos de Al y Fe oxalato, los cuales a valores de pH de este suelo (pH=4,68) están cargados positivamente, al igual que la azitromicina. Por tanto, en estas condiciones, los compuestos de baja cristalinidad no contribuirían significativamente a la retención de este antibiótico. No obstante, los grupos –COOH de la materia orgánica comenzarían a desprotonarse, favoreciendo interacciones electrostáticas con los grupos cargados positivamente de la AZM. Sin embargo, a este valor de pH, la carga negativa global del sistema sigue siendo limitada, lo que se traduce

en una menor adsorción en el suelo más ácido en comparación con aquellos de pH más elevado. En este contexto, los otros dos antibióticos podrían competir con la AZM por los sitios de adsorción, lo que daría lugar a una reducción en su retención respecto al sistema simple. En el resto de los suelos (que presentan valores de adsorción superiores al 80 % en el sistema ternario), el contenido de minerales no cristalinos es menor que en el suelo F. No obstante, debido a su rango de pH más elevado (entre 6,04 y 8,02, salvo en el caso de VP1), estos compuestos presentan cargas negativas. Esta circunstancia, junto con el incremento de la carga negativa de la materia orgánica a pH más altos, reduce la competencia entre antibióticos por los sitios de adsorción, lo que da lugar a una disminución mínima de la adsorción de AZM en comparación con el sistema simple. Balarak et al. (2021) también encontraron que la adsorción de AZM en carbón activado hecho a partir de *Azolla Filiculoides* se incrementaba progresivamente con el aumento del pH.

En cuanto a la CFX, se observaron diferencias mínimas entre los distintos suelos cuando este antibiótico se añadió de forma individual. Sin embargo, en el sistema ternario (AMX + CFX + AZM), sí se apreciaron diferencias en la adsorción entre los distintos suelos. Concretamente, los suelos con valores de pH elevados, el suelo C (pH 8,02) y el suelo VP3 (pH 7,27), presentaron los mayores porcentajes de adsorción de CFX, con valores comprendidos entre el 73 % y el 100 % para todas las concentraciones añadidas, especialmente en el sistema ternario. A estos niveles de pH, la cefuroxima se encuentra en forma zwitteriónica, lo que permite que sus grupos amino con carga positiva interactúen electrostáticamente con las superficies del suelo cargadas negativamente, mientras que sus grupos aniónicos (COO^-) pueden unirse a dichas superficies mediante puentes catiónicos (Legnoverde et al., 2014). Sin embargo, el suelo VO presenta una adsorción relativamente baja si se considera su pH (6,04), lo que probablemente se deba a que es el suelo con el menor contenido de materia orgánica (3,05 %) y también con las concentraciones más bajas de compuestos no cristalinos. Por otro lado, cabe destacar el comportamiento de este antibiótico en el suelo F (pH=4,68), ya que a pesar de su pH ácido presentó valores de adsorción superiores al 80%, lo que puede ser debido a su alto contenido en materia orgánica (12,33%), cuyos grupos -COOH ya estarían disociados a pH<5 (Yu et al., 2011), como se indicó a lo largo de este trabajo.

En cuanto a la AMX, este fue el antibiótico que presentó los valores de adsorción más bajos, en comparación con la CFX y AZM, tanto en el sistema simple, donde la adsorción osciló entre 50% y 80% al añadir la concentración más alta (50 $\mu mol\ L^{-1}$), como en el sistema ternario, donde el porcentaje de adsorción osciló entre 40% y 90%. Al comparar el sistema ternario y el sistema simple, se observaron tanto efectos sinérgicos como antagónicos de los otros dos antibióticos (CFX y AZM) sobre la adsorción de AMX, dependiendo de las propiedades del suelo. En los dos suelos con los valores de pH más elevados (C y VP3), la presencia de los otros antibióticos tuvo un efecto antagónico, reduciendo la adsorción de AMX, probablemente debido a la competencia por los sitios de adsorción, un fenómeno que se intensifica a concentraciones más altas. Por el contrario, en los otros cuatro suelos se observó un efecto sinérgico, en el que la presencia de CFX y AZM favoreció la adsorción de AMX. Además, un análisis estadístico indicó que el pH y la adsorción de AMX se correlacionan significativa y negativamente ($r=-0,953$; $p<0,01$). En cuanto a los mecanismos de unión, en el suelo forestal (pH < 5), los grupos carboxilo desprotonados de la AMX pueden unirse a las cargas positivas del suelo (minerales no cristalinos y grupos amino de la materia orgánica) mediante interacciones electrostáticas, y además los grupos $–COO^-$ de la materia orgánica podrían interactuar con las cargas positivas de la AMX. Los suelos con pH entre 5 y 6,5 (VO y VP1) presentarán una mayor carga negativa que el suelo F, lo que permite interacciones con los

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grupos protonados de la AMX. En los suelos con pH > 7 (C, VP2 y VP3), predominarán las cargas negativas, especialmente en el suelo C (pH = 8,02), y la AMX mostrará una mayor proporción de grupos desprotonados, favoreciendo su adsorción mediante puentes catiónicos, lo que hará que se produzca competencia entre la AMX y los otros dos antibióticos por los sitios de adsorción.

Por otro lado, al analizar el sistema binario entre este antibiótico y el CIP, se observa que, aunque la presencia de CIP incrementa la adsorción de AMX en todos los suelos, este efecto es especialmente notable en los suelos con pH elevado, al contrario de lo que sucede en el sistema ternario CFX+AMX+AZM. Esto puede ser debido a que, en los suelos con los valores de pH más altos, la AMX actúa como una especie aniónica con los grupos amino y carboxilo deprotonados, mientras que el CIP se encuentra en forma zwitteriónica, lo que permite interacciones electrostáticas entre los grupos cargados positivamente del CIP y la AMX aniónica. Además, estos suelos presentan un elevado contenido de materia orgánica (entre 6,14% y 8,58%), la cual adquiere carga negativa a estas condiciones de pH. Como consecuencia, en el sistema individual se producirían repulsiones electrostáticas entre la AMX y el suelo, por lo que sería necesaria la formación de un puente catiónico para favorecer la adsorción; en este contexto, el CIP podría actuar como un componente que facilite la formación de dicho puente.

5.5.3. Desorción de los antibióticos por los suelos en sistemas competitivos

La AMX fue el antibiótico que presentó los porcentajes más elevados de desorción, tanto en el sistema simple como en el sistema ternario CFX+AMX+AZM; comparando estos ambos sistemas, se observa que la desorción es mayor en el sistema ternario (entre 0% y 37%) que en el sistema simple (entre 0% y 14%). En el caso de la AZM, la desorción fue nula en todos los casos excepto en el sistema ternario en el suelo F, donde la desorción alcanzó un máximo del 10%. Estos resultados coinciden con los obtenidos en estudios previos para tres tetraciclinas (Conde-Cid et al., 2019a) y para tres sulfonamidas (Conde-Cid et al., 2020c), donde se obtuvieron valores de desorción más altos cuando se añadieron de forma simultánea. Sin embargo, en la CFX ocurrió el efecto contrario, presentando los valores de desorción más elevados en el sistema simple, aunque la desorción fue siempre muy baja en ambos sistemas, no superando el 8% en ningún caso.

Estos datos indican que la adsorción de CFX y AZM es más fuerte que la adsorción de la AMX. Esto puede ser debido a que cuando la AMX está en presencia de la CFX y AZM, estos dos antibióticos ocupan los lugares de mayor energía, mientras que la AMX se adhiere a los sitios de menor energía. Chen et al. (2020) encontraron que al comparar dos sulfonamidas (sulfametozol y sulfametazina), estas ocupaban sitios adsorbentes de diferente energía, concluyendo que la energía de adsorción es un factor decisivo en la competencia entre los antibióticos.

Además, comparando los suelos, el que presentó el pH más bajo (suelo F) mostró los mayores porcentajes de desorción para CFX y AZM, mientras que el suelo con el pH más alto (suelo C) presentó los valores de desorción más bajos. Este comportamiento es opuesto al observado en el proceso de adsorción, en el que la mayor retención se produjo en los suelos con pH más elevado, lo que sugiere que en estos suelos los enlaces formados son altamente estables y, en gran medida, irreversibles.

5.5.4 Estudios de competencia en bioadsorbentes

En la figura 31 se muestran las curvas de adsorción de la CFX, AMX y AZM, tanto en el sistema simple como en el sistema ternario, para los tres bioadsorbentes (ceniza, corteza de pino y concha de mejillón), mientras que, en la figura 32 se muestran las curvas

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de adsorción de la AMX y el CIP, tanto en el sistema simple como en el binario para los mismos bioadsorbentes.

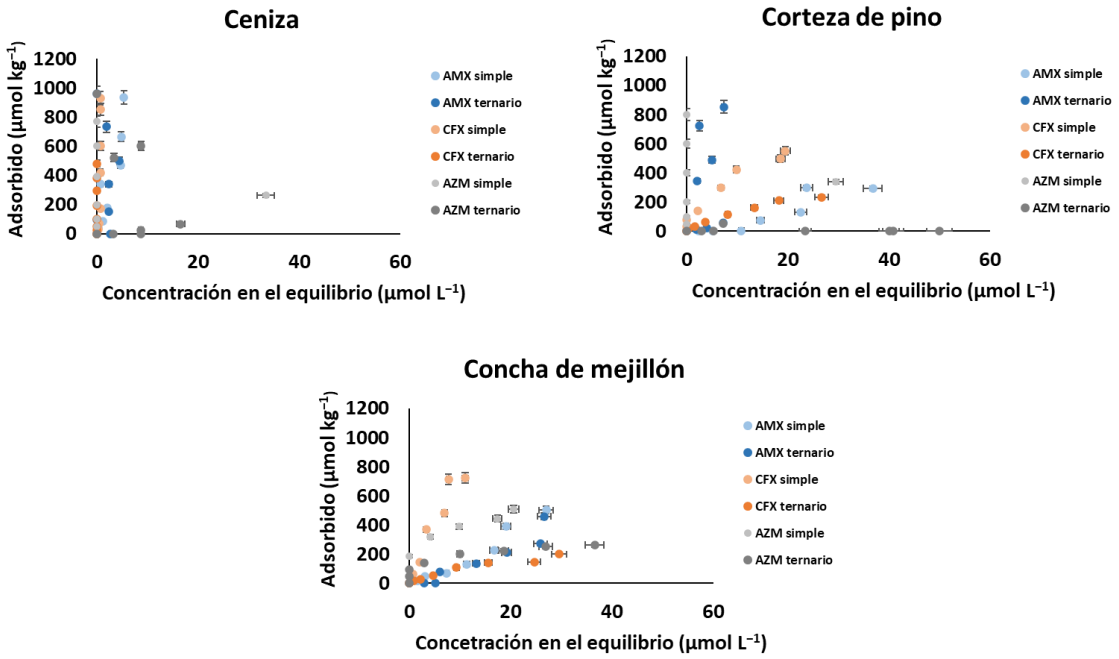

Figura 31. Curvas de adsorción obtenidas para cada antibiótico en el sistema simple y en el ternario CFX+AMX+AZM por los bioadsorbentes. AMX: amoxicilina; CFX: cefuroxima; AZM: azitromicina.

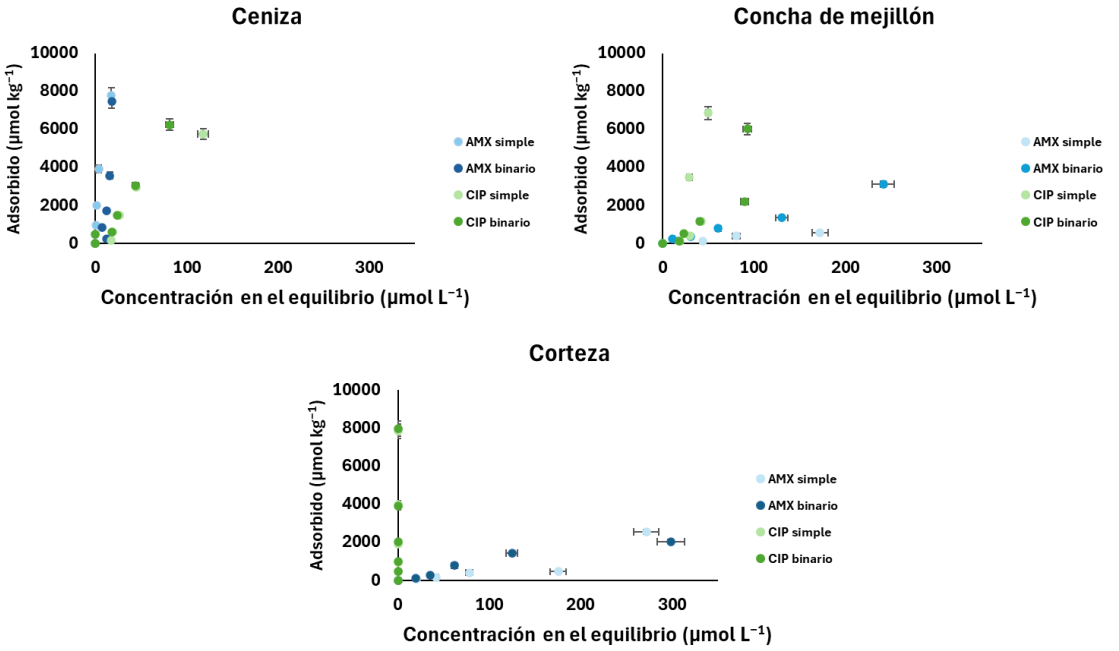

Figura 32. Curvas de adsorción obtenidas para cada antibiótico en el sistema simple y en el binario AMX+CIP por los bioadsorbentes. AMX: amoxicilina; CIP: ciprofloxacino.

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Los datos de adsorción obtenidos en el sistema ternario CFX+AMX+AZM fueron ajustados al modelo Lineal y al de Freundlich, pero solo la concha de mejillón presentó un buen ajuste (R^2>0,9) a estos dos modelos. En cuanto a los valores de adsorción obtenidos en el sistema binario AMX+CIP solo fueron ajustados al modelo de Freundlich, obteniendo un buen ajuste (R^2>0,9) para los tres bioadsorbentes.

El valor del coeficiente de afinidad de Freundlich (KF) en el sistema ternario se situó en 111,004 L^n kg^{-1} μmol^{1-n} para la corteza, 37,496 L^n kg^{-1} μmol^{1-n} para la concha de mejillón y en el caso de la ceniza no se obtuvo valor ya que no se pudo realizar el ajuste. En el sistema binario AMX+CIP, los valores de KF se situaron en 56,746 L^n kg^{-1} μmol^{1-n} en la ceniza y en 83,643 L^n kg^{-1} μmol^{1-n} en la corteza de pino, mientras que en el caso de la concha de mejillón no se pudo realizar el ajuste. Sin embargo, Conde-Cid et al. (2019b) obtuvieron valores de KF más altos para tres tetraciclinas en un sistema ternario para los mismos bioadsorbentes. En cuanto al valor de n, en el sistema ternario este fue menor que 1 (0,517 para la corteza de pino y 0,691 para la concha de mejillón), mientras que en el sistema binario el valor de n fue mayor que 1 en el caso de la ceniza (1,128), sin embargo, en el caso de la corteza de pino este valor fue de 0,843. Esto indica que, excepto en la ceniza en el sistema binario la adsorción tiene lugar en una matriz heterogénea, en la cual los sitios de mayor energía son ocupados preferentemente. Conde-Cid et al. (2019b) obtuvieron para el sistema ternario de tres tetraciclinas valores de n<1 para la corteza de pino y para la ceniza, sin embargo, para la concha de mejillón este valor fue mayor que 1.

Respecto al modelo lineal, la constante de distribución (Kd) alcanzó 14,887 L kg^{-1} para la corteza de pino y 10,015 L kg^{-1} para la concha de mejillón. Estos valores fueron generalmente inferiores a los obtenidos para el sistema simple, lo que indica una menor capacidad de adsorción cuando los tres antibióticos se añadieron conjuntamente.

5.5.5. Influencia de las propiedades fisicoquímicas de los bioadsorbentes

La ceniza fue el bioadsorbente que presentó los valores más altos de adsorción para todos los antibióticos en el sistema ternario CFX+AMX+AZM, especialmente en el caso de CFX y AMX. Concretamente, la ceniza adsorbió prácticamente el 100% del antibiótico añadido en la mayoría de los casos, tanto en el sistema simple como en el ternario CFX+AMX+AZM, aunque en este último los valores de adsorción fueron ligeramente más altos. La naturaleza claramente alcalina de este material y su abundancia en componentes de carga variable, específicamente Fe y Al no cristalinos (Fe_{ox}, Al_{ox}), en teoría no favorecerían la retención de antibióticos, ya que este alto pH provocaría una alta densidad de carga negativa tanto en los antibióticos como en los componentes de carga variable presentes en el adsorbente, lo que impediría la unión mediante atracciones electrostáticas (Conde-Cid et al., 2019b). Sin embargo, otros mecanismos, como la adsorción mediante puentes catiónicas, podrían seguir desempeñando un papel importante (Wang y Wang, 2015), probablemente utilizando Ca como elemento de enlace.

En el caso de la corteza de pino (excepto para la AMX) y la concha de mejillón en el sistema ternario sucede lo contrario que en la ceniza, siendo la adsorción más baja en el sistema ternario que en el simple, mostrando que existe una competencia entre los tres antibióticos por los sitios de adsorción. La menor adsorción de AZM y CFX en la corteza de pino se debe a su pH ácido, ya que como se comentó anteriormente estos antibióticos presentan mayor adsorción a pH más elevados. En condiciones fuertemente ácidas, la corteza de pino presenta una carga superficial negativa reducida, lo que limita la adsorción de la AZM (que está cargada positivamente) y de los grupos catiónicos de la CFX. Por ello, en los sistemas simples y a bajas concentraciones, el porcentaje de adsorción de estos

antibióticos es elevado. Sin embargo, en sistemas multicomponentes, la superficie del adsorbente se satura y se genera competencia entre los antibióticos por los sitios disponibles. Sin embargo, en el caso de la AMX sucedió lo contrario, con una mayor adsorción en condiciones más ácidas y una adsorción favorecida por un efecto sinérgico con los otros antibióticos en la corteza de pino. En cuanto a la concha, a pesar de tener un pH similar al de la ceniza, presenta una menor capacidad de adsorción debida a su menor contenido en minerales no cristalinos, así como en Ca, que es un elemento de enlace de gran importancia. En el sistema binario AMX+CIP, se observó un efecto sinérgico en la adsorción entre ambos antibióticos en los dos bioadsorbentes y especialmente en la corteza de pino. Autores como Ringot et al. (2007) y Foo y Hameed, (2010) relacionaron este efecto sinérgico con el modelo de adsorción cooperativa, que indica que cuando un soluto es retenido por un sitio en una superficie adsorbente homogénea, puede influir en los sitios activos consecutivos de esa superficie, promoviendo nuevas adsorciones y retenciones más fuertes.

Por otro lado, en sistema binario AMX+CIP se apreciaron diferencias entre los tres bioadsorbentes. La ceniza presentó valores más altos de adsorción para la AMX que para el CIP, mientras que la concha de mejillón y especialmente la corteza de pino mostraron el efecto contrario, presentando el CIP valores de adsorción más elevados que la AMX. En el sistema binario AMX+CIP cuando se añaden las concentraciones bajas de antibiótico, la adsorción de AMX por la ceniza es más baja que en el sistema simple, lo que indica que el CIP compite con la AMX por los sitios de adsorción. Sin embargo, a altas concentraciones de antibiótico añadidas, la adsorción es similar en ambos sistemas. Chandrasekaran et al. (2020) encontraron que la adsorción de AMX por *Prosopis juliflora* en el sistema binario AMX+CIP fue más baja que en el simple, lo que confirma que el CIP tiene efectos antagonistas/competitivos en la adsorción de AMX. Esto puede ser debido a que al igual que la AMX, el CIP también está cargado negativamente al pH de la ceniza, por lo que ambos necesitan formar puentes catiónicos para unirse a la ceniza, mostrando el CIP mayor afinidad que la AMX para formar este tipo de enlaces.

5.5.6 Desorción por los bioadsorbentes en sistemas competitivos

En la figura 33 se muestran los porcentajes de desorción obtenidos para la CFX, AMX y AZM, tanto en el sistema simple como en el ternario, por los tres bioadsorbentes estudiados (ceniza de roble, corteza de pino y concha de mejillón) y en la figura 34 se representan los porcentajes de desorción obtenidos para la AMX y CIP en los sistemas simple y binario por los tres bioadsorbentes.

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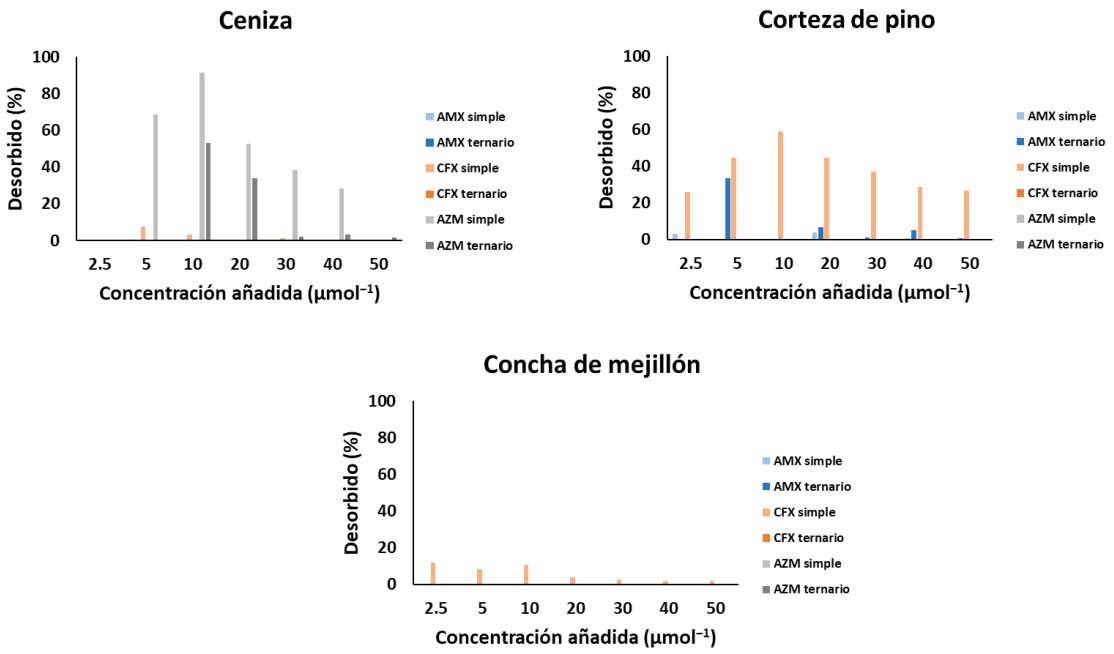

Figura 33. Porcentaje de desorción de la CFX, AMX y AZM, tanto en el sistema simple como en el ternario, por los diferentes bioadsorbentes. AMX: amoxicilina; CFX: cefuroxima; AZM: azitromicina.

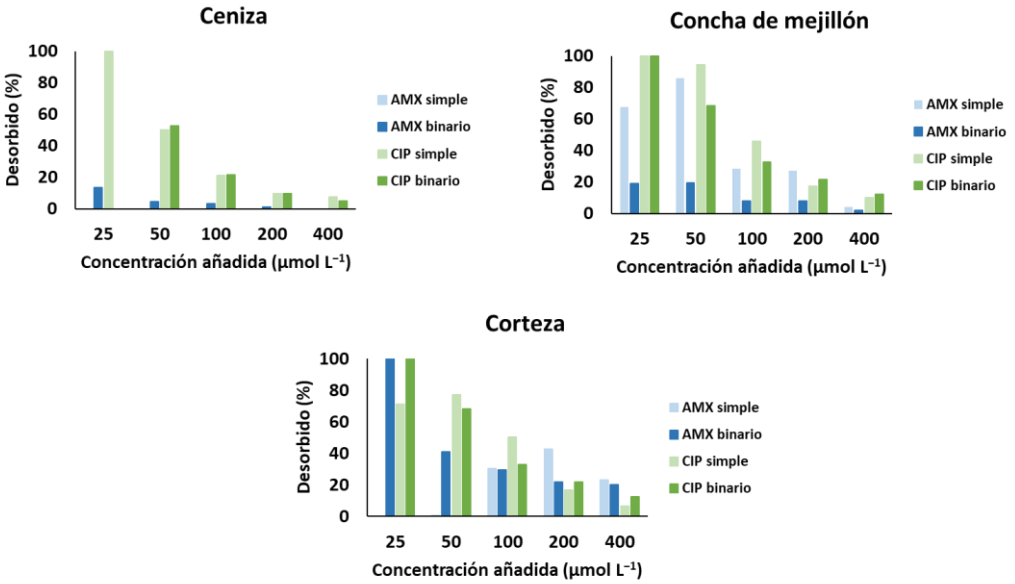

Figura 34. Porcentaje de desorción de la AMX y CIP, tanto en el sistema simple como en el binario, por los diferentes bioadsorbentes. AMX: amoxicilina; CIP: ciprofloxacino

La concha de mejillón fue el bioadsorbente que presentó los valores más bajos de desorción en el sistema ternario para todos los antibióticos, al ser prácticamente nula en todos los casos, excepto para la CFX en el sistema simple donde alcanzó valores del 12%. Sin embargo, en el caso del sistema binario AMX+CIP, la concha de mejillón presentó valores de desorción elevados para ambos antibióticos, especialmente a bajas concentraciones añadidas, donde se desorbió el 100% del CIP, mientras que en la AMX este porcentaje decreció hasta el 20%.

Por el contrario, la ceniza fue el bioadsorbente que presentó los valores de desorción más elevados en el sistema ternario CFX+AMX+AZM. La AZM alcanzó un 53% de desorción cuando se añadieron 10 μmol L^{-1}, y este porcentaje fue decreciendo a medida que iba aumentando la dosis hasta alcanzar el 2% al añadir la máxima concentración de antibiótico. Sin embargo, tanto en la AMX como en la CFX la desorción fue nula en el sistema ternario. La AMX también presentó bajos porcentajes de desorción por la ceniza en el sistema binario AMX+CIP, no superando el 20% en ningún caso. Sin embargo, el CIP alcanzó porcentajes de desorción de hasta el 50%.

Tanto la AZM como la CFX presentaron nula desorción en la corteza de pino en el sistema ternario, mientras que la AMX alcanzó valores de desorción del 33%. Resultados similares se obtuvieron en el sistema binario, donde la AMX presentó valores altos de desorción, oscilando estos porcentajes entre el 20% y el 100%, mientras que la desorción del CIP fue nula para todas las concentraciones de antibiótico añadidas.

Comparando estos resultados con los obtenidos en los sistemas simples de cada antibiótico se observó que en el caso del sistema ternario los porcentajes de desorción fueron más bajos que en los sistemas simples, excepto en la AMX por la corteza de pino, donde en el sistema simple alcanzó un máximo de adsorción del 4%, mientras que en el sistema ternario este porcentaje ascendió al 33%.

Los resultados muestran que la ceniza de roble es el bioadsorbente más efectivo para AMX y CFX, tanto en sistemas simples como ternarios, evidenciando las mayores eficiencias de adsorción y bajas tasas de desorción. No obstante, para AZM no resulta adecuado, ya que, aunque presenta una adsorción elevada (especialmente en el sistema simple y a las concentraciones más altas), los porcentajes de desorción aumentan notablemente a concentraciones intermedias, lo que limita su uso práctico. La corteza de pino retuvo bien la AZM en el sistema simple (hasta 40 μmol L^{-1}), pero su eficacia disminuyó en el sistema ternario. Además, mostró baja efectividad para CFX a dosis altas, con adsorciones inferiores al 60% y desorciones alrededor del 20%. Sin embargo, para AMX, la corteza de pino ofreció los mejores resultados en el sistema ternario, con una adsorción elevada y estable. Por último, la concha de mejillón presentó una baja capacidad de adsorción para los tres antibióticos, especialmente a concentraciones elevadas, con porcentajes de adsorción generalmente por debajo del 60%, lo que limita su potencial como bioadsorbente bajo las condiciones evaluadas.

En el caso del sistema binario, la desorción de AMX y CIP suele ser más elevada cuando se encuentran por separado que cuando se encuentran juntos, especialmente a concentraciones bajas, confirmando la adsorción cooperativa de estos antibióticos por los bioadsorbentes en el sistema binario. Además, al comparar los tres bioadsorbentes entre sí se observa que la corteza de pino es la que muestra la mayor capacidad de retención, mientras que la concha de mejillón es la que muestra la mayor desorción. Resultados similares fueron obtenidos por Conde-Cid et al. (2019b) para el sistema ternario de tres tetraciclinas, donde encontraron que la concha de mejillón fue el bioadsorbente que obtuvo los porcentajes de desorción más altos, mientras que la ceniza y corteza de pino presentaron una adsorción irreversible.

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6. CONCLUSIONS

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6. CONCLUSIONS

1. Antibiotic occurrence in sludge showed clear seasonal variation, with higher detection rates in spring, likely reflecting increased usage. Sludges treated by waste management companies contained fewer antibiotics at lower concentrations, confirming the effectiveness of waste processing. Antibiotics were present in 12% of analysed soils, and none were detected in plant tissues, suggesting limited transfer to the crops under the studied conditions.

2. When each antibiotic was added separately to the soil, azithromycin (AZM) demonstrated the highest affinity, with adsorption rates up to ten times greater than those observed for cefuroxime (CFX) and amoxicillin (AMX), and no measurable desorption; this suggests a minimal risk of AZM leaching into water bodies or entering the food chain. In contrast, AMX posed the greatest environmental and human health risk, showing the lowest adsorption and the highest desorption (up to 17%), indicating a high potential for mobility.

3. The competitive interactions observed in multi-antibiotic systems highlight the complex dynamics governing antibiotic behaviour in soils. While AMX adsorption increased in ternary and binary systems, particularly in high-pH soils and at elevated concentration, its concurrent rise in desorption suggests a binding to lower-energy sites, limiting retention. AZM and CFX adsorption and desorption were lower in the ternary system. These results emphasize the importance of considering co-contaminant presence when assessing the environmental mobility and risk of antibiotics.

4. Soil properties such as pH and organic matter (OM) content had the strongest influence on antibiotic adsorption in single-compound systems. Alkaline pH favoured CFX and AZM retention, although forest soils with low pH but high OM also showed strong CFX adsorption. AMX adsorption was higher in acid soils with elevated OM content. The ternary system further confirmed the role of pH, while other properties such as non-crystalline mineral content and the pKa of the antibiotics also contributed to adsorption behaviour, underscoring the complexity of adsorption mechanisms in heterogeneous soil matrices.

5. The good fit of the adsorption data to the Freundlich model ($R^2 > 0.9$) in all systems indicates heterogeneous adsorption and suggests that soil surfaces are unlikely to reach saturation with the antibiotics studied. The poor fit to the Langmuir and linear models, emphasizes that multilayer and energetically variable interactions dominate the retention mechanisms.

6. Regarding the residual materials in the single-component systems, oak ash was the most effective bioadsorbent for all three antibiotics, while mussel shell was primarily effective for CFX. Eucalyptus leaves and pine needles showed high adsorption for AMX. When added to soils, these bioadsorbents enhanced AZM adsorption to nearly 100% in all cases. CFX adsorption improved significantly with ash and mussel shell additions, while pine bark resulted in the greatest increase in AMX retention.

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7. In the ternary system, oak ash exhibited the highest efficiency in retaining the three antibiotics, due to its elevated pH, abundant non-crystalline minerals, and high exchangeable calcium content, which all together promoted strong and stable adsorption

with minimal desorption. In the binary system AMX+CIP, ash also showed excellent adsorption capacity for AMX, while pine bark was most effective for CIP.

8. The adjustments to the adsorption models of the three antibiotics by the bioadsorbents in the simple systems showed that Freundlich was the best-fit model for AMX and CFX adsorption across most materials, with exceptions observed for ash (CFX) and eucalyptus leaves and pine needles (AMX). For AZM the results were more variable: mussel shell fitted well to the Freundlich model and ash to the linear model, but pine bark did not fit well with either model.

9. In the ternary system, only mussel shell presented a good adjustment to both Freundlich and linear models. In the binary system (AMX+CIP), all three bioadsorbents exhibited good fits to the Freundlich model, suggesting consistent multilayer adsorption mechanisms.

10. Overall, AZM posed the lowest environmental risk due to its high retention and negligible desorption across soils and systems. In contrast, CFX and particularly AMX represent a potential threat to environmental and human health. The latter showed the lowest adsorption and the highest desorption values, both individually and in combination with other antibiotics. The materials evaluated (especially pine bark, oak ash, and mussel shell) demonstrated high sorption capacity and can be considered suitable bioadsorbents for the antibiotics studied. Their incorporation into soils significantly enhanced the retention capacity for these compounds, indicating their potential application in soil remediation, but also in water treatment processes to improve the removal efficiency of wastewater treatment plants.

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7. REFERENCIAS

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Se analiza la presencia de antibióticos de uso humano en lodos de depuradora, suelos y cultivos. También se estudia la capacidad de suelos de Galicia con distintas propiedades fisicoquímicas para retener amoxicilina (AMX), cefuroxima (CFX) y azitromicina (AZM), así como el potencial de materiales residuales sostenibles para mitigar su impacto. La AZM es el fármaco que supone menor riesgo ambiental, sin embargo, la CFX y, especialmente, la AMX, representan una posible amenaza para la salud ambiental y humana. Los materiales estudiados pueden considerarse bioadsorbentes idóneos para reducir la contaminación por estos antibióticos.