

1 **Simultaneous Adsorption of Amoxicillin and Ciprofloxacin on Agricultural Soils**
2 **and By-products Used as Bio-adsorbents: Unraveling the Interactions in Complex**
3 **Systems**

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14
15 **Abstract**

16 The presence of pharmaceuticals in agricultural soils, like amoxicillin (AMX) and
17 ciprofloxacin (CIP), poses a significant environmental challenge with potential
18 implications for ecosystems and human well-being. This study explores the simultaneous
19 adsorption of AMX and CIP on crop soils and bio-adsorbents, focusing on competitive
20 adsorption dynamics. Tests were conducted with varying pharmaceutical concentrations
21 in six soils and three bio-adsorbents. CIP consistently exhibited higher adsorption than
22 AMX, particularly at higher concentrations. In the binary system, AMX's adsorption
23 exceeded the individual system at higher concentrations, implying a synergistic effect.
24 Bio-adsorbents, especially pine bark and oak ash, displayed superior adsorption capacities
25 compared to soils. Some soils exhibited enhanced adsorption and retention of both
26 antibiotics simultaneously, aligning with the cooperative adsorption model. Freundlich's
27 adsorption model described the competitive adsorption systems well. These findings have
28 implications for addressing antibiotic contamination in agricultural ecosystems, offering
29 insights into complex interactions in soil environments amid rising pharmaceutical
30 concerns.

31 **Keywords:** Antibiotics pollution; crop soils; emerging pollutants; release; retention;
32 synergistic adsorption
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48 **1. Introduction**

49 The extensive utilization of antibiotics in recent years has resulted in their dissemination
50 and subsequent detection in water bodies and cultivated soils, posing a significant threat
51 to both human health and ecosystems [1]. Given that up to 90% of these biocides are
52 excreted from the body through feces and urine (due to not complete absorption in the
53 intestine), they accumulate in farm solid excreta and effluents or get released into
54 wastewaters and sludge generated from wastewater treatment plants (WWTP) after their
55 use in human medicine [2]–[4]. Regrettably, most WWTPs are not adequately equipped
56 to inactivate or remove antibiotics effectively, leading to their substantial release in
57 effluents and accumulation in sludge [5], [6].

58 The incorporation of WWTP sludge and/or wastewater effluents into soil to enhance
59 fertility can inadvertently introduce these pollutants into crop soils. This may result in the
60 emergence of resistant bacteria, ecotoxicity, and the potential entry of antibiotics into the
61 food chain through water and crops, classifying these compounds as emerging pollutants
62 with adverse effects on human and animal health [7]–[9].

63 Adsorption processes stand out as simple, cost-effective, highly efficient, eco-friendly,
64 and renewable approaches to improve the retention of antibiotics in soils [10]–[13].
65 Employing adsorption to retain antibiotics in soils could present a viable and affordable
66 solution to control the dispersion of these pollutants into the food chain. Other methods
67 like advanced oxidation, hydrolysis, photodegradation and biodegradation have been
68 explored as alternatives to adsorption. However, most of these processes generate toxic
69 by-products, and lack sufficient reliability for antibiotic removal [14], [15].

70 The concurrent presence of amoxicillin and ciprofloxacin in crop soils holds considerable
71 scientific importance due to its potential environmental ramifications and impact on
72 agricultural systems. Once introduced into the soil, these antibiotics may exhibit
73 prolonged persistence and interact with soil components, thereby exerting long-term
74 effects on soil quality, microbial communities, and nutrient cycling processes [16], [17].
75 Moreover, the coexistence of both antibiotics could give rise to synergistic or antagonistic
76 effects, altering their adsorption behavior and mobility, and consequently influencing
77 their bioavailability to plants and potential uptake by crops [18]. Understanding the
78 simultaneous adsorption of amoxicillin and ciprofloxacin in crop soils is pivotal in
79 comprehending their environmental fate and the associated risks of entering the food
80 chain. This underscores the significance of employing sustainable agricultural practices
81 to mitigate potential adverse effects on human and ecosystem health.

82 Drawing from this context and our prior research works [19]–[22], the primary aim of
83 this study is to investigate the retention capacity of six crop soils, namely VO5, M1, FC,
84 VP1, VP6, and VP7, along with three distinct bio-adsorbents materials (pine bark, mussel
85 shell, and oak ash), concerning amoxicillin and ciprofloxacin adsorption. Additionally,
86 we seek to explore the simultaneous adsorption behavior of both antibiotics within these
87 systems. This comprehensive approach ensures a realistic representation of real-world
88 scenarios, enabling an in-depth analysis of the intricate interactions among the
89 pharmaceuticals, soils, and bio-adsorbents. Through elucidating the underlying
90 mechanisms, this study significantly could contribute to the understanding of the
91 functioning of such real systems and their potential implications.

92
93 **2. Materials and methods**

94 *2.1. Screening and selection of soils samples and bio-adsorbents*

95 The soil sampling procedure encompassed the collection of 60 distinct soil samples
96 distributed across Galicia, a geographic area in northwestern Spain. Among these, 27
97 samples were derived from corn crops, and 33 samples originated from vineyard soils.

98 Additionally, three samples were acquired from forest soils due to their differential
99 organic matter content and pH values compared to crop soils, both of which play pivotal
100 roles in adsorption/desorption processes (as depicted in Table S1, Supplementary
101 Material).

102 The sampling process involved obtaining ten sub-samples from each plot within the
103 topsoil layer (0-20 cm) using an Edelman probe in a zig-zag pattern. These ten sub-
104 samples were combined to form a comprehensive and representative sample.
105 Subsequently, the collected samples were transported to the laboratory and subjected to
106 oven drying (at a specific temperature) until a constant weight was achieved. Following
107 this, the samples were sieved to a 2-mm size and properly stored.

108 From the initial pool of 60 soil samples, a subset of six soils was selected based on their
109 respective pH and organic matter contents. These soils were designated as M1, VO5, VP1,
110 VP6, VP7, and FC.

111 The study also employed biosorbents sourced from the forestry and agro-food industry.
112 Specifically, phyto mass ash was obtained from oak log combustion at a combustion
113 boiler in Lugo, Spain. The uncalcined mussel shell, with a particle size smaller than 1
114 millimeter, was provided by Abonomar S.L. on Isla de Arousa, Pontevedra, Spain. Lastly,
115 the Geolia trademark pine bark from Madrid, Spain, was crushed and sieved to a particle
116 size of 0.63 mm.

117

118 *2.2. Chemicals and reagents*

119 The antibiotic ciprofloxacin (CIP) was of 98% purity while amoxicillin trihydrate (AMX)
120 was of >95% purity, all of them supplied by Sigma-Aldrich (Madrid, Spain). All the
121 reagents needed for the quantification of the antibiotics were of HPLC grade, provided
122 by Sigma-Aldrich (Madrid, Spain). A Millipore system (Madrid, Spain) was used to
123 provide ultrapure water.

124

125 *2.3. Characterizations of soils and bio-adsorbents*

126 The granulometry of the soils was determined following the Robinson International
127 Pipette Method [23], which allowed for the separation of sand, silt, and clay fractions.
128 The soil textures were then classified using the Soil Taxonomy approach [24].

129 The pH measurement was conducted in water and KCl solutions using a soil-to-liquid
130 ratio of 1:2.5 [25]. The pH readings were performed using a CRISON model 2001 pH-
131 meter (Spain), with a contact time of ten minutes for water and two hours for KCl. To
132 analyze organic matter (OM) and nitrogen contents, elemental analysis was employed,
133 utilizing a TRUSPEC CHNS equipment from LECO model (USA). For the determination
134 of exchange cations, a 1M NH₄Cl extract was utilized, with a soil-to-solution ratio of
135 1:10, and the extraction process was conducted over 12 hours [26]. Atomic
136 adsorption/emission spectrophotometry was employed, with the addition of 1% LaCl₃ to
137 prevent any potential interferences. The elements Al, Ca, Mg, Na, and K were quantified
138 through this method. To assess the effective cation exchange capacity (eECE), the sum of
139 the exchange cations was calculated [27]. For the non-crystalline iron (Fe_{ox}) and
140 aluminum (Al_{ox}) fractions, an extraction procedure was carried out using an ammonium
141 oxalate buffered solution at pH=3 [28]. Additionally, the fractions bound to organic matter
142 (Fe_{pir} and Al_{pir}) were obtained using a sodium pyrophosphate solution at pH=10 [29]. The
143 quantification of these fractions was performed using atomic absorption
144 spectrophotometry. Tables S1 and S2 (Supplementary Material) provide an overview of
145 the characteristic physicochemical parameters of the six soils and three bio-adsorbents
146 studied.

147

148 *2.4. Simple and binary adsorption/desorption tests and antibiotics quantification*

149 Batch experiments were conducted in simple and binary systems, each containing either
150 one of the antibiotics (amoxicillin/ciprofloxacin) or both and were exposed to five
151 different concentrations of the respective antibiotics (ranging from 0 to 400 $\mu\text{mol L}^{-1}$). To
152 initiate the tests, 4 g of the soils (0.5 g in the case of bio-adsorbents) were mixed with 10
153 mL of a 0.005 M CaCl_2 solution, which served as the background electrolyte to maintain
154 a constant ionic strength. The antibiotic concentrations were varied independently for
155 each individual antibiotic. The mixture was stirred for 48 hours, a duration determined
156 based on prior kinetics experiments, to ensure that equilibrium was reached [20], [22].
157 Following the adsorption phase, the samples were subjected to centrifugation at 4000 rpm
158 for 15 minutes, and the resulting supernatants were filtered through 0.45 μm nylon-type
159 syringe filters.

160 The quantification of antibiotic concentrations was performed using High-Performance
161 Liquid Chromatography (HPLC) with a Thermo-Fisher Model LPG 3400 SD equipment
162 (USA) coupled with a HPG-3400 quaternary pump (USA), a WPS3000 autosampler
163 (USA), a thermostated compartment for the TM-120 column, and an ultraviolet-visible
164 detector of the UltiMate 3000 series (USA). Data processing was facilitated using the
165 Chromeleon software, and a Luna C18 column (150 mm long; 4.6 mm internal diameter;
166 5 μm particle size) provided by Phenomenex (Madrid, Spain) was used, along with a
167 pre-column (4 mm long; 2 mm internal diameter; 5 μm particle size) packed with the
168 same material as the column. The injection volume was set at 50 μL , and the flow rate
169 was maintained at 1.5 mL min^{-1} . Regarding the mobile phase, acetonitrile (phase A) and
170 0.01 M phosphoric acid at pH=2 (phase B) were used for amoxicillin, while for
171 ciprofloxacin, the phases remained the same, but with a different linear gradient, varying
172 from 5% to 32% of phase A and from 95% to 68% of phase B over 10.5 minutes. The
173 wavelengths used for quantification were 212 nm for CIP and 230 nm for AMX.

174 After the adsorption step, desorption tests were conducted. The remaining material post-
175 adsorption was weighed, and 10 mL of 0.005 M CaCl_2 was added. The samples were
176 stirred for 48 hours, followed by centrifugation and filtration using the same conditions
177 as in the adsorption process. The concentration of the corresponding antibiotic in the
178 equilibrium solution was then determined using a similar procedure as that used in the
179 adsorption tests.

180
181 *2.5. Freundlich's adsorption modelling for simple and binary systems*

182 To describe the individual adsorption process of each antibiotic, the Freundlich model
183 was used (eq. 1).

184
185
$$q_e = K_f C_e^n \quad (1)$$

186
187 Where q_e ($\mu\text{mol kg}^{-1}$) is the amount of antibiotic adsorbed per unit mass of adsorbent
188 (difference between the added amount and the amount remaining at equilibrium); C_e
189 ($\mu\text{mol L}^{-1}$) is the equilibrium concentration of the antibiotic in the solution, K_f ($\text{L}^n \text{kg}^{-1}$
190 μmol^{1-n}) is the Freundlich constant related to the adsorption capacity, and n
191 (dimensionless) is a Freundlich parameter related to the adsorption intensity [30].

192 Furthermore, to study a possible competition between antibiotics for adsorption sites, the
193 Freundlich equation was adapted (eq. 2) following the literature [2], focusing on the total
194 amount adsorbed for both antibiotics (eq. 2).

195
196
$$(Q_{aCIP} + Q_{aAMX}) = K_f (C_{eqCIP} + C_{eqAMX})^n \quad (2)$$

197

198 Where Q_a is the individual amount adsorbed for each antibiotic; C_{eq} is the equilibrium
199 concentration of each antibiotic; K_F and n are the parameters mentioned above.

200

201 3. Results and discussion

202 3.1. Adsorption of amoxicillin and ciprofloxacin in a binary system by different soils and 203 bio-adsorbents

204 Fig. S1 (Supplementary Material) depicts the adsorption capacity (in $\mu\text{mol kg}^{-1}$) of AMX
205 and CIP in individual and binary systems of the six soils under investigation, while Fig.
206 S2 (Supplementary Material) represents the adsorbed amount as a percentage.

207 The addition of the two or even all three lower concentrations (25, 50, and $100 \mu\text{mol L}^{-1}$)
208 generally does not show significant differences in the adsorbed amount, neither between
209 the two antibiotics (AMX and CIP) nor between the two systems (individual and binary)
210 for each antibiotic (Fig. S1, Supplementary Material). This behavior changes for the two
211 higher concentrations added (200 and $400 \mu\text{mol L}^{-1}$), where generally a higher adsorption
212 of CIP than AMX is observed in both systems. Furthermore, in the case of AMX, there is
213 a higher retention in the binary compared to the individual system (Fig. S1,
214 Supplementary Material).

215 By expressing the data as a percentage of adsorption relative to the amount added, it is
216 generally observed that the adsorption rate of CIP is higher than that of AMX in both
217 systems, especially when the two higher concentrations are added. In the case of AMX,
218 the adsorption rate is higher in the binary than in the individual system, particularly at the
219 two higher concentrations. However, this rate decreases as the added concentrations
220 increase, particularly in the individual system. As for CIP, the behavior is less defined.
221 Some soils, such as M1, adsorb almost all of the added concentrations in both systems,
222 while others, as VO5, show an increasing adsorption percentage with the added
223 concentration. Soils such as FC and VP6 exhibit higher adsorption rates in the binary
224 system (between 80% and 100%) than in the individual system (between 40% and 100%),
225 with the percentage increasing as the added concentration increases. Finally, soils like
226 VP1 and VP7 show higher adsorption in the individual than in the binary system, with
227 the former adsorbing 100% of the added concentrations, while the latter shows an
228 increasing retention with concentration, reaching 100% for the two highest doses (Fig.
229 S2, Supplementary Material).

230 The higher adsorption of CIP compared to AMX in both the individual and binary systems
231 (Fig. S1, Supplementary Material) can be related to the fact that CIP has various
232 functional groups (hydroxyl, carboxyl, and fluoride) that play an important role in the
233 adsorption process [31]. These groups are found in cationic or zwitterionic form within
234 the pH range of the studied soils [32], electrostatically interacting with negatively charged
235 soil components. The non-crystalline components, which have a high presence in the
236 studied soils, contribute to the strong affinity of these substances. In contrast, AMX exists
237 in zwitterionic or anionic form [33], resulting in less favorable electrostatic interactions
238 with soil components. Several authors have also reported higher adsorption of CIP
239 compared to AMX [34], [35].

240 The fact that AMX is generally more adsorbed in the presence of CIP becomes more
241 noticeable at higher concentrations, especially at $400 \mu\text{mol L}^{-1}$, where AMX adsorption
242 in the binary system can increase up to 2.7 times in soils M1 and VP7, and between 50%
243 and 75% in the rest of the soils, except for VP1. This synergistic relation in the binary
244 system, favoring AMX adsorption in the presence of CIP, is more significant in soils M1
245 and VP7, which have higher pH values (8.02 and 7.27, respectively). At these pH values,
246 AMX acts as an anionic species with deprotonated amino and carboxyl groups, while CIP
247 exists as a zwitterionic species, allowing for electrostatic attractions between the

248 positively charged groups of CIP and the anionic AMX. This suggests that the presence
249 of CIP adsorbed in the soils enhances the incorporation of AMX onto the adsorbent. Due
250 to the high amount of organic matter in both soils (8.58% for M1 and 6.14% for VP7),
251 which carries a negative charge at basic pH values, electrostatic repulsions between AMX
252 and the soils are expected in the individual system, leading to the adsorption of a certain
253 amount of the antibiotic possibly through cationic bridges due to the high amounts of
254 exchangeable calcium in these soils [36](39.44 $\mu\text{mol kg}^{-1}$ for M1 and 10.53 $\mu\text{mol kg}^{-1}$ for
255 VP7). The high affinity of soil M1 for adsorbing CIP at all concentrations promotes the
256 incorporation of AMX in the binary system, resulting in AMX adsorptions also
257 approaching 100%. Thus, a ternary complex of soil-CIP-AMX is formed, where CIP
258 provides positive charges for the binding of the anionic AMX. This synergistic
259 phenomenon between antibiotics has been described by other authors in the binary
260 adsorption of tetracycline and sulfadiazine [37].

261 Regarding CIP, the adsorption in the binary system shows a different behavior compared
262 to the individual system, depending on the concentrations added. For the two highest
263 concentrations, no differences are observed between the two systems (Fig. S2,
264 Supplementary Material), indicating that AMX has no influence on the adsorption of CIP.
265 On the other hand, at the two lower concentrations, two different behaviors are observed.
266 In soils FC and VP6, there is higher adsorption of CIP in the binary system compared to
267 the individual system, and it is also higher than that of AMX. However, in soils VP1 and
268 VP7, the adsorption is higher in the individual system, and in the binary system, it is lower
269 than that of AMX (Fig. S2, Supplementary Material). Therefore, at low concentrations,
270 AMX sometimes has a synergistic effect on CIP adsorption, while in other cases, it has
271 an antagonistic effect. Some authors have found that at low concentrations, CIP is more
272 adsorbed in individual systems than in binary systems, indicating that it cannot efficiently
273 compete with AMX. Conversely, at high concentrations, greater adsorption in the binary
274 system than in the individual system has been observed, which is interpreted as a decrease
275 in mass transfer resistance for the adsorption of CIP species in this binary system [38].

276 Fig. S3 (Supplementary Material) shows the adsorption of CIP and AMX by the bio-
277 adsorbents in both the individual and binary systems, in terms of $\mu\text{mol kg}^{-1}$ and as a
278 percentage. It is worth noting that all three bio-adsorbents used exhibit much higher
279 adsorption capacities than the soils in the present study (Figs. S1 and S3, Supplementary
280 Material). It can be observed that, generally, both in the individual and binary systems,
281 mussel shell and especially pine bark adsorb more CIP than AMX, while oak ash shows
282 the opposite trend (Fig. S3, Supplementary Material). Therefore, oak ash is an excellent
283 adsorbent for AMX, and pine bark is efficient for CIP in both systems. Expressing the
284 data as percentage values, it can be observed that the adsorption of CIP, both in binary
285 and single systems, is 100% onto pine bark. In mussel shell and oak ash, similar
286 adsorptions of CIP are obtained, while AMX is adsorbed more than CIP only onto oak
287 ash.

288 Comparing the single and binary systems, different behaviors are observed depending on
289 the antibiotic and bio-adsorbent used. In the case of AMX, it is found that in oak ash, at
290 low concentrations added, the adsorption is higher in the single than in the binary system,
291 indicating that CIP competes favorably with AMX under these conditions. However, at
292 higher concentrations, the adsorption is similar in both systems. In contrast, in pine bark
293 and especially in mussel shell, the effect of CIP on AMX is synergistic (Fig. S3,
294 Supplementary Material).

295 Chandrasekaran et al. [35] found that the adsorption of AMX by *Prosopis juliflora*, in a
296 binary system AMX + CIP, was lower than in the single system, indicating that CIP has
297 antagonistic/competitive effects on the adsorption of AMX, as observed in the case of the

298 oak ash used in the current research. This may be due to both antibiotics being negatively
299 charged at the pH of oak ash, leading to the most probable mechanism being cationic
300 bridges established by the abundant exchangeable calcium with the soil components, as
301 mentioned before. This suggests that CIP has a higher affinity than AMX for forming
302 cationic bridges.

303 Regarding the synergistic effect of CIP on AMX observed in pine bark and especially in
304 mussel shell, this can be related to the cooperative adsorption model proposed by several
305 authors [39], [40]. According to this model, when a solute is retained by a site on a
306 homogeneous adsorbent surface, it can influence the consecutive active sites of that
307 surface, promoting new adsorptions and stronger retentions.

308 As for CIP, the presence of AMX generally affects its adsorption very little in oak ash and
309 pine bark, while in mussel shell, it decreases the adsorption in the binary system (at high
310 concentrations), indicating that in this case, AMX competes with CIP (Fig. S3,
311 Supplementary Material).

312
313 *3.2. Adsorption isotherms in the amoxicillin-ciprofloxacin binary system for soils and bio-*
314 *adsorbents*

315 Table 1 presents the values obtained after applying the Freundlich model for the binary
316 system of AMX with CIP for each soil.

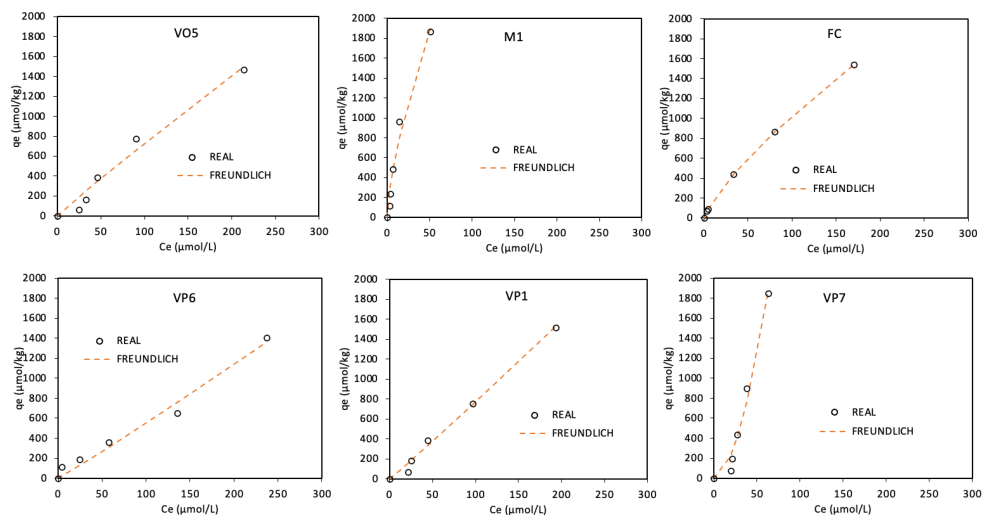
317
318

319 Table 1. Freundlich's model adsorption parameters for the binary system CIP+AMX and
 320 simple systems in soils (K_F , in $L^n \text{ kg}^{-1} \mu\text{mol}^{1-n}$, and n , dimensionless); -: error value too
 321 high for fitting

Soil	Antibiotic	Freundlich				R2
		K_F	Error	n	Error	
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

322
 323 Fig. 1 shows the relation between equilibrium concentration ($\mu\text{mol L}^{-1}$) and the adsorbed
 324 amount of AMX and CIP ($\mu\text{mol kg}^{-1}$) by the different soils used. The data is fitted to the
 325 Freundlich model.
 326

327



328

329 Fig. 1. Adsorption curves for AMX and CIP in the binary system onto the studied soils,
330 both real and according to the Freundlich model

331

332 As can be observed in Table 1, the Freundlich model for the binary system AMX+CIP fits
333 well, with high R^2 values. Therefore, as mentioned earlier, the Freundlich model can be
334 satisfactorily adapted to describe these competitive adsorption systems.

335 The obtained K_F values for the binary system AMX+CIP ranged from 1.12 to 129.80 Ln kg^{-1}
336 μmol^{1-n} (Table 1), which are lower than those found in a previous study for the
337 binary adsorption of tetracycline+sulfonamide (ranging from 34.30 to 1130.45 Ln kg^{-1}
338 μmol^{1-n}) in six cultivation soils [41]. In the binary system of AMX+CIP, the K_F values
339 were higher than for the individual systems of each antibiotic in FC, VP6, and M1 soils,
340 indicating that the simultaneous presence of both antibiotics favors their adsorption onto
341 these three soils, probably due to the previously described synergistic effects.

342 Regarding the parameter n for the binary system, the VP7 soil presents a value of 1.79
343 (the highest among all the studied soils), which indicates chemical adsorption between
344 the simultaneously present antibiotics and the soil. If we compare the value of n in the
345 AMX+CIP system for this soil with the respective values of the individual systems, it can
346 be appreciated that the presence of both antibiotics may cause modifications in the
347 adsorption mechanisms with the soil surface [41]. Other authors found values of $n > 1$ for
348 binary adsorption systems of chlortetracycline+sulfonamide, suggesting strong
349 interactions between the antibiotics and the adsorption sites of the adsorbents [42]. For
350 the rest of the soils of the current research, the n values were close to 1 (linear adsorption,
351 Fig. 1) or less than 1, indicating the presence of heterogeneous adsorption surfaces, with
352 the highest energy sites being occupied first.

353 Regarding the bio-adsorbents, Table 2 presents the values obtained for each of them after
354 applying the Freundlich model for the binary system AMX-CIP.

355

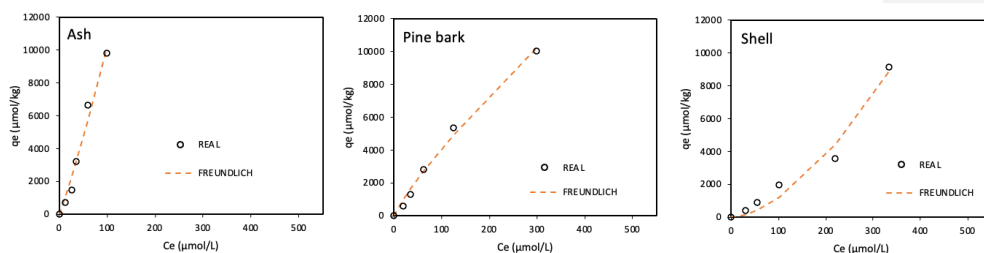
356

357 Table 2. Freundlich's model adsorption parameters for the binary system CIP+AMX and
 358 simple systems in bio-adsorbents (K_F , in $L^n \text{ kg}^{-1} \mu\text{mol}^{1-n}$, and n , dimensionless); -: error
 359 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	$2.767 \cdot 10^{-6}$	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

Con formato: Superíndice

360
 361 In Fig. 2, the relation between equilibrium concentration ($\mu\text{mol L}^{-1}$) and the adsorbed
 362 amount of AMX and CIP ($\mu\text{mol kg}^{-1}$) by the different bio-adsorbents used is depicted,
 363 both real and according to the Freundlich model.
 364



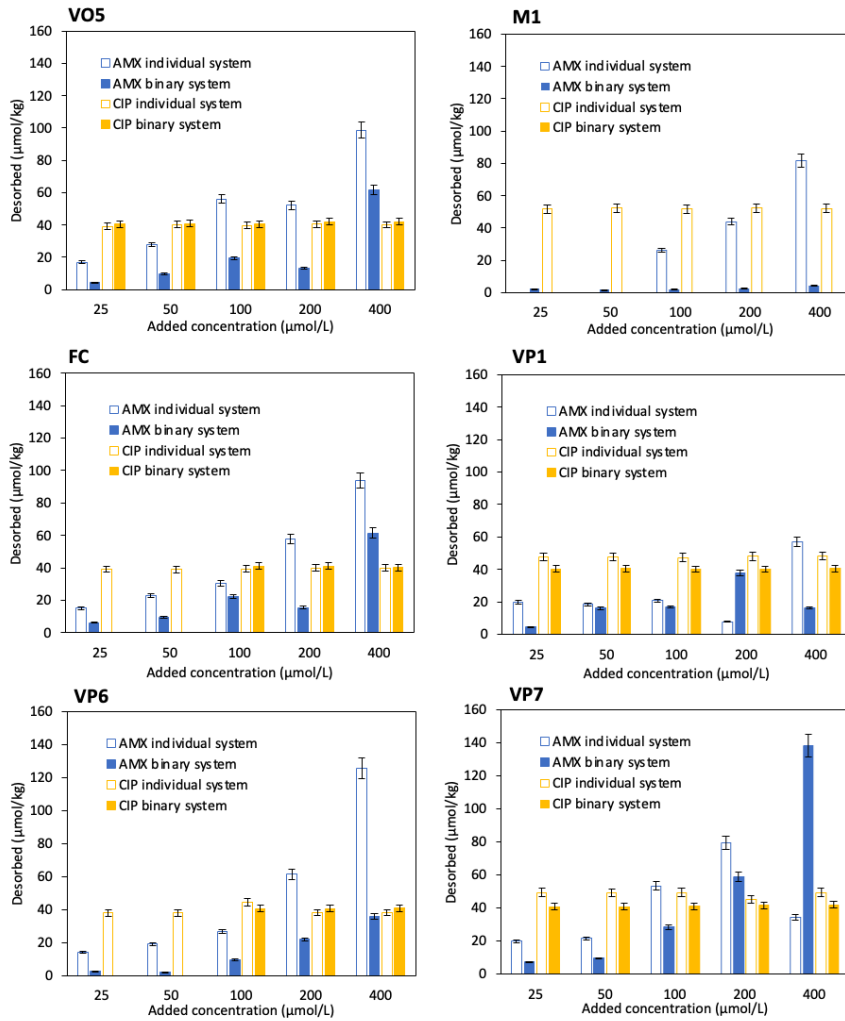
365
 366 Fig. 2. Adsorption of AMX and CIP in the binary system in bio-adsorbents, real and
 367 according to the Freundlich model
 368

369 As can be seen in Table 2, the R^2 values in all cases exceed 0.97, indicating a good fit of
 370 the AMX and CIP adsorption in the binary system to the Freundlich model for all three
 371 bio-adsorbents.

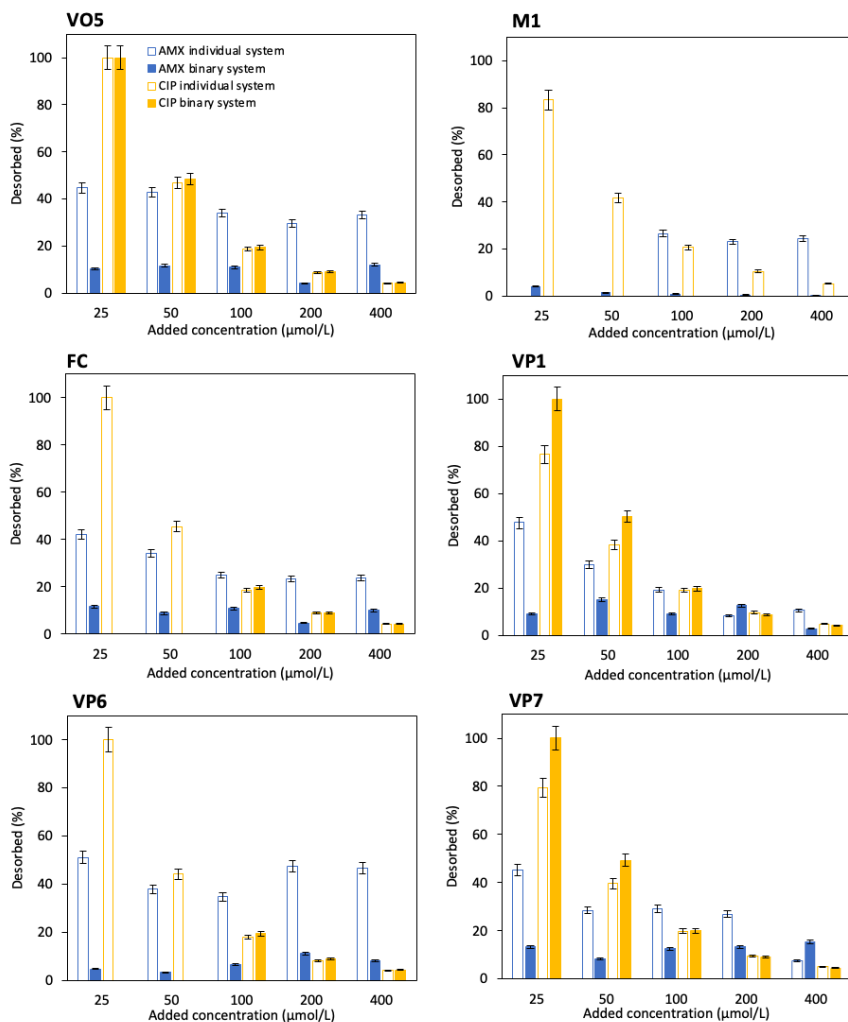
372 The values of the Freundlich affinity coefficient (K_F), related to the adsorption capacity
 373 of the adsorbent, range from ~~56.7460-582~~ to 83.643 $L^n \mu\text{mol}^{1-n} \text{ kg}^{-1}$ for the binary
 374 adsorptions. The significantly higher values are obtained in oak ash and pine bark, which
 375 align with their high adsorption capacity. Other authors found K_F values for the binary
 376 adsorption of tetracycline+sulfonamide on these same bio-adsorbents between 35.15-
 377 123.29 $L^n \mu\text{mol}^{1-n}$ [41], higher than those in the present study.

378 Regarding the adsorption intensity (n), it takes values below 1 for pine bark, indicating
 379 the presence of heterogeneous adsorption surfaces and high-energy adsorption sites,
 380 which are occupied first [30]. In the case of mussel shell and oak ash, n takes values of
 381 1.657 and 1.128, respectively, suggesting a favorable intensity model for adsorption as
 382 the added concentration of antibiotics increases [43]. Studies conducted by Cela-
 383 Dablanca et al. [41] regarding the adsorption of tetracycline with sulfadiazine by the bio-
 384 adsorbents used in this study also reported values of n greater than 1 for oak ash.
 385

386 3.3. Binary desorption of amoxicillin and ciprofloxacin by different soils and bio-
 387 adsorbents
 388 Fig. 3 displays the desorption, in $\mu\text{mol kg}^{-1}$, in both the single and binary systems of AMX
 389 and CIP by the soils used in this study. In Fig. 4, these results are expressed as percentage.
 390



391 Fig. 3. Simple/binary desorption of amoxicillin and ciprofloxacin as $\mu\text{mol/kg}$ from the six
 392 soils of study
 393
 394



395
 396 Fig. 4. Simple/binary desorption of amoxicillin and ciprofloxacin as percentages from the
 397 six soils of study
 398

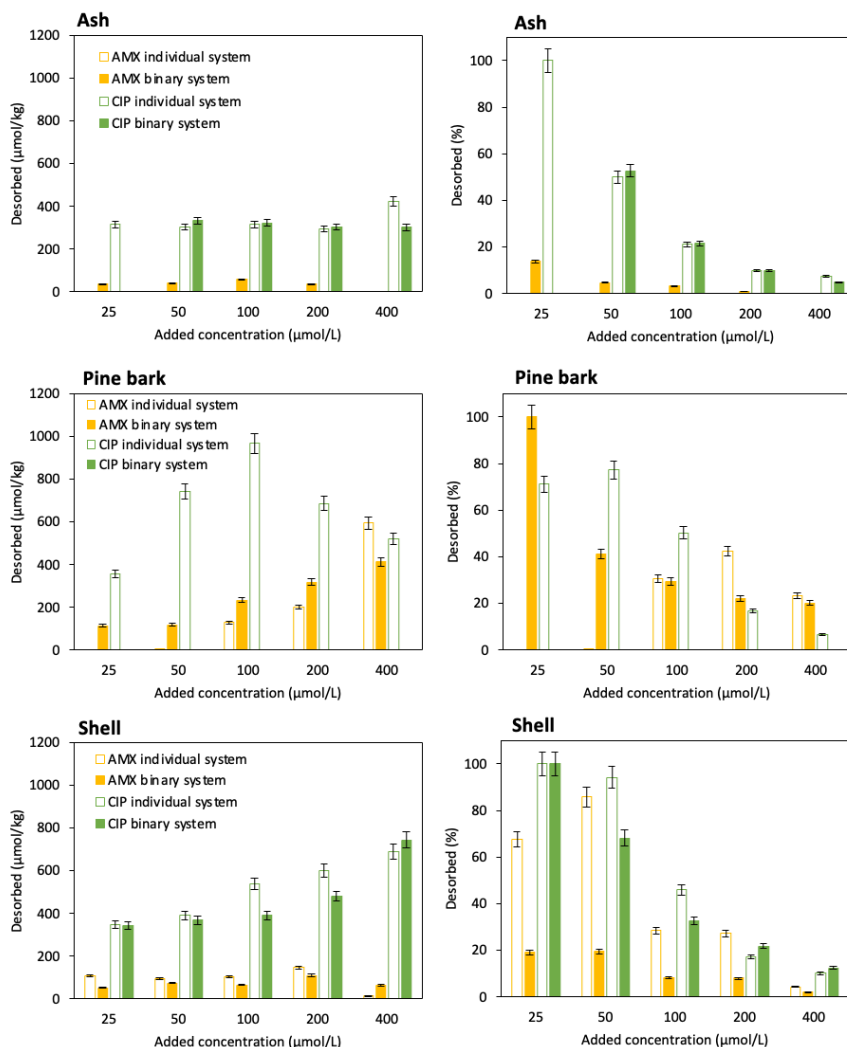
399 In general, it can be observed that the desorption rate of CIP and, especially, AMX is
 400 higher in the individual system than in the binary system, particularly at lower added
 401 concentrations (Fig. 4). CIP desorbs more than AMX at lower concentrations, while the
 402 opposite occurs at higher concentrations. Previously, Chandrasekaran et al. [35] found
 403 higher desorption of CIP than AMX in *Prosopis juliflora*.

404 Looking at the binary system, it can be noted that the desorption percentage for AMX is
 405 always below 10% in all soils, being particularly low from M1, FC, and VP6 soils. These
 406 soils also retain CIP very strongly, as its desorption in the binary system is very low (<5%)
 407 for all added concentrations, while in the other three soils (VO5, VP1, and VP7), there is
 408 significant desorption when the two lower concentrations are added (up to 100% of the
 409 adsorbed), being slightly higher than in the single system (Fig. 4). Therefore, particularly

410 in M1, FC, and VP6 soils, the simultaneous presence of both antibiotics not only has a
411 synergistic effect on increasing the adsorption of both pollutants, but it also enhances their
412 retention strength, resulting in less release into the environment. This can be explained
413 by the cooperative adsorption model proposed by several authors [39], [40] as mentioned
414 before, which assumes that when a solute is retained by a site on an adsorbent surface, it
415 can influence consecutive active sites on that surface, promoting new adsorptions and
416 stronger retentions.

417 Regarding the bio-adsorbents, the desorption of AMX and CIP in the single and binary
418 systems is shown in Fig. 5, expressed in $\mu\text{mol kg}^{-1}$ and as a ~~adsorption-desorption~~
419 percentage.

420



422 Fig. 5. Simple/binary desorption of amoxicillin and ciprofloxacin in $\mu\text{mol/kg}$ (left) and
 423 as percentages (right) from the three bio-adsorbents used
 424

425
 426 It can be observed that the highest desorption rate generally occurs for the lowest added
 427 concentrations, both in the individual and binary systems (Fig. 5). The behavior is
 428 different for each antibiotic and each bio-adsorbent, similar to what happens in
 429 adsorption. For instance, in the case of AMX, oak ash, which had the highest retention
 430 capacity, exhibits very low desorption, negligible at high concentrations. Thus, this by-
 431 product is capable of irreversibly retaining a significant amount of AMX. The other two
 432 bio-adsorbents have low retention in both systems (less than 60% in mussel shell and less
 433 than 30% in pine bark), and the release at low concentrations can reach 70% in mussel
 434 shell in the individual system, and close to 100% in pine bark in the binary system.

435 Regarding CIP, when low concentrations are added, the desorption is very high in the
436 single system for all three bio-adsorbents (up to 100% of the adsorbed), and it is also high
437 in the binary system for oak ash and mussel shell (Fig. 5). Pine bark, which showed 100%
438 retention in both systems and for any concentration, retains this antibiotic strongly in
439 binary systems, while in the individual system, desorption is high, especially at the lowest
440 added concentrations (Fig. 5). Once again, the cooperative adsorption model can explain
441 the stronger adsorptions both in the binary system and when increasing the antibiotic
442 concentrations [39], [40].

443 In studies conducted by Cela-Dablanca [41] on the same bio-adsorbents with tetracycline
444 and sulfadiazine, it was found that pine bark did not desorb tetracycline but did desorb
445 sulfadiazine in the binary system, similar to what occurs in this case where it does not
446 desorb CIP but does desorb AMX. The high retention of CIP by pine bark could be
447 attributed to its high organic matter content, with an abundance of adsorption sites with a
448 high affinity for CIP compounds. As for mussel shell, it can be seen that it is the bio-
449 adsorbent that exhibits the highest CIP desorption and some AMX desorption, besides
450 being the bio-adsorbent with the lowest adsorptions of these antibiotics. These results are
451 comparable to those obtained by Conde-Cid et al. [44] in a binary system of
452 chlortetracycline+tetracycline and chlortetracycline+oxytetracycline. These authors
453 indicate that mussel shell is the by-product that presented the highest desorption
454 percentages in the binary system, being also the bio-adsorbent with the lowest adsorption
455 capacity for these antibiotics. In the same study, Conde Cid et al. [44] also mention that
456 oak ash has a very good retention capacity for the three aforementioned tetracyclines, as
457 it barely showed desorption for these antibiotics, similar to what happens in this case with
458 AMX.

459 **4. Conclusions**

461 The adsorption capacity of the antibiotics AMX and CIP in individual and binary systems
462 exhibits variations based on the concentration of antibiotics and the specific soil or bio-
463 adsorbent being examined. Notably, CIP demonstrates consistently higher adsorption than
464 AMX in both individual and binary systems, particularly at elevated concentrations of the
465 pollutant. In the binary system, AMX's adsorption surpasses that of the individual system
466 at higher concentrations, especially in soils with higher pH levels, implying a synergistic
467 effect for AMX and CIP that enhances AMX adsorption. The presence of CIP in the binary
468 system shows either synergistic or antagonistic behavior on AMX adsorption, depending
469 on the soil type and antibiotic concentrations. Among the bio-adsorbents, oak ash and
470 pine bark stand out for their superior adsorption capacities for CIP compared to AMX,
471 both in individual and binary systems. Conversely, AMX and CIP desorption tends to be
472 higher in the individual than in the binary system, particularly at lower antibiotic
473 concentrations. The bio-adsorbents also exhibit varying degrees of desorption for AMX
474 and CIP in both individual and binary systems, with pine bark displaying the highest
475 retention capability and mussel shell exhibiting the highest desorption. The application of
476 the Freundlich model provided satisfactory descriptions for the competitive adsorption
477 systems of AMX and CIP in the investigated soils and bio-adsorbents. The findings
478 highlight the potential influence of the simultaneous presence of multiple antibiotics on
479 their adsorption mechanisms and retention strength in the examined environmental
480 contexts. Consequently, considering the coexistence of multiple antibiotics in
481 environmental systems becomes essential for a comprehensive understanding of their fate
482 and potential impact on the environment.

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