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Tese de doutoramento

Mercury in human bones and burial context: an osteoarchaeological approach

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TESE DE DOUTORAMENTO

MERCURY IN HUMAN BONES AND BURIAL CONTEXT: AN OSTEOARCHAEOLOGICAL APPROACH

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ESCOLA DE DOUTORAMENTO INTERNACIONAL DA UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

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Mercury in human bones and burial contexts: an osteoarchaeological approach

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A toda a xente que me aturou nesta aventura,

un toxiño conciso.

RESUMO

Palabras clave: mercurio, contaminación, esqueletos, osteoarqueoloxía, solo/sedimentos, diaxénese, PLS-R, PLS-SEM

O mercurio é un elemento potencialmente tóxico sen función biolóxica coñecida que se considera un problema de saúde pública mundial. O seu uso remóntase á prehistoria, a primeira evidencia de contaminación por mercurio data do 3250 BCE (Idade do cobre) no sur da Península Iberia. Debido á falta de sumidoiros a longo prazo, o mercurio herdado xoga un papel clave no seu ciclo actual, polo que é fundamental coñecer as tendencias e os patróns do pasado. O obxectivo desta tese doutoral é avaliar, dende unha perspectiva de paleocontaminación, a relación entre os niveis ambientais de mercurio, as actividades humanas e a contaminación humano-ambiental. A hipótese principal é que os esqueletos humanos conteñen información sobre a exposición ó mercurio en períodos pasados. Para probalo, investigamos: i) a variabilidade do contido en mercurio en individuos que viviron en dous períodos históricos diferentes cunha exposición ambiental ó mercurio distinta; ii) a variabilidade inter e intraesquelética do contido en mercurio; e iii) o papel dos compoñentes óseos no contido/acumulación de mercurio nos ósos. Os ósos arqueolóxicos poden verse afectados polo ambiente de enterramento e o proceso de descomposición do corpo, polo tanto, tamén abordamos: i) a distribución do mercurio no solo/sedimentos do enterramento e os procesos que hai detrás; e ii) a relación entre os ósos e o contido de mercurio do solo/sedimentos do enterramento. Ademais, dado que os humanos, en vida, actúan como sumidoiros temporais de mercurio que eventualmente terminan enterrados, tamén consideramos o papel dos esqueletos e o solo/sedimentos do enterramento no ciclo do mercurio. Con este obxectivo, eliximos o xacemento arqueolóxico da Lanzada (NO de España). Este sitio representa unha oportunidade única para investigar o impacto da contaminación atmosférica por mercurio, xa que: i) proporciona información sobre dous períodos con distintos niveis de contaminación atmosférica (romano e tardoantigo); ii) está relativamente afastado das fontes de emisión; iii) era un asentamento rural sen evidencia de bens que contivesen mercurio; iv) investigacións previas suxiren que todos os esqueletos son locais; v) estudos previos en diaxénese ósea concluíron que tanto os individuos romanos coma os tardoantigos tiñan un grao similar de alteración ósea; e vi) o contido de mercurio do solo é moi baixo e descartouse como fonte de mercurio para os esqueletos. Esta investigación confirmou os restos humanos osteoarqueológicos coma arquivos adecuados para o estudo da

exposición humana no pasado. Descubrimos cá variabilidade na concentración de mercurio intra e interesquelético vese afectada pola exposición *ante-mortem*. Ademais, a estrutura ósea *ante-mortem* afecta á dispoñibilidade e retención ósea. Confirmouse cós corpos humanos son unha fonte de mercurio para o solo e cá distribución do mercurio na tumba está influenciada polo contexto de enterramento e a localización dos órganos diana (é dicir, atópanse concentracións máis altas preto da área torácica e abdominal). O contido de mercurio dos esqueletos arqueolóxicos ten unha orixe tanto *ante-mortem* (vía primaria) como *post-mortem* temperán (vía secundaria, é dicir, descomposición). Ademais, os compoñentes do solo/sedimentos parecen ter unha importancia menor no contido de mercurio nos ósos. Por último, os esqueletos e os solos funerarios desempeñan un papel no ciclo do mercurio como sumidoiros durante períodos de tempo relativamente longos.

RESUMEN

Palabras clave: mercurio, contaminación, esqueletos, osteoarqueología, suelo/sedimentos, diagénesis, PLS-R, PLS-SEM

El mercurio es un elemento potencialmente tóxico sin función biológica conocida que se considera un problema de salud pública mundial. Su uso se remonta a la prehistoria, la primera evidencia de contaminación por mercurio data del 3250 BCE (Edad del Cobre) en el sur de la Península Iberia. Debido a la falta de sumideros a largo plazo, el mercurio heredado juega un papel clave en su ciclo actual, por lo que es fundamental conocer las tendencias y los patrones del pasado. El objetivo de esta tesis doctoral es evaluar, desde una perspectiva de paleocontaminación, la relación entre los niveles ambientales de mercurio, las actividades humanas y la contaminación humano-ambiental. La hipótesis principal es que los esqueletos humanos contienen información sobre la exposición al mercurio en períodos pasados. Para probarlo, investigamos: i) la variabilidad del contenido en mercurio en individuos que vivieron en dos períodos históricos diferentes con una exposición ambiental al mercurio distinta; ii) la variabilidad inter e intraesquelética del contenido en mercurio; y iii) el papel de los componentes óseos en el contenido/acumulación de mercurio en los huesos. Los huesos arqueológicos pueden verse afectados por el ambiente de enterramiento y el proceso de descomposición del cuerpo, por lo tanto, también abordamos: i) la distribución del mercurio en el suelo/sedimentos del enterramiento y los procesos que hay detrás; y ii) la relación entre los huesos y el contenido de mercurio del suelo/sedimentos del enterramiento. Además, dado que los humanos, en vida, actúan como sumideros temporales de mercurio que eventualmente terminan enterrados, también consideramos el papel de los esqueletos y el suelo/sedimentos del enterramiento en el ciclo del mercurio. Con este objetivo, elegimos el yacimiento arqueológico de A Lanzada (NO de España). Este sitio representa una oportunidad única para investigar el impacto de la contaminación atmosférica por mercurio, ya que: i) proporciona información sobre dos períodos con distintos niveles de contaminación atmosférica (romano y tardoantiguo); ii) está relativamente lejos de las fuentes de emisión; iii) era un asentamiento rural sin evidencia de bienes que contuvieran mercurio; iv) investigaciones previas sugieren que todos los esqueletos son locales; v) estudios previos en diagénesis ósea concluyeron que tanto los individuos romanos como los tardoantiguos tenían un grado similar de alteración ósea; y vi) el contenido de mercurio del suelo es muy bajo y se descartó como fuente de mercurio para los esqueletos. Esta investigación confirmó los restos humanos osteoarqueológicos como archivos adecuados para

el estudio de la exposición humana en el pasado. Descubrimos que la variabilidad de la concentración de mercurio intra e interesquelético se ve afectada por la exposición *ante-mortem*. La estructura ósea *ante-mortem* afecta a la disponibilidad y retención ósea. Se confirmó que los cuerpos humanos son una fuente de mercurio para el suelo. La distribución del mercurio en la tumba está influenciada por el contexto de enterramiento y la ubicación de los órganos diana (es decir, concentraciones más altas cerca del área torácica y abdominal). El contenido de mercurio de los esqueletos arqueológicos tiene un origen tanto *ante-mortem* (ruta primaria) como *post-mortem* temprano (ruta secundaria, es decir, descomposición). Además, los componentes del suelo/sedimentos parecen tener una importancia menor en el contenido de mercurio en los huesos. Por último, los esqueletos y los suelos funerarios desempeñan un papel en el ciclo del mercurio como sumideros durante períodos de tiempo relativamente largos.

ABSTRACT

Keywords: mercury, pollution, skeletons, osteoarchaeology, soil/sediments, diagenesis, PLS-R, PLS-SEM

Mercury is a potentially toxic element with no known biological function that is considered a global public health concern. There is evidence of its use since prehistory, the first evidence of mercury pollution dating back to 3250 BCE (Cooper Age) in South Iberia. Due to the lack of long-term sinks, legacy mercury plays a potential key role in the current mercury cycle, making it essential to understand past trends and patterns. The goal of this doctoral thesis is to assess, from a paleo-pollution perspective, the relationship between environmental mercury levels, human activities and human-environmental pollution. The main hypothesis is that human skeletons contain information about mercury exposure in past periods. To prove it, we investigated: i) the mercury content variability in humans that lived in two different periods with contrasting mercury environmental exposure; ii) the inter- and intra-skeletal variability in mercury content; and iii) the role of bone components in mercury content/accumulation in bone. Archaeological bones can be affected by the burial environment and body decomposition after burial, therefore we also addressed: i) the mercury distribution in burial soil/sediments and the processes behind it; and ii) the relationship between bone and burial soil/sediments mercury content. Furthermore, as humans act as temporary sinks of mercury during their lifetime that eventually end buried, we also considered the role of skeletons and burial soil/sediments in the mercury cycle. With this aim, we chose the archaeological site of A Lanzada (NW Spain). This site represents a unique opportunity to investigate the impact of mercury atmospheric pollution as it: i) provides information about two periods of contrasting atmospheric pollution (Roman and post-Roman); ii) is relatively far from emission sources; iii) was a rural settlement with no evidence of mercury-containing goods; iv) previous research suggests that all skeletons are locals; v) previous studies in bone diagenesis concluded that both Roman and post-Roman individuals had a similar degree of bone alteration; and vi) soil mercury content is naturally very low and was discarded as a significant source of mercury to the skeletons. This research confirmed osteoarchaeological human remains as suitable archives of human exposure in the past. We found that intra- and inter-skeletal mercury concentration variability is affected by *ante-mortem* exposure. *Ante-mortem* bone structure affects bone availability and retention. Human bodies were confirmed as a source of mercury to the soil. Mercury distribution in the burial environment is influenced

by the burial context and the location of the target organs (i.e., higher concentrations near the thoracic and abdominal area). The mercury content of archaeological skeletons has both an *ante-mortem* (primary path) and early *post-mortem* (secondary path, i.e., decomposition) origin. In addition, soil/sediment components seem to have a minor importance on bone mercury content. Lastly, skeletons and burial soils play a role in the mercury cycle as sinks for relatively long time spans.

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1 | SUMMARY

1.1 | Resumo

O mercurio atópase na natureza en diferentes formas químicas que se poden resumir en tres categorías: mercurio elemental (Hg^0), sales de mercurio (Hg^{2+}, Hg_2^{2+}) e compostos orgánicos (ex., etil-, metil-, dimetil-). Non se coñece ningunha función biolóxica para este metal e algunhas das súas formas son tóxicas mesmo en doses moi baixas. Ó ser un veleno sistémico, pode danar potencialmente calquera órgano, tecido ou estrutura subcelular. A intoxicación aguda pode danar gravemente múltiples órganos e mesmo provocar a morte. Con todo, o principal risco para a saúde radica na súa exposición crónica, sendo especialmente preocupantes os danos que provoca durante o desenvolvemento embrionario. A toxicoloxía do mercurio é complexa xa cás súas diferentes formas químicas non comparten necesariamente vías de absorción ou comportamento dentro do organismo. Ademais, a súa toxicoloxía depende da dose, o tempo de exposición e as diferenzas fisiolóxicas individuais. O mercurio elemental absórbese facilmente por inhalación sendo o sistema nervioso central o órgano máis sensible. Os sales de mercurio penetran por inxestión ou contacto cá pel, e os riles e o tracto intestinal son os órganos críticos. Os compostos orgánicos asimílanse principalmente por inxestión e distribúense de forma uniforme no organismo. Esta variabilidade da lugar a unha ampla gama de órganos diana e a unha extensa sintomatoloxía clínica. Debido ás propiedades fisicoquímicas das distintas formas de mercurio, este atópase entre os principais elementos considerados de risco para a saúde pública por organismos internacionais como a Organización Mundial da Saúde, a Axencia Europea do Medio Ambiente ou a Axencia do Goberno de EE.UU. para Substancias Tóxicas e Rexistro de Enfermidades. Esta preocupación mundial sobre o impacto do mercurio na saúde e o medio ambiente traduciuse no tratado da Convención de Minamata sobre o Mercurio, asinado por 128 países.

O uso do mercurio por parte dos humanos remóntase, polo menos, ó Neolítico na súa forma de mineral de sulfuro vermello (é dicir, cinabrio). Tamén hai evidencia do uso do cinabrio por parte das antigas culturas exipcia e romana, e na antiga China, Xapón e México. Baixo a República e o Imperio Romano, utilizouse para extraer outros minerais preciosos coma o ouro e a prata. Esta práctica aínda se usa hoxe en día na minería artesanal e de pequena escala do ouro. O mercurio usábase como medicina ou tratamento. Este uso documentouse por primeira vez na antiga China e India e despois aparece nas culturas do Antigo Exipto, Grecia, Roma e Islam. En Europa, durante o período medieval e posmedieval, aplicáronse medicamentos que contiñan mercurio no tratamento da sífilis. Esta práctica estivo en uso ata mediados do século XX. A pesar da toxicidade do mercurio, aínda está presente nas amálgamas dentais ou nalgúns cosméticos. En agricultura empregouse como pesticida, o que provocou importantes desastres para a saúde humana con mortes masivas. O mercurio tamén foi clave na ciencia e a industria, debido ó seu amplo rango de aplicación dadas as súas particulares propiedades fisicoquímicas.

O mercurio é liberado ó ambiente tanto por fontes antropoxénicas como non antropoxénicas. As fontes non antropoxénicas comprenden procesos xeoxénicos e bioxeoxénicos, mentres cás actividades antropoxénicas cambiaron co tempo dende a minería e metalurxia ata a combustión de carbón e a extracción de ouro artesanal e a pequena escala. Durante o século pasado, as actividades antropoxénicas aumentaron significativamente as emisións de mercurio dende os reservorios estables á circulación global, aínda que se propuxo có primeiro punto de inflexión ocorreu ó redor do ano 2000 BCE. O mercurio ten longos tempos de residencia nos océanos e solos e relativamente longos na atmósfera. Debido á súa volatilidade e a falta de reservorios a longo prazo, unha vez liberado ós reservorios globais, pode estar en circulación durante máis de 1000 anos. Este comportamento xoga un papel importante no seu ciclo bioxeoquímico. Xunto co impacto das liberacións antropoxénicas, isto levou ó concepto de mercurio herdado. É dicir, o mercurio liberado de fontes antropoxénicas que é reciclado de reservorios superficiais.

A atmósfera é clave para o transporte e a redistribución global do mercurio. O comportamento e destino do mercurio na atmósfera depende da forma do mercurio liberado (mercurio elemental, óxido mercúrico ou asociado a partículas). As concentracións de mercurio e a especiación na atmósfera dependen de varios factores, como a proximidade da fonte, a dispoñibilidade de oxidantes, o tipo e a concentración do aerosol, a meteoroloxía e as condicións da superficie. A especie máis abundante neste sistema é o mercurio elemental e deposítase prin-

cipalmente como óxido mercúrico e mercurio asociado a partículas. Despois da deposición, unha fracción do mercurio pode reducirse a mercurio elemental e volver emitirse á atmósfera.

A deposición atmosférica de mercurio é unha fonte importante de mercurio para o solo, sendo predominante en áreas afastadas das fontes de emisión e non enriquecidas de forma natural. Acumúlase nos horizontes superiores do solo e o seu destino está determinado polas especies de mercurio e as propiedades fisicoquímicas do chan. A especie de mercurio que se atopa con maior frecuencia nos solos é o óxido mercúrico. Este óxido forma facilmente sales e minerais inorgánicos ou compostos orgánicos de Hg. Ten unha alta afinidade pola materia orgánica e os compostos minerais arxilosos do solo. O mercurio no solo pode removilizarse debido á xestión da terra e os bosques, os incendios forestais ou o cambio climático. Os solos poden actuar como fonte ou sumidoiro de mercurio dependendo de varios factores. Para comprender o papel dos ecosistemas terrestres no ciclo bioxeoquímico global do mercurio, é esencial comprender o comportamento do mercurio nos solos. Os cemiterios tamén poden ser fontes de mercurio para o solo cando a inhumación do corpo é parte do ritual funerario, xa cós corpos liberan o mercurio acumulado en vida. Cando o número de corpos é alto, o mercurio liberado pode causar efectos adversos nos ecosistemas circundantes e, por tanto, o estudo da dinámica do mercurio nestes ambientes é de particular interese.

A minería e a metalurxia prehistóricas no sur da Península Ibérica dan conta da primeira evidencia de contaminación por mercurio, ó redor do 3250 BCE (Idade do cobre). Dado có mercurio herdado desempeña un papel importante no ciclo do mercurio, comprender as tendencias e patróns pasados tamén é un aspecto clave. A dinámica do mercurio no pasado pódese abordar a través de paleoarquivos como sedimentos lacustres e mariños, turba, xeo ou aneis de árbores. Cada un deles proporciona distinta información sobre o ciclo do mercurio e ten as súas propias vantaxes e desvantaxes. Recentemente, propuxemos os restos osteoarqueolóxicos humanos como paleoarquivos adecuados, xa que brindan información sobre a exposición ó mercurio en poboacións humanas pasadas, xa sexa a través de fontes culturais ou ambientais.

A investigación no campo do mercurio en restos osteoarqueolóxicos humanos non é moi ampla. O número de publicacións aumentou dende 2010, pero non hai unha tendencia constante dende a primeira publicación en 1995. Estes traballos tratan tanto a incorporación *ante-mortem* como *post-mortem* do mercurio ó esqueleto. A incorporación *post-mortem* do mercurio pode ser consecuencia de rituais funerarios que involucren pigmentos de mercurio, ou enriquece-

mento de mercurio derivado da descomposición dos tecidos brandos. Mentres que se observou cá incorporación *ante-mortem* pode deberse a: i) exposición a altas concentracións ambientais de mercurio derivadas de actividades volcánicas, xeotérmicas, mineiras e metalúrxicas, e industriais; ii) uso e procesado do cinabrio; iii) medicamentos, cosméticos, amalgamas, intoxicacións; e iv) incluso á dieta, a través de alimentos e bebidas contaminados. O foco da investigación centrouse principalmente en individuos moi afectados debido ó seu estilo de vida ou prácticas culturais. Prestandoselle menos atención ás doses baixas e a exposición crónica, que é a máis común.

Os restos humanos foron propostos como arquivos naturais adecuados, nun estudo de investigación estreitamente relacionado con esta tese e baseado nalgunhas das mostras empregadas no primeiro artigo, abrindo a posibilidade de comprender millor o impacto da contaminación por mercurio no pasado. Ademais, este traballo tamén expuxo novas preguntas sobre a variación intra e interesquelética do contido en mercurio. Con todo, hai outros dous aspectos a considerar: i) os ósos non son o órgano diana do mercurio, e ii) os ósos poden verse afectados pola diaxénese na contorna do enterramento. Por tanto, é fundamental comprender o comportamento do mercurio no ambiente do enterramento para lograr unha boa interpretación dos datos proporcionados polos esqueletos. De acordo cá literatura, a incorporación de mercurio *post-mortem* pode estar relacionada con rituais funerarios, o solo do enterramento en áreas con minerais de mercurio ou por transferencia dende órganos diana durante a descomposición do corpo. Con todo, estes traballos son esencialmente descritivos, explicando o mercurio só, ignorando a relación con outros factores xeoquímicos e sen profundar no comportamento do mercurio, os procesos subxacentes e como estes poden relacionarse co contido de mercurio. Para avaliar a dinámica do mercurio nos solos funerarios, é esencial comprender a súa relación co tecido óseo, tanto *ante-mortem* como *post-mortem*. Para iso, é necesario estudar os solos dos enterramentos e a variabilidade intraesquelética do mercurio.

O obxectivo desta investigación foi avaliar, cunha perspectiva de paleocontaminación, a relación entre os niveis ambientais de mercurio, as actividades humanas e a contaminación humano-ambiental. Con este obxectivo seleccionáronse restos osteoarqueolóxicos humanos da Lanzada (NO de España, Galicia). Ademais, realizouse unha revisión detallada da literatura no campo do mercurio en restos osteoarqueolóxicos humanos. Os individuos da Lanzada representan unha oportunidade única para investigar a exposición atmosférica ó mercurio xa que: i) brindan información sobre dous períodos con concentracións de mercurio atmosférico dis-

tinta (romano e tardoantigo); ii) vivían relativamente lonxe das fontes de emisión; iii) vivían nun asentamento rural sen evidencia de bens que contivesen mercurio; iv) investigacións previas suxiren que todos os esqueletos son de individuos locais; v) tamén existen estudos previos que avalían a diaxénese ósea que conclúen que tanto os esqueletos romanos como os tardoantigos sufriron un grao similar de alteración ósea; e vi) o contido en mercurio no solo é moi baixo (5 ng g^{-1}) e descartouse como fonte de mercurio para os esqueletos xa cás concentracións de Hg na capa funeraria están por baixo de ∼1,5 ng g^{-1}, mentres que nos ósos a mediana foi de 36,0 ± 51,7 ng g^{-1}, concordando con investigacións previas que suxiren cá incorporación posdeposicional de mercurio é insignificante en ausencia de fontes xeoxénicas.

En investigacións previas documéntanse varios casos de incorporación *post-mortem* de mercurio ó esqueleto relacionada con rituais funerarios, como a pintura corporal con pigmentos que conteñen mercurio, restrinxida a grupos de alto status, en Xapón e culturas prehispánicas mexicanas. O ritual de pintura corporal tamén se documentou para o Calcolítico no Sur da Península Ibérica. Os usos do mercurio restrinxidos ós estratos de alto status tamén se atoparon na Rusia medieval, onde as tsarinas e as princesas usaban cosméticos a base de mercurio. Con todo, na Lanzada non hai constancia destas prácticas. Ademais, de non atoparse ningunha conexión cos indicadores de status social (é dicir, bens funerarios e tipoloxía de tumbas); hai que sinalar cá poboación estudada era de status medio-baixo e non se esperan membros de clase alta. O contido de mercurio osteoarqueolóxico tamén se relacionou con usos médicos e de envelenamento en poboacións medievais, como sacerdotes alemáns e daneses, individuos sifilíticos e leprosos, e individuos como o astrónomo Tycho Brahe ou o santo italiano Francesco Caracciolo. Na Lanzada, a pesar dalgúns casos de infeccións crónicas inespecíficas, diagnosticadas por avaliación paleopatolóxica, a variabilidade do mercurio intraesquelético non suxire un uso médico ou como veleno do mercurio. O alto contido de mercurio en individuos arqueolóxicos tamén se relacionou cá exposición ocupacional (por exemplo, minería e metalurxia) en lugares como La Encantada en Ciudad Real (SE, España), Ranas en Querétaro (México) e a Catedral da Trindade de Moscova (Rusia). Pero non se atopou unha relación directa con este tipo de fontes de exposición para a comunidade da Lanzada nos períodos estudados (romano e tardoantigo). Baixas concentracións de mercurio, coma as atopadas na Lanzada, documentáronse en sociedades e individuos sen uso directo de mercurio, coma as poboacións rurais medievais e posmedievales de Alemaña, Dinamarca e Italia.

O estudo de 143 mostras de óso cortical recuperadas na necrópole da Lanzada mostrou diferen-

zas significativas entre os dous períodos estudados, estando a cohorte romana máis enriquecida en mercurio (54 ± 60 ng g^{-1}) fronte á tardoantiga (21 ± 23 ng g^{-1}). Isto permitiunos confirmar os restos óseos humanos como arquivos adecuados de paleocontaminación por mercurio cando a exposición cultural é baixa, xa que: i) todos os esqueletos procedían da mesma área de enterramento; ii) as diferenzas cronolóxicas entre os dous períodos son máis curtas có tempo cós restos humanos estiveron baixo terra expostos á alteración diaxenética; iii) descartáronse os solos/sedimentos como fonte de mercurio; e iv) non se atopou relación entre a alteración ósea e o contido de mercurio. A contaminación ambiental (atmosférica) identificouse como a fonte de mercurio para a poboación, dado que: i) non hai evidencia de exposición cultural e non hai conexión cás características individuais *ante-/post-mortem* (é dicir, a variabilidade interesquelética); e ii) observouse evidencia de contaminación atmosférica afectando a esta zona durante a época romana a partir dun rexistro de turba de Penido Vello (NE de Galicia).

Ademais, no Artigo II analizáronse mostras dunha mostraxe detallada do solo (n = 46; unha práctica pouco común) de dous enterramentos tardoantigos, realizada por membros de Eco-Past na última campaña de escavación (2016 - 2017). O obxectivo era investigar a distribución e o comportamento do mercurio no solo/sedimentos de enterramento aplicando a regresión por mínimos cadrados parciais (PLS-R). No Artigo III, combinámolas con mostras de óso cortical (n = 73 mostras de óso + 37 mostras de solo) considerando diferentes taxas de recambio óseo, modelando con ecuacións estruturais por mínimos cadrados parciais (PLS-SEM) e segmentación orientada á predición por mínimos cadrados parciais (PLS-POS) para estudar a variabilidade intraesquelética tendo en conta o efecto solo/sedimentos.

O estudo do solo/sedimentos destas dúas tumbas mostrou cá fracción terra fina (< 2 mm) ten baixo contido en mercurio, mentres cá fracción limo e arxila está enriquecida, estando de acordo con estudos en solos non contaminados e co feito de cá maioría dos silicatos primarios carecen da capacidade de reter mercurio. Co modelo PLS-R explicouse > 72 % da variabilidade do mercurio, identificando tres procesos subxacentes principais cá controlan. (1) A diferenza entre o interior (12 a 39 ng g^{-1}) e o exterior (6 a 23 ng g^{-1}) dos enterramentos explicou a maior parte da súa variabilidade. Os nosos datos confirman os corpos como fonte de mercurio á contorna do enterramento, así como fontes de materia orgánica que tamén contribúe á fixación do mercurio, mentres có chan foi descartado como fonte de mercurio para os esqueletos. (2) As diferenzas relacionadas co contexto de enterramento foron a fonte secundaria de variación. Diferenzas que poden explicarse polo número de individuos por fosa, xa cá masa corporal está

relacionada co contido de mercurio no solo do enterramento; unha das tumbas contiña dous individuos masculinos, un adolescente e un adulto maduro, mentres cá outra era unha soa tumba dunha anciá. Tamén deben considerarse as diferenzas *ante-mortem* de cada individuo, como a exposición ambiental ou o estilo de vida, xa que poden ter un efecto na variabilidade do mercurio. (3) A proximidade ás áreas onde hai unha maior masa de tecidos brandos (é dicir, a rexión torácica e abdominal) produciu un maior enriquecemento local en mercurio, moi probablemente debido á súa liberación dos órganos diana (é dicir, os riles e o fígado) e a súa retención nas fraccións reactivas do chan (materia orgánica e arxila). Os nosos achados resaltan a importancia de analizar o solo/sedimentos dos enterramentos, xa que brindan información complementaria que pode ser clave para comprender os procesos relacionados cás interaccións corpo/solo.

Cos modelos PLS-SEM + PLS-POS da distribución do mercurio nos enterramentos, combinando datos de ósos e solo/sedimentos, conseguiuse explicar unha alta proporción da variabilidade do mercurio na hidroxiapatita ósea (> 90 %) e unha proporción moderada da súa variabilidade no coláxeno óseo (∼60 %). Este esforzo de modelado revelou seis aspectos principais. (1) A variabilidade do mercurio no ambiente de enterramento está influenciada pola estrutura ósea *ante-mortem*, o que reflicte diferenzas na fracción ósea que controla o contido de mercurio nos ósos con respecto á relación hidroxiapatita/coláxeno; pequenos aumentos no coláxeno poden ter un gran impacto na acumulación e retención de mercurio na estrutura ósea. Debido á presenza de grupos tiol na estrutura do coláxeno, existe a posibilidade de que se formen compostos de Hg-S, que son moi estables. Por tanto, a liberación de mercurio ó chan pode ser moi baixa ata cá estrutura do coláxeno estea altamente degradada. (2) O papel dos compoñentes óseos (é dicir, coláxeno e hidroxiapatita) na retención do mercurio depende do grao de cristalinidade ósea. A cristalinidade da hidroxiapatita ten un dobre papel na acumulación do mercurio no tecido óseo, afectando tanto á liberación de mercurio óseo (fonte primaria/secundaria ó chan) coma á retención/acumulación (sumidoiro; non se pode discriminar entre mercurio incorporado *ante-mortem* e *post-mortem* como resultado da interacción cos tecidos brandos durante a súa descomposición). En ósos arqueolóxicos, a cristalinidade da hidroxiapatita aumenta cá alteración ósea. Por tanto, espérase unha relación negativa co coláxeno óseo. Dado que, cando o mercurio óseo está controlado polo coláxeno, a liberación de mercurio aumentará cá cristalinidade da hidroxiapatita. Aínda que, cando a hidroxiapatita controla o contido de mercurio nos ósos, a dobre función da cristalinidade da hidroxiapatita relaciónase: i) cun maior contido de mercurio, que actúa como indicador do estado *ante-mortem* do tecido óseo;

e ii) cá liberación de mercurio como consecuencia da alteración ósea *post-mortem*. (3) O modelado confirmou cós corpos humanos (tecidos brandos e óso) son fontes de mercurio para o solo. (4) A concentración de mercurio no esqueleto depende do mercurio acumulado durante a vida (vía primaria), pero tamén durante a descomposición *post-mortem* temperá dos tecidos brandos (vía secundaria), actuando tanto como fonte e sumidoiro. (5) Nos enterramentos estudados, os compoñentes do solo/sedimento parecen desempeñar un papel menor no contido de mercurio nos ósos dos esqueletos humanos arqueolóxicos, en ausencia de minerais que conteñan mercurio. (6) Os modelos tamén suxiren cá importancia dos ósos como fonte primaria de mercurio para o solo está relacionada cá estrutura ósea *ante-mortem* (grao de madurez ósea, ex., contido de coláxeno), mentres cá súa función como fonte/sumidoiro secundario está asociada cá cristalinidade da hidroxiapatita. Isto destaca a relevancia de comprender como os tecidos contribúen ó ciclo do mercurio nos enterramentos, xa que poden ser fontes e sumidoiros simultaneamente. Estes achados teñen implicacións osteoarqueolóxicas, xa que non se pode supoñer que todo o mercurio contido nos ósos arqueolóxicos proveña dunha incorporación *ante-mortem*. Hai unha fracción que se pode incorporar *post-mortem* durante a descomposición do corpo. Isto debe terse en conta durante o deseño da mostraxe, xa có recambio óseo e a proximidade á rexión torácica/abdominal, especialmente preto dos principais órganos diana (é dicir, riles e fígado), poden afectar as concentracións de mercurio. A pesar disto, o contido de mercurio nos ósos arqueolóxicos é representativo da exposición *ante-mortem*, xa que todo o mercurio provén dos individuos.

Ademais, os nosos resultados revelan có estudo de individuos pouco contaminados produce información de alta calidade que complementa a de ambientes altamente contaminados. Esta investigación destaca a importancia de comprender a dinámica dos posibles contaminantes nas contornas de enterramento (cemiterios/tumbas), mesmo cando a exposición ambiental ó contaminante e o número de corpos enterrados no 'cemiterio' era baixo. Segundo os nosos datos, e de acordo cá literatura, os solos/sedimentos dos cemiterios poden estar significativamente enriquecidos en mercurio en comparación cos solos de áreas próximas. Isto debe terse en conta ó describir as propiedades do solo e cando esas áreas estean a experimentar cambios no uso da terra (por exemplo, a un uso agrario), xa que isto pode afectar á saúde humana e dos ecosistemas debido á liberación de contaminantes coma o mercurio. Isto reforza a importancia de coñecer como se distribuían no pasado os usos do solo, en particular os cemiterios, para comprender tamén como están a afectar o presente.

1.2 | Resumen

El mercurio se encuentra en la naturaleza en diferentes formas químicas que se pueden resumir en tres categorías: mercurio elemental (Hg^0), sales de mercurio (Hg^{2+}, Hg_2^{2+}) y compuestos orgánicos (ej., etil-, metil-, dimetil-). No se conoce ninguna función biológica para este metal y algunas de sus formas son tóxicas incluso en dosis muy bajas. Al ser un veneno sistémico, puede dañar potencialmente cualquier órgano, tejido o estructura subcelular. La intoxicación aguda puede dañar gravemente múltiples órganos e incluso provocar la muerte. Sin embargo, el principal riesgo para la salud radica en su exposición crónica, siendo especialmente preocupantes los daños que provoca durante el desarrollo embrionario. La toxicología del mercurio es compleja ya que las diferentes formas químicas no comparten necesariamente rutas de absorción o comportamiento dentro del organismo. Además, su toxicología depende de la dosis, el tiempo de exposición y las diferencias fisiológicas individuales. El mercurio elemental se absorbe fácilmente por inhalación siendo el sistema nervioso central el órgano más sensible. Las sales de mercurio penetran por ingestión o contacto con la piel, y los riñones y el tracto intestinal son los órganos críticos. Los compuestos orgánicos se asimilan principalmente por ingestión y se distribuyen de forma uniforme en el organismo. Esta variabilidad da lugar a una amplia gama de órganos diana y a una extensa sintomatología clínica. Debido a las propiedades fisicoquímicas de las distintas formas de mercurio, este se encuentra entre los principales elementos considerados de riesgo para la salud pública por organismos internacionales como la Organización Mundial de la Salud, la Agencia Europea del Medio Ambiente o la Agencia del Gobierno de EE.UU. para Sustancias Tóxicas y Registro de Enfermedades. Esta preocupación mundial sobre el impacto del mercurio en la salud y el medio ambiente se tradujo en el tratado de la Convención de Minamata sobre el Mercurio, firmado por 128 países.

El uso del mercurio por parte de los humanos se remonta, al menos, al Neolítico en su forma de mineral de sulfuro rojo (es decir, cinabrio). También hay evidencia del uso de cinabrio por parte de las antiguas culturas egipcia y romana, y en la antigua China, Japón y México. Bajo la República e Imperio Romano, se utilizó para extraer otros minerales preciosos como el oro y la plata. Esta práctica todavía se usa hoy en día en la minería artesanal y de pequeña escala del oro. El mercurio también se usó como medicina o tratamiento. Este uso se documentó por primera vez en la antigua China e India y luego aparece en las culturas del Antiguo Egipto, Grecia, Roma e Islam. En Europa, durante el período medieval y posmedieval, se aplicaron medicamentos que contenían mercurio en el tratamiento de la sífilis. Esta práctica estuvo en

uso hasta mediados del siglo XX. A pesar de la toxicidad del mercurio, todavía está presente en las amalgamas dentales o en algunos cosméticos. En agricultura se utilizó como pesticida, lo que provocó importantes desastres para la salud humana con muertes masivas. El mercurio también fue clave en la ciencia y la industria, debido a su amplio rango de aplicación dadas sus particulares propiedades físicoquímicas.

El mercurio es liberado al ambiente tanto por fuentes antropogénicas como no antropogénicas. Las fuentes no antropogénicas comprenden procesos geogénicos y biogeogénicos, mientras que las actividades antropogénicas han cambiado con el tiempo desde la minería y metalurgia hasta la combustión de carbón y la extracción de oro artesanal y en pequeña escala. Durante el siglo pasado, las actividades antropogénicas han aumentado significativamente las emisiones de mercurio desde los reservorios estables a la circulación global, aunque se propuso que el primer punto de inflexión ocurrió alrededor del año 2000 BCE. El mercurio tiene largos tiempos de residencia en los océanos y suelos, y relativamente largos en la atmósfera. Debido a la volatilidad del mercurio y la falta de reservorios a largo plazo, una vez liberado a los reservorios globales, puede estar en circulación durante más de 1000 años. Este comportamiento juega un papel importante en el ciclo biogeoquímico del mercurio. Junto con el impacto de las liberaciones antropogénicas, esto conduce al concepto de mercurio heredado. Es decir, el mercurio liberado de fuentes antropogénicas que es reciclado de reservorios superficiales.

La atmósfera es clave para el transporte y la redistribución global del mercurio. El comportamiento y destino del mercurio en la atmósfera depende de la forma del mercurio liberado (mercurio elemental, óxido mercúrico o asociado a partículas). Las concentraciones de mercurio y la especiación en la atmósfera dependen de varios factores, como la proximidad de la fuente, la disponibilidad de oxidantes, el tipo y la concentración del aerosol, la meteorología y las condiciones de la superficie. La especie más abundante en este sistema es el mercurio elemental y se deposita principalmente como óxido mercúrico y mercurio asociado a partículas. Después de la deposición, una fracción del mercurio puede reducirse a mercurio elemental y volver a emitirse a la atmósfera.

La deposición atmosférica de mercurio es una fuente importante de mercurio para el suelo, siendo predominante en áreas alejadas de las fuentes de emisión y no enriquecidas de forma natural. Se acumula en los horizontes superiores del suelo y su destino está determinado por las especies de mercurio y las propiedades fisicoquímicas del suelo. La especie de mercurio que

se encuentra con mayor frecuencia en los suelos es el óxido mercúrico. Este óxido forma fácilmente sales y minerales inorgánicos o compuestos orgánicos de Hg. Tiene una alta afinidad por la materia orgánica y los componentes minerales arcillosos del suelo. El mercurio en el suelo puede removilizarse debido a la gestión de la tierra y los bosques, los incendios forestales o el cambio climático. Los suelos pueden actuar como fuente o sumidero de mercurio dependiendo de varios factores. Para comprender el papel de los ecosistemas terrestres en el ciclo biogeoquímico global del mercurio, es esencial comprender el comportamiento del mercurio en los suelos. Los cementerios también pueden ser fuentes de mercurio para el suelo cuando la inhumación del cuerpo es parte del ritual funerario, ya que los cuerpos liberan el mercurio acumulado en vida. Cuando el número de cuerpos es alto, el mercurio liberado puede causar efectos adversos en los ecosistemas circundantes y, por lo tanto, el estudio de la dinámica del mercurio en estos ambientes es de particular interés.

La minería y la metalurgia prehistóricas en el sur de la Península Ibérica dan cuenta de la primera evidencia de contaminación por mercurio, alrededor del 3250 BCE (Edad del Cobre). Dado que el mercurio heredado desempeña un papel importante en el ciclo del mercurio, comprender las tendencias y patrones pasados también es un aspecto clave. La dinámica del mercurio en el pasado se puede abordar a través de paleoarchivos como sedimentos lacustres y marinos, turba, hielo o anillos de árboles. Cada uno de ellos proporciona distinta información sobre el ciclo del mercurio y tiene sus propias ventajas y desventajas. Recientemente, propusimos los restos osteoarqueológicos humanos como paleoarchivos adecuados, ya que brindan información sobre la exposición al mercurio en poblaciones humanas pasadas, ya sea a través de fuentes culturales o ambientales.

La investigación en el campo del mercurio en restos osteoarqueológicos humanos no es muy amplia. El número de publicaciones ha aumentado desde 2010, pero no hay una tendencia constante desde la primera publicación en 1995. Estos trabajos tratan tanto la incorporación *ante-mortem* como *post-mortem* de mercurio al esqueleto. La incorporación *post-mortem* del mercurio puede ser consecuencia de rituales funerarios que involucren pigmentos de mercurio, o enriquecimiento de mercurio derivado de la descomposición de los tejidos blandos. Mientras que se ha observado que la incorporación *ante-mortem* puede deberse a: i) exposición a altas concentraciones ambientales de mercurio derivadas de actividades volcánicas, geotérmicas, mineras y metalúrgicas, e industriales; ii) uso y procesado del cinabrio; iii) medicamentos, cosméticos, amalgamas, intoxicaciones; y iv) incluso la dieta, a través de alimentos y bebidas

contaminados. El foco de la investigación se ha centrado principalmente en individuos muy afectados debido a su estilo de vida o prácticas culturales. Prestándosele menos atención a las dosis bajas y la exposición crónica, que es la más común.

Los restos humanos han sido propuestos como archivos naturales adecuados, en un estudio de investigación estrechamente relacionado con esta tesis y basado en algunas muestras utilizadas en el primer artículo, abriendo la posibilidad de comprender mejor el impacto de la contaminación por mercurio en el pasado. Además, este trabajo también planteó nuevas preguntas sobre la variación intra e interesquelética del contenido de mercurio. Sin embargo, hay otros dos aspectos a considerar: i) los huesos no son el órgano diana del mercurio, y ii) los huesos pueden verse afectados por la diagénesis en el entorno de enterramiento. Por lo tanto, es fundamental comprender el comportamiento del mercurio en el ambiente de enterramiento para lograr una buena interpretación de los datos proporcionados por los esqueletos. De acuerdo con la literatura, la incorporación de mercurio *post-mortem* puede estar relacionada con rituales funerarios, el suelo del enterramiento en áreas con minerales de mercurio o por transferencia desde órganos diana durante la descomposición del cuerpo. Sin embargo, estos trabajos son esencialmente descriptivos, explicando el mercurio solo, ignorando la relación con otros factores geoquímicos y sin profundizar en el comportamiento del mercurio, los procesos subyacentes y cómo estos pueden relacionarse con el contenido de mercurio. Para evaluar la dinámica del mercurio en los suelos funerarios, es esencial comprender la relación entre el mercurio y el tejido óseo, tanto *ante-mortem* como *post-mortem*. Para ello, es necesario estudiar los suelos de enterramiento y la variabilidad intraesquelética del mercurio.

El objetivo de esta investigación fue evaluar, con una perspectiva de paleocontaminación, la relación entre los niveles ambientales de mercurio, las actividades humanas y la contaminación humano-ambiental. Con este objetivo se seleccionaron restos osteoarqueológicos humanos de A Lanzada (NO de España, Galicia). Además, se realizó una revisión detallada de la literatura en el campo del mercurio en restos osteoarqueológicos humanos. Los individuos de A Lanzada representan una oportunidad única para investigar la exposición atmosférica al mercurio ya que: i) brindan información sobre dos períodos de concentraciones de mercurio atmosférico distinta (romano y tardoantiguo); ii) vivían relativamente lejos de las fuentes de emisión; iii) vivían en un asentamiento rural sin evidencia de bienes que contuvieran mercurio; iv) investigaciones previas sugieren que todos los esqueletos son de individuos locales; v) también existen estudios previos que evalúan la diagénesis ósea que concluyen que tanto los

esqueletos romanos como los tardoantiguos sufrieron un grado similar de alteración ósea; y vi) el contenido de mercurio en el suelo es muy bajo (5 ng g^{-1}) y se descartó como fuente de mercurio para los esqueletos ya que las concentraciones de Hg en la capa funeraria están por debajo de ∼1,5 ng g^{-1}, mientras que en los huesos la mediana fue de 36,0 ± 51,7 ng g^{-1}, concordando con investigaciones previas que sugieren que la incorporación posdeposicional de mercurio es insignificante en ausencia de fuentes geogénicas.

En investigaciones previas se documentan varios casos de incorporación *post-mortem* de mercurio en el esqueleto relacionado con rituales funerarios, como la pintura corporal con pigmentos que contienen mercurio, restringida a grupos de alto estatus, en Japón y culturas prehispánicas mexicanas. El ritual de pintura corporal también se documentó para el Calcolítico del Sur de la Península Ibérica. Los usos del mercurio restringidos a los estratos de alto estatus también se encontraron en la Rusia medieval, donde las zarinas y las princesas usaban cosméticos a base de mercurio. Sin embargo, en A Lanzada no hay constancia de estas prácticas. Además, de no encontrarse ninguna conexión con los indicadores de estatus social (es decir, bienes funerarios y tipología de tumbas); hay que señalar que la población estudiada era de estatus medio-bajo y no se esperan miembros de clase alta. El contenido de mercurio osteoarqueológico también se relacionó con usos médicos y de envenenamiento en poblaciones medievales, como sacerdotes alemanes y daneses, individuos sifilíticos y leprosos, e individuos como el astrónomo Tycho Brahe o el santo italiano Francesco Caracciolo. En A Lanzada, a pesar de algunos casos de infecciones crónicas inespecíficas, diagnosticadas por evaluación paleopatológica, la variabilidad del mercurio intraesquelético no sugiere un uso médico o como veneno del mercurio. El alto contenido de mercurio en individuos arqueológicos también se relacionó con la exposición ocupacional (por ejemplo, minería y metalurgia) en lugares como La Encantada en Ciudad Real (SE, España), Ranas en Querétaro (México) y la Catedral de la Trinidad de Moscú (Rusia). Pero no se encontró una relación directa con este tipo de fuentes de exposición para la comunidad de A Lanzada en los periodos estudiados (romano y tardoantiguo). Bajas concentraciones de mercurio, como las encontradas en A Lanzada, se documentaron en sociedades e individuos sin uso directo del mercurio, como las poblaciones rurales medievales y posmedievales de Alemania, Dinamarca e Italia.

El estudio de 143 muestras de hueso cortical recuperadas de la necrópolis de A Lanzada mostró diferencias significativas entre los dos periodos estudiados, estando la cohorte romana más enriquecida en mercurio (54 ± 60 ng g^{-1}) frente a la tardoantigua (21 ± 23 ng g^{-1}). Esto nos per-

mitió confirmar los restos óseos humanos como archivos adecuados de paleocontaminación por mercurio cuando la exposición cultural es baja, ya que: i) todos los esqueletos procedían de la misma área de enterramiento; ii) las diferencias cronológicas entre los dos períodos son más cortas que el tiempo que los restos humanos estuvieron bajo tierra expuestos a la alteración diagenética; iii) se descartaron suelos/sedimentos como fuente de mercurio; y iv) no se encontró relación entre la alteración ósea y el contenido de mercurio. La contaminación ambiental (atmosférica) se identificó como la fuente de mercurio para la población, dado que: i) no hay evidencia de exposición cultural y no hay conexión con las características individuales *ante-/post-mortem* (es decir, la variabilidad interesquelética); y ii) se observó evidencia de contaminación atmosférica afectando a esta zona durante la época romana a partir de un registro de turba de Penido Vello (NE de Galicia).

Además, en el Artículo II se analizaron muestras de un muestreo detallado del suelo (n = 46; una práctica poco común) de dos enterramientos tardoantiguos, realizado por miembros de Eco-Past en la última campaña de excavación (2016 - 2017). El objetivo era investigar la distribución y el comportamiento del mercurio en el suelo/sedimentos de enterramiento aplicando la regresión por mínimos cuadrados parciales (PLS-R). En el Artículo III, las combinamos con muestras de hueso cortical (n = 73 muestras de hueso + 37 muestras de suelo) considerando diferentes tasas de recambio óseo, modelando con ecuaciones estructurales por mínimos cuadrados parciales (PLS-SEM) y segmentación orientada a la predicción por mínimos cuadrados parciales (PLS-POS) para estudiar la variabilidad intraesquelética teniendo en cuenta el efecto suelo/sedimentos.

El estudio del suelo/sedimentos de estas dos tumbas mostró que la fracción tierra fina (< 2 mm) tiene bajo contenido en mercurio, mientras que la fracción limo y arcilla está enriquecida, en concordancia con estudios en suelos no contaminados y con el hecho de que la mayoría de los silicatos primarios carecen de la capacidad de retener mercurio. Con el modelo PLS-R se explicó > 72 % de la variabilidad del mercurio, identificando tres procesos subyacentes principales que la controlan. (1) La diferencia entre el interior (12 a 39 ng g^{-1}) y el exterior (6 a 23 ng g^{-1}) de los enterramientos explicó la mayor cantidad de variabilidad del mercurio. Nuestros datos confirman los cuerpos como fuente de mercurio al entorno de enterramiento, así como fuentes de materia orgánica que también contribuye a la fijación del mercurio, mientras que el suelo fue descartado como fuente de mercurio para los esqueletos. (2) Las diferencias relacionadas con el contexto de enterramiento fueron la fuente secundaria de variación. Difer-

encias que pueden explicarse por el número de individuos por fosa, ya que la masa corporal está relacionada con el contenido de mercurio en el suelo del enterramiento; una de las tumbas contenía dos individuos masculinos, un adolescente y un adulto maduro, mientras que la otra contenía a una anciana. También se deben considerar las diferencias *ante-mortem* de cada individuo, como la exposición ambiental o el estilo de vida, ya que pueden tener un efecto en la variabilidad del mercurio. (3) La proximidad a las áreas donde hay una mayor masa de tejidos blandos (es decir, la región torácica y abdominal) produjo un mayor enriquecimiento local en mercurio, muy probablemente debido a su liberación de los órganos diana (es decir, los riñones y el hígado) y su retención en las fracciones reactivas del suelo (materia orgánica y arcilla). Nuestros hallazgos resaltan la importancia de analizar el suelo/sedimentos de los enterramientos, ya que brindan información complementaria que puede ser clave para comprender los procesos relacionados con las interacciones cuerpo/suelo.

Con los modelos PLS-SEM + PLS-POS de la distribución de mercurio en los enterramientos, combinando datos de huesos y suelo/sedimentos, se consiguió explicar una alta proporción de la variabilidad del mercurio en la hidroxiapatita ósea (> 90 %) y una proporción moderada de su variabilidad en el colágeno óseo (∼60 %). Este esfuerzo de modelado reveló seis aspectos principales. (1) La variabilidad del mercurio en el ambiente de enterramiento está influenciada por la estructura ósea *ante-mortem*, lo que refleja diferencias en la fracción ósea que controla el contenido de mercurio en los huesos con respecto a la relación hidroxiapatita/colágeno; pequeños aumentos en el colágeno pueden tener un gran impacto en la acumulación y retención de mercurio en la estructura ósea. Debido a la presencia de grupos tiol en la estructura del colágeno, existe la posibilidad de que se formen compuestos de Hg-S, que son muy estables. Por lo tanto, la liberación de mercurio al suelo puede ser muy baja hasta que la estructura del colágeno esté altamente degradada. (2) El papel de los componentes óseos (es decir, colágeno e hidroxiapatita) en la retención de mercurio depende del grado de cristalinidad ósea. La cristalinidad de la hidroxiapatita tiene un doble papel en la acumulación de mercurio en el tejido óseo, afectando tanto la liberación de mercurio óseo (fuente primaria/secundaria al suelo) como la retención/acumulación (sumidero; no se puede discriminar entre mercurio incorporado *ante-mortem* y *post-mortem* como resultado de la interacción con los tejidos blandos durante su descomposición). En huesos arqueológicos, la cristalinidad de la hidroxiapatita aumenta con la alteración ósea. Por tanto, se espera una relación negativa con el colágeno óseo. Dado que, cuando el mercurio óseo está controlado por el colágeno, la liberación de mercurio aumentará con la cristalinidad de la hidroxiapatita. Si bien, cuando la hidroxiap-

atita controla el contenido de mercurio en los huesos, la doble función de la cristalinidad de la hidroxiapatita se relaciona con: i) un mayor contenido de mercurio, que actúa como indicador del estado *ante-mortem* del tejido óseo; y ii) liberación de mercurio como consecuencia de la alteración ósea *post-mortem*. (3) El modelado confirmó que los cuerpos humanos (tejidos blandos y hueso) son fuentes de mercurio para el suelo. (4) La concentración de mercurio en el esqueleto depende del mercurio acumulado durante la vida (ruta primaria), pero también durante la descomposición *post-mortem* temprana de los tejidos blandos (ruta secundaria), actuando tanto como fuente como sumidero. (5) En los enterramientos estudiados, los componentes del suelo/sedimento parecen desempeñar un papel menor en el contenido de mercurio en los huesos de los esqueletos humanos arqueológicos, en ausencia de minerales que contengan mercurio. (6) Los modelos también sugieren que la importancia de los huesos como fuente primaria de mercurio para el suelo está relacionada con la estructura ósea *ante-mortem* (grado de madurez ósea, ej., contenido de colágeno), mientras que su función como fuente/sumidero secundario está asociada con la cristalinidad de hidroxiapatita. Esto destaca la relevancia de comprender cómo los tejidos contribuyen al ciclo del mercurio en los enterramientos, ya que pueden ser fuentes y sumideros simultáneamente. Estos hallazgos tienen implicaciones osteoarqueológicas, ya que no se puede suponer que todo el mercurio contenido en los huesos arqueológicos provenga de una incorporación *ante-mortem*. Hay una fracción que se puede incorporar *post-mortem* durante la descomposición del cuerpo. Esto debe tenerse en cuenta durante el diseño del muestreo, ya que el recambio óseo y la proximidad a la región torácica/abdominal, especialmente cerca de los principales órganos diana (es decir, riñones e hígado), pueden afectar a las concentraciones de mercurio. A pesar de esto, el contenido en mercurio de los huesos arqueológicos es representativo de la exposición *ante-mortem*, ya que todo el mercurio proviene de los individuos.

Además, nuestros resultados revelan que el estudio de individuos poco contaminados produce información de alta calidad que complementa la de ambientes altamente contaminados. Esta investigación destaca la importancia de comprender la dinámica de los posibles contaminantes en los entornos de enterramiento (cementerios/tumbas), incluso cuando la exposición ambiental al contaminante y el número de cuerpos enterrados en el 'cementerio' era bajo. Según nuestros datos, y de acuerdo con la literatura, los suelos/sedimentos de los cementerios pueden estar significativamente enriquecidos en mercurio en comparación con los suelos de áreas cercanas. Esto debe tenerse en cuenta al describir las propiedades del suelo y cuando esas áreas estén experimentando cambios en el uso de la tierra (por ejemplo, a un uso agrario),

ya que esto puede afectar a la salud humana y de los ecosistemas debido a la liberación de contaminantes como el mercurio. Esto refuerza la importancia de conocer cómo se distribuían en el pasado los usos del suelo, en particular los cementerios, para comprender también cómo están afectando el presente.

1.3 | Abstract

Mercury occurs in nature in different chemical forms that can be summarised in three categories: elemental mercury (Hg^0), mercury salts (Hg^{2+}, $Hg_2{}^{2+}$), and organic compounds (e.g., ethyl-, methyl-, dimethyl-Hg). No biological function is known for this metal and some of its forms are toxic even at very low dose. As a systemic poison it can potentially harm any organ, tissue, or subcellular structure. Acute intoxication can severely damage multiple organs and even lead to death. However, the main health risk relies on mercury chronic exposure, with special concern regarding the damage that causes during embryonic development. Mercury toxicology is complex as different chemical forms do not necessarily share absorption paths or behaviour inside the organism. Furthermore, its toxicology depends on dose, time of exposure, and individual physiological differences. Elemental mercury is easily absorbed by inhalation, with the central nervous system being the most sensitive organ. Mercuric salts enter via ingestion or skin contact, and kidneys and the intestinal tract are the critical organs. Organic compounds are mainly assimilated by ingestion and rather evenly distributed in the organism. This variability leads to a wide range of target organs and extensive clinical symptomatology. Due to the physico-chemical properties of mercury forms, mercury is among the top elements considered of public health concern by international organisations such as the World Health Organisation, the European Environment Agency and the US Government Agency for Toxic Substances and Disease Registry. This global concern regarding mercury impact on health and environment was translated into the treaty of the Minamata Convention on Mercury, signed by 128 countries.

Mercury use by human's dates back, at least, to the Neolithic on its red sulphide ore form (i.e., cinnabar). There is also evidence of the use of cinnabar by ancient Egyptian and Roman cultures, but also in ancient China, Japan, and Mexico. Under the Roman Republic and Empire, it was also used to extract other precious ores such as gold and silver. This practice is still in use today in small-scale and artisanal gold mining. Mercury was also used as medicine or treatment. This use was first documented in ancient China and India and then it

also appears in Ancient Egyptian, Greek, Roman, and Islamic cultures. In Europe, during the medieval and post-medieval period, mercury-containing medicines were applied in the treatment of syphilis. This practice was in use until the middle 20th century. Despite mercury toxicity, it is still present in dental amalgams, and cosmetics. It was also used in agriculture as a pesticide, leading to important human health disasters with massive deaths. Mercury was also key in science and industry, due to its wide range of applications given its particular physico-chemical properties.

Mercury is released to the environment both from anthropogenic and non-anthropogenic sources. Non-anthropogenic sources comprised geogenic and biogeogenic processes, while anthropogenic activities have changed over time from mining and metallurgy to coal combustion and artisanal and small-scale gold mining. Over the past century, anthropogenic activities have significantly increased mercury outputs from stable reservoirs to global circulation, although the first inflexion point was proposed to have occurred $\sim$2000 BCE. Mercury has long residence times in oceans and soils and a relatively long residence in the atmosphere. Due to mercury volatility and lack of long-term reservoirs, once released to global reservoirs it can be in circulation up to > 1000 years. This behaviour plays an important role in the mercury biogeochemical cycle. Together with the impact of anthropogenic releases, this leads to the concept of legacy mercury; that is, the mercury released from anthropogenic sources that is recycled from surface reservoirs.

The atmosphere is key to mercury transport and global redistribution. Mercury's behaviour and fate in the atmosphere depends on the form of mercury released (elemental mercury, mercuric mercury, or associated to particulate matter). Mercury concentration and speciation in the atmosphere depend on several factors such as source proximity, oxidants availability, aerosol type and concentration, meteorology and surface conditions. The most abundant specie in this system is elemental mercury (Hg^0) and it is mainly deposited as mercuric mercury (Hg^{2+}) and particulate mercury. After deposition, a fraction of the mercury can be reduced to elemental mercury and reemitted to the atmosphere.

Atmospheric mercury deposition is an important source of mercury to the soil, being predominant in areas far from emission sources and not naturally enriched. It is accumulated in the uppermost soil horizons and its fate is determined by mercury speciation and soil physico-chemical properties. The most common mercury specie found in soils is mercuric mercury. Mercuric mercury readily forms inorganic salts and minerals or organo-Hg compounds. It has

a high affinity to organic matter and mineral clay soil components. Mercury stored in soil can be remobilised due to land and forest management, forest fires, or climate change. Soils can act both as source or sink of mercury depending on several factors. To understand the role of terrestrial ecosystems in the global biogeochemical mercury cycle it is essential to understand mercury behaviour in soils. Another unique source of soil mercury is cemeteries, which is the focus of this thesis. Cemeteries can be sources of mercury to the soil when body inhumation is part of the funerary ritual, as bodies release the mercury accumulated in life. When the number of bodies is high the released mercury may cause adverse effects in the surrounding ecosystems and, thus, the study of mercury dynamics on these environments is of particular interest.

Prehistoric mining and metallurgy in South Iberia account for the first evidence of mercury pollution, around 3250 BCE (Cooper Age). As legacy mercury plays an important role in the mercury cycle, understanding past trends and patterns is also a key aspect. Mercury dynamics in the past can be approached through paleoarchives such as lake and marine sediments, peat, ice, or tree rings. Each of them provides different information about the mercury cycle and has its own advantages and disadvantages. Recently, we proposed human osteoarchaeological remains as another suitable paleoarchive as they bring information about mercury exposure in past human populations, whether through cultural or environmental sources.

There is no broad research in the field of mercury in human osteoarchaeological remains. The number of publications has increased since 2010, but there is no consistent trend since the first publication in 1995. These works deal both with *ante-* and *post-mortem* incorporation of mercury to the skeleton. Mercury *post-mortem* incorporation can be the consequence of burial rituals involving mercury pigments, or mercury enrichment derived from the soft tissues' decomposition. While *ante-mortem* incorporation was reported to be due to: i) exposure to high environmental concentrations of mercury derived from volcanic, geothermal, mining and metallurgic activities, and gilding vapours and industry; ii) use and processing of cinnabar; iii) medicines, cosmetics, amalgams, poisoning; and iv) even diet, through polluted food and drinks. The focus of research has been mainly on heavily affected individuals due to their lifestyle or cultural practices. But less attention has been paid to low dose and chronic exposure, which is the most common.

Human remains have been proposed as suitable natural archives, in a research study closely related with this thesis and based on some samples used in the first paper, which have opened

the possibility to better understand the impact of mercury pollution in the past. In addition, this work also posed new questions about intra- and inter-skeletal variation of mercury content. However, there are two further aspects to consider: i) bones are not the target organ of mercury, and ii) bones can be affected by diagenesis in the burial environment. Therefore, it is fundamental to understand mercury behaviour in the burial environment to achieve a good interpretation of the data provided by the skeletons. According to literature, mercury *post-mortem* incorporation can be related to funerary rituals, burial soil in areas with mercury minerals, or by transfer from target organs during body decomposition. However, these works are essentially descriptive, explaining mercury alone, ignoring the relationship with other geochemical factors, and do not deepen on mercury behaviour, the processes behind and how these can relate back on mercury content. To assess mercury dynamics in burial soils it is essential to understand the relationship between mercury and bone tissues, both *ante-* and *post-mortem*. To do so, it is necessary to study the burial soils and the mercury intra-skeletal variability.

The goal of this research was to assess, with a paleo-pollution perspective, the relationship between environmental mercury levels, human activities and human-environmental pollution. With this aim, A Lanzada (NW Spain, Galicia) human osteoarchaeological remains were selected. In addition, a detailed review of literature in the field of mercury in human osteoarchaeological remains was carried out. A Lanzada individuals represent a unique opportunity to investigate the atmospheric exposure to mercury as: i) they provide information about two periods of contrasting atmospheric mercury concentrations (Roman and post-Roman); ii) they lived relatively far from emission sources; iii) they lived in a rural settlement with no evidence of mercury containing goods; iv) previous research suggests that all the skeletons are from local individuals; v) there are also previous studies assessing bone diagenesis that concluded that both Roman and post-Roman skeletons suffered similar degree of bone alteration; and vi) soil mercury content is very low (5 ng g^{-1}) and it was discarded as a source of mercury to the skeletons as Hg concentrations in the burial layer are below $\sim$1.5 ng g^{-1}, while in bone the median was 36.0 ± 51.7 ng g^{-1}, being in agreement with previous research in that post-depositional incorporation of mercury is negligible in the absence of geogenic sources.

Previous research reported several cases of mercury *post-mortem* incorporation into the skeleton related to funerary rituals, such as body painting involving mercury-containing pigments, restricted to high status groups, in Japan and pre-Hispanic Mexican cultures. The body paint-

ing ritual was also reported for Chalcolithic South Iberia. Mercury uses restricted to high status strata were also found in Medieval Russia, where czarinas and princesses used mercury-based cosmetics. However, in A Lanzada there is no evidence of these practices. Furthermore, any connection with social status indicators (i.e., grave goods and grave typology) was not found; but it must be noted that the studied population was middle-low status and no high-class members are expected. Osteoarchaeological mercury content was also linked to medical and poisoning uses in medieval populations, such as German and Danish priests, syphilitic and leprosies individuals, and individuals such as the astronomer Tycho Brahe or the Italian saint Francesco Caracciolo. In A Lanzada, despite a few cases of chronic unspecific infections, diagnosed by paleopathological assessment, the intra-skeletal mercury variability does not suggest a medical/poisoning use of mercury. High mercury content in archaeological individuals was also related to occupational exposure (e.g., mining and metallurgy) in places such as La Encantada in Ciudad Real (SE, Spain), Ranas in Querétaro (México) and the Trinity Cathedral of Moscow (Russia). But no direct relationship with these kinds of exposure sources was found for the A Lanzada community in the studied periods (Roman and post-Roman). Low mercury concentrations, such as those found in A Lanzada, were reported in societies and individuals with no direct use of mercury, such as Medieval and post-Medieval rural populations from Germany, Denmark and Italy.

The study of 143 cortical bone samples recovered from the necropolis of A Lanzada showed significant differences between the two studied periods, with the Roman cohort enriched in mercury (54 ± 60 ng g^{-1}) when compared to the post-Roman (21 ± 23 ng g^{-1}). This allowed us to confirm skeletal human remains as suitable paleo-pollution archives of mercury when cultural exposure is low as: i) all the skeletons came from the same burial area; ii) the chronological differences between the two periods are shorter than the time the human remains were under earth exposed to diagenetic alteration; iii) soil/sediments were discarded as source of mercury; and iv) there is no relationship between bone alteration and mercury content. Environmental pollution (atmospheric) was identified as the source of the mercury for the population because: i) there is no evidence of cultural exposure and no connection with *ante-/post-mortem* individual characteristics (i.e., inter-skeletal variability); and ii) there is evidence of atmospheric pollution affecting this area during Roman times from a peat record from Penido Vello (NE Galicia).

Additionally, samples from a detailed soil sampling (n = 46; an uncommon practice in osteoar-

chaeology) of two post-Roman burials, carried out by EcoPast members in the last excavation campaign (2016 - 2017), was analysed in Paper II. The aim was to investigate mercury distribution and behaviour in the burial soil/sediments applying Partial Least Squares Regression (PLS-R). In Paper III, we also combined them with cortical bone samples (n = 73 bone samples + 37 soil samples) considering different bone turnover rates, modelling with Partial Least Squares - Structural Equations (PLS-SEM) and Partial Least Square – Prediction Oriented Segmentation (PLS-POS) to study intra-skeletal variability taking into account the soil/sediments effect.

The study of the soil/sediments of these two graves showed that the fine earth (< 2 mm) fraction has low mercury content, while the silt and clay fraction is enriched, in agreement with studies in non-polluted soils and with the fact that most of the primary silicates lack the capacity to retain mercury. With the PLS-R model > 72 % of the mercury variability was explained, identifying three main underlying processes driving it. (1) The difference between inside (12 to 39 ng g^{-1}) and outside (6 to 23 ng g^{-1}) the burials accounted for the largest amount of mercury variability. Our data confirm bodies as a source of mercury to the burial environment, as well as sources of organic matter which also contributes to mercury fixation, while soil was discarded as a source of mercury to the skeletons. (2) Differences related to the burial context was the secondary source of variation. Differences that can be explained by the number of individuals per grave, as body mass is related to mercury content in the burial soil; one of the graves contained two male individuals, an adolescent and a mature adult, while the other was a single grave of an elder woman. Individual's *ante-mortem* differences, such as environmental exposure or lifestyle, should also be considered, as they can have an effect on mercury variability. (3) The proximity to areas where larger soft tissues mass was placed (i.e., thoracic and abdominal region) produced a further local enrichment in mercury, most likely due to its release from target organs (i.e., kidneys and liver) and its retention in the reactive soil fractions (organic matter and clay). Our findings remark the importance of analysing the burial soil/sediments as they provide complementary information that can be key to understand processes related to the body/soil interactions.

The PLS-SEM + PLS-POS modelling of mercury distribution in the burials, combining bone and soil/sediment data, showed high to moderate explanation of mercury variability in bone hydroxyapatite (> 90 %) and bone collagen (∼60 %). This modelling effort indicated six main aspects. (1) Mercury variability in the burial environment is influenced by the bone *ante-*

mortem structure, reflecting differences in the bone fraction controlling bone mercury content regarding the ratio hydroxyapatite/collagen; small increases in collagen can have a large impact on mercury accumulation and retention in the bone structure. Due to the presence of thiol groups in the collagen structure there is the potential for the formation of Hg-S compounds, which are highly stable. Therefore, the release of mercury to the soil may be very low until the collagen structure is strongly degraded. (2) The role of bone components (i.e., collagen and hydroxyapatite) in mercury retention depends on the degree of bone crystallinity. Hydroxyapatite crystallinity has a dual role in the accumulation of mercury in bone tissue, affecting both bone mercury release (primary/secondary source to the soil) and retention/accumulation (sink; it cannot be discriminated between mercury incorporated *ante-mortem*, and *post-mortem* as the result of the interaction with the soft tissues during their decomposition). In archaeological bones, hydroxyapatite crystallinity increases with bone alteration. Therefore, a negative relationship with bone collagen is expected. Given that, when bone mercury is controlled by collagen, mercury release will increase with hydroxyapatite crystallinity. While, when hydroxyapatite controls bone mercury content the dual role of hydroxyapatite crystallinity relates to: i) higher mercury content, acting as a proxy of the bone tissue *ante-mortem* status; and ii) release of mercury as a consequence of *post-mortem* bone alteration. (3) Modelling confirmed that human bodies (flesh and bone) are mercury sources to the soil. (4) Mercury concentration in the skeleton depends on the mercury accumulated while living (primary path), but also during early *post-mortem* decomposition of body tissues (secondary path), acting both as source and sink. (5) In the studied burials, soil/sediment components seem to play a minor role for bone mercury content in archaeological human skeletons, in the absence of mercury containing minerals. (6) The models also suggest that the importance of bone as primary source of mercury to the soil is related to *ante-mortem* bone structure (degree of bone maturity, e.g., collagen content), while its function as a secondary source/sink is associated with hydroxyapatite crystallinity. This highlights the relevance of understanding how body tissues contribute to mercury cycling in burials, as they can be sources and sinks simultaneously. These findings have osteoarchaeological implications, as it cannot be assumed that all the mercury contained in archaeological bones comes from *ante-mortem* incorporation. There is a fraction that can be incorporated *post-mortem* during the body decomposition. This should be kept in mind during the sampling design, because bone turnover and proximity to the thoracic/abdominal region, specially of those near the main target organs (i.e., kidneys and liver), may affect mercury concentrations. Despite this,

mercury content in archaeological bones can still be representative of *ante-mortem* exposure, as all mercury came from the studied individuals.

In addition, our results reveal that the study of low contaminated individuals produces high quality information that can complement that reported in more contaminated environments. This research highlightes the importance of understanding the dynamics of potential pollutants on burial environments (cemeteries/graves), even when the environmental exposure to the pollutant and the number of bodies buried in the 'cemetery' was low. According to our data, and in agreement with the literature, cemeteries' soil/sediments can be significantly enriched in mercury when compared with soils from nearby areas. This should be noted when describing the soil properties and when those areas are experiencing changes in land use (e.g., to an agrarian use), as this can affect human or ecosystem health due to the release of contaminants such as mercury. This strengthens the importance of knowing how land uses, cemeteries in particular, were distributed in the past to also understand how they are affecting the present.

2 | INTRODUCTION

Mercury is a transition metal represented by the symbol Hg. Its atomic number is 80 and it has an atomic weight of 200.592. This element presents a liquid physical state at 20 °C and it is highly volatile. It has various chemical species that occur naturally in the environment; including elemental mercury (Hg^0), inorganic mercurous (Hg_2^{2+}) and mercuric (Hg^{2+}) salts, as well as organic compounds such as methyl- and ethyl-mercury (Berlin et al., 2015). Due to its physico-chemical properties, it is considered, in general terms, as the most toxic non-radioactive element (Pushie et al., 2014). Therefore, the mercury cycle and its toxicology are widely researched. Furthermore, it is a worldwide distributed pollutant. Mercury is, according to the World Health Organisation (WHO, 2020), a global public health concern. It is harmful even at very low dose and has no known biological function. There are both anthropogenic and non-anthropogenic sources to the environment, and once bioavailable it bioaccumulates and biomagnifies in food chains and is very persistent in ecosystems (Evers, 2018; Morel et al., 1998; Tang et al., 2020).

2.1 | Mercury toxicology

Mercury is a systemic poison. The primary toxic effects of mercury are caused by its capacity to bind to sulfhydryl groups, and, to a lesser extent, to hydroxyl, carboxyl, and phosphoryl groups. This ability to bind to such compounds affects non-proteins containing thiol groups, reduced glutathione, and proteins containing cysteine, in addition to its interactions with ionic channels, transporters, and enzymes (Silva-Filho et al., 2021; Tchounwou et al., 2003). Therefore, mercury can potentially impair function of any organ, or any subcellular structure (Bernhoft, 2012). Mercury acute intoxication in humans can permanently damage several organs and tissues and in the worst of the cases it can lead to death. However, acute intoxication is rare and the main health risk is related to chronic exposure, which is of major

concern, among other reasons, because of the damage that it can cause in embryonic development (Liu et al., 2011). When exposure is due to ambient environmental levels (i.e., low dose and chronic), mercury mainly accumulates in kidneys and liver (García et al., 2001), while in acute or occupational exposure high concentrations can also be found in the central nervous system (Clarkson and Magos, 2006). At the molecular level, it is assumed that toxicity is determined by the 'molecular dose' (i.e., the amount of mercury bounded to the receptor or target molecules), which is determined by the amount of mercury accumulated in cells, tissues, and whole organisms. Therefore, mercury toxicity will be determined by the maximum levels of mercury expressed in terms of dose to the organism/tissue/cell (Clarkson, 1997). Mercury's different chemical forms do not necessarily share absorption paths or behaviour once they are inside an organism, which makes mercury toxicology complex (Berlin et al., 2015; Clarkson, 1997).

2.1.1 | Elemental mercury

Elemental mercury is mainly absorbed by inhalation (80% of inhaled Hg^0 is retained). Some absorption has been shown to take place through the skin, but about half of the absorbed mercury is lost in the exfoliated skin cells and the systemic uptake for all the skin areas is ∼1% of the uptake from inhalation of the exposure to gas. Elemental mercury is poorly absorbed from the gastrointestinal tract, being this path considered of non-toxicological importance (Berlin et al., 2015; Bernhoft, 2012; Clarkson, 1997). It is highly diffusible and lipid soluble, making it deeply mobile in the body (Clarkson, 1997). It is absorbed in the lung and then carried to all tissues dissolved in the bloodstream - in serum or adhered to red blood cell membranes. It easily penetrates the blood brain barrier and the placenta; being deposited on the central nervous system (CNS), placenta and foetal tissues (Bernhoft, 2012; Clarkson, 1997). Once Hg^0 is inside the cell it is oxidised to Hg^{2+}, this process takes place quickly making Hg^0 lifetime limited (Berlin et al., 2015; Clarkson, 1997). Therefore, Hg^{2+} cation is assumed to be the proximate toxic species (Clarkson, 1997). However, the oxidation of Hg^0 does not occur as quickly as to prevent a considerable uptake by the CNS (Bernhoft, 2012). After oxidation, the pattern of distribution and toxicological effects start to change to that seen in mercury salts, with the kidneys becoming the major site of accumulation (Berlin et al., 2015; Clarkson, 1997). The retention time of accumulated mercury varies widely among different organs (those with longest retention times are the brain, kidneys, and testicles) and the half-life vary from a few days to months (Berlin et al., 2015). Hg^0 is mainly excreted as Hg^{2+} in the urine and faeces. It was also

demonstrated that small quantities of mercury vapour can be exhaled, being a fraction the result of the reduction of Hg^{2+} stored in the tissues. The rate of excretion is dose dependent and there are considerable interspecies differences. It is estimated that $\sim$ 80% of the mercury accumulated in the human body is excreted with a half-life of 60 days, but the half-life of the mercury stored in the brain may exceed several years (Berlin et al., 2015; Bernhoft, 2012; Clarkson, 1997).

Acute exposure to mercury vapour can cause erosive bronchitis and bronchiolitis with interstitial pneumonitis potentially leading to respiratory failure that may be accompanied by CNS symptoms such as tremor or erethism. Chronic exposure to toxic levels of mercury vapour usually produces neurological and immune dysfunction. Higher exposure levels are associated with mercurial tremor (i.e., fine muscle fasciculations punctuated every few minutes by coarse shaking), erethism (i.e., severe behaviour and personality changes, emotional excitability, loss of memory, insomnia, depression, fatigue, and in severe cases delirium and hallucination), gingivitis and copious salivation. At low-level exposures, nonspecific symptoms like weakness, fatigue, anorexia, weight loss, and gastrointestinal disturbance have been described. Symptoms may regress with cessation of exposure, but not necessarily, persistent neurological symptoms being common (Berlin et al., 2015; Bernhoft, 2012). Foetal exposure to mercury vapour may cause developmental issues, with special regard to the brain (Berlin et al., 2015).

2.1.2 | Mercury salts

Mercury salts are mainly absorbed by ingestion or skin contact (Bernhoft, 2012; Clarkson, 1997). The Hg^{2+} ion is assumed to be the proximate toxic species for all compounds of inorganic mercury and differences in toxicity between them probably reflect differences in the rates of dissolution of Hg^{2+} (Clarkson, 1997). Mercurous mercury salts (e.g., calomel) are poorly absorbed due to their low water solubility (Bernhoft, 2012; Clarkson, 1997). The bond between the mercury atoms in the $Hg_2{}^{2+}$ form is unstable in the presence of organic ligands, leading to the production of Hg^0 and Hg^{2+} (Berlin et al., 2015; Clarkson, 1997). Therefore, its toxicity is expected to behave as the mercuric mercury (Bernhoft, 2012; Clarkson, 1997). However, it is doubtful whether mercurous mercury survives in the organism (Berlin et al., 2015; Clarkson, 1997). Among inorganic mercury compounds, the solubility of HgS and HgSe are very low, making them, for practical reasons, non-toxic substances. On the contrary, soluble mercuric mercury salts, such as $HgCl_2$, are highly toxic (Clarkson, 1997). Mercuric mercury, like elemen-

tal mercury, is distributed through the body dissolved in the blood stream (Bernhoft, 2012; Clarkson, 1997). The primary target organs are the kidneys, followed by the liver. It is also accumulated in mucous membranes, intestinal tract, the epithelium of the skin, the spleen, and the interstitial cells of the testicles. It does not cross efficiently the blood brain barrier, but it does accumulate in quantity in the placenta, foetal tissues, and amniotic fluid. Excretion of mercuric mercury is mainly through urine and stool, although significant amounts are shed through sweat, tears, breast milk, and saliva (Berlin et al., 2015; Bernhoft, 2012).

Acute poisoning with mercuric salts (commonly $HgCl_2$) generally targets the gastrointestinal tract – leading to corrosive damages – and the kidneys – resulting in a shutdown of renal blood flow, ischemia, and cessation of kidney function (Bernhoft, 2012; Clarkson, 1997). Extensive precipitation of enterocyte proteins occurs, with abdominal pain, vomiting, and bloody diarrhoea with potential necrosis of the gut mucosa, which may produce death either from peritonitis or from septic or hypovolemic shock. Patients that survive commonly develop renal tubular necrosis with anuria (Bernhoft, 2012). Chronic poisoning is rare, usually also involving occupational exposure to mercury vapour (Bernhoft, 2012; Clarkson, 1997). It may lead to kidney toxicity involving either/both renal tubular necrosis or autoimmune glomerulonephritis, immune dysfunction including hypersensitivity reactions to mercury exposure (e.g., asthma, dermatitis, various types of autoimmunity, suppression of natural killer cells and disruption of various other lymphocyte subpopulations), thyroid dysfunction, hormone alteration in females with changes in reproductive behaviour, infertility and ovarian failure, spermatogenesis inhibition, decrease in spermatozoa motility and increase in pathological changes, atrophy and capillary damage in thigh muscle, and brain dysfunction but less evident than with other forms of mercury (Bernhoft, 2012; Massányi et al., 2020).

2.1.3 | Mercury organic compounds

Mercury organic compounds can be absorbed by inhalation, ingestion or skin contact (Bernhoft, 2012). Among them there is monomethyl-mercury, the major source of human mercury exposure nowadays, it is found in fish tissues and other aquatic organisms and it is relatively stable (Bernhoft, 2012; Clarkson, 1997). Monomethyl-mercury is well absorbed from food, ∼95% of the oral intake. The biochemistry of the monomethyl-mercury cation (CH_3Hg^+) is similar to the inorganic cation (Hg^{2+}) (Clarkson, 1997). Once in the blood-stream it adheres to sulfhydryl groups, particularly to those in cysteine, and it is deposited throughout the body, being taken up by all tissues – the equilibrium between blood and body occurs ∼4 days after

exposure (Bernhoft, 2012; Clarkson, 1997). It readily crosses the blood brain and the placental barriers, and is deposited in the adult and foetal brain, being of special concern in the second as the prenatal period appears to be the most susceptible stage of the life cycle. The selective damage to the CNS (both in developed or developing brains) is a remarkable behaviour given mercury's high mobility and reactivity in the body, the peripheral nervous system may become involved in the damage but only in the most severe cases (Clarkson, 1997). Mercury is also deposited in liver, kidneys, placenta, peripheral nerves and bone marrow, as well as other foetal tissues than the brain (Bernhoft, 2012). Once deposited in the different tissues it is slowly demethylated to inorganic mercury, this process plays a key role in the elimination of monomethyl-mercury from the body (Bernhoft, 2012; Clarkson, 1997). The excretion half-life in humans is about 70 days and takes place primarily by the faecal route, mainly as inorganic mercury (Bernhoft, 2012; Clarkson, 1997). Ethyl-mercury has a similar behaviour as monomethyl-mercury at the cellular level and produces similar signs and symptoms, but its half-life in the organism is at most one third (Bernhoft, 2012). Dimethyl-mercury is produced in the environment (similar to monomethyl-mercury) and it is also efficiently absorbed through the skin, but environmental human exposure is exclusive to monomethyl-mercury (Bernhoft, 2012; Clarkson, 1997). Despite this fact, a death of a scientist caused by minimal skin contact with dimethyl-mercury was reported (Bernhoft, 2012).

Organic mercury poisoning symptoms relate more to the magnitude of retention than to the rate of deposition (Bernhoft, 2012). Several mass disasters account to the potential risk of monomethyl-mercury exposure. The damage includes extensive loss of neuronal cells in the granular layer of the cerebellum, in the visual cortex, and other focal areas. The signs of damage include ataxia and constriction of the visual fields. The effect that appears first at the lowest dose is paraesthesia in the extremities of the hands and feet and circumorally (Clarkson, 1997). Acute exposure tends to have a latency period of one or more weeks; once acquired, toxic doses are cleared slowly (Bernhoft, 2012; Clarkson, 1997). The combination of non-specific symptoms with the extensive and variable latent period makes difficult an early diagnosis. Exposure during embryonic phase differs from exposure of developed organism, as basic processes of the brain development are inhibited (i.e., neuronal cell division and migration). In severe cases it can cause microencephaly. The biochemical basis appears to be the selective action of monomethyl-mercury on microtubules in the cytoskeleton of the neuronal cell (element needed for the cell division and migration) breaking them down, as the tubulin protein monomers contain thiol groups (Clarkson, 1997). Actually, in cases of severe prenatal

poisoning a form of cerebral palsy may be induced (Bernhoft, 2012; Clarkson, 1997). Lesser prenatal doses have been associated with neurodevelopmental delays and cognitive deficits (Bernhoft, 2012).

2.2 | Mercury global situation and regulations

Anthropogenic activities were estimated to have increased atmospheric mercury concentrations by three to five-fold and three-fold mercury in surface ocean waters (less than 200 m deep) over the past century (UNEP, 2013). Anthropogenic emissions to the atmosphere are estimated to total ∼2.5 kilotons per year (kt y^{-1}), with a further ∼7.5 kt y^{-1} released to surface waters and the terrestrial environment (Outridge et al., 2018). In 2015, ∼2.2 kt of mercury were released globally from 17 key sectors. From 2010 to 2015 the global anthropogenic emissions increased by ∼20%. These data reflect a modest decrease in emissions in North America and in the European Union, while an increase in Asia. The emissions pattern has not changed much between 2010 and 2015. Most of the emissions are still being released in Asia (49%; primarily S and SE Asia), followed by South America (18%) and Sub-Saharan Africa (16%). Artisanal and small-scale gold mining are the major source with almost 38% of the global total. This activity is the main contributor to South American and Sub-Saharan Africa releases, while in other regions the major sources are energy production (∼24% of global emission being from fossil fuels and biomass combustion, 21% coal burning) and industrial processes (non-ferrous metal production, 15% of global inventory; cement production, 11%; ferrous metal production, 2%). Emissions from waste comprise about 7% of the 2015 global inventory (UNEP, 2019).

Mercury pollution is such important issue that the WHO classifies mercury among one of the top 10 chemicals or groups of chemicals of major public health and environmental concern (WHO, 2022). It is also ranked as the third most toxic element to human health by the US Government Agency for Toxic Substances and Disease Registry (ATSDR). The general population is mainly exposed to methyl-mercury through fish and seafood consumption. The other main source of mercury exposure is mercury vapour inhalation in work-environments during industrial processes (Prüss-Üstün et al., 2016). Global concern based on mercury impact on health and the environment lead to the Minamata Convention on Mercury. This is a global treaty, signed by 128 countries, that aims to protect environmental and human health from mercury and mercury compounds emissions while promoting sustainable development (UNEP, 2013). It entered into force in August 2017 and is legally binding as well as the main

international policy instrument for managing anthropogenic mercury emissions. The foundation for its successful implementation is the use of recent policy based on relevant scientific advances that have furthered our understanding of mercury biogeochemical cycling (see a description in Gustin et al. (2020)). The proposed measures include: i) promote monitoring of mercury uses and emissions; ii) reduce availability, use, releases and impacts; and iii) improve knowledge about mercury. Examples of these main goals are: quantify national emissions and releases per source, limit mercury trade and prohibit primary mercury mining, limit mercury use in gold mining and phase out use of mercury in industrial processes, implement safe management of waste mercury, develop strategies to reduce releases, identify contaminated sites and remediation options, reduce human exposure through awareness raising and education, improve knowledge about environmental and health impacts of mercury, as well as about mercury-free alternatives (Agency, 2018; UNEP, 2013).

Parallel to this international convention, local efforts worldwide are in progress to remediate mercury contaminated areas (Chen and Driscoll, 2018). Europe has always been a consumer and emitter of mercury, being especially significant since the mid-19th century CE. Despite its high use in the past, the regulation to control mercury impacts here has gone further than the Convention's requirements, making a significant progress in the last few decades in limiting mercury emissions. Among these actions are: the banning of several mercury-containing products (e.g., thermometers, batteries, switches and blood pressure monitors), the requirement of management and remediation of mercury contaminated sites, limit the mercury content of light lamps, require dentist to install high-efficiency filters to prevent mercury releases, limit the use of mercury-based fillings in dentistry and assessing the feasibility of an completely future ban, and banning all industrial processes using mercury. There are also more local actions among the EU members, such as food safety advice. For example, in Spain, Ireland and the Netherlands, pregnant, breastfeeding and women who may become pregnant, as well as very young children, should avoid consuming certain predatory fish species (Agency, 2018). The economic benefit of preventing mercury exposure and the associated neurotoxicity effects in the EU is estimated to be ∼9000 € million per year (Bellanger et al., 2013).

2.3 Use and perception of mercury along History

Mercury has been used by human populations at least since the Neolithic (Parsons and Percival, 2005; Steinnes, 2013). It is hypothesised that these populations used it attracted by the

red colour of cinnabar (the red mercury ore). Cinnabar was used in funerary rituals, being evident in ancient Egyptian, Mexican and Japanese cultures (Emslie et al., 2015; Parsons and Percival, 2005; Yamada et al., 1995). It was also used as pigment in everyday life at least from 3000 BCE in China and later by Egyptian, Roman and Medieval societies. During Roman rule, this red ore was an appreciated pigment known as vermillion. During the 1st century BCE the Romans started to extract elemental mercury by distillation and then used it to extract other ores, such as silver and gold, by amalgamation. This process is still in use nowadays in artisanal and small-scale gold mining (Parsons and Percival, 2005; Steinnes, 2013). In the Middle Ages, mercury was employed by alchemists in their aim to transform base metals into gold. Another use of mercury is in the process of gilding objects (Parsons and Percival, 2005).

Mercury had its place in medicine too. Apparently, the first usage as a medicine was in China and India and later by Egyptians, Arabs, Greeks and Romans. The first documented evidence of medical use comes from Hippocrates (460 – 377 BCE). In Pliny the Elder's time, cinnabar was used in medicines and possibly as early treatment for treponematosis (e.g., syphilis). The treatment of syphilis with mercury was generalised since the late 16th century CE until the middle 20th century. During the 17th and 18th centuries mercury was widely used in medicine, with a peak during the mid-18th century when it was seen as a panacea for many diseases. During the 20th century, mercurials continued to be used as diuretics, ointments, antiseptics, and contraceptives. Despite its high toxicity, mercury is still present in dental amalgams, cosmetics and some cultural practices (e.g., Asiatic traditional medicine). Mercury was also used in scientific, industrial, medical and agricultural applications. It has played a direct or indirect role in the discovery of 22 elements, including noble gases and the alkaline earth elements, as well as some acids and salts. In 1643 it was used by Torricelli in the development of the barometer and by Fahrenheit, in 1720, in the thermometer. It was also used in paintings, batteries, switches, fluorescent lights, explosives, in chlor-alkali production, dentistry, and in pharmaceutical applications (Parsons and Percival, 2005; Steinnes, 2013).

Despite its multiple uses through history, the dangerous health effects of high dose exposure have been recognised for centuries (Clarkson, 1997; Holmes et al., 2009). In 1953, probably the best-known case of a massive mercury poisoning took place in Minamata (Japan) due to the consumption of fish with high mercury content. This epidemic showed the consequences of monomethyl-mercury in human health and the resulting disorders associated with monomethyl-mercury poisoning became known as 'Minamata disease'. After Minamata, a

similar fish-mediated outbreak occurred in Niigata (Japan) in 1964-1965 (Clarkson, 1997; Guzzi and La Porta, 2008). Polluted fish consumption was not the only source of massive mercury poisoning, in the 1970s the use of Hg-based fungicides ended with similar poisoning consequences in Iraq, Ghana and Pakistan (Guzzi and La Porta, 2008; Langford and Ferner, 1999). Research has beguchn to consider low dose and chronic exposure, but more efforts are needed to better understand mechanisms and effects (Holmes et al., 2009). In these circumstances, mercury has important consequences for health, such as chronic intoxication and increased risk of developmental disorders in children (Budnik and Casteleyn, 2019; Ha et al., 2017). Low environmental levels are ultimately linked – in most cases – with anthropic activities, which means that the environmental impact of humans and their health consequences are closely related. This cause-effect interaction has been proposed as a promising tool in Environmental Health studies (Pasetto et al., 2016).

2.4 | Mercury cycle

The mercury cycle results from complex interactions between emission sources, deposition and reemission, until it is eventually removed from surface systems through burial in ocean sediments (Outridge et al., 2018). There are primary and secondary emission sources. Primary sources are those where mercury is transferred from long-term lithospheric reservoirs to the atmosphere, increasing the global mercury pool in surface reservoirs. While, secondary sources comprise the reemission processes that exchange mercury among surface reservoirs through the atmosphere (Driscoll et al., 2013). Mercury has a long residence time in oceans and soils and relatively long (∼1 year) in the atmosphere (Outridge et al., 2018; Streets et al., 2011). Once an atom of Hg is released to the atmosphere it can remain in circulation up to > 1000 years, this is the result of mercury volatility and the lack of long-term sinks (Amos et al., 2013). Its long residence times in natural systems play an important role in its cycle. Legacy mercury is anthropogenic Hg released and deposited, that is then recycled via reemission from terrestrial and aquatic reservoirs (see an explanation in Cooke et al. (2020)). Currently, 60% of the annual emissions to the atmosphere come from mercury previously deposited on soils and water (Li et al., 2022).

Mercury cycles naturally through geochemical reservoirs (Figure.1). It is released from deep mineral deposits by non-anthropogenic and anthropogenic processes (Amos et al., 2013). While non-anthropogenic sources comprise geogenic and bio-geogenic emissions, anthro-

pogenic onsets have changed from mining and metallurgy being dominant in the past to current emissions mainly derived from coal combustion and artisanal gold mining (Cooke et al., 2020). Anthropogenic sources have significantly increased the mercury fluxes from the deep mineral reservoir to the atmosphere for millennia (Amos et al., 2013). Before anthropogenic emissions started to play a key role in the mercury cycle, the major mercury atmospheric sources included volcanic eruptions, emissions from rocks and soils enriched in Hg, and reemission of mercury previously deposited to surfaces, coal burning events, and forest fires; and the major sink would have been vegetation uptake. With the beginning of ore mining by humans a new significant source appeared (Gustin et al., 2020). The inflexion point was proposed to be 2000 BCE by Amos et al. (2013) and lasts until now. Pre- and early 20th century CE anthropogenic emissions were primarily from silver, gold, and cinnabar mining and were mainly deposited near to sources, rather than circulated globally to remote regions (Fitzgerald et al., 2018; Outridge et al., 2018). Currently, the main sources of anthropogenic mercury emissions to the atmosphere are considered to be artisanal gold mining > fossil fuel combustion > nonferrous metal and cement production (UNEP, 2019). Once released, mercury cycles between the atmosphere and surface reservoirs (i.e., ocean and terrestrial ecosystems) on timescales of years to decades, until it is eventually transferred to recalcitrant soil pools and the deep ocean over centuries and back to deep mineral reservoir over millennia (Amos et al., 2013).

The atmosphere plays a key role on mercury transport and global redistribution from sources to sinks (Driscoll et al., 2013). This system is the main entrance of mercury to the environment (Bindler, 2003). Non-anthropogenic sources release mercury to the atmosphere in its elemental form, while anthropogenic activities release it as elemental mercury, mercuric mercury and associated with particulate matter (Hg_p). Elemental mercury is the most abundant form of mercury in the atmosphere (Selin, 2009). It is relatively inert and is globally transported via the atmosphere before it is oxidised and deposited or taken up by plants or soils (Ariya et al., 2015; Driscoll et al., 2013; Lyman et al., 2020). Atmospheric mercury concentrations and speciation depend on source proximity, oxidants availability, aerosol types and concentrations, meteorology and surface conditions (Lyman et al., 2020). Hg^0 atmospheric oxidation is driven by several compounds. This is a key process in the biogeochemical cycle as an increase in the velocity of deposition is a critical step for the removal of mercury from this system (Ariya et al., 2015). Mercury is mainly deposited as Hg^{2+} and Hg_p, as they are more soluble in water than Hg_0. The atmospheric residence time of Hg_0 is ~0.5-1 year, while the residence time of Hg^{2+} and Hg_p is of days to weeks. Differences in lifetime affect mercury's spatial redistribution;

longer residence times allow for long-range spatial distribution, while shorter times relate to regional deposition patterns (Selin, 2009).

Atmospheric deposition is a significant source to the aquatic and soil environments (Si and Ariya, 2018). Ultimately, mercury is removed from the atmosphere by wet and dry deposition (Driscoll et al., 2013). However, after being deposited on surface reservoirs, Hg^{2+} can be reduced – photochemically and/or biochemically – to Hg^0 and reemitted to the atmosphere (Selin et al., 2008). Elemental mercury exchanges dynamically with plant stomata and soils, while oxidised species – both in gas and aerosol phase – are taken up by atmospheric water and deposited by precipitation or undergo dry deposition to surface reservoirs (terrestrial and aquatic). Vegetation plays a key role as sink. It has been estimated that ∼1/3rd of the atmospheric mercury is dropped by vegetative uptake each year (Arnold et al., 2018). As a consequence of this uptake of mercury and the litterfall process, mercury content has increased by up to 15-20% in forest soils around the globe over the last century (Webster et al., 2016). From the terrestrial environment, mercury can also reach aquatic ecosystems (Lyman et al., 2020). Once in the aquatic environment it can be methylated; this is of especial concern as it can be biomagnified up food-chains to concentrations that can pose threats to ecosystems and humans (Eagles-Smith et al., 2018).

A significant proportion of the annual mercury emitted is deposited on terrestrial ecosystems (see for example Nóvoa-Muñoz et al. (2008)). The global amount of Hg mass accumulated in soils is estimated to be in the range of 250-1000 Gg (Obrist et al., 2018). Total Hg natural background concentrations in non-polluted soils are < 0.1 $\mu g\ g^{-1}$ (Xin and Gustin, 2007). Soil deposition sources are different among geographical locations. These include vegetation senescence, direct wet and dry deposition, and weathering of the soil parent material (Gómez-Armesto et al., 2021b; Wang et al., 2019). The predominant source in areas far from emission sources and not naturally enriched in mercury is the atmosphere. Atmospheric mercury is primarily accumulated in the uppermost soil horizons (up to 30-50 cm), mainly bonded to the organic matter sulphur groups (Khwaja et al., 2006; Skyllberg et al., 2006). Mercury in soils exists in different species with different properties that affect their fate and transport. Furthermore, soil physico-chemical properties (i.e., pH, cation exchange capacity, grain size distribution, organic matter type and content and the presence of different clay types and fractions) affect interactions with mercury (Gómez-Armesto et al., 2020b; O'Connor et al., 2019; Stein et al., 1996). Among the three oxidation states of mercury, the mercurous form is rarely

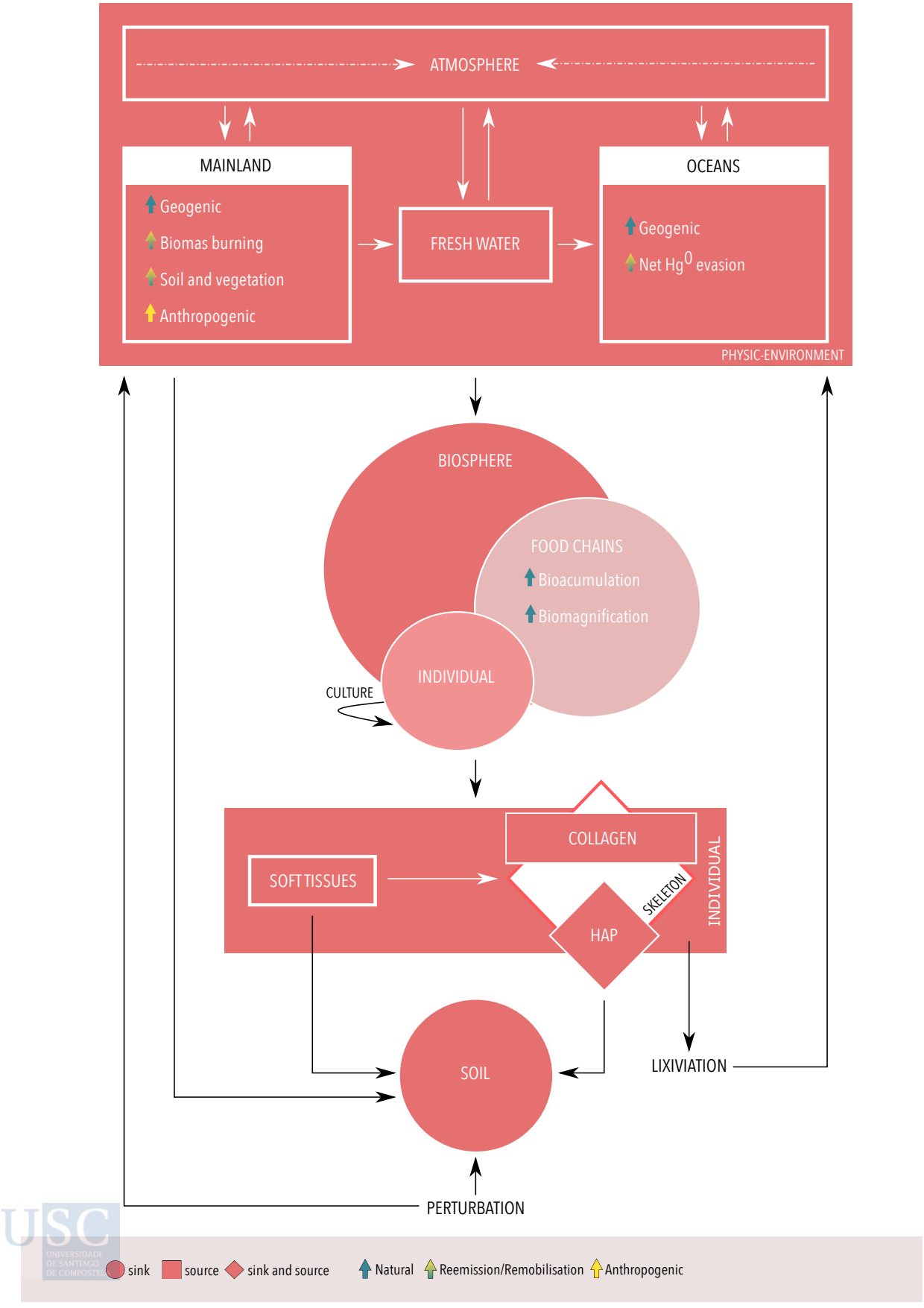

Figure 1. Mercury cycle. **HAP**: hydroxyapatite.

observed as it is not stable under typical environmental conditions, while elemental mercury is typically only present in the atmosphere, due to its high volatility and susceptibility to oxidation, but it can be found in heavily polluted soils. This makes mercuric mercury the species more commonly found in soils. The deposition of atmospheric Hg^0 to soils occurs over large temporal and spatial scales, while atmospheric Hg^{2+} is almost always quickly deposited to the pedosphere by wet or dry deposition. Once in the soil, Hg^{2+} readily forms inorganic mercury salts and minerals or organo-Hg compounds. Mercury is known to be relatively immobile as it can bind strongly with soil constituents - Hg sorption is rapid and strongly irreversible. Hg^{2+} has a high affinity for soil organic matter (O'Connor et al., 2019). Soil clay content also plays an important role in soil-Hg binding due to its high specific surface area and high cation exchange capacity. Furthermore, clay in soil is also an effective sorbent of organic matter (Gómez-Armesto et al., 2020a; O'Connor et al., 2019). Mercury mobility with depth is influenced by the soil properties, but also by the mercury species and compounds. Its depth distribution suggests that, when bound to soluble organic matter, it is more prone to migrate to deeper soil layers (∼20 cm) than reactive Hg or weak Hg complexes (Biester et al., 2002).

Soil mercury remobilisation can be mediated by land management, forestry practices, forest fires or climate change (Obrist et al., 2018). Land use and land cover change are factors with the potential to modify terrestrial and aquatic mercury distribution. Changes in land management and forestry practices can drive processes such as erosion that may contribute to increased mercury exchange from terrestrial pools to surface waters; this is of special concern when the soils are deforested (Gómez-Armesto et al., 2021a; Obrist et al., 2018). The knowledge about mercury behaviour in soils (i.e., presence, distribution and fate) is fundamental to understand the role of terrestrial ecosystems in the global biogeochemical cycle of this element. They can be acting as sources or sinks depending on the balance between inputs and emissions (Gómez-Armesto et al., 2021b). This will determine their function on the Hg exchanges among biosphere components worldwide.

Among the mercury sources to the soil cemeteries have also to be considered, especially when the inhumation of bodies was part of a funerary ritual (Amuno, 2013; Jonker and Olivier, 2012; Mohammed and Abudeif, 2020; Prestes da Silva et al., 2020; Spongberg and Becks, 2000; Uslu et al., 2009; WHO, 1998). Human bodies act as temporary sinks of mercury during life, as they incorporate mercury from the environment by inhalation, ingestion, etc. (Liu et al., 2011). Once bodies are buried, they act as sources of mercury – among other elements – to the soil,

and leave a fingerprint on it (García-López et al., 2022; Sobocká, 2004). When many bodies are buried in the same place (e.g., long-used cemeteries), the transfer of mercury is significant and could have an adverse impact on surrounding ecosystems. This is why how mercury behaves in these environments is of particular interest.

2.5 | Mercury in paleoarchives

Due to the importance of legacy mercury, understanding its cycle behaviour in the past is crucial (Cooke et al., 2020; Guédron and Acha, 2021; Outridge et al., 2018; Streets et al., 2011). Past trends and patterns of this metal have been studied at different time-scales through natural archives, such as lake and marine sediments, peat, ice, and tree rings. Lake and marine sediments, peat, and ice are archives of atmospheric Hg deposition, while tree rings record atmospheric concentrations of Hg^0 (Arnold et al., 2018; Cooke et al., 2020). They have been studied to different degrees and in the case of ice cores are limited to specific areas of the globe. Lake sediment cores account for the greatest number of records to date (Cooke et al., 2020). Decades of research on this field have revealed that all of these archives report information about different aspects of the mercury cycle and that they are also affected by a range of processes that should be taken into account when interpreting the data (Cooke et al., 2020; Gustin et al., 2020). Therefore, it is necessary to understand how each archive accumulates and retains mercury to reconstruct the information it contains (see a review in Cooke et al. (2020)). Despite this issue, they provide a consistent picture of the atmospheric mercury variations throught time (Gustin et al., 2020). However, the analysed archives provide little information about the direct impact of mercury pollution on biota, which is the most affected by mercury toxicity.

The first evidence of anthropic mercury pollution dates back at least to 3250 BCE (Copper Age), due to mining of metal sulphide ores in South Iberia (Leblanc et al., 2000), while the first evidence of atmospheric pollution derived from human activities dates to c. 1400 BCE from regional cinnabar mining in the Peruvian Andes (Cooke et al., 2009). There is also evidence of mercury mining in Almadén (SE Spain) - the largest mercury mine known in Antiquity and until recently - from BCE 5300 (Hunt Ortiz et al., 2011). In our region of study (NW Spain), a reconstruction of the atmospheric mercury levels suggested that atmospheric mercury pollution started by 500 BCE (Martínez-Cortizas et al., 1999), right near the onset of Iron Age mercury mining in Spain. It also revealed an intense impact of the Roman Em-

pire economy in atmospheric mercury pollution – due to extensive mining and metallurgy – and a later decrease after the Germanic invasions (post-Roman period). Mercury atmospheric pollution derived from anthropogenic sources in NW Spain showed a steady increase coeval wth the Islamic period (8^{th} to 11^{th} centuries CE) that lasted until the Modern era, when pollution increased exponentially. This research (Martínez-Cortizas et al., 1999) and later investigations using short peat cores spanning the last 150 years (Martínez Cortizas et al., 2012), showed that the highest pollution levels occurred during the Industrial Period (i.e., last 300 - 350 years), reached a maximum around 1980s CE and then started to decrease. A similar trend was also found in other peat records in Greenland and Denmark (Shotyk et al., 2003), Switzerland (Roos-Barraclough and Shotyk, 2003), and Sweden (Bindler et al., 2011), in lake sediment cores in southern England (Yang, 2010), Swiss Alps (Thevenon et al., 2011), and Sweden (Bindler et al., 2012, 2011, 2009; Lindeberg et al., 2007), in Posidonia oceanica mats along the northwest Mediterranean coast (Serrano et al., 2013), and in coastal lagoon sediment core in South East France (Elbaz-Poulichet et al., 2011).

2.6 | Mercury in archaeological human remains

To our knowledge, only a handful of studies have dealt with mercury in archaeological human remains (Alexandrovskaya and Alexandrovskiy, 2005; Ávila et al., 2014; Biehler-Gomez et al., 2021; Bocca et al., 2018; Cervini-Silva et al., 2018, 2013; Emslie et al., 2022, 2019, 2015; Kepa et al., 2012; Lombardo et al., 2022; Ochoa-Lugo et al., 2017; Panova et al., 2018; Rasmussen et al., 2021, 2020, 2017, 2015, 2013a,b, 2012, 2008; Torino et al., 2015; Walser et al., 2019; Yamada et al., 1995; Zuckerman, 2016); for a geographical distribution overview see Figure.2. Although more publications have appeared from 2010s onwards, there is no consistent increase and the research effort was variable from the first well-known project published in 1995 (Yamada et al., 1995). These works deal with *post-* and *ante-mortem* mercury (see a summary in Table.1).

The first study of mercury in archaeological bones is included among those studying *post-mortem* incorporation: Yamada et al. (1995) analysed two sites (Tokushima and Matsuyama, Japan) for two different periods (6^{th} - 7^{th} and 12^{th} - 17^{th} centuries). They found significant differences in mercury content between sites and periods. Furthermore, the authors discarded the soil as the mercury source as the soil samples analysed showed none or low mercury concentrations. The differences were attributed to the burial ritual, as during the Kofun period men of power were buried painted with a mercury-containing paint. The use of mercury-

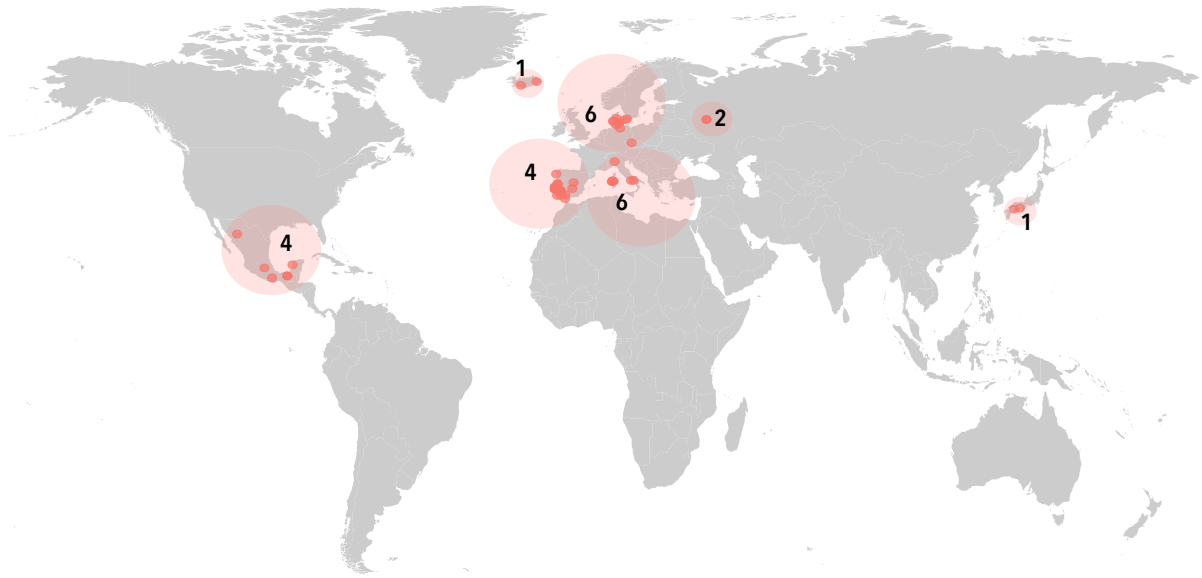

Figure 2. World's map of mercury studies in human bone remains. The numbers represent the number of papers in the highlithed area.

containing pigments in the funerary ritual was also reported in studies from México. Cervini-Silva et al. (2018, 2013) researched the use of cinnabar during the burial ritual of royal members in the Temple of Palenque, pre-Hispanic times Mayan culture. These works, together with Ochoa-Lugo et al. (2017), were focused on the effect of cinnabar in bone preservation. Ochoa-Lugo et al. (2017) compared two vertebrae from the Temple of Palenque, one from inside and the other from outside a sarcophagus. They interpreted the use of cinnabar in burial ritual for the remains inside the sarcophagus and attributed the mercury levels of the other individual to *ante-mortem* exposure to high mercury concentrations (i.e., use/processing of cinnabar, mining activities, etc.). Regarding the impact of cinnabar in bone preservation, the three studies concluded that cinnabar had a positive effect due to its antibacterial activity. Ávila et al. (2014) also assessed the presence of mercury in Mesoamerican bones, but from a different perspective. They analysed remains from four sites (Monte Albán, Ranas, Jaina, and Tlatelolco) and found that some of them were exposed *ante-mortem* to high concentrations (i.e., geologically rich mercury area, mining activities, etc.) but for most of them the origin of mercury was *post-mortem* due to the use of cinnabar to cover/paint the bodies. In Europe, the use of cinnabar in burial rituals was also reported in the Iberian Peninsula during the Chalcolithic (Emslie et al., 2022, 2015) and will be explained below.

Works assessing *ante-mortem* incorporation show varied casuistic, mainly but not only by anthropogenic causes. Natural *ante-mortem* exposure was reported by Walser et al. (2019) in Ice-

land (1000 - 1554 CE) due to volcanic and geothermal activity. The older report of human exposure to mercury is from South Spain (BCE 4221 - 3708), where mercury content was attributed to diet as well as the use and processing of cinnabar (Emslie et al., 2019). In this area (South-Eastern Iberia Peninsula), the analyses of bones from BCE 4221 to 400 CE also reported mercury *ante-mortem* incorporation through diet, use and processing of cinnabar and environmental exposure (Emslie et al., 2022, 2019, 2015). As the authors highlight, the studied sites are all near the Almadén mine and close to or in the trade path of mercury during the late prehistory in Iberia. In medieval Europe, one of the most common ways of mercury exposure was medical treatment (Rasmussen et al., 2013a), in many cases related to syphilis and leprosy (Biehler-Gomez et al., 2021; Rasmussen et al., 2020, 2017, 2012, 2008; Walser et al., 2019). Associated with mercury-containing medicines, Rasmussen et al. (2008) found some human remains of monks that may have been exposed to mercury through the handling and/or use of red ink (HgS) but also by producing/administering mercury-containing medicines. Bocca et al. (2018) studied the two rural (14^{th} - 18^{th} centuries CE Bisarcio and 15^{th} century CE Geridu) and two urban (12^{th} - 14^{th} centuries CE Sassaari and 13^{th} - 16^{th} centuries CE Alghero) regions in Sardinia (Italy) to assess socio-environmental differences. In this study, urban populations showed higher mercury content than rural ones. They attributed the result to some point sources and differences in lifestyle - urban populations would have easier access to medicines, pigments/paints, amalgams, and cosmetics. In the urban area of Alghero, the authors also point to fish consumption as a likely source. Alexandrovskaya and Alexandrovskiy (2005) and Panova et al. (2018) reported differences between the general population from Moscow and royal members (14^{th} - 17^{th} centuries CE Kremlin and 14^{th} - 17^{th} centuries CE Trinity Cathedral). In the first case some of them were probably exposed to mercury through gilding vapours and industry, while royal members were exposed through medical treatments, poisoning, cosmetics and dyes and together with their service were affected by gilding vapours and by an open barometer inside the Kremlin. There are also studies reporting exposure to non-specific sources of mercury in normal populations, such as Bocca et al. (2018), Lombardo et al. (2022), Rasmussen et al. (2015, 2008), and Torino et al. (2015).

Overall, research has focused on populations or individuals heavily affected by mercury due to their way of living or cultural practices. Little attention has been paid to the effects of environmental pollution, that is the most frequent kind of exposure. Chronic environmental pollution is the topic demanding a larger effort to understand the impact of mercury today (Holmes et al., 2009) and in the past. In a recent study, closely related with this thesis, we

proposed human bones as a suitable natural archive (López-Costas et al., 2020). This opens the possibility for a new approach to get a better understanding of the impact of pollution on humans from past populations. This first work, based on some of the samples used on the first paper of the present thesis, we analysed lead and mercury concentrations and lead isotopic composition in the osteoarchaeological remains to support the hypothesis of metal atmospheric incorporation derived from a polluted environment. Approaching in this case the impact of low dose and chronic environmental exposure. The results of this investigation indicated that mercury was key - together with lead isotopic composition - to determine the most probable sources of the pollution metals, since no geogenic mercury sources were present in the area and because the mercury cycle is dominated by atmospheric deposition (Selin, 2009). In addition, López-Costas et al. (2020) have opened new questions regarding the distribution in the population and skeletal variation. Preserved archaeological human remains are mainly skeletons that have been buried for long periods of time - much longer than the individual's life. Therefore, two aspects must be considered: (1) bones are not the target organ for mercury, and (2) bones can be affected by diagenetic processes in the burial environment.

In this regard, it is important to understand how mercury behaves in the burial environment to achieve a good interpretation of the data that skeletons provide. To do so, the burial soil has also to be considered. Moreover, one of the major issues when studying trace elemental composition in archaeological human remains is diagenesis. Bone diagenesis comprises those changes experienced from the time of death until the recovery of the remains (Hedges, 2002). Regarding *post-mortem* incorporation, mercury can be related to funerary rituals involving mercury-containing pigments, from soil in burial areas with mercury ores or by transfer from target organs during body decomposition. However, soil has only been studied in this kind of work to exclude mercury incorporation from the soil into the bone. Yamada et al. (1995) and Emslie et al. (2015) analysed discrete soil samples adhered to bones and other authors analysed punctual samples from the site (Kepa et al., 2012; Rasmussen et al., 2017; Walser et al., 2019; Zuckerman, 2016). There are only two studies with a more detailed analysis of the burial soil. The first one is that accomplished by Rasmussen et al. (2008). To study three individuals from a medieval cemetery in Denmark, soil samples vertically below and horizontally away from the femur surface were taken at 1, 2, 5, 10, and, when possible, 20 cm (n_v = 5 - 6, n_h = 5 - 6, per individual). Rasmussen et al. (2013c), also published a second study focused on the soil surrounding the skeletons. They took ∼10 samples horizontally away from the femur of

7 individuals, with distances of 1 cm between them. In addition, they also sampled the areas where the kidneys, liver, and lung would have been located. As a result of these investigations, the authors concluded that there was no evidence of diagenetic incorporation of mercury in bone from the soil, when there was no geogenic mercury source. Despite this result, these investigations are, in essence, descriptive and do not dig deeper on mercury behaviour, the processes behind the observed results and how these can relate back to skeletal mercury content. To our knowledge, previous works do not consider the variation in Hg content in relation to sources and the soil components involved in Hg retention. Mercury in these studies is usually explained alone, ignoring the relationship between elements in geochemical studies. Furthermore, the potential impact that past human demography and funerary rituals may have had on the soil current state remains to be investigated. To do so, it is important to address the relationship between mercury and bone tissue, both *ante-* and *post-mortem*, to be able to understand the role of skeletons on mercury dynamics in the surrounding soil.

To fully understand the mercury behaviour and the factors controlling its distribution in the burial environment it is also important to assess the intra-skeletal variability. However, most of the research carried out in mercury content in human remains has been based on one, or more rarely a few, bones from a skeleton. Alexandrovskaya and Alexandrovskiy (2005), Cervini-Silva et al. (2018), and Yamada et al. (1995), did not specify the number and type of bone analysed. Cervini-Silva et al. (2013) and Ochoa-Lugo et al. (2017) only analysed one vertebra per individual. Ávila et al. (2014) chose the samples based on the more reddish areas of the selected bones and teeth. They analysed 2 molars, 1 incisive, 1 skull, 1 tibia, 1 femur, 1 fibula, and 1 humerus fragment. Emslie et al. (2015) focused mainly on the humerus, but also included a non-systematic selection of long-bones and ribs (maximum 2 samples per skeleton). Emslie et al. (2019) sampled humerus, femur, and/or tibia. Emslie et al. (2022) mainly analysed the humerus with few exceptions where they sampled femur, tibia and radius. Walser et al. (2019) and Panova et al. (2018) only studied ribs. Rasmussen et al. (2020, 2015, 2008) and Torino et al. (2015) chose preferentially femur, but if not present they sampled tibia or humerus. Rasmussen et al. (2013b) carried out a first approach to the mercury intra-skeletal variability analysing femur, tibia, humerus, ulna, ilium, sacrum, ribs, vertebra, and tubular bone from the foot of two medieval individuals. They found that in one of the individuals, mercury distribution was homogeneous and hypothesised, based on the other individual, that an excess of mercury is more likely to be incorporated in compact bone tissue at sites with active remodelling. This work was extended in Rasmussen et al. (2017), where instead of 2 individuals

they studied 10 individuals from 5 different sites, 2 per site. They sampled 20 femora, 19 tibias, 20 humeri, 10 ulnas, 7 vertebrae, 9 sacra, 9 ilia, 20 ribs and they also indicated that they sampled trabecular bone (femora, tibia, humeri and metacarpal/tarsal). In their interpretation, they focus on the trabecular bone and propose the post-depositional incorporation of mercury from soft tissues as the cause of the higher mercury concentrations in the bones from the abdominal area. Bocca et al. (2018) sampled long bones. Lombardo et al. (2022) analysed two long bone fragments and three dentin samples from the first permanent molars. Rasmussen et al. (2012) studied the remains of San Francesco Caracciolo and they sampled soft tissue (from the ilium and inside the cranium), a fragment of iliac trabecular bone, and another fragment of tibia. Biehler-Gomez et al. (2021) analysed the crania of two individuals. Therefore, there is still a gap of knowledge in mercury intra-skeletal variability that needs to be fulfilled.

This doctoral thesis aimed to understand the relationship between mercury environmental levels, human activities and human-environmental pollution with a paleo-pollution perspective. This means that we needed an archive with information about mercury exposure in past human populations. At centennial and millennial scales, the only way to get this information is to research natural archives (i.e., peat, lakes sediments, polar ice, etc.) and, among them, those with direct information are the osteological remains.

The A Lanzada archaeological site represents a unique opportunity to study differences in atmospheric exposure to mercury as: i) it has a necropolis with two funerary areas covering about 700 years of occupation that contain information about two historical periods of contrasting atmospheric mercury concentrations (Roman and post-Roman); ii) it is relatively far from emission sources; iii) it was occupied by a rural population with no evidence of presence of mercury-containing goods (associated to a more luxury lifestyle) that could add complexity to the data and it is also located far from any urban centre (∼100 km); iv) previous studies suggested that the individuals were probably born and buried in the same place (López Costas, 2012); v) skeletal diagenesis was previously studied and showed that both Roman and post-Roman skeletons suffered a similar intensity in bone diagenesis with no significant differences in their elemental composition - bone constituents (López-Costas et al., 2016) and organic bone fraction (Kaal et al., 2016; López-Costas and Müldner, 2016); and vi) local soil mercury content is very low (5 ng g^{-1}).

All these facts allowed us, in Paper I, to assess if skeletal human remains are suitable paleo-

pollution archives of mercury and to identify the mercury source of exposure for the populations. An additional advantage comes from the fact that a detailed human remains and soil sampling was done during the last archaeological campaigns on A Lanzada by members of EcoPast group. The two supervisors of this thesis were involved in the extraction of the bone remains from two post-Roman burials and carried out a detailed soil sampling (an uncommon practice). These burials were researched in this thesis to provide information about mercury distribution and behaviour in the burials soil/sediments (Paper II) and to study intra-skeletal variability taking into account the soil/sediments effect (Paper III).

Table 1. Summary of mercury studies in bone remains from past populations.

	Country	Period	Site	n	Source	[Hg] (ng g^{-1})	Description	Reference
ASIA	Japan	6th - 7th	Tokushima	8	*post*	$\leq$ 1700 x 10^3	Probably men of	Yamada et al. (1995)
			Matsuyama	8		$\leq$ 200 x 10^3	power (Kofun)	
		12th - 17th	Tokushima	6	-	8200 ± 3300 *	-	
			Matsuyama	8		8200 ± 3300 *		
AMERICA	Mexico	AC 700	Temple XIII, Palenque	5	*post*	301060 ± 78056 *	Royal member	Cervini-Silva et al. (2013)
		AC 1360 ± 30	Temple of the Scriptures, Palenque	10	*post*	10.02 x 10^8 ± 30.38 x 10^8	Royal member	Cervini-Silva et al. (2018)
		BC 3100 ± 30	Temple XIII, Palenque	36		16.13 x 10^8 ± 28.83 x 10^8		
		BP 5050 ± 30	Temple XIII, Palenque	5	*post*	30 x 10^7 ± 8.1 x 10^7 *	-	Ochoa-Lugo et al. (2017)
		BP 5440 ± 30		5	*pre*[1]	25.5 x 10^7 ± 2 x 10^7 *		
		BC 2000 - AD 200	Monte Albán	1	*post*	1.02 **	-	Ávila et al. (2014)
		AD 200/300 - 1300	Ranas	5	*post* + *pre*[2]	0 (outlier: 1.13) **		
		AD 300 - 900	Jaina	1	*post*	0.62 **		
		AD 1337 - 1521	Tlatelolco	1		0 **		
EUROPE	Spain	BC 4221 - 3708	Campo de Hockey	47	*pre*[11]	315 ± 583	-	Emslie et al. (2019)
		BC 1850 - 1450	La Encantada	21	*pre*[12]	2170 ± 2280		
		BC 3200 - 2500	Santa Rita	14	*post*	130490 ± 58090 *	-	Emslie et al. (2022)
		BC 3185 - 2880	Montelirio	1		44740 ± 5260 *		
		BC 3005 - 2630		2		3925 ± 3259 *		
		BC 3005 - 2320		14		500 ± 410 *		
		BC 2915 - 2575		12		9380 ± 1457 *		
		BC 2875 - 2590		1		415880 ± - *		
		BC 1200 - 500		5		320 ± 520 *		
		BC 2455 - 2200	Las Mayores	1	*pre*[12]	7390 ± 310 *		
				2		362200 ± 191625 *		
		AD 1st - 4th	A Lanzada	68	*pre*[12]	53.57 ± 60.43	-	Paper I
		AD 5th - 7th		75		20.64 ± 23.48		
		AD 5st - 7th	A Lanzada	3	*pre*[12]	5.98 ± 6.82	-	Paper III
	Portugal	BC 3020 - 2420	Monte Canelas I	6	*pre*[11]	3852 ± 1773 *	-	Emslie et al. (2019)
		BC 2455 - 2411	Monte da Comenda	6		39162 ± 4365 *		
		BC 4080 - 4670	Sobreira de Cima	5	*post* + *pre*[11]	52260 ± 48250 *	-	Emslie et al. (2015)
		BC 3400 - 2100	Perdigões	31		52800 ± 60833		
		BC 3020 - 2420	Monte Canelas I	6	*pre*[12]	3850 ± 1777 *		
		BC 3645 – 2495	Tholos of Cabeço Arruda II	15	*post*	7350 ± 6190 *	-	Emslie et al. (2022)
		BC 3635 – 3095	Dolmen Estrada Ansião	4	-	18710 ± 17970 *		
		BC 3635 – 2205	Cova da Moura	13		14650 ± 14900 *		
		BC 3510 – 3110	Outeiro Alto 2	6		229230 ± 97250 *		
		BC 3400 – 2900	Monte Malheiro 2	2		96790 ± 96070 *		
		BC 3090 - 2900	Pedra Furada	4		130 ± 60 *		
		BC 3075 - 2474	Tholos of Paimogo I	17	*post*	1540 ± 2690 *		
		BC 2905 - 1950	São Paulo II	10	-	9890 ± 8490 *		
		BC 2890 - 2670	Perdigões	5	*pre*[11]	2100 ± 1180 *		
		BC 2620 - 2350		2		1060 ± 910 *		
		BC 2560 - 2040		2		0.36 ± 0.11 *		
		BC 2900 - 2200	Torre Velha 3	5	-	26 ± 13 *		
			Eira Pedrinha	5		190 ± 90 *		
		BC 1890 - 1250	Torre Velha 3	22		21 ± 11 *		
		BC 1740 - 1450		9		260 ± 580 *		
		BC 1660 - 1500	Outeiro Alto 2	19		140 ± 150 *		
		BC 550 - 600	Vinha das Caliças 4	30		90 ± 110 *		
		AD 100 - 400	Miroiço	16		44 ± 29 *		
		-	Monte da Nora	15		41 ± 28 *		

Iceland	AD 1000 - 1104	Skeljastaðir	14	pre^{3}	1508 ± 7932	-	Walser et al. (2019)
	AD 1494 - 1554	Skriðuklaustur	36	pre^{4}	233 ± 333		
Germany	AD 1000 - 1200	St. Clements Kirche	19	pre^{5}	41 ± 34	Rural	Rasmussen et al. (2015)
	AD 1060 - 1205	Rathaus Markt	37		134 ± 177	Urban	
Prague	AC 1546 - 1601	Church of our Lady	2	pre^{4}	64 ± 14.14	Astronomer	Rasmussen et al. (2013a)
Denmark	AD 1150 - 1350	Tirup	23	pre^{5}	18 ± 16	Rural	Rasmussen et al. (2015)
	AD 1200 - 1450	Nybøl	17		18 ± 13		
	AD 1250 - 1536	Skt. Alberts Kapel	28		18 ± 16		
	AD 1350 - 1536	Ole Worms Gade	30		21 ± 21	Urban	
	AC 12th - 19th	*Various* *	14	pre^{4}	180.36 ± 47.83 *	Leprosy	Rasmussen et al. (2008, 2013b)
			12	pre^{5}	34.3 ± 15.02 *	Leprosy control	
			20	pre^{4}	49.55 ± 113.17	Syphilis	
			17	pre^{5}	52.8 ± 38.6	Syphilis control	
		Svendborg Franciscan Friary	12		31.7 ± 66.05	Laymen	
			13		20.9 ± 17.6	Friars	
		Øm Cistertian Abbey	10		45.8 ± 17.27	Laymen	
			13	pre^{7}	68.4 ± 232.3	Monks	
	AC 13th - 19th	Tirup	16	pre^{4}	14.08 ± 19.37	Rural	Rasmussen et al. (2017)
		Skt. Alberts Kapel	13		31.47 ± 52.77		
		Svendborg Franciscan Friary	14		269.9 ± 465.16	Urban	
		Ribe	13		323.3 ± 334.7		
		Svendborg Sct. Nicolai Church	10		3415 ± 4998		
	AC 17th - 18th	Svendborg Franciscan Friary	7	pre^{4}	103 ± 115 *	Nobelty	Rasmussen et al. (2020)
			14	-	63.0 ± 38.0 *	Friars and villagers	
Italy	AD 1222 - 1524	Convento di Sn. Francesco a Folloni	147	pre^{5}	34.7 ± 3.5	-	Torino et al. (2015)
	AC 12th - 14th	Sassari	16	pre^{6}	740 ± 1190	Urban	Bocca et al. (2018)
	AC 13th - 16th	Alghero	27	pre^{6}	420 ± 1500		
	AC 14th - 18th	Bisarcio	14	pre^{5}	130 ± 290	Rural	
	AC 15th	Geridu	15		150 ± 560		
	AC 16th	Alghero	1	pre^{5}	7.64 ± 4.31 *		Lombardo et al. (2022)
	AC 1563 - 1608	Cathedral of Naples	2	pre^{4}	8800 ± 5515	Priest, leprosy and unknown illness	Rasmussen et al. (2012)
	AC 1637 - 1697	Ca' Granda, Milan	2	pre^{4}	1025 ± 385	Syphilis	Biehler-Gomez et al.
			1	-	380	Control	(2021)
	AC 17th - 18th	Convento di Sn. Francesco a Folloni	17	pre^{4}	34.4 ± 26.8 *	Nobelty	Rasmussen et al. (2020)
			34	-	10.5 ± 3.48 *	Friars and villagers	
Russia	AC 14th - 17th	Trinity Cathedral, Moscow	9	pre^{8}	300 ± 0	Urban, men	Alexandrovskaya
			5		300 ± 0	Urban, women	and Alexandrovskiy
			8		250 ±125	Urban, children	(2005)
	AC 15th - 17th	Kremlin, Moscow	6	pre^{9}	900 ± 4325	Princess and czarinas	
			2		600 ± 300	Monks	
			7		900 ± 721 *	Children	
	AC 16th - 17th	Kremlin, Moscow	3	pre^{10}	5720 ± 26740 *	Czars and Czarinas	Panova et al. (2018)

Site: *various* *: Øm Cistertian Abbey, Nordby, Odense Franciscan Friary, Svendborg Franciscan Friary.

n: number of samples not individuals.

Source: post is always associated to burial rite; **pre:** [1]Ceramic ware, elaboration of paintings/murals, exposure to vapours from mining or ingestion of contaminated food or water; [2]Exploitation of cinnabar mines or poisoning through polluted dishes or toxic food; [3]Chronical exposure to volcanogenic and geothermal emissions; [4]Medical treatment; [5]No specific source; [6]Some point sources and lifestyle (drugs, pigments, paints, amalgams, cosmetics) and also in Alghero fish consumption; [7]Handling and/or using red ink (HgS) and production/administration of Hg-containing medicine; [8]Gilding vapours and industry; [9]Medical treatment, gilding vapours, open mercury barometer and also for Princess and Czarinas cosmetics and dyes; [10]Medical treatment and poisoning; [11]Diet and use/processing of cinnabar; [12]Environmental pollution.

[Hg] (ng g^{-1}): Median ± IQR, except when *: Average ± SD and **: Hg/S (in cinnabar ca. 1.0).

3 HYPOTHESIS AND OBJECTIVES

The main hypothesis of this thesis is that mercury content in the human skeleton can inform us about environmental conditions, culture, and individual characteristics, since mercury accumulation in bone tissue may be affected by these aspects. This means human skeletons are archives of information of past periods. To prove it, we break it down into the following specific objectives:

1. To determine mercury content variability between populations from different periods with contrasting Hg environmental exposure (Paper I).
2. To assess both inter- and intra- skeletal variability in mercury content (Paper I and III).
3. To determine the role of bone components in mercury content/accumulation in bone (Paper III).

As our source of information is archaeological bone, the mercury content can also be influenced by the burial environment (i.e., the physico-chemical conditions of the burial soil/sediment) and the process of body decomposition. Therefore, the following specific objectives needed to also be addressed:

4. To determine mercury distribution in burial soils/sediments (i.e., Necrosol) and the processes behind it (Paper II).
5. To evaluate the relationship between bone and burial soils/sediments mercury content (Paper III).

Furthermore, as humans accumulate mercury during their lifespan, they act as temporary sinks that, eventually, end buried. Therefore, as a last objective we aimed to:

6. Consider the role of skeletons and burial soil/sediments in the mercury cycle (Paper I, II and III).

4 | METHODOLOGY

4.1 | Samples

4.1.1 | The site

All samples (bone and soil/sediments) used in this investigation were recovered from the archaeological site of A Lanzada (Sanxenxo, Pontevedra, NW Spain, 42º25'46''N 8º52'25''W) (Figure.3). The site was discovered in the 18th century CE and was mainly excavated during the 1960s and 1970s. It has several occupational phases. The most relevant ones to this thesis are a long-used habitational area (Bronze to post-Roman times), to the West, and a necropolis, to the East (Lopez-Costas, 2015; Rodríguez Martínez, 2017). The population of this settlement has been described as rural and of low-middle class status; no cultural goods related with mercury were found. The necropolis has been widely studied (see among others Blanco Freijeiro et al. (1967, 1961); López Costas (2012); Lopez-Costas (2015); López-Costas et al. (2020, 2016); López-Costas and Müldner (2016)). Geologically, A Lanzada site is on dune material (López-Costas et al., 2016) that was subject to anthropic alteration - at least since the BCE 2nd century - that reduced its dimensions (Rodríguez Martínez, 2017).

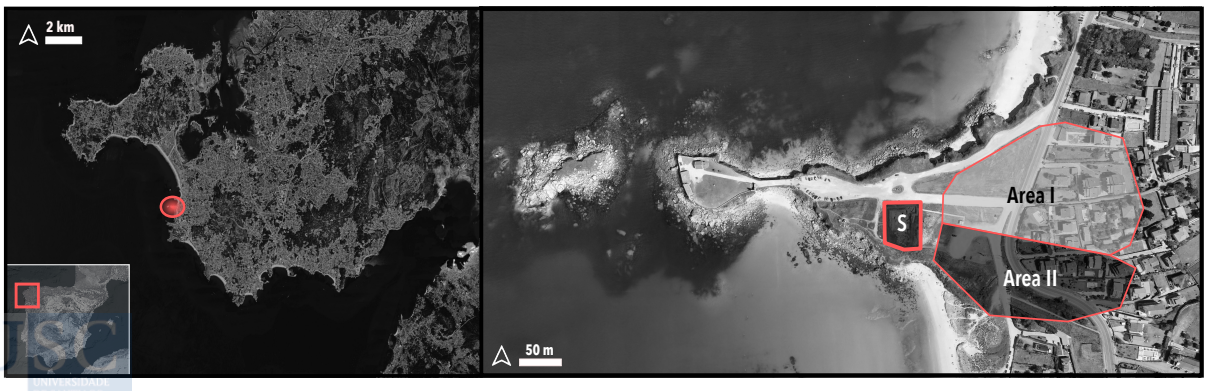

Figure 3. Aerial view of A Lanzada site with the approximated location of the necropolis and the settlement area, modified from Earth (2019). **S:** settlement area; **Area I**: Roman necropolis; **Area II**: post-Roman.

Most of the effort during the earlier excavations was done in the necropolis that is located on the East side of the site, a few tens of meters from the sea, and which contains two funerary areas - Roman and post-Roman (Lopez-Costas, 2015). Full studies about demography, burial typology, paleopathology and dietary reconstructions (collagen C/N and C and N stable isotopes) are published elsewhere (López Costas, 2012; Lopez-Costas, 2015; López-Costas and Müldner, 2016). Bone diagenesis was assessed in previous investigations about elemental (bulk bone) and molecular organic (collagen) composition showing moderate skeletal alterations (Kaal et al., 2016; López Costas, 2012; López-Costas et al., 2016; Martínez Cortizas and López-Costas, 2020). However, neither a pedological study nor soil/sediment sampling was developed during those campaigns and information about the soil/sediment characteristics where bodies were inhumated was based on photos and colour changes in the bones (see López-Costas et al. (2016)). Skeletons were found in both dune sands and acidic soils independently of the funerary area (López-Costas et al., 2016).

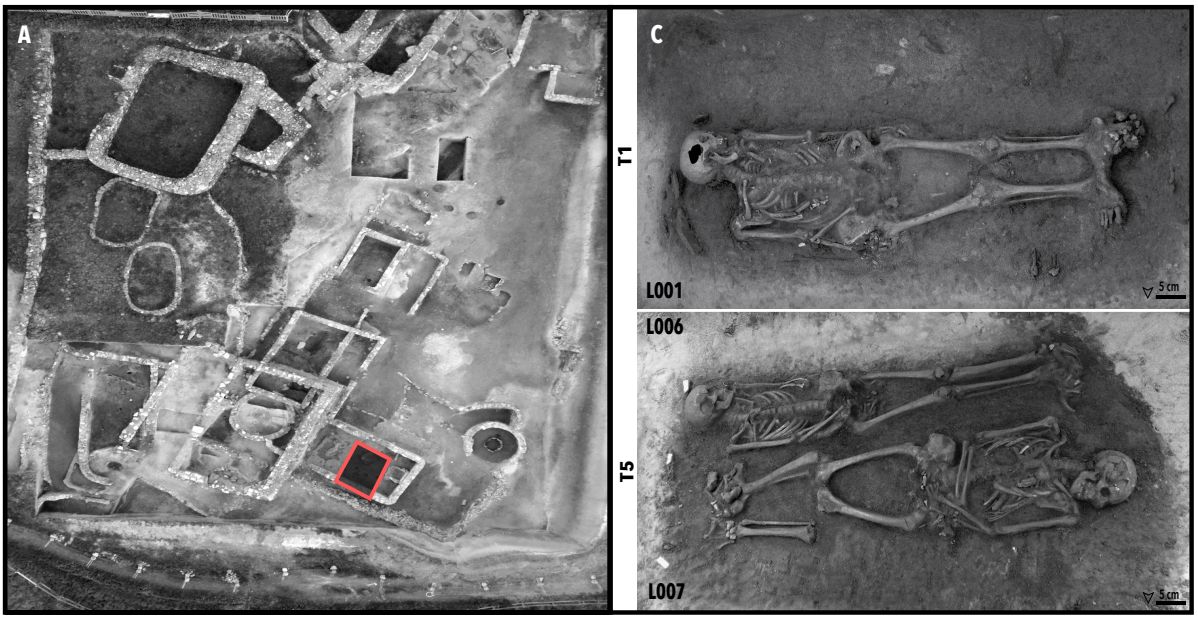

Figure 4. A. Aerial view of A Lanzada site settlement area with the aproximate location of the graves, modified from Rodríguez Martínez (2017). **B.** T1 and T5 graves.

The last excavation campaigns (2010 and 2016 - 2017) were performed in the settlement area, placed on the West side of the site. The last one (2016 - 2017) comprised the settlement area with houses occupied from the Late Bronze Age ($\sim 8^{th}$ century BCE) to the Late Iron Age-Early Roman Period ($\sim 1^{st}$ century BCE-CE). On the East margin of the excavation area, a structure with more monumental walls was discovered and described as a possible church (Rodríguez Martínez, 2017). According to the archaeological material it was dated to post-Roman

times (∼6th - 7th century CE). Two graves, named as T1 and T5, were found close to this structure and were stratigraphically located in the same archaeological layer (Figure.4). They were West-East oriented and close to each other. Bodies were probably deposited in wood boxes (i.e., coffins) since a clear colour pattern of rectangular shape was evident in both burials and nails were found at the edges. Radiocarbon dating indicates that the individuals died between the 5th to the 6th centuries cal. CE. They were excavated from dune sands. The grain size of the burial soil (Necrosol)/sediments is dominated by sand (85% in the Necrosol and 95% in the sediment outside the burials; (García-López et al., 2022)). The Necrosol showed an enrichment in P and a decrease in alkalinity (from pH ∼9 to 8), compared to the soil located outside the burials. T1 and T5 were not directly related to the funerary areas of the necropolis previously found, so the large number of bodies of the necropolis was not expected to have greatly influenced the geochemistry of these two burials.

4.1.2 | The human osteoarchaeological remains

Bulk bone (cortical) was micro-sampled using a dentist's drill, after the superficial layer was removed to avoid contamination. Due to the importance of preserving archaeological human skeletons, a small amount was sampled (∼30 to 60 mg). Sex was estimated following established criteria on the innominate and cranial bones (see a summary in Buikstra and Ubelaker (1994)). Age was estimated using standard identification criteria for innominate bone (auricular surface and pubic symphysis morphology), fourth rib and epiphyseal fusion (for a summary see Buikstra and Ubelaker (1994)), and Iberian methods for growth and maturity of postcranial bones (López Costas, 2012; Rissech et al., 2013). Statures were calculated using the humerus and femur maximum length (De Mendonça, 2000). Abrasion degree (average for the whole preserved bony parts) was estimated according to Brickley and McKinley (2004).

The necropolis – Roman and post-Roman funerary areas

For this research we analysed the vast majority of the well-preserved skeletons from the necropolis (abrasion scale mainly between 0 and 3). We assessed the mercury content of 143 bone samples from 76 skeletons, 43 Roman (1st - 4th CE) and 33 post-Roman (5th - 7th CE). They were the same, finely milled, cortical bone samples of a previous work on bone diagenesis (López-Costas et al., 2016), plus 15 additional new ones. Individuals under 12 years were excluded for ethical (more fragile skeletons) and preservation (they were not collected in earlier excavations) reasons. Male/female ratios were almost 1:1 for both Roman (22/17 and 4 unknown sex) and post-Roman (14/11 and 8 unknown sex) groups. Three types of bones were

analysed when possible (Figure.5: i) type 1: thoracic bones, ribs; ii) type 2: long bones, mainly femur; iii) type 3: cranial bones, neurocranium and jaw. Most Roman skeletons were recovered during the 1960s and at that moment only long bones and crania were collected. Therefore, we had for Roman times: 33 long bones and 35 crania; and for post-Roman period: 24 ribs, 28 long and 23 cranial bones. Highly taphonomic altered and pathological pieces were avoided.

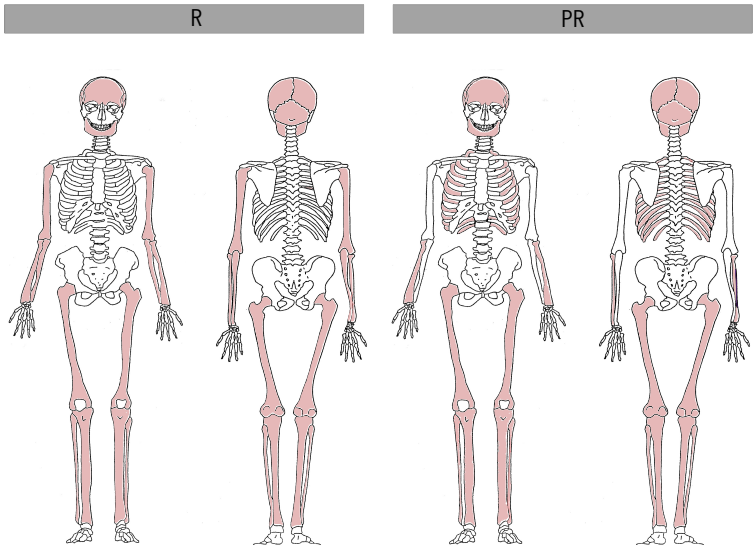

Figure 5. Sampling design for the Roman and post-Roman individuals of the necropolis.

T1 and T5 - isolated burials

T1 is a single burial containing the skeleton of an elderly (> 60 years) female (L01) of short stature (152 cm) when compared to Iberian archaeological populations from Roman and Medieval times (López Costas, 2012; López Costas et al., 2017). T5 is a multiple coetaneous burial containing two male skeletons, an adolescent (15 - 19 years) placed in West-East orientation (L06) and a mature adult (40 - 50 years) placed in East-West orientation (L07). Both had above-average statures (175 cm and 170 cm, respectively) when compared to archaeological populations from the same period (López Costas, 2012; López Costas et al., 2017). These skeletons were in supine position with stretched arms and legs (hands placed at both sides of the body or over the pelvic area). All bone remains were well-preserved, presenting the following abrasion degrees: L01 degree 3, L06 degree 2, L07 degree 2. L06 and L07 had intense physical (pressure) and chemical alteration in localised areas (L06: face and feet, L07: long bones epiphyses), that resulted in the loss of some of the bones/bone areas despite their low degree of superficial abrasion and being well preserved (López Costas et al., 2017).

In T1 and T5, four types of bone were analysed when possible: i) type 0a: vertebrae (spine)

and ilium; ii) type 1a: ribs; iii) type 2a: long bones; and iv) type 3a: crania. This classification was a modification from previous studies investigating intra-skeletal variability from the large necropolis area, including the first paper of this thesis (Álvarez Fernández et al., 2020; López-Costas et al., 2016), which was already described in this section. The sampling strategy followed a multisampling approach to cover most of the skeleton intra-variability (Figure.6). For ethical reasons the number of samples in L01 was reduced due to the low bone density, related to severe osteopenia (possibly a case of senile osteoporosis). A total of 73 bulk powder bone samples were analysed: 11 for L01, 31 for L06 and 31 for L07. Bulk bone sampling was intended to be in harmony with the soil/sediment sampling design.

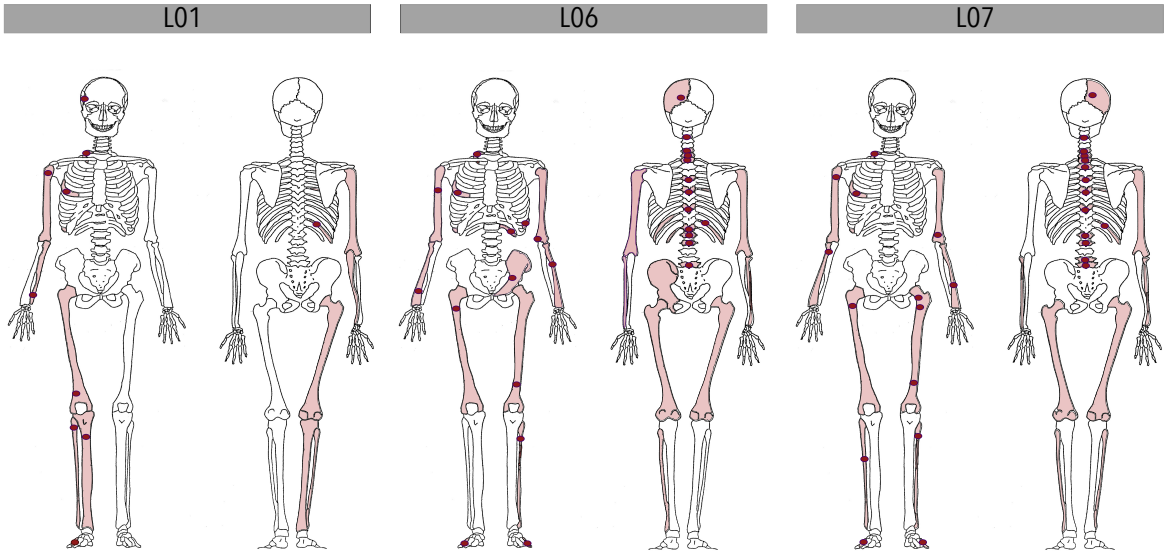

Figure 6. L001, L006, and L007 cortical bone sampling design.

4.1.3 | The soil/sediment samples

To determine the mercury content in the archaeological area we also analysed a pedo-sedimentary sequence (LSQ1, collected during the 2016 - 2017 excavation campaign). Thirty-four continuous samples of 5 cm in thickness were taken to a depth of 2.05 m (LSQ1.S01 - S34) in a freshly open cut. Samples comprised between LSQ1.S27 and LSQ1.S34 correspond to the same sand dune layer in which T1 and T5 burials were done. Fine-milled (< 50 µm) subsamples were processed for mercury concentration. Other physico-chemical properties of the soils/sediments have been published elsewhere (López Costas et al., 2017).

The soil/sediment sampling design carried out in T1 and T5 consisted of one longitudinal and one transverse transect on each individual plus some additional samples taken from inside the crania, and on L001 coxal/innominate bone area (Figure.7). Samples were taken in the field

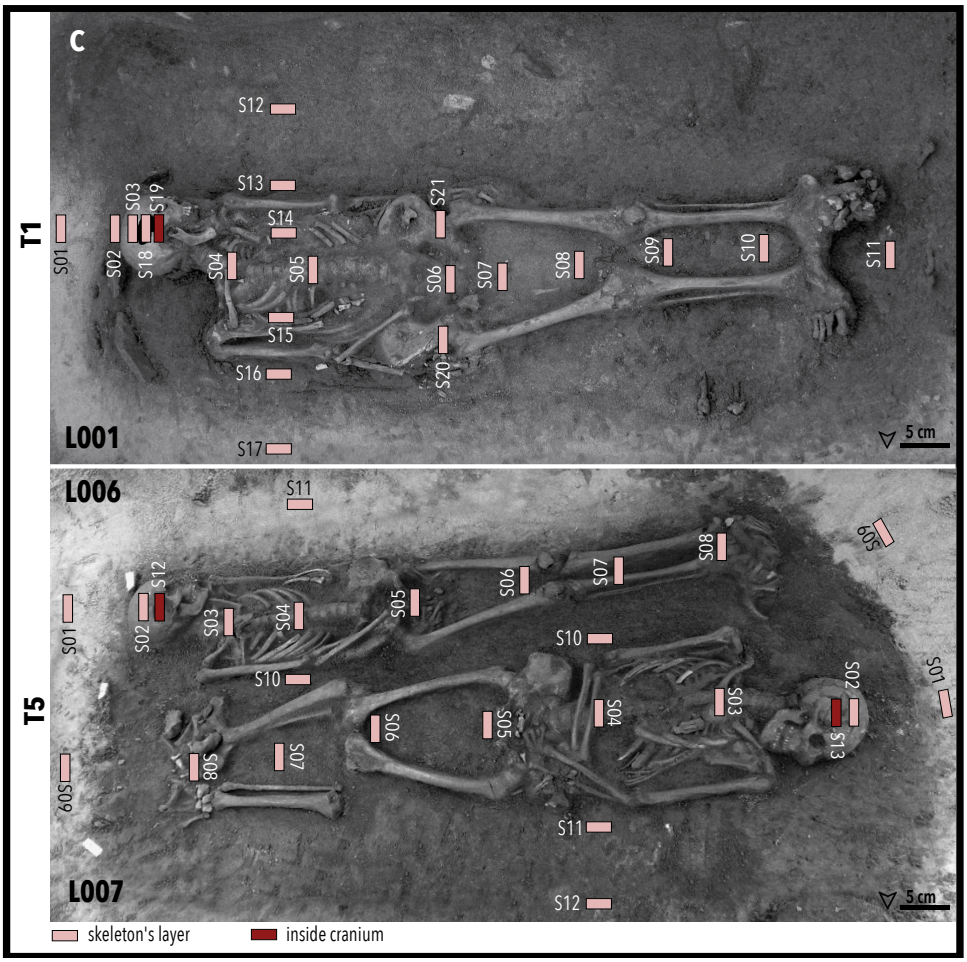

Figure 7. T1 and T5 soil/seiment sampling design.

except for the additional ones, which were collected from soil adhered to the bones once in the laboratory. In total 46 soil/sediment samples were collected and analysed (both fine earth and silt+clay fractions). Given that sample position is susceptible to affect mercury concentration, it was recorded using the longitudinal axis of each skeleton. Sample coordinates were referred to this axis and module was used as predictive variable.

4.2 | Biogeochemical analysis

4.2.1 | Mercury analyses

Mercury concentrations were determined using a DMA-80 (Milestone), by thermal decomposition, Hg amalgamation, and detection by atomic absorption. The equipment is hosted at the laboratory of the *Ecotoxicoloxía e Ecofisioloxía Vexetal research group (Departamento de Bioloxía Funcional, Universidade de Santiago de Compostela)*. The protocol was described in Paper I and, briefly, is as follows: ∼20 mg of dried and finely milled bone and/or soil/sediment sample, and

standard reference materials (SRM) were placed in Ni holders in the auto-sampler and 200 µl of ultrapure (Type 1) water was added. At the start, four ultrapure water blanks and the SRM were run, and thereafter one blank and one SRM were analysed every ten samples. In the case of soil/sediment samples from T1 and T5, mercury content was analysed in two fractions, fine earth (< 2 mm, FE) and silt+clay (< 0.05 mm, SC). Four SRM were used: GBW07601a (670 ± 100 ng g^{-1}), NIST 1486 (2.3 ± 1.4 ng g^{-1}) for bone, and CRM 277 (1770 ± 60 ng g^{-1}), BCR27R (128 ± 17 ng g^{-1}) for soil/sediment. For bone, SRM NIST 1486 - bone matrix – only provides Reference Mass Fraction Value, so GBW07601a was also used. The quantification limits were 0.24 ng g^{-1} for bone and 0.08 ng g^{-1} for soil/sediment in Paper I, 0.51 for soil/sediment in Paper II, and 0.26 ng g^{-1} for bone in Paper III. Mean recoveries for reference materials were 100% GBW07691a (685 ± 62.76 ng g^{-1}), 93% NIST 1486 (2.1 ± 1.4 ng g^{-1}), 75% CRM 277 (1325 ± 98 ng g^{-1}), and 91% BCR27R (116 ± 17 ng g^{-1}). In the study of the necropolis populations (Paper I) 124 duplicates were done, reporting a stabilised relative error of 5% (data can be found in supplementary material Table S1). During the analysis of the mercury distribution in the Necrosol (Paper II), 13 quality controls were done reporting a relative error average of 6.4% for silt+clay. When the intra-skeletal variability was assessed (Paper III), 29 quality controls were done, reporting an average difference of 1.9 ng g^{-1}.

4.2.2 | Soil/sediment elemental composition analyses

Elemental composition analysis was carried out, as described in Paper II, on dried (25°C) silt+clay samples using two energy dispersive X-ray fluorescence (EMMA-XRF) analysers hosted at the RIAIDT facility of the *Universidade de Santiago de Compostela* (USC). XRF is a non-destructive technic that allowed us to obtain measurements of 10 elements (P, S, Ca, Ti, Mn, Fe, Cu, Zn, Sr, and U; see Table SM 2 in Paper II supplementary material for detection limits). Carbon and N were measured using a LECO (TruSpec CHNS) analyser hosted at the Elemental Analysis Service of the RIAIDT in *Campus Terra* (USC).

Nitrogen was selected as a direct proxy for organic matter content (OM); Cu and Zn as indicators of metal enrichment in soil fine fractions bound to OM and clay; Ca and Sr as proxies of biogenic carbonates, due to the presence of mollusc shells; the association of Ca, P and C is an indicator of the presence of bone, as these elements are the major constituents of the bone mineral component (i.e., hydroxyapatite); S was used as proxy for OM forming strong bindings with Hg; Fe as major soil component; Ti as proxy for fine grain fractions; Mn as a proxy for redox conditions; and U, that presents high affinity for phosphate groups (i.e., bone mineral

matrix), was used as marker of the local geology of the area.

4.2.3 | Spectroscopic characterisation by FTIR-ATR

Bone and soil samples were analysed by total attenuated reflectance Fourier-transform infrared spectroscopy (FTIR-ATR) using a spectrometer Agilent Cary 630 FTIR coupled with an ATR module, located at EcoPast laboratories (*Facultade de Bioloxía*, USC). Spectra were acquired in the mid-infrared region (MIR) 4000 - 400 cm^{-1}, by averaging 100 scans at a resolution of 4 cm^{-1}. The equipment was cleaned, and a background was collected before every measurement. Peak identification was done using the {andurinha} R package (Álvarez Fernández and Martínez Cortizas, 2020) following the second derivative sum spectrum method after Z score standardisation of the spectra. Assignment of compounds related to vibrations is based on those reported in the literature (see references in Table SM 2 in Paper III supplementary material), taking into account the limitations imposed on IR interpretation of complex samples (Coates, 2000; Larkin, 2017; Simonescu, 2012; Socrates, 2004).

Several indices related to the bone structure were calculated: i) the infrared splitting factor (IRSF), which is related to hydroxyapatite crystallinity (Weiner and Bar-Yosef, 1990), as the sum of the $\nu_4(PO_4)$ peaks intensities at 600 and 559 cm^{-1} divided by the intensity of the valley between them (587 cm^{-1}); ii) the mineral maturity index (MMI), which represents the progressive transformation of poorly crystallised non-apatite domains into well crystallised apatite (Farlay et al., 2010), as the ratio 1019/1111 cm^{-1} of $\nu_3(PO_4)$; iii) the CO_3/PO_4 (C/P) ratio, which indicates the carbonate content of hydroxyapatite, dividing the $\nu_3(CO_3)$ peak intensity at 1409 cm^{-1} by the $\nu_3(PO_4)$ peak intensity at 1019 cm^{-1} (Grunenwald et al., 2014); and iv) the collagen content estimated through the Amide I/PO_4 ratio (AmP), dividing the peak intensity of ν_1(Amide I) band at 1638 cm^{-1} by the $\nu_3(PO_4)$ band at 1019 cm^{-1} (Trueman et al., 2004).

4.3 | Statistical methods

Statistical analyses and graphs were made using the software R (R Core Team, 2021), with the exception of PLS-SEM modelling that was run using the SmartPLS software (Ringle et al., 2015). The R packages used were: {robCompositions} for zero imputation using 'lmrob' method (Filzmoser et al., 2018), {rgr} for clr transformation (Garrett, 2018), {plsRglm} for PLSR model (Bertrand and Maumy-Bertrand, 2019), {ggplot2} + {ggpubr} for graphics (Kassambara, 2020; Wickham, 2016).

4.3.1 | Descriptive methods

Normality was assessed using the Shapiro-Wilk test, as it is the most robust even when the sample size is relatively small (Yap and Sim, 2011). Given the non-parametric nature of the data, descriptive analysis was based on median and interquartile ranks (IQR). To detect significant differences among groups, Mann-Whitney-Wilcoxon was applied when comparing two groups, and Kruskal-Wallis combined with pairwise Mann-Whitney-Wilcoxon when more than two groups were involved. The p-values were considered significant when $p < 0.05$. Correlations were done using Kendall test, which is specific for non-normal distributed data. Z-score (95% confidence level) method has been used to handle outliers as well as visual methods. In the study of the necropolis populations (Paper I) for tests based on age groups, only young and mature adults were considered due to the small size of the other categories. Violin plots are provided to show variability between populations for individual variables.

4.3.2 | Partial Least Squares Regression Modelling

To understand mercury distribution in the soil we used Partial Least Squares Regression (PLSR) (Wold et al., 2001). The elemental composition data set (elemental concentrations in the silt+clay fraction; see explanation in the results section) was used as predictors while Hg concentration was set as the response variable. Zero imputation has been performed for those cases where the element concentration was below the detection limit, following the robust method proposed by Martín-Fernández et al. (2012). Due to the compositional nature of the data the elemental data set was transformed to centered log ratios (clr) (Egozcue et al., 2003) and scaled (Wold et al., 2001). The main purpose of the PLSR modelling was the identification of the latent variables (i.e., processes/compounds) involved in Hg distribution and their weight in the soil samples. The model was performed using leave one out cross-validation without splitting the data in training and test sets to avoid the loss of relevant information and explanatory power.

See Paper II supplementary material for: PLSR data set, $\hat{y}$ values, T matrix and W* matrix.

4.3.3 | Partial Least Squares Structural Equation Modelling

To assess mercury intra-skeletal distribution, Partial Least Squares Structural Equation Modelling (PLS-SEM) was applied. This method enables the estimation of complex models following a causal-predictive approach without imposing distributional assumptions on the data matrix (Hair et al., 2019a; Sarstedt et al., 2017), while aiming to maximise the explained vari-

ance of the dependent latent constructs (Hair et al., 2019b). The inner and outer models are represented in Paper III Figure 3. The latent constructs can be classified into three groups. 1) Those related to bone components, collagen, and hydroxyapatite (HAP), to assess their role on the skeleton mercury content, and the degree of crystallinity of the mineral phase (HAPc), as it can affect the release of mercury from the bone tissue to the surrounding soil. 2) Soil components in the silt+clay fraction, among which mercury (Hg), primary silicates (p-silicates) and clay were selected to assess the degree of interaction between bone and soil, as the bone was found to be a potential source of mercury to the soil. As soil/sediment samples were collected in the field, an exact match was not possible for all the bone samples, so the closest soil/sediment sample to each bone sample was used. Median distance between bone and soil samples was less than 10 cm in all cases. Unfortunately, the other skeletons belonging to the large necropolis area were excavated more than 50 years ago without collecting soil samples. 3) Location, as it is susceptible to affect mercury content.

Measured variables included: i) for collagen the FTIR-ATR bands at 3280 (Amide A and –OH vibration), 1638 (Amide I) and 1545 cm^{-1} (Amide II) (Goormaghtigh et al., 2006; Martínez Cortizas and López-Costas, 2020; Trueman et al., 2004); ii) for HAP bands at 870 ($\nu_2(CO_3)$), 712 ($\nu_4(CO_3)$), 690 ($\nu_4(PO_4)$ and –OH), and 470 cm^{-1} ($\nu_2(PO_4)$) (Baxter et al., 1966; Fleet, 2009; Rey et al., 2011; Zammel et al., 2021); iii) for HAPc the band at 1019 cm^{-1} characteristic of $\nu_3(PO_4)$ (Dal Sasso et al., 2016; Rey et al., 1991); iv) for soil Hg, concentrations obtained in a previous work on the same graves were used (Álvarez Fernández et al., 2021); v) for p-silicates the bands at 606 (Si, Al tetrahedral deformation), 585 (Si tetrahedral breathing) and 531 cm^{-1} (Si – O – H vibrations) (McKeown, 2005; Pérez-Rodríguez et al., 2016); vi) for clay, bands at 3694 and 3621 cm^{-1} characteristic of kaolinite –OH stretching (Vaculíková et al., 2011); and vii) for location, the longitudinal axis (x-axis) of each skeleton setting as zero the pelvic area (approximately at S3 sacral vertebra). All measured variables were set on reflective mode.

Given the compositional nature of the manifest variables for latent constructs, bone Hg and soil Hg concentrations were previously transformed by natural logarithm. The data matrix (Table SM 3 in Paper III supplementary material) was standardised and the path method was chosen for the weighting scheme (Hair et al., 2017). Two-tailed bootstrapping was applied to test the statistical significance of path coefficients choosing the bias-corrected and accelerated (BCa) method for the confidence intervals (Hair et al., 2017). The significance level was set to 0.05. Partial Least Square Prediction-Oriented Segmentation (PLS-POS) was also used to

identify segments (i.e., groups of samples) in the data set (Becker et al., 2013).

5 | DISCUSSION

5.1 | Hg in archaeological bones, A Lanzada in a global context

Mercury is not a main topic in bio-osteoarchaeology, but some studies have dealt with its use and incorporation into the human body in ancient times (Table.1). This thesis provides, to our knowledge, the first detailed revision of the study of mercury in human remains from archaeological contexts. To our knowledge, Yamada et al. (1995) were the first to report mercury concentrations in archaeological bone. They found significant differences between two Japanese sites (Tokushima and Matsuyama) from two periods (6 - 7th and 12 - 17th centuries), which they related to differences in burial customs. During the Kofun period, men of high status were painted with paints made of mercury minerals before inhumation and high concentrations were found in the skeletons and the surrounding soil. We did not find any evidence of the use of mercury as *post-mortem* decoration in A Lanzada. The low amount of mercury obtained in our research also supports the hypothesis that mercury was not used *post-mortem* as funerary ritual. In contrast, burial customs using mercury (i.e., body painting) were described for pre-Hispanic Mexican populations, but they were restricted to high status groups (Ávila et al., 2014; Cervini-Silva et al., 2018, 2013; Ochoa-Lugo et al., 2017). For Royal members of pre-Hispanic societies, high mercury content on skeletal surfaces was connected with post-mortem body painting, while exposure in lower status groups was linked to the use of ceramic ware, paintings elaboration, mining and consumption of polluted food and water. Similar practices have been described for Chalcolithic South Iberia, where there is evidence of mercury use since 3000 BCE (Emslie et al., 2022, 2019, 2015). The mechanisms for mercury incorporation proposed in these works were: i) taphonomic, due to decoration of the dead body with cinnabar; ii) *post-mortem* incorporation from burial soil in areas rich in mercury minerals; and iii) *ante-mortem* incorporation related to continuous mining and metallurgy practices and consumption of polluted food/beverages. This combination of sources can be found in

areas with mercury ores, such as SW Iberia, but not in A Lanzada. In addition, we have so far not found similar rituals for the elite or non-elite groups of those societies under Roman rule. However, since mercury was a rare product, its use could be connected to social status during Roman times as it happened with the use of cosmetics based on mercury in the case of czarinas and princesses from Medieval Russia. Mercury-based cosmetics can also lead to high mercury contents through its absorption by skin-contact (Alexandrovskaya and Alexandrovskiy, 2005). However, in A Lanzada we have not found any connection with the indicators of social status (i.e., grave goods and grave typology), but we also consider that our populations were middle-low status (Lopez-Costas, 2015), so no high class members are expected.

Mercury was used for medical purposes by Romans and Greeks and was probably used as treatment for *lues venerea* (syphilis) for the first time in Pliny the Elder's time (Parsons and Percival, 2005). In the Middle Ages, it was commonly used to treat chronic diseases with skin-involving symptoms such as leprosy, tuberculosis and syphilis (Crane-Kramer, 2000; Dracobly, 2004; Philip and Ozuah, 2000). This medical use was reported as the cause of high mercury content in several osteoarchaeological studies (Table.1). Despite having in A Lanzada a few cases of chronic unspecific infections (diagnosed by paleopathological assessment), the inter-skeletal variability does not seem to respond to a specific pathology, so any high mercury content related to disease was not found. In contrast, Rasmussen et al. (2017, 2012, 2008) have related to medical treatment the slight increase in mercury in long bones and the higher values in thoracic bones found in individuals with pathological signs of infectious diseases. Panova et al. (2018) found high mercury concentrations in czars and czarinas and although some could result from poisoning, the others were more likely the result of medical treatment. In the post-Medieval community of Skriðklaustur in Iceland, medical treatment was also a major source of the metal incorporation (Walser et al., 2019). Biehler-Gomez et al. (2021) reported two cases of tertiary syphilis from the 17th century from the Ca' Granda hospital in Milan with high mercury levels that they attribute to the treatment of this disease.

High mercury concentrations are usually related to *post-mortem* burial customs and *ante-mortem* medical treatment and poisoning. However, occupational exposure has also been linked to elevated values in populations involved with mining and metallurgy, such as La Encantada in Ciudad Real (Spain; Emslie et al. (2019)), Ranas from Querétaro (México; Ávila et al. (2014)) and Trinity Cathedral of Moscow (Russia; Alexandrovskaya and Alexandrovskiy (2005)). But no direct relationship with these kind of metal sources was found for the whole

community of A Lanzada in any of the studied periods (Roman and post-Roman).

Low concentrations were found in societies/individuals with no direct use of mercury (Bocca et al., 2018; Rasmussen et al., 2015). Research concerning urban and rural populations from Germany, Denmark (Rasmussen et al., 2015), and Italy (Bocca et al., 2018) concluded that individuals from Medieval and post-Medieval urban sites had higher concentrations than those from rural areas. The use of cultural items rich in mercury is presented as the major source: drugs, pigments, paints, amalgams, cosmetics and even fish consumption in the case of the Sardinian village of Alghero. Mercury concentrations in A Lanzada Roman and post-Roman individuals (54 ± 60 ng g^{-1} and 21 ± 23 ng g^{-1}, respectively) are slightly higher than those obtained for rural medieval Denmark (14 ± 19.37 to 41 ± 34 ng g^{-1}) (Rasmussen et al., 2015, 2017), but much lower than urban and rural Sardinia (130 ± 290 to 150 ± 560 ng g^{-1}) (Bocca et al., 2018). One of the differences with previous works, which makes A Lanzada so special, is that we studied the same area and population through time (two periods of use over 700 years).

5.2 | Human osteoarchaeological remains as natural paleo-archive of Hg cycle

First of all, as our osteoarchaeological remains were inhumated, soil had to be excluded as a potential source of mercury. To do so, we followed the most common approach in these kinds of studies (Kepa et al., 2012; Rasmussen et al., 2017; Walser et al., 2019; Zuckerman, 2016) and mercury concentrations were measured in the fine earth fraction of a pedo-sedimentary sequence associated to the archaeological site (Paper I). Mercury content was found to be low, in agreement with the local geology that shows no mercury ores (IGME, 1982). The vertical distribution indicated no downward lixiviation of mercury. The samples corresponding to the same soil/sediment layer of the burials showed Hg concentrations below ∼1.5 ng g^{-1}, while in bone the median was 36.0 ± 51.7 ng g^{-1}. This suggested that post-depositional incorporation from the soil/sediments to the skeleton was unlikely. This is in agreement with previous research where mercury incorporation from the soil was excluded when there was no geogenic mercury (Emslie et al., 2015; Kepa et al., 2012; Rasmussen et al., 2008, 2013c, 2017; Walser et al., 2019; Yamada et al., 1995; Zuckerman, 2016).

Significant differences were observed between the individuals of the two studied periods. Mercury concentrations were significantly higher in the Roman cohort when compared to the post-Roman. Therefore, given that: i) all the skeletons came from the same necropolis and the two funerary areas are placed close by; ii) the chronological difference between the

two periods is much shorter than the time the skeletons were under earth and exposed to diagenetic modifications; iii) the burial environment was excluded as the source of mercury to the individuals; and iv) no relationship was found between a skeleton's mercury content and bone preservation (i.e., C/N and abrasion). Skeleton mercury concentrations must contain information about the *ante-mortem* mercury content of the individuals (i.e., mercury exposure). There is no evidence of cultural exposure to this metal as well as no connection with *ante-/post-mortem* characteristics (see subsection 5.3.1 Inter-skeletal variability); therefore, the ultimate source of mercury should be environmental pollution. The more likely source is atmospheric mercury. A Lanzada is located relatively far from the emissions sources during that time but there is evidence of differences in mercury atmospheric pollution affecting this area between Roman and post-Roman times. A peat record from Penido Vello shows an increase in atmospheric mercury during the Roman rule and a decrease in post-Roman times (Martínez-Cortizas et al., 1999). This trend was also reported by Serrano et al. (2013) in the Mediterranean coast of NE Spain in a Posidonia oceanica mat core, by Elbaz-Poulichet et al. (2011) in a SE France coastal lagoon core and by Thevenon et al. (2011) in the Swiss Alps in a lake core. Results that are in agreement with our previous study, which also considered lead and lead isotopes but in a more limited sample size (López-Costas et al., 2020). This confirms human osteoarchaeological remains as suitable paleo-archives of Hg cycling, which will be further supported in the next points of the discussion.

5.3 | Inter- and intra-skeletal variability

5.3.1 | Inter-skeletal variability

The results from the necropolis (Paper I) suggested a homogeneous degree of exposure, as: i) similar mercury levels were found for females and males and among all age groups, indicating that the population dynamics and structure were not main factors controlling mercury content; and ii) no relationship was found with social status, approached by burial typology and grave goods. This is in line with studies assessing the relationship between mercury concentration in bone tissue and age that found no significant correlation (García et al., 2001; Yoo et al., 2002; Zaichick et al., 2011; Zioła-Frankowska et al., 2017). Zioła-Frankowska et al. (2017) reported a significant increase in mercury concentration in the neck of the femur for women over 50 years and for men over 80 years old. But the mechanism related to age-driven accumulation in bone tissue is not yet totally understood. One of the proposed mechanisms is linked

to a reduction in liver and renal functioning, which may lead to impaired mercury excretion (Skalnaya et al., 2014). Unfortunately, in our data set we do not have a representative group for ages > 50, neither women nor men.

Mercury content was neither dependent on the amount of food consumption nor on the trophic level. No correlation was found with dietary proxies (i.e., collagen stable isotopes, including trophic position $\delta^{15}N_{col}$) among individuals of the same chronology. It was known, from a previous study (López-Costas and Müldner, 2016) that the post-Roman individuals of A Lanzada consumed more marine resources and C_4 plants (i.e. millets) than the Roman individuals. Today, the consumption of marine resources, mainly large predatory fish, due to the methylation process that takes place in aquatic environments and the long marine food-chains, is related to increased mercury exposure (i.e., through bioaccumulation and biomagnification; Liu et al. (2011)). However, our results do not seem to show this trend. A Lanzada post-Roman people, with a diet enriched in fish, have significant less mercury in their bones than the Roman individuals, whose diet included fish in a lower proportion. This may be related to the less polluted environment of the post-Roman period.

In T1 and T5 (Paper III), mercury content varied among individuals and decreased with age (L01 < L07 < L06). However, the small sample size (n = 3 individuals) does not allow us to discard a spurious relationship, as many other factors (e.g., exposure during life) could also be responsible for this pattern. In the necropolis, no relationship was found between mercury content and age in any of the two cohorts (Paper I), in line with results from recent research from biopsies (Babuśka-Roczniak et al., 2021; Zioła-Frankowska et al., 2017) and autopsies (Domingo et al., 2017; Yoo et al., 2002). Variation in mercury concentrations among the three analysed individuals (L01, L06, and L07) may be explained by individual differences in *ante-mortem* exposure and accumulation.

We think that, in A Lanzada, mercury exposure was mainly related to atmospheric pollution during both Roman and post-Roman times. Exposure to larger doses of mercury was very unlikely as the median concentration was ∼6 ng g^{-1} for L01, L06, and L07, and 21 ± 23 ng g^{-1} for the post-Roman cohort from the necropolis. L06 and L07 were likely to share a similar environmental exposure as they were buried in a double non-consecutive burial that indicates that both died at the same time or within a few days of each other. L01 could have lived a few decades earlier or later and this individual could have had different environmental exposure. Differences in environmental exposure could explain in part the slight differences observed

here, as well as those found in the individuals buried in the necropolis (Paper I). In contrast, mercury accumulation appears to be related to individual factors such as daily life habits, occupational activities, and body mass index (Bjørklund et al., 2017; Zioła-Frankowska et al., 2017). So, differences in mercury content among the studied individuals may be explained by non-measured characteristics, such as body mass. If stature is used as a proxy for body mass (notwithstanding any limitations), the same trend as that observed in mercury content is found: L01 (152 cm) < L07 (170 cm) < L06 (175 cm). L06 and L07, both males, had higher stature, taller than the Iberian average for the period, thus may have had higher body mass than L01, an elderly woman of shorter stature. L01 also suffered from advanced osteopenia, probably as a side effect of her age (> 60 years-old) that could have affected mercury accumulation/release (Lanocha et al., 2013; Zioła-Frankowska et al., 2017). Old age could also have prevented her from participating in active occupational activities and she may have had a more sedentary lifestyle compared to the two men - or at least less related to metalworking. There is an ongoing debate regarding the association between mercury and osteopenia, with contrasting results. Some studies suggest mercury affects bone metabolism (Suzuki et al., 2004; Tang et al., 2022), while others did not find any significant association between mercury exposure and this pathology (see a review in Jalili et al. (2020)). There is only one study where mercury was found to disrupt calcium metabolism, in goldfish scales, and mercury dose was high (10^{-7} M methylmercury exposure during 2, 4, and 8 days in Suzuki et al. (2004)). As final consideration, it is unlikely that mercury exposure was the cause of osteopenia in L01.

A Lanzada was a rural site with no Hg material culture, typical of a Roman/post-Roman middle-low class settlement, which agrees with our mercury results of no clear group distribution within each period. Burial customs involving mercury as well as *ante-mortem* exposure due to cosmetic and cultural items was not likely, neither suggested by our data (Paper I). Despite this fact, in the necropolis, seven individuals presented values over the median and were considered outliers. Two causes can explain the elevated concentrations: i) the individuals were migrants coming from a more polluted area (e.g., urban sites), or ii) they were involved in activities related to metal extraction and manufacture (even women, since they could be exposed to indoor air pollution in the home environment). Medical treatment does not seem to be a suitable explanation here since all bones from these individuals have high concentrations but no markers of chronic pathologies - however, we might consider the presence of a soft tissue disease treated with mercury. Current information about the studied individuals does not allow to discard or accept one option or another of the above list. However, pre-

vious morphometric studies suggest homogeneity among A Lanzada individuals that can be connected to low mobility (López-Costas et al., 2012; López Costas, 2012). In addition, in López-Costas et al. (2020), where lead isotopic analyses were included, excluded migrants. Regarding the second option, no large facilities related to metal transformation were found in the A Lanzada Roman settlement, although they may have been located elsewhere. In addition, outlier individuals do not show any characteristic *ante-mortem* or *post-mortem* features that could be related to activities with high Hg exposure. More research about paleo-mobility will help to explain these outliers.

Regional to local variations in atmospheric pollution in the past can affect the mercury values observed in skeletons. Apart from our previous study in A Lanzada (López-Costas et al., 2020), only Walser et al. (2019) point to atmospheric pollution as a primary source of mercury exposure in the Middle Ages in an Icelandic community. Skeljastaðir individuals lived close to the Hekla volcano, which erupted during the occupation of the settlement, as well as the geothermal emissions that affected the whole environment. No natural metal emissions are expected in A Lanzada vicinity. However, intense human activities such as mining and metallurgy can produce anthropogenic atmospheric pollution. Variations in atmospheric mercury pollution can be approached studying natural archives such as glacial ice, lakes or peatlands. In North-Western Spain, Martínez-Cortizas et al. (1999) analysed mercury in a peat core from the Penido Vello peatland, and found consistent differences between Roman and post-Roman times that reflected variations in atmospheric mercury pollution. A Lanzada human bones fit nicely with these findings, with Roman individuals having significantly higher mercury concentrations than post-Roman ones. Mercury mines such as Almadén (Ciudad Real) were intensely exploited during Roman rule to obtain vermillion (red dye) for wall painting, make-up and togas staining (Higueras et al., 2011). Almadén was mentioned by Pliny the Elder (Higueras et al., 2003). Mercury was also used for gold amalgamation. One of the largest Roman gold mines in Iberia, Las Médulas, lies 237 km to the east of A Lanzada. It is known that after the socioeconomic decline linked to the fall of the Western Roman Empire, mining activity decreased and turned more local, especially considering that gold mercury mines were under the Emperor's control (Ott, 2009). The use of mercury was later restricted to medical and pharmaceutical uses (Steinnes, 2013), which agrees with the lower values of atmospheric mercury found in Penido Vello peatland (Martínez-Cortizas et al., 1999) and the decrease in mercury concentrations observed in A Lanzada bones. Thus, historical and natural records as well as the inter-skeletal comparison support the hypothesis that the main mercury source af-

fecting A Lanzada population during 700 years was atmospheric pollution, in agreement with our previous results for this archaeological site (López-Costas et al., 2020).

5.3.2 | Intra-skeletal variability

Mercury content

Aiming to evaluate intra-skeletal variability, we considered bones with a priori different turnover rates: from ribs, with high turnover rate (around 10 years for full turnover), to femur (long bone), in which a substantial proportion of adult bone was synthesised during adolescence (Hedges et al., 2007). On one hand, due to bone type availability and ethical reasons, we had to restrict the type of sampled bone and the number of samples per individual when analysing individuals from the necropolis area (Paper I). On the other hand, for T1 and T5 (Paper III), specially T5, we could do a more extensive analysis, i.e., sampling a larger number of bones. In the study of the necropolis three bone types were considered (type 1: thoracic bones mostly ribs, type 2: long bones and type 3: crania). For T1 and T5, initially four types were considered (type 0a: vertebrae (spine) and ilium; type 1a: ribs; type 2a: long bones; and type 3a: crania), but after the evaluation of significant differences among them they were reduced into two groups (type 1: spine, ilium, and ribs; type 2: long bones and crania).

In the necropolis (Paper I), no clear trend was found between the three bone types analysed, although long bones seemed to be slightly enriched. This was in agreement with the results reported for cortical bones in medieval Denmark populations (Rasmussen et al., 2013b). Note that only long bones and crania could be sampled for Roman individuals, because thoracic bones were not collected during 1960s excavations. This fact could have biased our results since the Roman period included the skeletons with a larger concentration of mercury. When we extended the research to include a greater number of samples per individual by analysing T1 and T5 (Paper III), bone type showed significant differences in mercury content. Bone type 1 (spine, ilium, and ribs) had higher mercury concentrations than bone type 2 (long bones and crania). In a previous investigation on bone alteration due to diagenesis, based on (essentially) the same skeletal remains in Paper I, significant differences were found regarding elemental composition (López-Costas et al., 2016). Ribs were enriched in elements representative of the soil mineral matrix (Fe, Al, Si) and relatively depleted in elements representative of the bone mineral component (Ca, P, Sr), i.e., more altered; while long bones and crania had a similar behaviour between them and were found to be less altered than ribs. This study also concluded

that lead concentrations did not significantly vary between bone types. Paper I indicated a similar trend for mercury distribution, in agreement with López-Costas et al. (2020). The differences between Paper I and Paper III can be explained by the sampling design, in Paper I a maximum of 3 bones per individual where analysed while in Paper III the samples numbers were n = 11 for L01, n = 31 for L06, and n = 31 for L07. We think that the more exhaustive sampling allowed to identify the individual intra-skeletal variability, while in Paper I it was probably masked by the sampling design. However, as we will explain later, soil is not the source of this variability, like it was for diagenetic alteration in López-Costas et al. (2016).

Previous studies in osteoarchaeological mercury content found that ribs were higher enriched in mercury compared to long bones (Rasmussen et al., 2017), and the same with humeri when compared to femora and tibiae (Emslie et al., 2019, 2015). In the case of Rasmussen et al. (2017), the studied individuals were treated with mercury remedies and showed mercury concentrations between 7 and 24715 ng g^{-1} in cortical bones. They proposed two causes for the ribs mercury enrichment: i) the bone turnover, which is quicker in short bones, and ii) the collapse of Hg-enriched soft tissues contained in the thorax. The second option was based in the higher trabecular bone content in the rib samples, even when in these bones the trabecular component is small. We consider the second hypothesis but excluding the influence of the trabecular bone. Therefore, both causes are possible and may co-exist as *ante-* and *post-mortem* mechanisms, and account for mercury variability between the analysed bone types. Paper I supported the first interpretation: differences between bone types are only visible when exposure occurred early or late in life. If this exposure is stable through life (i.e., chronic and low dose), we might expect to find no difference, as Paper I suggested for A Lanzada. In Paper III, type 1 bones (spine, ilium, and ribs) also had a priori higher turnover rates than type 2 bones (long bones and crania), particularly long bones such as the femur (Hedges et al., 2007). The higher Hg content in bone type 1 may be related to an increased exposure to mercury in the years before death, and it may be the main cause of variability. A large proportion of an adult's femur is formed during growth spurts (Hedges et al., 2007), and it could have been produced when the individual might have been less engaged in activities related to metal exposure (i.e., metallurgy). In the case of Emslie et al. (2019, 2015), they hypothesised that the higher mercury content in humeri, when compared to femora and tibiae, was due to bone remodelling rates, relating them to biomechanical regulation as a consequence of the use of heavy tools and other daily life activities. However, they did not explain why skeletal markers were more expressed in humeri than on lower limbs. Since they only analysed long bones

(humeri, femora, and tibiae), the comparison with small bones from thoracic and abdominal areas was not possible. We have proposed an alternative hypothesis: the secondary mercury enrichment of bones from the body trunk occurred during early *post-mortem*, due to the decomposition and collapse of the thoracic and abdominal soft tissues (note that during the breakdown of soft tissues the body mass creates anoxic conditions; Janaway et al. (2009)). Depending on the buried body position, ulna and radius may be parallel to the legs rather than over the abdomen (body position in A Lanzada included stretched arms; Lopez-Costas (2015), see also Figure.4). Therefore, the influence of the thoracic and abdominal area could be extended to the long bones placed near them, especially the humerus. Interestingly, in Paper III we found that L07.20 (radius over the thorax at the ∼L1 vertebra) and L06.22 (humerus) showed higher mercury concentrations than the average of bone type 2 (long bones and crania). The main target organs for mercury, when exposure is low and chronic, are placed in the abdomen and thorax (i.e., kidneys and liver) and lungs and digestive apparatus can also accumulate some mercury (Lech and Sadlik, 2004). Rasmussen et al. (2013c) also measured mercury content in the soil close to the kidneys, liver and lung ($n_{kidneys}$ = 3, n_{liver} = 4, n_{lungs} = 4 individuals) revealing the same trend.

Therefore in Paper I the sampling design most probably masked the thoracic effect on Hg content ruling out the *post-mortem* incorporation from soft tissues, as suggested by Rasmussen et al. (2017). However, in Paper II and III we showed that the thoracic area has a positive effect on mercury content, both in soil and bones. It is worthy to note here that the influence of this region varies among the thoracic bones; for example, the first rib/vertebra shows no influence while ribs/vertebrae closer to the kidneys area show this effect. This means that there is a gradient that can only be seen with a sampling design as that applied in Papers II and III, but that may escape a sampling design such as that applied in Paper I.

Bone structure indices

Bone IR indices related to hydroxyapatite mineral structure (IRSF and MMI) correlated negatively with mercury content, suggesting that mercury tends to be higher in newly-formed bone tissue, and lower in mature bone tissue. The relationship with the mineral maturity index (MMI) and the infrared splitting factor (IRSF) indicates that Hg content increased in bone areas that were recently created or remodelled (Farlay et al., 2010). Hydroxyapatite content and crystal size increase with bone maturity, negatively affecting Hg retention in the bone structure since large crystals have lower specific reactive surface for binding. As expected,

carbonate/phosphate (CP) and amide I/phosphate (AmP) ratios correlated positively with bone mercury. This is consistent with their negative correlation with IRSF and MMI. New-formed and remodelled bone tissues have a higher proportion of collagen fibres and higher rates of $C0_3/PO_4$ (Akkus et al., 2003), since they are less mineralised. Differences observed between new and remodelled bone (high mercury) and mature bone (low mercury) could respond to two different processes: i) an *ante-mortem* process: mercury is released or diluted due to bone tissues maturation and mineralization, since both large crystals and lower collagen content limit mercury retention; ii) a *post-mortem* process: mercury released during body decomposition is easily accumulated in new and remodelled bone due to its larger collagen content and smaller hydroxyapatite crystal size.

The indices IRSF and MMI showed no differences regarding individuals, consistent with previous research showing independence of the degree of bone mineralisation with respect to the age and sex of adults, and more mature individuals (Boivin and Meunier, 2002). Differences in the degree of mineralisation seem to be related to the age of the tissue (Bala et al., 2013). For bone tissue, the degree of mineralisation depends on the turnover rate (Boivin, 2007). However, differences were not found for IRSF and MMI between bone types 1 and 2, that have contrasted turnover rate (Hedges et al., 2007). There are no divergences in AmP between L06 and L07 skeletons, in agreement with studies from biopsies (Danielsen et al., 1994) and autopsies (Wang et al., 2002), in which differences in collagen structure - in non-pathological individuals - were only found for individuals older than ∼60 years. These studies indicated that changes in collagen are age-independent and that modifications in the turnover rate may exert some control. Pathological or age-dependent features like osteopenia modify the bone turnover rate and may alter collagen content (Bala et al., 2013; Paschalis et al., 1997). This fact could explain why AmP is higher in L06 and L07 compared to L01, as L01 was an elder woman with osteopenia and L06 and L07 were a juvenile and a mature adult with no visible paleopathological features. AmP is also higher in bone type 1 than in bone type 2, perhaps also related to their different turnover rates (Akkus et al., 2003), and the fact that long bones are largely mineralized with a thick cortex, especially those skeletal pieces which are related to locomotion. Some consideration may also be given to the micro-sampling strategy and to the fact that no metaphyseal areas were sampled for L06, so small differences between samples can be related to bone variability.

5.4 | The role of bone components in Hg content

The three PLS-SEM models in Paper III (G0, G1, and G2) were applied to improve the certainty of results. They showed high to moderate explanation of mercury variability in bone hydroxyapatite (> 90 %) and bone collagen (∼60 %). G1 and G2 showed differences in predictive power for soil mercury with 17 % and 60% explanation, respectively. Bone mercury had a non-significant effect on soil mercury in G1, but a significant positive effect in G2. Differences in bone ossification and surface chemical alteration (abrasion) were found to be significant factors in G1 and less significant in G2. G2 was the model most similar to G0, suggesting sample dominance in the general model (G0). The bone mineral phase had a significant impact on bone Hg in all models, while bone collagen was only relevant in G1. Soil mercury was affected by the bone mineral phase in opposite ways in G1 and G2. Thus, the POS models seem to capture intra-skeletal variability in mercury accumulation based on the weight of bone components.

On average, bones are composed of ∼70 % HAP, ∼20 % collagen (type I), and ∼8 % water per weight (Augat and Schorlemmer, 2006; Currey, 2008). In G0 and G2, hydroxyapatite (HAP) had a positive effect on bone mercury, while collagen was not relevant. In contrast, in G1, HAP effect has a negative effect and collagen was the dominant factor controlling bone mercury variability. This suggests that the different PLS-POS groups responded to differences in the bone fraction controlling mercury content. Therefore, the organic fraction (i.e., collagen) was the main factor controlling in G1, while the inorganic fraction (i.e., HAP) was the main factor dominating the mercury content in G2.

This difference in the bone fraction controlling bone mercury content reflects a different behaviour related to the *ante-mortem* status of the bone tissue structure. Bones of type 1 are expected to have higher turnover rates, thereby implying that the proportion of HAP/collagen will be lower than in group 2 (Akkus et al., 2003). Thus, small increases in collagen in type 1 bones could have a larger impact on mercury accumulation, emphasizing the contribution of the less abundant component. Mercury tends to bind to soft bases like S, P, and N; bonds with S-containing groups being especially stable (Schuster, 1991). HAP can stabilise Hg as $(Hg)_3(PO_4)_2$ (Cervini-Silva et al., 2021). Bone collagen structure is characterised by a repeating amino acid motif (Gly-X-Y), where X and Y can be any amino acid (Hulmes, 2008). All amino acids contain N-groups, but Cysteine (-SH, Cys) and Methionine (-S-CH_3, Met) also contain S-groups. The amount of Cys and Met per 100 g of collagen in bovine bone were estimated to

be 0.32 g and 1.07 g, respectively (Gauza-Włodarczyk et al., 2017). Hence, there is the potential to form Hg-S compounds, with an origin that may be either *ante-* or *post-mortem*. To our knowledge, the mechanisms of mercury incorporation into the bone during life are currently unknown, but this pathway cannot be ruled out. Meanwhile, during the main phase of soft tissues breakdown in the *post-mortem* span, the reducing conditions needed to form Hg-S compounds are met (Janaway et al., 2009; Schuster, 1991). The presence of compounds that bind mercury strongly is consistent with the fact that the pathway from bone mercury to soil is not significant when collagen controls bone mercury content, i.e., the release of mercury to the soil may be very low until the collagen structure is strongly degraded.

The models suggest a complex relationship between bone hydroxyapatite crystallinity (HAPc) and bone Hg, with the relationship being both negative and positive in the three models. This suggests that HAPc plays a dual role in the accumulation of Hg in bone tissue. The models suggest that HAPc affects both bone Hg release (primary/secondary source) and retention/accumulation (sink; we cannot discern retention of bone *ante-mortem* content from mercury incorporated as the result of the interaction with soft tissues during their decomposition). This can be related to the potential of the skeleton to be both primary/secondary source and sink (Paper II). In archaeological bone, as hydroxyapatite crystallinity increases bone structure is altered (Nielsen-Marsh and Hedges, 2000) and, thus, as found in Paper III, a negative relationship with collagen and bone hydroxyapatite structure is expected. In G1, HAPc has a direct negative effect on bone mercury, as bone mercury is controlled by collagen. But there is also a slightly significant positive indirect effect, related to HAPc as a proxy of bone alteration. Despite bone mercury being controlled by collagen, it is very likely that HAP also contains mercury and as HAP is altered, bone mercury will be lost. In G2, HAPc has a direct positive effect on bone mercury, reflecting that HAP controls mercury content in this group. When considering the *ante-mortem* condition of these bone samples, the higher the HAPc, the higher the proportion of HAP (Akkus et al., 2003). However, when HAPc increases as a result of *post-mortem* bone alteration, any accumulated mercury in HAP can be released.

5.4.1 | Osteoarchaeological implications

Intra-skeletal variability of mercury in archaeological skeletons can provide valuable insights into the fate of this pollutant when people were alive, as well as any potential occupational and environmental exposure, use of mercury-containing medicines, use of mercury-rich artefacts, dietary factors, poisoning and funerary rituals involving mercury painting. Previous studies

have been conducted in this area, such as Alexandrovskaya and Alexandrovskiy (2005); Álvarez Fernández et al. (2020); Ávila et al. (2014); Bocca et al. (2018); Cervini-Silva et al. (2013); López-Costas et al. (2020); Ochoa-Lugo et al. (2017); Panova et al. (2018); Rasmussen et al. (2017, 2013c); Walser et al. (2019); and Yamada et al. (1995). However, in order to understand exposure, it is necessary to better comprehend the mercury dynamics in the burial environment.

Understanding the role of diagenesis is foremost in osteoarchaeological studies as the soil is a potential source for major, minor and trace elements to bone (e.g. Emslie et al. (2019); López-Costas et al. (2016)). Previous works report that, unless soils are naturally enriched, bone mercury is not related to diagenesis (Emslie et al., 2015; Kepa et al., 2012; Rasmussen et al., 2008; Walser et al., 2019; Yamada et al., 1995). Our results (Paper II and III) suggest that human bodies are, indeed, sources of mercury to the soil environment - and possibly the main source in necrosols (soils formed due to funerary deposit). Furthermore, our results also indicate that not all mercury in archaeological bone comes from *ante-mortem* accumulation, as there is a fraction that is incorporated *post-mortem* due to the release of mercury from soft tissues during body decomposition. This factor was only considered before for trabecular bone (Rasmussen et al., 2017, 2008), and the secondary enrichment should be taken into account when comparing Hg data obtained from archaeological skeletons (decomposed in situ) with those obtained from autopsies and biopsies, since no or minimal decomposition took place on the latter.

Despite this issue, mercury contents in archaeological bones can still be representative of *ante-mortem* exposure, as all mercury came from the studied individuals. Given that the accumulation of this metal is tissue-dependent (Berlin et al., 2015), it needs to be kept in mind that intra-skeletal variability exists and it is related to bone turnover (*ante-mortem*) and to the proximity of the bone to target organs (*post-mortem*). Rasmussen et al. (2013b) approached mercury heterogeneity in the skeleton by analysing several samples in two individuals (n = 73), concluding that mercury was distributed almost uniformly in compact bone. Their study was mainly focused on cortical pieces of long bones (n = 59). The authors attributed the higher mercury content found in the thoracic/pelvic bones (n = 14) to the presence of higher trabecular bone on the samples. Our study develops this idea including a detailed statistical design and knowledge of the bone structure and composition, allowing us to show that mercury dynamics in the burial environment is complex and may vary depending on the *ante-mortem* status of the bone matrix and proximity to the target organs.

5.5 | Hg distribution in the burial environment

The low mercury concentrations of the fine earth samples of the soil/sediment are in agreement with previous studies in non-polluted soils (do Valle et al., 2005; Fiorentino et al., 2011; Gómez-Armesto et al., 2018; Roulet et al., 1998) and are consistent with the predominantly sandy grain size of the samples and the absence of mercury mineral phases. The silt+clay fraction, on the other hand, is usually naturally enriched in organic matter and metals (Qin et al., 2014).

The PLSR model obtained for the silt+clay fraction revealed three main underlying processes (i.e., latent variables; LV) that account for a significant proportion (> 72 %) of the variation in mercury concentrations in the two burials. The difference in mercury concentrations between inside and outside the burials explained the largest amount of mercury variability (LV1). Followed by the differences between the two burials (LV2) and the burial variability related to the proximity of the soil samples to areas where larger body mass (i.e., thoracic) was placed (LV3). Residuals showed no-clear pattern and can be linked to micro-scale soil variability.

The LV1 variables with positive loadings (Cu, Zn, N) are related to the abundance of organic matter in the silt+clay fraction. Copper and Zn are known to bind to organic matter (OM) and clay (Acosta et al., 2011) and N can be related to the total amount of OM (Janaway et al., 2009; Obrist et al., 2009). Indicators with negative loadings included elements related to carbonate content (Ca and Sr) that can be linked to the presence of mollusc shell remains (note that the area is by the sea), and location within the burial (module). The positive regression coefficient for this LV indicates that mercury content increases with organic matter content, which is clearly higher inside the burials. Many investigations have shown that mercury in soils and sediments is correlated to organic matter content (Cooke et al., 2020; Schuster, 1991). This can be seen as a sort of fingerprint of the individuals that also affected the mercury content. The buried bodies most probably were both the source of the organic matter (together with the wood coffin) and the source of the mercury, which has accumulated in the finest and most reactive soil fractions (i.e., OM and clay minerals). The original substrate is a dune dominated by silicic sand with biogenic carbonates that lacks the capacity to retain mercury. This is consistent with the low Hg concentrations in the samples outside the burial environment (both in the fine earth: median = 1.6, IQR = 0.8 ng g^{-1}; and the silt+clay fraction: median = 12.1, IQR = 3.6 ng g^{-1}).

The LV2 indicators with positive loadings are P, C, and Ca, all constituents of the bone mineral component (i.e., hydroxyapatite), and S, which is a proxy of body decomposition. Among the indicators with negative loadings, Ti is indicative of soil fine fractions (i.e., clay) (Taboada et al., 2006a). Iron is representative of major soil minerals (e.g., biotite), but also increases in the clay fraction as secondary oxy-hydroxides. LV2 scores are systematically positive in the double burial, T5, with the exception already mentioned before (distal samples in the L07 transect). This means that the fine soil fraction has higher concentrations of elements typical of the bone mineral matrix and S containing compounds than the same fraction in T1 burial. This difference most probably reflects the fact that T5 contains two individuals (an adolescent and a mature adult) - with a priori higher bone and soft tissue mass interacting with the soil components - and T1 contains only one individual (a single burial). The weight of LV2 in the samples is systematically positive in L06 individual, while it is negative in L01 and shows an irregular pattern in L07. The regression coefficient for LV2 is positive meaning that mercury content is higher in T5 burial compared to T1. The link between mercury and the other elements can be chemical bone alteration and sulphur compounds availability during early body decomposition.

Sulphur-containing amino acids (i.e., cysteine, cystine, and methionine) are subjected to desulphydralation by gastrointestinal microorganisms (i.e., sulphate-reducing bacteria) during body decomposition, producing the release of hydrogen sulphide gas, sulphides, ammonia, thiols, and pyruvic acids. Given that during the main phase of tissues breakdown the tissue mass keeps anoxic (Janaway et al., 2009) and Hg tends to precipitate as its sulphide form (Schuster, 1991), HgS compounds can be expected in these environments. Rasmussen et al. (2015) also hypothesised about this mechanism, but provided no explanation for it. HgS compounds have extremely low solubility (Schuster, 1991), preventing from lixiviation and contributing to Hg retention in the burial environment. Thus, soft tissue decomposition may play a role in Hg release, as primary source, but is also key in providing compounds involved in Hg retention and accumulation in the burial environment.

Bone tissue acts as a sink for some elements during body decomposition (Hedges, 2002) and then as a source to the burial environment, altering the elements that are released and accumulated in the finer, more reactive, soil fractions. Indeed, bone is a potential primary and/or secondary source of mercury to the soil of the burial (i.e., nercosol); while soft tissues are the primary source both to soil and bone. A fact that is in agreement with the recent finding that

mercury can be stabilised in bioapatite as $(Hg)_3(PO_4)_2$ (Cervini-Silva et al., 2021). Furthermore, it could explain why mercury concentrations in archaeological bones are higher than the concentration found in actual bones from autopsies and biopsies (Babuśka-Roczniak et al., 2021; Domingo et al., 2017) - although lower contents in recent bone can also be due to lower exposure as today environmental loads are much more controlled. The higher the bone mass the higher the transfer of mercury to the soil. Rasmussen et al. (2008) also suggested that mercury distribution in the soil could be affected by burial context when graves are placed closed by as interactions between them cannot be excluded. In our case, T1 and T5 burials were far apart (∼4 - 5 m) and with no other graves over or close to them. Another factor that should also be considered is that the overall mercury content of the individual in T1 compared to that of the individuals of T5 may have been different depending on environmental exposure during life. In Paper I, bone mercury content for individuals of the same period as those studied here varied between 4.6 and 300 ng g^{-1}.

The LV3 indicators with positive loadings are U and S. Uranium can be related to the geology of the area and has a high affinity for bone phosphates (López-Costas et al., 2016; Taboada et al., 2006b), while S is a proxy for early stages of body decomposition, as previously mentioned in LV2 interpretation. Indicators with negative loadings are P, an indicator of bone alteration (López-Costas et al., 2016), and Mn, a proxy for redox conditions (Windmöller et al., 2015). Most samples with positive LV3 scores are those collected in the thoracic area of the three skeletons. The weight of LV3 tends to be positive in samples of burial T5 while it does not show the same pattern in burial T1. This can be reflecting differences between burials. As already commented, the most important mercury target organs (kidneys, liver) are located close to the thoracic area (Berlin et al., 2015), which also contains the higher body mass - in comparison for example with limbs or head. Thus, differences in mercury content between soil close to the thoracic area and soil close to the rest of the body would be expected. Furthermore, proteins forming the kidneys and liver start its putrefactive changes at early stages of body decomposition, while epidermis and muscles are more resistant to breakdown (Janaway et al., 2009). It is also likely that the higher content of Hg in this area is related to the higher availability during early body decomposition of both mercury and S compounds. Since the process takes place in the earliest stages of body decomposition the non-putrefied tissues would produce a confined environment and decrease mercury leaching. The negative loadings of P in this LV may also support this interpretation, as the soft tissue/bone ratio should also be higher in this body area and thus with lower net transfer of P to the soil. We speculate

that the "thoracic area" effect may be even more pronounced in graves with high mercury levels (i.e., periods with higher mercury levels of pollution and/or individuals which suffered an acute intoxication, among other causes). As already commented, the model residuals showed no clear pattern and can reflect soil microscale heterogeneity.

Diagenesis is of major concern when elemental composition is studied in archaeological bone, as there is the potential for the soil to be a source of elements for bone (Emslie et al., 2019; López-Costas et al., 2016). However, works which assessed mercury diagenetic incorporation into bone in archaeological burial environments concluded that mercury bone concentrations were not related to diagenesis, unless the soils were naturally enriched in mercury (Emslie et al., 2015; Kepa et al., 2012; Rasmussen et al., 2008; Walser et al., 2019; Yamada et al., 1995). In Paper I we reported very low mercury contents in soil (a pedo-sedimentary sequence provided an average of 0.9 ± 0.7 ng g^{-1}) in comparison to bones of individuals of the same period as those of the present study (20.6 ± 23.5 ng g^{-1}). Our PLSR model also supports the idea that the soil was not a significant source of Hg for buried bones. The situation seems to be rather the opposite; the buried individuals were most probably the source of the mercury accumulated in the soil. In agreement, we included an interaction from the "Hg bone" to the "Hg soil" when building the SEM-PLS model in paper III. The decomposition of the buried bodies seems to have triggered a number of processes - developing a positive feedback mechanism - that led to the rapid evolution of the parent soil material (i.e., sand dune), contributing to mineral weathering (i.e., decarbonation), as well as clay formation and organic matter enrichment (García-López et al., 2022), and finally creating the necessary conditions for the retention of the mercury released during the decomposition of the target organs (i.e., liver and kidney).

5.6 | Bone-Necrosol interaction and Hg content

Most soil primary silicates lack the capacity to retain mercury (Schuster, 1991); a fact that is consistent with their dilution effect on bone and soil mercury content in the PLS-SEM models (Paper III). The higher the amount of primary silicates the fewer soil components with the potential to retain mercury. The silt+clay fraction is a reactive component of the soil and tends to be enriched in metals (Qin et al., 2014). Therefore, a higher silt+clay content leads to an increase in mercury concentrations (Álvarez Fernández et al., 2021; Cooke et al., 2020; Schuster, 1991) that limits the amount of mercury that remains available to be stabilised in bone. However, the weight of the clay in the three PLS-SEM models (G0, G1, and G2) is low

(-0.13 G0, 0.01 G1, -0.17 G2), what is more, it has a slightly negative effect on bone mercury content.

Bone mercury shows a significant direct positive effect on soil mercury in G0 and G2. This supports the results of Paper II which indicate that skeletons are potential sources of mercury to the soil/sediments in the burial environment. It also supports the contention that bone mercury concentrations are not diagenetic when the geological materials of the area do not contain mercury ores, as it was found in several investigations on archaeological skeletons (Emslie et al., 2015; Kepa et al., 2012; Rasmussen et al., 2013b; Walser et al., 2019; Yamada et al., 1995). This is also likely the case for the burials studied here, because soil/sediment mercury concentrations are on average 0.9 ± 0.7 ng g^{-1} in the dune sands layer where the skeletons were buried (Paper I). The models also suggest that the crystallinity of the mineral component of bone (HAPc) indirectly controls the release of bone mercury from the hydroxyapatite (HAP) into the soil/sediment.

Location (i.e., the sample position regarding the pelvic area) has a negative effect both on bone and soil mercury content. Samples close to the thoracic/abdominal area had higher mercury concentrations compared to those far away. This is consistent with what has been already discussed in section 5.5, and related to the location of the main target organs for mercury in the thoracic/abdominal area (Berlin et al., 2015; García et al., 2001). It is known that bones may act as sink of some elements during body decomposition and then release them to the burial environment (Hedges, 2002). The relationship between location of the sample and bone mercury content strongly suggests that bones act as a temporary sink for mercury.

5.7 | The role of skeletons and cemeteries in the mercury cycle

Humans are exposed through culture and/or environment to mercury, incorporating this element to their bodies during life (UNEP, 2019; WHO, 2022). This makes us a temporary sink at least until our death. At that time, the funerary ritual will determine the fate of this mercury. Cremation will release mercury to the atmosphere and it was already pointed out as an issue for mercury emissions (Mari and Domingo, 2010). In the case of inhumation rituals, mercury interaction with the buried environment (i.e., the body, buried items such as the wooden coffin, and the soil) will condition this metal dynamic. Papers I, II, and III showed that even after ~2000 year there is still mercury in the skeletons and the burial soils. Therefore, these environments can act as sinks of mercury unless they are perturbed. Research in contempo-

rary cemeteries have shown that the soils used with this purpose are significantly enriched in mercury compared with nearby ones (Amuno, 2013; Jonker and Olivier, 2012; Mohammed and Abudeif, 2020; Prestes da Silva et al., 2020; Spongberg and Becks, 2000; Uslu et al., 2009; WHO, 1998). In Paper II we report the same results and confirm that this difference persists over long timescales, i.e., centuries. Indeed, burial soils can play a role as sinks of mercury for relatively long time spans.

In order to develop this role, the body decomposition process is key as it can create the necessary conditions to generate bonds between mercury and sulphur, creating very stable compounds (Janaway et al., 2009; Schuster, 1991). The sulphur compounds, possibly related to body's amino acids, like methionine and cysteine, are able to contribute to Hg fixation. A necessary step in future research will be to analytically find these HgS compounds. HgS compounds are highly stable, limiting mercury release to the environment - even when the total amount is small. Therefore, HgS compounds can help to prevent mercury release in individuals/cemeteries with high mercury concentrations.

Furthermore, Papers I and III together with other research in osteoarchaeological remains (Alexandrovskaya and Alexandrovskiy, 2005; Ávila et al., 2014; Biehler-Gomez et al., 2021; Bocca et al., 2018; Emslie et al., 2022, 2019, 2015; Lombardo et al., 2022; Ochoa-Lugo et al., 2017; Panova et al., 2018; Rasmussen et al., 2020, 2017, 2015, 2013a, 2012, 2008; Torino et al., 2015; Walser et al., 2019) show that skeletons also have a role as sinks for relatively long timespans. Paper III reported a double role of bones as they can be acting as sources and sinks in the burial environment (Figure.1), something that has to be considered when interpreting mercury behaviour.

6 | CONCLUSIONS

This research has confirmed osteoarchaeological human remains as suitable archives of human exposure to mercury in the past. Furthermore, it has highlighted some aspects that should be considered when researching mercury in archaeological skeletons.

1) Archaeological bones can be considered a suitable environmental archive of mercury pollution when mercury cultural exposure is low (Paper I). Mercury content measured in osteoarchaeological remains is possibly more complex than previously thought, with different *ante-* and *post-mortem* sources interacting. The literature presents the use of cultural items rich in Hg as the major source of exposure in past populations. But in A Lanzada the more likely source of mercury exposure was the diachronic variation in – regional to local – atmospheric Hg pollution. The analyses discard the soil as source of mercury and show significant differences in Hg content between Roman and post-Roman populations, two periods with contrasting Hg environmental concentrations in NW Iberia. But more research is needed to consolidate the use of this archive in different environments and periods, or in populations with stronger cultural dependence of mercury. Therefore, we recommend broadening the research of mercury content in archaeological human bones to all kinds of necropolises even if they are far from mines or urban areas, since low-dose chronic pollution, when is researched with other bone properties, can be used as a good proxy for environmental pollution along History.

2) Intra- and inter-skeletal variability in mercury content is affected by *ante-mortem* exposure (Paper I and III). Inter-skeletal differences may be affected by non-easily approached characteristics, such as body mass index, life habits, and occupational activities. Environmental exposure determines mercury inter-skeletal variability, as those individuals exposed to higher environmental mercury content show higher mercury concentrations. Intra-skeletal variability is dependent on the soft tissues; bones and soil/sediment near the thoracic area display higher mercury concentrations. The sampling design may mask this effect, as there is a gra-

dient among those bones affected by the thoracic region. Therefore, it is important to have this fact in mind when designing the sampling, depending on the objectives of the research - as well as clearly specify the selected sampled bone. More research is still needed to fully understand intra- and inter-skeleton variation, especially in bones located close to kidneys (main body mercury reservoir) and including more sensitive population strata, i.e., babies, a more detailed study of the outliers, as well as other skeletal parts such as teeth. We also encourage not to compare directly the mercury content in bones close (e.g., humerus) and far (e.g., tibia, distal femur) from the thoracic area, without considering the possible bias due to the proximity to Hg target organs.

3) Bone components affect mercury content in bone (Paper III). Bone *ante-mortem* structure influences the availability of mercury in the buried skeletons and the role of bone components (i.e., collagen and hydroxyapatite) in mercury retention depends on the degree of bone crystallinity that may change *ante-mortem* and *post-mortem*.

4) Mercury distribution in burial soils/sediments (i.e., Necrosol) is influenced by human bodies (flesh and bone) for two reasons: 1) they are a direct mercury source to the soil, 2) they are a source of organic matter which also contributes to mercury fixation. The burial context affects mercury distribution; we found differences that could be explained by the number of individuals buried together. The body mass (i.e., soft tissues and bones) seems also to be related to mercury content in the burial soil. The thoracic and abdominal area produces a further local enrichment in Hg, most probably due to its release from target organs and its retention in the reactive soil fractions (organic matter and clay). The obtained findings remark the importance of analysing soil/sediment associated to the burials in order to provide complementary information that can be key to understand some processes. Regarding the analytical procedure, we encourage to analyse the finer (silt + clay) soil fractions when researching sandy soil/sediments, because this fraction is the most reactive and has better potential to contain information related to the body/soil interactions. In addition, the study of individuals/sites with low mercury levels can produce high quality results and complement those made in high polluted environment. Furthermore, according to our results, soil/sediments from cemeteries can be significantly enriched in mercury compared to nearby ones. A fact that should be taken into account when describing soil properties or when those areas are affected by land use changes (e.g., a new agrarian use). In addition, to assess the mercury content of an archaeological soil from a necropolis the proximity to the thoracic area of the skeletons can bias

the obtained results (Paper II and III).

5) The archaeological skeleton acquired the mercury during the life of the individuals (primary path), but also during early *post-mortem* decomposition of body tissues (secondary path), acting both as source and sink to the burial soils/sediments. In addition, soil/sediment components seem to play a minor role on bone mercury content in archaeological human skeletons, in the absence of mercury containing minerals. The importance of bone as a primary source of mercury to the soil is related to *ante-mortem* bone structure (degree of bone maturity, e.g., collagen content), while its function as secondary source/sink is associated with hydroxyapatite crystallinity. Further research is needed to elucidate how bone components control mercury behaviour in burials (Paper II and III).

6) Skeletons and burial soils play a role in mercury cycle as sinks for relatively long time spans. The burial conditions will determine the fate of mercury. Further research in mercury on soil/sediments associated to skeletons could help to achieve a better understanding of how mercury is distributed in these environments. Other questions such as how different burial contexts can affect mercury distribution in graves and how soft tissues affect it, remain open. This research highlights the importance of understanding the dynamics of potential pollutants on burial environments (cemeteries/graves), even when the environmental exposure to the pollutant and the number of bodies buried in the 'cemetery' was low. It also highlights the relevance of understanding how body tissues contribute to mercury cycling in burials, as they can be sources and sinks simultaneously. The results also suggest that when land use changes occur in former burial areas, this can affect human or ecosystems health due to the release of contaminants, such as mercury. This strengthens the importance of knowing how land-use, cemeteries in particular, were distributed in the past to also understand how they are affecting the present.

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9 | PAPERS

Paper I

Atmospheric mercury pollution deciphered through archaeological bones.

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CONTRIBUTION

Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing – Original Draft, Visualization, Supervision, Project administration.

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Paper II

Approaching mercury distribution in burial environment using PLS-R modelling.

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Paper III

Structural equation modelling of mercury intra-skeletal variability on archaeological human remains.

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CONTRIBUTION

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The relationship between humans and mercury pollution is investigated from a paleo-pollution perspective. We study the mercury content variability in Roman and post-Roman individuals, the skeletal mercury variability, the role of bone components in bone mercury content, the burial soil mercury distribution and the processes behind it, the bone-soil mercury relationship, and the role of skeletons and burial soil in mercury cycle. We confirmed skeletons as suitable paleo-archives, bodies as sources of mercury to the soil, that ante-mortem exposure affects intra- and inter-skeletal mercury variability, that context and location affect mercury burial distribution, the ante- and post-mortem origin of skeletal mercury, the minor role of soil on bone mercury, and that skeletons and burial soils play a role in mercury cycle.