UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

Evaluación de la utilidad de la gaviota patiamarilla (*L. michahellis*) en estudios de contaminación ambiental: análisis de contaminantes y biomarcadores.

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Doctorado en Salud Pública y Animal

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Índice

Resumen.

La posibilidad de evaluar las concentraciones de metales pesados y de contaminantes orgánicos persistentes en el medio ambiente y en los seres vivos, así como los potenciales efectos de estas sustancias en los organismos, desde el punto de vista toxicológico, es de especial relevancia para mantener un control sobre la Salud Ambiental. El objetivo principal de la presente tesis es la evaluación de las concentraciones de metales pesados y metaloides (Hg, Cd, Pb, Se y As) y de contaminantes orgánicos persistentes bioacumuladas en muestras de gaviota, más concretamente en la especie *Larus michahellis*. Las muestras provinieron de tres zonas del noroeste de España: Pontevedra, A Coruña y Gijón. También se ha realizado una profunda y exhaustiva revisión bibliográfica, donde se han recopilado numerosas investigaciones en las que se han determinado concentraciones de metales pesados en hígado, riñón, y pluma de diferentes especies de gaviotas y de otras aves marinas por todo el mundo. Los datos obtenidos son de gran relevancia, dado que permiten conocer si las concentraciones acumuladas en los animales de estos contaminantes podrían ser lo suficientemente elevadas como para poder producir efectos dañinos en los mismos. Para evaluar este efecto nocivo se ha determinado la posible alteración de los niveles de seis biomarcadores de estrés oxidativo (MDA, GSH, GST, CAT, GR y GPx). Tanto los niveles de contaminantes como los niveles de los biomarcadores fueron evaluados en diversos tejidos y órganos (hígados, riñones, tejido adiposo y plumas), permitiendo identificar cual de ellos podría mostrar una mayor sensibilidad, así como evaluar la utilidad de muestras no invasivas (ej. plumas) frente a los órganos internos para realizar estudios de biomonitorización. Además de cuantificar los niveles de contaminantes en los animales, se ha tratado de identificar la implicación del sexo y la edad, como factores endógenos, y la zona de muestreo, como factor exógeno, en los patrones de bioacumulación, así como en los potenciales efectos aparecidos en los biomarcadores de estrés oxidativo. Los resultados obtenidos muestran que la edad pudo ser relevante al realizar el estudio, ya que se encontraron diferencias estadísticamente significativas de los individuos adultos con respecto a los pollos, encontrándose concentraciones más elevadas en los individuos adultos en la mayoría de los contaminantes estudiados, tanto en los metales (Hg y Cd en hígado y Hg, Cd, Pb y Se en riñón) como en los contaminantes orgánicos persistentes (PCBs 28, 101, 138, 153 y 180, y 4,4´-DDE). Sin embargo, el sexo pareció no ser tan influyente a la hora de realizar el estudio, ya que fueron muy pocos los casos en los que se encontraron diferencias significativas entre machos y hembras. En concreto, se hallaron dichas diferencias en dos casos, en uno de ellos relacionadas con en el Hg acumulado en las plumas y en el otro en relación con el contaminante 4,4´-DDE en el tejido adiposo de las gaviotas, en ambos casos los niveles fueron superiores en las hembras que en los machos. Tras determinar las concentraciones de los contaminantes evaluados y realizar el estudio de las posibles correlaciones con los biomarcadores de estrés oxidativo, se puede concluir que los niveles de los contaminantes en los animales evaluados no fueron lo suficientemente elevados para causar daños serios en estos animales.

Palabras clave: Gaviota, metales, biomarcadores, contaminantes, contaminates organoclorados, sexo, edad, zona de muestreo.

Abstract.

The possibility of evaluating the concentrations of heavy metals and persistent organic pollutants in the environment and in living beings, as well as the potential effects of these substances on organisms, from a toxicological point of view, is of special relevance for maintaining control over Environmental Health. The main objective of this thesis is to evaluate the bioaccumulated concentrations of heavy metals and metalloids (Hg, Cd, Pb, Se and As) and persistent organic pollutants in seagull samples, more specifically in the species Larus michahellis. The samples analysed came from three areas in northwest Spain: Pontevedra, A Coruña and Gijón. A deep and exhaustive bibliographic review has also been carried out, where numerous investigations have been identified reporting concentrations of heavy metals in the liver, kidney, and feather of different species of seagulls and other seabirds throughout the world. This data is of great relevance as it allowed us to identify if the concentrations accumulated in the animals of these contaminants were at high enough levels to cause harmful effects. To evaluate this harmful effect, possible alterations in the levels of six oxidative stress biomarkers (MDA, GSH, GST, CAT, GR and GPx) have been determined. Both the levels of contaminants and the levels of the biomarkers were determined in various tissues and organs (liver, kidneys, adipose tissue and feathers). This allowed us to identify which sampled tissue/organ showed greater sensitivity, as well as to evaluate the usefulness of non-invasive samples (e.g. feathers) compared to internal organs in biomonitoring studies. In addition to quantifying the levels of contaminants in the animals, an attempt has been made to identify the involvement of age and sex as endogenous factors, and the sampling area as an exogenous factor in the bioaccumulation patterns and effects on biomarkers of oxidative stress. The results obtained show that age seemed relevant at the time of the study, since statistically significant differences were found between adult individuals and chicks. Indeed, high concentrations were found in adult individuals for most of the contaminants studied, including metals (Hg and Cd in liver, and Hg, Cd, Pb and Se in kidney), and persistent organic pollutants (PCBs 28, 101, 138, 153 and 180, and 4,4´-DDE). However, sex did not seem to be so influential when carrying out the study, since there were very few cases in which significant differences were found between males and females. Specifically, these differences were found in two cases, in one of them related to the Hg accumulated in the feathers and in the other in relation to the contaminant 4,4'-DDE in the adipose tissue of the seagulls. In both cases the levels were higher in females than in males. After determining the concentrations of the evaluated contaminants and carrying out the study of possible correlations with the biomarkers of oxidative stress, it can be concluded that the levels of the contaminants in the evaluated animals were not high enough to cause serious harmful effects in these animals.

Keywords: Seagull, metals, biomarkers, pollutants, organochlorine contaminants, sex, age, sampling area.

Presentación de la tesis como compendio de publicaciones.

La presente tesis doctoral, que lleva por título "Evaluación de la utilidad de la gaviota patiamarilla (*Larus michahellis*) en estudios de contaminación ambiental: análisis de contaminantes y biomarcadores" realizada dentro del Programa de Doctorado "Salud Pública y Animal" se presenta como compendio de publicaciones, de acuerdo con el artículo 33 de la Resolución de 14 de diciembre de 2021, del Rector, por la que se ejecuta el acuerdo adoptado por el Consejo de Gobierno por el que se aprueba la Normativa de Doctorado de la Universidad de Extremadura. Por acuerdo del Consejo de Gobierno con fecha 20 de julio de 2021 se aprueba el nuevo Reglamento de la Escuela Internacional de Doctorado, por lo que procede a adaptar la normativa a la atribución de las competencias en materia de doctorado establecidas en el Real Decreto 99/2011, a la Escuela Internacional de Doctorado (DOE 28 de diciembre de 2021).

Las publicaciones que se presentan recogen todos los resultados que han sido obtenidos en los diferentes trabajos de investigación desarrollados con el fin de alcanzar los objetivos marcados en la presente tesis y que se engloban en cuatro artículos publicados, los cuales se comentan a continuación:

1. "Mercury (Hg), Cadmium (Cd), Lead (Pb), Selenium (Se), and Arsenic (As) in Liver, Kidney, and Feathers of Gulls: A Review".

En esta primera publicación se ha realizado una exhaustiva recopilación de los datos de diferentes artículos publicados en los últimos años del siglo XX y las primeras dos décadas del siglo XXI, en referencia a los niveles de cinco metales (Hg, Cd, Pb, As y Se) aparecidos en tres tejidos (hígado, riñón y pluma) en numerosas aves marinas (específicamente especies de gaviotas) a lo largo de todo el planeta. El objetivo de la revisión bibliográfica es generar una base de datos a la que poder recurrir cuando se pretendan realizar futuros estudios de biomonitorización. Una de las conclusiones extraídas de la revisión crítica de los datos define la edad como un factor intrínseco relevante en la biomonitorización de metales.

2. "Heavy metals and metalloid levels in the tissues of yellow-legged gulls (*Larus michahellis*) from Spain: sex, age, and geographical location differences".

La segunda publicación de la presente tesis versa sobre el estudio de monitorización de metales y metaloides en gaviotas patiamarillas. En esta publicación se ha determinado la concentración de cinco elementos metálicos (Hg, Cd, Pb, Se y As) en hígado, riñón y plumas de *Larus michahellis* procedentes de tres zonas del noroeste de España (A Coruña, Pontevedra y Gijón), evaluando si factores como el sexo, la edad o la zona geográfica pueden influir en la acumulación de dichos contaminantes. Las concentraciones de metales detectadas durante el presente estudio se encontraron siempre dentro del rango, o incluso por debajo, de los niveles encontrados para otras gaviotas, siendo niveles que no llegaron a provocar letalidad en los animales seleccionados para este estudio. Con respecto a los tres factores analizados, se ha comprobado que el sexo puede influir significativamente, dado que se han encontrado mayores niveles de Hg en pluma de machos que de hembras. Este hecho, puede estar justificado por la posible transferencia de Hg de la madre a los huevos. Otra posible explicación para estas diferencias podría ser el tamaño más pequeño de las hembras, implicando una menor ingesta y, por tanto, adquiriendo concentraciones más bajas de Hg. Los resultados también parecen señalar una asociación entre

la zona geográfica donde habitan las aves y la acumulación de varios metales en los órganos analizados (hígado y riñón). Así, se pueden observar diferencias en todos los metales/metaloides analizados entre unas zonas y otras. Los mayores niveles de metales hallados en ambos tejidos procedentes de animales de Pontevedra, comparados con los de A Coruña y Asturias, sugieren una mayor contaminación de dicha área con respecto a las otras estudiadas.

3. "Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (*Larus michahellis*) from Spain: role of gender and age".

La tercera publicación se ha centrado en la determinación de la concentración de contaminantes clorados en gaviotas. Los compuestos analizados fueron siete congéneres de bifenilos policlorados (PCBs) (PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180) y once plaguicidas organoclorados (OCPs), incluyendo metabolitos (4,4´-DDE, HCB, Hepatocloro epóxido, 4,4´-DDD, Endrin, Endosulfán, γ-HCH, Endosulfán sulfato y Dieldrín). Todos ellos fueron evaluados en el tejido adiposo de las gaviotas en las se analizaron los niveles de metales/metaloides, considerando igualmente la posible influencia del sexo y la edad de los animales. Únicamente se encontraron diferencias significativas en uno de los contaminantes: 4,4'-DDE, donde los niveles en tejido adiposo de hembras fueron significativamente superiores que en el de machos. La edad también influyó en los resultados, puesto que los niveles de PCBs 180, 138, 101, 28 y 153, así como del pesticida 4,4´-DDE, fueron significativamente superiores en animales adultos que, en los jóvenes, pudiendo establecer una relación directamente proporcional entre los niveles de COPs y la edad del ave.

4. "Biochemical effects of heavy metals and organochlorine compounds accumulated in different tissues of yellow-legged gulls (*Larus michahellis*)".

La tesis finaliza con la cuarta publicación, en la cual se han evaluado los niveles de seis biomarcadores de estrés oxidativo (MDA, GSH, CAT, GST, GR y GPx) en hígado y riñón de gaviotas, estudiándose también posibles relaciones con las variables edad, sexo y zona de muestreo. Las asociaciones entre los efectos y los niveles de los contaminantes mencionados en las anteriores publicaciones también han sido estudiadas. El objetivo de esta publicación fue determinar si las concentraciones de contaminantes, tanto inorgánicos como orgánicos, determinadas en las gaviotas eran suficientemente altas como para dar lugar a un efecto a nivel bioquímico. Como resultado, se comprobó que las concentraciones de los metales y los PCBs detectadas en los diferentes tejidos de la gaviota patiamarilla no fueron lo suficientemente elevadas como para poder causar alteraciones significativas en los biomarcadores de estrés oxidativo. De las variables estudiadas, exclusivamente la zona de muestreo pareció influir significativamente en los niveles de los biomarcadores de estrés oxidativo. También se estudiaron las posibles correlaciones entre los contaminantes y los biomarcadores, encontrando una asociación entre el Hg y la actividad enzimática GST en el hígado y, en segundo lugar, entre el As y las actividades enzimáticas GPx y GR en el riñón, siendo en ambos casos una correlación positiva.

En definitiva, los resultados obtenidos en la presente tesis, tras la realización de una exhaustiva revisión y tras la determinación de los niveles de metales/metaloides y COPs, junto con los datos relativos a los análisis de los biomarcadores de estrés oxidativo en diversos tejidos de la gaviota patiamarilla, permiten concluir que las concentraciones de los contaminantes

analizados detectadas en los animales no son lo suficientemente elevadas como para producir daño a las aves. Sin embargo, hay que considerar como preocupante la aparición de este tipo de sustancias, ya que su uso se ha visto reducido en los últimos años. Es más, en ciertos casos, como el de los PCBs, su uso ha sido prohibido por completo. Por todo ello, los resultados del presente trabajo contribuirán a ampliar información sobre valores basales de sustancias químicas y parámetros bioquímicos, siendo muy útiles como referencia en futuros estudios de biomonitorización en diferentes matrices ambientales.

1. Introducción general.

1.1 Ecotoxicología y biomonitorización.

Muchos compuestos químicos se encuentran en el medio ambiente de manera habitual. Como fuente principal se encuentran las actividades humanas, sobre todo a raíz de la revolución industrial, influyendo de manera significativa en el aumento de las cantidades de estos compuestos químicos y, por tanto, incrementándose el riesgo de efectos adversos sobre los seres vivos. En las últimas décadas se han realizado campañas de sensibilización al respecto, orientadas a intentar reducir las emisiones industriales y, desde los organismos reguladores, se ha prohibido el uso de determinados productos comprobadamente nocivos, produciéndose así una mayor conciencia ambiental.

Una tarea fundamental en el conocimiento de la presencia de contaminantes en los ecosistemas es la realización de estudios de biomonitorización ambiental.

La biomonitorización se define como aquel proceso que se encarga de determinar la cantidad de los agentes químicos, sus metabolitos o sus productos de reacción en cualquier tipo de muestra biológica (grasa, pelo, uñas) de la población que se desee estudiar (Ibarluzea et al., 2016). Es una herramienta básica y fundamental para llevar a cabo la evaluación de riesgo para los seres vivos derivado de la presencia de contaminantes y residuos en el medio ambiente (aire, agua, suelo, alimentos, …).

Los contaminantes ambientales emitidos por el ser humano más frecuentemente encontrados en la naturaleza son los metales pesados y los compuestos organoclorados, debido a que tienen una tasa baja de degradación, por lo que no se eliminan fácilmente y son transportados a través de la cadena trófica. La biomonitorización permite conocer cómo actúa un contaminante en la especie objeto de estudio, es decir, determinar si sigue un patrón de evolución. Dada la importancia de los resultados obtenidos, en las últimas décadas, han sido varios los países que han puesto en marcha programas de monitorización ambiental, particularmente de biomonitorización, con el objetivo de conocer los niveles de contaminantes ambientales presentes en el medio ambiente, el grado de exposición en los seres vivos y los efectos que pueden causar a largo plazo. En su conjunto, las tareas de biomonitorización están orientadas a conocer el estado de un ecosistema; de forma que, si se establece que los niveles de los contaminantes encontrados son elevados, se podrían tomar medidas para tratar de reducir las actividades que produzcan las emisiones de dichos contaminantes (García-Fernández y María-Mojica, 2000).

Para llevar a cabo estas tareas de monitorización ambiental, se utilizan diversas herramientas, que pueden ser resumidas en dos grandes grupos: bioindicadores y biomarcadores.

1.1.1 Bioindicadores.

Para llevar a cabo la biomonitorización se utilizan los bioindicadores, que se definen como “aquellos organismos vivos que poseen requerimientos particulares con relación a uno o a un conjunto de variables físicas o químicas, tal que los cambios de presencia/ausencia, número, morfología o de conducta de esa especie en particular, indiquen que las variables físicas o químicas consideradas, se encuentran cerca de sus límites de tolerancia” (Egwumah et al., 2017). Es decir, son las respuestas biológicas que se observan y se originan en dicho ser vivo debido a

la acumulación de una sustancia frente a un cambio ecológico y permite conocer y/o evaluar las variaciones en el medioambiente que se producen, en resumen, contienen parte de la información ambiental que los rodea. De esta forma, reflejan el estado del medio ambiente, representando el impacto de los cambios ambientales en su hábitat o ecosistema (Fox, 2001; Iqbal et al., 2021). La utilidad de las especies bioindicadoras viene demostrada por su capacidad para permitir: evaluar el impacto de la actividad humana sobre el medio ambiente, conocer si existen cambios ambientales, evaluar los efectos de los tóxicos y, por último, identificar la riqueza de especies presentes en un sitio.

Los bioindicadores deben reunir una serie de características para ser considerados buenos indicadores de contaminación ambiental que son las siguientes: (Li et al., 2010; Holt y Miller, 2011) a) Suficientemente sensible para advertir del estado del medio ambiente, b) aportar información biológicamente relevante, c) capacidad de advertir no solo del propio peligro sino del peligro de todo el ecosistema, d) los cambios que ocurren se producen muy poco tiempo después de originarse la alteración en el medio ambiente, e) abundancia máxima del individuo de estudio para poder tomar muestras cuando sea necesario sin comprometer la especie, f) poca movilidad para relacionarlo con el causante del problema, g) resistentes para poder ser manipulados en el laboratorio, i) ampliamente distribuidos para poder establecer relaciones entre distintos lugares.

Los ecosistemas acuáticos han sido estudiados durante muchos años en todo el planeta, más concretamente en España. Es por ello que las aves poseen un gran valor ecotoxicológico. En este sentido, las aves han desempeñado un papel importante en la determinación de los contaminantes ambientales en los últimos años, evidenciándose este hecho principalmente en estudios con aves rapaces y aves marinas (Gragnaniello et al., 2001; Naccari et al., 2009). El interés en usar las aves como bioindicador de contaminación ambiental ha aumentado debido a su amplia sensibilidad a los cambios ambientales y a su elevada posición en la cadena trófica, mostrando ser muy sensibles a los cambios en el medio ambiente y a las alteraciones antropogénicas, llegando a acumular altas concentraciones de cualquier contaminante en sus tejidos (Ek et al., 2004; Kuzyk et al., 2003; Swaileh y Sansur, 2006). Las aves marinas se utilizan comúnmente como centinelas de la salud de los ecosistemas debido a que son ampliamente utilizadas y estudiadas en relación con los contaminantes, ya que ocupan una gran cantidad de ecosistemas, y tienen la capacidad de adaptarse al entorno fácilmente (Elliott y Elliott, 2013). Además, hay gran cantidad de estudios donde se muestran datos sobre la ecología y comportamiento de las aves, mostrando como algunas conductas se pueden ver alteradas, como es el caso de la incubación y crianza o incluso los patrones de movimientos. Conociendo todas estas características se podría conocer si existe cualquier cambio en el comportamiento de estos animales y si es debido a la exposición de algún contaminante (Bustnes et al., 2001). Otra característica importante que poseen muchas aves es su longevidad, por lo que son más susceptibles a los fenómenos de bioacumulación de contaminantes (Braune y Gaskin, 1987; Lewis et al., 1993; Stewart et al., 1994; Ali and Khan, 2019). En muchos estudios se diferencian las aves jóvenes de las adultas por la alimentación diferente que presentan. En este sentido, por ejemplo, los pollos son alimentados por las madres, que obtienen la comida de los sitios más cercanos y accesibles, por lo que es un punto de información óptima para conocer qué contaminación local existe en ese momento y en ese ecosistema. Además, la toma de muestra de los pollos siempre es más eficaz y menos costosa que la toma de muestra de los adultos

(Gómez-Ramírez et al., 2012). Las aves se pueden encontrar expuestas a dosis bajas de cualquier contaminante de forma crónica, pudiendo provocar trastornos en su comportamiento y salud, pudiendo provocar un declive en poblaciones silvestres de estos animales tanto en ecosistemas marinos como en terrestres (Köhler y Triebskor, 2013). Dado que la dieta es la principal fuente de contaminación en las aves marinas, va a ser fundamental conocer la alimentación de las gaviotas para poder comprender los patrones de actuación y la presencia de contaminantes en el organismo (Hebert et al., 2009; Lato et al., 2021).

1.1.2 Biomarcadores.

Un biomarcador es aquel cambio biológico que se produce en un organismo en respuesta a un cambio en el medio exterior, producido por algún tipo de contaminante químico que se acumula en el interior de este organismo, y que puede ser medido en el mismo a nivel genético, enzimático, fisiológico o biológico (Markert et al., 2013). En resumen, cualquier alteración en cualquiera de los componentes moleculares, procesos celulares, bioquímicos y fisiológicos que ocurren dentro de un organismo vivo después de la exposición a un contaminante podrá ser utilizado como biomarcador. Los efectos de contaminantes en niveles más bajos de organización biológica (p. bioquímicos, celulares, fisiológicos) en general, ocurren más rápidamente que aquellos en niveles más altos (por ejemplo, efectos ecológicos) y, por lo tanto, pueden proporcionar una alerta temprana más sensible de efectos toxicológicos dentro de las poblaciones (Clements, 2000).

El biomarcador ideal debe reunir las siguientes condiciones: (i) que se pueda medir en tejidos de fácil obtención o mediante una técnica no invasiva y que se pueda relacionar con la exposición y/o grado de daño al organismo; (ii) que se pueda relacionar directamente con el mecanismo de acción de los contaminantes; (iii) que sea mensurable por técnicas muy sensibles que requieran cantidades mínimas de muestra y que sean fáciles de llevar a cabo y rentables económicamente; y (iv) que su análisis pueda ser aplicado en diferentes especies (Fossi, 1994).

Los biomarcadores se dividen en tres clases (Gil y Pla, 2001):

- Biomarcadores de Exposición: Los programas de biomonitorización y la mayoría de los estudios utilizan fundamentalmente estos biomarcadores ya que permiten conocer las concentraciones de un determinado compuesto tóxico en el organismo mediante el análisis del mismo o de sus metabolitos. Es decir, nos va a indicar si un organismo ha experimentado una exposición a un contaminante y ofrecer una señal temprana de exposición a micro contaminantes. Por otro lado, podrán proporcionar estimaciones cualitativas y cuantitativas de exposición a diversos compuestos. Los biomarcadores de exposición pueden tener el potencial de ofrecer una alternativa a algunos análisis químicos ya que pueden ser útiles para medir los efectos de los productos químicos de vida corta, así como para dar una indicación de exposición más relevante desde el punto de vista biológico (Hagger et al., 2006).

Un claro ejemplo de este tipo de biomarcadores es la detección y cuantificación de Hg, el cual puede introducirse a través de la dieta a base de pescado, aire contaminado o vía dérmica y se puede determinar a través de la sangre, orina o pelo (Roca Marugán y Yusà, 2013).

- Biomarcadores de Efecto: son las mediciones bioquímicas, fisiológicas o cualquier tipo de alteración en los tejidos o fluidos corporales de un organismo que puede ser reconocida o

asociada a en efecto nocivo para la salud, es decir, refleja la interacción del producto químico con los receptores biológicos. En resumen, pueden permitir la posibilidad de detectar alteraciones tempranas en el estado de salud de los organismos, que, si se mantiene, puede producir consecuencias patológicas. Por ejemplo, la carboxihemoglobina es un biomarcador de efecto, para la cual se ha confirmado una correlación significativa con exposición ambiental a monóxido de carbono, reflejando además una dosis interna unida al tejido blanco (Ramírez, 2006).

- Biomarcadores de Susceptibilidad: indican la capacidad que tiene un organismo para adaptarse a los cambios producidos por la exposición a una sustancia externa que produzca efectos en dicho organismo, ya sean cambios genéticos o cambios en los receptores que alteran la susceptibilidad de un organismo a un determinado compuesto. El polimorfismo de enzimas es un ejemplo, son enzimas que intervienen en el metabolismo de sustancias tóxicas, existiendo diferencias interindividuales en los genes codificantes de estos enzimas, lo que determina que dentro del organismo haya patrones diversos de metabolismo de algunos xenobióticos (Anderson et al., 2006)

La interpretación de las respuestas de los biomarcadores no siempre es sencilla, ya que puede dar lugar a conclusiones erróneas y confusas sobre los efectos de los contaminantes en los seres vivos o en el medio ambiente. Además, es imprescindible conocer los numerosos factores que pueden influir en la determinación y la selección de los biomarcadores más útiles y relevantes para así poder usarlo en la evaluación del impacto de los contaminantes en el medio ambiente, por lo que va a ser fundamental la utilización correcta de un biomarcador. Este tendrá que tener en cuenta numerosos factores como la gran variabilidad interespecífica de la especie a estudiar (sexo, edad, localización, alimentación, etc.), la sensibilidad del propio biomarcador, si responde a los contaminantes en función de la dosis o del tiempo, tiempo después de la exposición dura la respuesta, las relaciones dosis-respuesta, influencia de parámetros biológicos, parámetros ambientales sobre los valores de respuesta. Por eso una misma respuesta establecida para una especie no tiene por qué ser la misma para otra especie, aunque ambas sean muy similares (Fossi, 1994; Hagger et al., 2006). Sin embargo, en algunos casos, el creciente conocimiento de la respuesta del biomarcador permite comprender con mayor precisión su especificidad.

1.1.2.1 Biomarcadores de estrés oxidativo.

El estrés oxidativo es uno de los mecanismos que pueden provocar numerosos contaminantes como pueden ser los metales y los plaguicidas organoclorados, el estrés oxidativo se puede generar de manera directa generando especies reactivas de oxígeno (ROS) o de manera indirecta inhibiendo el sistema antioxidante celular (Abdollahi et al., 2004; Ercal et al., 2001). Las ROS son producidas como una consecuencia del metabolismo aeróbico fisiológico normal. El desbalance entre la producción de los ROS y el sistema de defensa antioxidante en los sistemas vivos ocasiona una ruptura de la función celular y daño. Hay que destacar que las ROS a bajas concentraciones pueden tener efectos beneficiosos para la salud porque participan en diferentes procesos fisiológicos como los sistemas de señalización celular, inducción de la respuesta mitogénica o la defensa contra microorganismos infecciosos (Valko et al., 2006). Pero cuando el desbalance es hacia la alta concentración de ROS se puede alterar la función celular normal y llegar a promover daño irreversible en macromoléculas (ácidos nucleicos, lípidos,

proteínas celulares) extrayéndoles un electrón, produciendo una oxidación y su posterior modificación generando nuevas ROS y poniendo en marcha una cascada de daños (Monagahn et al., 2009; Carvajal, 2019). Los seres vivos aerobios tienen la capacidad de defenderse del estrés oxidativo ya que poseen moléculas antioxidantes que son sustancias capaces de inhibir la generación de los radicales libres, eliminarlos, reducir la oxidación y el daño ocasionado por los mismos (Koivula y Eeva, 2010).

Las moléculas antioxidantes han sido ampliamente estudiadas. Entre ellas se incluyen diversas enzimas y productos: la enzima catalasa (CAT), que utiliza una molécula de peróxido de hidrógeno (H_2O_2) como sustrato donador de electrones y otra molécula de H_2O_2 como oxidante o aceptador de electrones para transformarlo en oxígeno (O_2) y agua. El H_2O_2 es una especie reactiva de oxígeno que puede llegar a dañar las proteínas, los lípidos y los ácidos nucleicos, por lo que hay que destacar la importancia de la enzima catalasa como sistema antioxidante de los organismos, evitando que se pueda formar el radical hidroxilo y el oxígeno singlete, ambos son especies de oxígeno muy reactivas. Esta enzima se encuentra en la mayoría de los organismos aerobios, donde se puede localizar en los peroxisomas de los eritrocitos, médula ósea, mucosas, riñón e hígado (Cedillo Jiménez y Hernández López, 2010). El glutatión reducido (GSH) es el tiol no proteico más abundante presente en concentraciones milimolares en los tejidos de los mamíferos que actúa como un antioxidante que protege a las células de las lesiones oxidativas provocadas por los peróxidos de lípidos, las especies reactivas de oxígeno y nitrógeno y los xenobióticos, uniéndose a las enzimas de defensa, ya que interviene como sustrato de estas enzimas (glutatión peroxidasa, transferasa, reductasa), destoxificando aldehídos y peróxidos tóxicos y neutralizando las especies reactivas de oxigeno (ROS). En la Fig. 1.1 se puede observar de manera simplificada el mecanismo de acción del glutatión y de las demás enzimas antioxidantes. Estudios recientes destacan la importancia de este compuesto en reacciones de transducción de señales como controlador de la diferenciación celular, apoptosis, proliferación, ferroapoptosis y función inmunitaria. Siendo en células sanas crucial para la eliminación y destoxificación de carcinógenos (Kennedy et al., 2020).

La enzima glutatión peroxidasa (GPx) es una enzima importante dentro del sistema antioxidante porque se encuentra en todos los tejidos y órganos siendo parte del sistema antioxidante del glutatión, por lo que se encuentra involucrada en la fisiopatología de varias enfermedades, su función principal es la catalización de la reacción de oxidación de GSH a glutatión disulfuro (GSSG) utilizando para ello H_2O_2 (Cisneros Prego et al., 1997); la enzima glutatión reductasa (GR) cataliza la reducción del GSSG para formar GSH en su forma sulfhidrilo, el cual es utilizado por la enzima GPx para la reducción del peróxido y de lipoperóxidos; por lo que va permitir que se mantengan concentraciones de GSH en la célula, que no solamente va ser utilizado por la GPx en la eliminación del H_2O_2, sino que se encargará de la recuperación de las vitaminas C y E y participará en la eliminación de radicales libres generados *in situ* o a distancia (Cisneros Prego, 1995).

La enzima glutatión-S-transferasa (GST), es una familia de enzimas de fase II (conjugación) altamente conservadas e implicadas en el metabolismo de muchos xenobióticos orgánicos, llevando a cabo la destoxificación de estos compuestos mediante la catalización de la conjugación del GSH a sustratos xenobióticos, pertenece a una superfamilia de enzimas versátiles que también posee una gama de otras funciones (Sheehan et al., 2001). Estas enzimas

tienden a la obtención de productos biológicamente menos reactivos que los inicialmente metabolizados, por lo que son sumamente importantes ya participan de lleno en la prevención de la peroxidación lipídica, habiéndose demostrado que se produce una inducción de las GSTs con ciertos xenobióticos tales como hidrocarburos aromáticos policíclicos (HAPs), PCBs y compuestos fenobarbitales (Cunha et al., 2005). La actividad de esta enzima se ha podido medir tradicionalmente usando GSH y clorodinitrobenceno (CDNB) como co-sustratos (Habig et al., 1974), ha sido ampliamente utilizada como biomarcador de exposición a HAPs, PCBs y trazas de metales tanto en peces, aves y mamíferos como en invertebrados (Funes et al., 2006; de Farias Araujo et al., 2022; Osman et al., 2022).

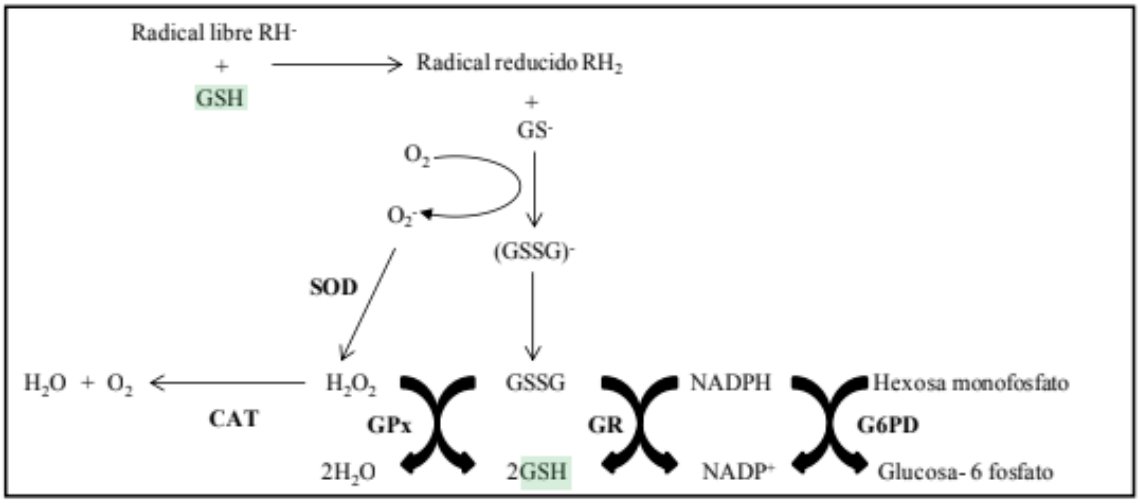


Fig. 1.1. Representación simplificada del mecanismo de acción del glutatión y de las principales enzimas antioxidantes. Adaptada de Martínez (2010).

Uno de los indicadores más comunes de estrés oxidativo es el malondialdehído (MDA). Esta es una molécula de bajo peso molecular que se genera por descomposición primaria y secundaria durante el proceso de oxidación de los lípidos en las membranas biológicas, provocando una alteración de la estructura de la misma y produciendo pérdida de fluidez y un mal funcionamiento, por lo que la determinación de este producto es un indicador que nos permite conocer el nivel oxidación lipídica de las membranas celulares. Además, la presencia en el organismo del MDA provoca que este se una a la guanina del ADN afectando a la función de la mitocondria y reduciendo la capacidad metabólica de las células. Esta molécula también es capaz de unirse a numerosos aminoácidos importantes (histidina, arginina, tirosina, metionina, lisina, prolina) mediante enlaces cruzados reduciendo el valor de la proteína original ya que al unirse llega a formar polímeros metabólicamente inactivos (Halamíčková et al., 2003).

1.2 Muestras ambientalmente relevantes: las aves como bioindicadores.

1.2.1 Las aves como bioindicadores.

Al analizar las concentraciones de contaminantes acumulados en los tejidos corporales de los animales, se obtiene información sobre la calidad del hábitat del que el animal es parte intrínseca. Sin embargo, la monitorización de los contaminantes en este tipo de muestras se ve dificultado por algunos factores como: la propia obtención de la muestra, la cantidad disponible

de muestra o los aspectos éticos que implican la posible necesidad de sacrificio del animal. Por estas razones, durante los últimos años se han realizado estudios de biomonitorización utilizando técnicas no invasivas de las especies estudiadas, en este caso las aves. De esta forma, muchos estudios han utilizado como muestra habitual la sangre (Martínez-López et al., 2005; Bjedov Et al., 2022), los huevos (Rani et al., 2022) o el aceite de la glándula uropigial (Díez-Fernández et al., 2023). Sin duda, la muestra por excelencia es la pluma, que ha sido usada en numerosos estudios de biomonitorización de metales, demostrando que es la gran alternativa a los estudios con tejidos internos, usándose en menor medida para evaluar efectos de compuestos orgánicos (Eulaers et al., 2011; Vizuete et al., 2022). Una de las grandes virtudes que presenta el uso de plumas, es que su obtención no genera ningún tipo de daño al animal, a diferencia de lo que ocurre con el muestreo de sangre (que puede provocar estrés en las aves). Además, para su muestreo y obtención no se requiere una técnica especial para su extracción y que tampoco requiere un almacenamiento para su conservación a bajas temperaturas (He et al., 2020; Solgi et al., 2020).

En general, hay que considerar la importancia de realizar estudios con animales que provengan de centros de recuperación, recogiendo muestras de aquellos que no se hayan podido recuperar o animales que aparezcan muertos por algún motivo fácilmente relacionable con la causa de esa muerte. Un claro ejemplo de ello es el estudio realizado por Rodríguez et al. (2007) en el que se analizaron las concentraciones de dos metales pesados tóxicos (plomo y cadmio) en hígado de dos especies de aves marinas (cormorán moñudo y alcatraz) afectadas por el vertido de crudo del "Prestige" en noviembre de 2002.

1.2.2 La gaviota patiamarilla como bioindicador de contaminación ambiental.

Múltiples son los factores a considerar que deberán cumplir los animales pertenecientes a una especie concreta para determinar si pueden ser usado como bioindicadores. En el presente trabajo se ha tratado de evaluar si la gaviota patiamarilla (*Larus michahellis*) podría ser un buen bioindicador de contaminación ambiental. A su favor tiene el hecho de ser una de las aves más abundantes del Mediterráneo; de hecho, en España se extiende alrededor de todas las zonas costeras del país llegando incluso a las zonas de interior (Fig. 1.2).

Fig. 1.2. Distribución de la gaviota patiamarilla en España.

La gaviota patiamarilla se distribuye ampliamente por la Península Ibérica y el norte de África, llegando hasta el oeste del Mar Negro. En el territorio español (Fig. 1.2) se encuentran tres subespecies: *michahellis,* que ocupa el litoral mediterráneo incluido Huelva y Cádiz; *atlantis,* distribuida por las costas atlánticas peninsulares y Canarias; y, por último, *lusitanicus,* que se extiende desde las costas gallegas hasta las de Euskadi. En toda su distribución esta gaviota presenta un comportamiento sedentario con desplazamientos considerables en algunas ocasiones. Hay que destacar que ha empezado a colonizar el interior de la península ibérica en algunos sitios como el embalse del Ebro, algún embalse de Ourense, Toledo y algunos ríos del interior de Cataluña (Muntaner et al., 1983; Román et al., 1996; Velasco et al., 1999).

Fig. 1.3. Individuos de gaviota patiamarilla, adulto (en el fondo de la imagen) y joven.

Esta gaviota (Fig 1.3) se adapta muy bien a cualquier hábitat, ya que su alimentación incluye una amplia variedad de productos y subproductos (peces, mamíferos pequeños, crustáceos, moluscos e incluso carroñas, donde destacan como principalmente como parte de la dieta de este animal el cangrejo patudo (*Polybius henslowii*), el percebe (*Pollicipes pollicipes*) y el mejillón gallego (*Mytilus galloprovincialis*) (Munilla et al., 2014). Las dos fuentes principales de alimentación durante el año son los vertederos de las grandes ciudades y los descartes producidos por los barcos de pesca de arrastre, con los que se encuentra estrechamente relacionada la gaviota. Duhem et al. (2003) demostraron en su estudio que los basureros son consistentemente el principal hábitat de alimentación de estas gaviotas. Además, la alta accesibilidad a los vertederos de basura conduce a una dieta poco diversificada dominada por la basura. Por el contrario, cuando la accesibilidad a los basureros es baja, las gaviotas patiamarillas amplían su nicho trófico, con una mayor explotación de hábitats alternativos de alimentación, como los hábitats terrestres. Estos cambios de hábitat también han sido comentados por Almeida et al. (2023), quienes sugirieron que las gaviotas patiamarillas acceden a alimentos terrestres/antropogénicos de mayor calidad, con menos desechos plásticos, en el momento en que los polluelos son más susceptibles a la calidad del alimento, lo que promueve la supervivencia y el crecimiento de los polluelos.

La población de este animal en los últimos años se ha visto estabilizada, hecho que puede estar asociado con la privación de alimentos que les ha supuesto el cierre de muchos basureros (Bosch et al., 1994; Munilla, 1997). Durante los periodos de cría prefiere habitar en acantilados marinos o islas cercanas a la costa. También es frecuente que puedan llegar a criar con éxito en edificaciones humanas (Seobirdlife, 2022). Sin embargo, las poblaciones de estas aves han mostrado un ligero aumento desde el año 2021, siendo importante determinar si este hecho puede ser debido a una fluctuación natural entre años o si las gaviotas han encontrado otros recursos de alimentación accesible (Mutillod et., al 2023).

No es infrecuente que, debido al gran número de animales de estas especies, en los centros de recuperación de fauna silvestre ubicados en diferentes regiones del país se reciban ejemplares con signos de intoxicación y/o lesiones (traumatismos que en ocasiones han

generado daños tan intensos que los animales no sobreviven). Los centros de recuperación de fauna salvaje (CRFS) ubicados en cada una de las provincias del territorio español tienen como finalidad la recogida diaria de animales heridos, para poder así realizar un tratamiento clínico adecuado con el fin de intentar solventar los problemas de estos en sus instalaciones. Entre sus funciones también se encuentran el análisis de muestras, necropsias, estudios toxicológicos o de zoonosis. Por todo ello, los animales recibidos, tras su fallecimiento, pueden llegar ser fuente relevante y muy valorada de muestras para llevar a cabo estudios de biomonitorización, con las que podrán a llegar a obtener datos interesantes sobre la presencia de contaminantes ambientales en el hábitat de dichos animales, así como posibles procesos de bioacumulación y efectos subletales.

1.3 Contaminantes ambientales persistentes.

1.3.1 Inorgánicos: Metales y metaloides.

Los metales han acompañado a la humanidad desde tiempos inmemoriales, dado que, debido a sus particulares propiedades físicas han sido usados para para fabricar herramientas, utensilios domésticos, estatuas o estructuras de todo tipo. Algunos metales han mostrado ser esenciales para la vida a determinadas concentraciones, llegando a ser dañinos cuando dichas concentraciones son excedidas. A pesar de que, en múltiples ocasiones, estos metales no son sintetizados directamente por el hombre, sí que es responsable del aumento de las concentraciones de estos elementos en el medio ambiente, sobre todo desde que se produjo la revolución industrial. El mercurio (Hg), el cadmio (Cd) y el plomo (Pb) son tres de los metales más peligrosos desde el punto de vista medioambiental y toxicológico (García-Fernández et al., 1996). Por otro lado, el zinc (Zn), el selenio (Se) o el hierro (Fe) son esenciales a ciertas concentraciones, y cada uno tiene una función concreta para los seres vivos. Sin embargo, como ya se ha comentado, a concentraciones más elevadas pueden llegar a ser dañinos (Saaristo et al., 2018).

En la Tabla 1.1 se describen las principales características de los metales y metaloides que serán objeto de estudio en la presente tesis doctoral. En la Publicación I de la presente tesis se puede encontrar información ampliada con respecto a los elementos estudiados, su comportamiento en el medio ambiente y el efecto provocado en aves.

1.3.1.1 Características de los metales y metaloides.

El **Hg** es un metal pesado muy singular, ya que se encuentra en forma líquida a temperatura ambiente. Debido a su volatilidad puede ser transportado a grandes distancias una vez que se ha incorporado a la atmosfera en su forma gaseosa. El Hg se encuentra de manera natural en el medio ambiente, este metal es emitido a través de las erupciones volcánicas, de la erosión natural de los minerales que lo contienen o de los incendios forestales y las lluvias que tienen la capacidad de movilizar el Hg en el medioambiente (Campos et al., 2015). Sin embargo, la mayor parte del mercurio presente en el medio ambiente hoy en día es de origen antropogénico, ya muchas actividades del ser humano emiten grandes cantidades de Hg al medio ambiente como, por ejemplo, la propia extracción primaria de mercurio, la minería y la metalurgia de otros metales, depuración de gas natural, actividad de las incineradoras, crematorios, combustión de combustibles fósiles en centrales térmicas, cementeras..., lo cual puede tener importantes

efectos adversos para la salud humana y el medio ambiente (Kim y Zoh, 2012). Las emisiones globales están aumentando, y se prevé que el calentamiento climático en curso exacerbe los niveles de mercurio, debido principalmente a la descongelación de los suelos de permafrost que contienen cantidades de este (Schuster et al., 2018).

Tabla 1.1. Características generales de los metales estudiados.

Metal	MERCURIO	CADMIO	PLOMO	SELENIO	ARSÉNICO
Símbolo	Hg	Cd	Pb	Se	As
Numero atómico	80	48	82	34	33
Peso atómico	220,59	112,4	207,2	78,96	74,92
Punto de fusión	-38,9°C	321°C	327,3°C	220°C	419,4°C
Punto de ebullición	356,58°C	321°C	327,4°C	648,8°C	613°C
Aspecto y características	Blanco plateado, líquido a temperatura ambiente	Plateado gris metálico, solido a temperatura ambiente	Blanco de plata, brillante, maleable y muy dúctil, solido a temperatura ambiente	Solido a temperatura ambiente, elemento muy abundante en la corteza terrestre, insoluble en agua	Metaloide de color gris, altamente venenoso, raramente se encuentra en estado sólido
Interés fisiológico	No esencial	No esencial	No esencial	Esencial. Funciones antioxidantes, regulación hormonal del tiroides, efectos anticancerígenos	Esencial. Déficit de arsénico puede ocasionar problemas

Uno de los mayores problemas generados por este metal, desde el punto de vista toxicológico, deriva de la metilación del Hg, transformándose en metilmercurio gracias a la actividad bacteriana en los sedimentos marinos, que es la forma más tóxica y con mayor biodisponibilidad en la que se encuentra el Hg en la naturaleza. La problemática viene dada por la capacidad tan extraordinaria de este para acumularse en los animales acuáticos, bioacumulándose el metal en los niveles más altos de la cadena trófica (Obrist et al., 2018). Lo que hace que los organismos que se alimentan en posiciones tróficas más altas sean particularmente susceptibles a efectos perjudiciales provocados por el metilmercurio (Whitney y Cristol, 2017). El metilmercurio ingerido en la dieta es absorbido prácticamente en su totalidad (90%) y vertido al torrente sanguíneo. A continuación, este compuesto es transportado y penetra en los tejidos formando un complejo de metilmercurio y cisteína. El Hg metálico o elemental se volatiliza a vapores de Hg a temperatura ambiente, siendo la vía inhalatoria la principal vía de absorción, muy por encima de la vía digestiva que es prácticamente nula. A continuación, el metal se distribuye a través del sistema circulatorio (Goyer y Clarkson, 2001; Gad, 2005b). Se estima que el Hg representa el 15% de los compuestos inorgánicos presentes en los alimentos. Este dato no resulta tan preocupante si se piensa que entre un 75-92% es excretado durante los 4-5 días posteriores a la ingestión. El Hg es excretado a través de la orina y las heces (Barregard et al., 2022). En el caso de las aves hay que añadir la pluma como importante vía de eliminación.

El **Cd** es un metal pesado considerado como uno de los elementos más tóxicos junto con el mercurio y el plomo, además presenta una problemática que es su larga vida media y la gran

capacidad que tiene para que ser acumulado en los seres vivos. Se encuentra ampliamente distribuido en la naturaleza, asociado principalmente a los depósitos de zinc en la corteza terrestre. Su movilidad en el medio depende de varios factores tales como el pH, el potencial redox, la cantidad de materia orgánica y la presencia de arcillas y óxidos de hierro. El Cd es emitido mayoritariamente por las actividades antropológicas, mayoritariamente actividades industriales, minería, metalurgia, fabricación y aplicación de fertilizantes de fosfatos y de la incineración de residuos urbanos (Capó, 2007). Se han descrito diversos efectos tóxicos en el ser humano provocados por la exposición a Cd, entre ellos se ha confirmado su actividad como agente carcinógeno de tipo I (IARC, 2012). La inhalación de grandes cantidades de aerosoles de óxido de cadmio puede producir neumonitis aguda y edema pulmonar. Ingerir alimentos con niveles muy altos de cadmio produce irritación grave del estómago, vómitos y diarrea, pudiendo causar la muerte en algunas ocasiones. Se ha observado que el Cd se comporta como una metalohormona y disruptor endocrino. Se ha comprobado in vivo que el cadmio puede inducir respuestas estrogénicas como la hiperplasia e hipertrofia del endometrio (Nordic Council of Ministers, 2003; Järup y Åkesson, 2009; ATSDR, 2012). La absorción del Cd viene dada por la vía respiratoria y por la vía digestiva, siendo la vía respiratoria más eficiente que la vía digestiva, la mayor parte de Cd acumulado en aves procede de los alimentos que consumen (García-Fernandez et al., 1996). El Cd es transportado en la sangre unido a eritrocitos y a proteínas plasmáticas, en concreto la albúmina (Gad, 2005c). La mayor carga de Cd se acumula en el hígado y riñón (Scheuhammer, 1987). El Cd es finalmente eliminado a través de la orina (Barregard et al., 2022), aunque también puede eliminarse a través de las plumas y durante la formación del huevo (Burger, 1994; Martínez-López et al., 2005).

El **Pb** es un metal blando con múltiples aplicaciones, considerado tóxico y que se encuentra presente de forma natural en la corteza terrestre. El Pb se acumula en los organismos acuáticos y terrestres, comenzando así el proceso de biomagnificación que genera el incremento de sus concentraciones en especies posicionadas en lo alto de la cadena trófica. Este metal es uno de los cuatro metales que tienen un efecto más dañino sobre la salud humana. Las principales fuentes de contaminación ambiental son la minería, metalurgia y el uso de pinturas y gasolinas con plomo; aunque el mayor consumo de Pb se da en la fabricación de baterías de plomo-ácido para vehículos de motor. Industrialmente, sus compuestos más importantes son los óxidos de plomo y el tetraetilo de plomo (Gad, 2005a; Restrepo et al., 1991). Los compuestos del plomo son tóxicos y han producido envenenamiento de trabajadores por su uso inadecuado y por una exposición excesiva a los mismos. Sin embargo, en la actualidad, el envenenamiento por Pb es raro debido a la realización de controles industriales y la higiene en los procesos de la ingeniera. La absorción de Pb se realiza por vía pulmonar y por vía gastrointestinal siendo esta ultima la vía más rápida (Pain y Rattner, 1998). El Pb es distribuido a través el torrente sanguíneo unido a la hemoglobina para llegar a acumularse en el hígado, en el riñón e incluso en tejidos blandos como el cerebro, si la acumulación es muy prolongada puede llegar a acumularse en los huesos (García-Fernández et al., 1997; Gad, 2005a). El Pb es eliminado a través de las heces, también es excretado a través de las plumas en la muda de las aves y, por último y menos frecuente, es eliminado a través de los huevos (Heuel et al., 2023). Los efectos que puede ocasionar el Pb en los seres humanos son muy variados, incluyendo daños a nivel renal o del cerebro (Abadin et al., 2007; Flora et al., 2012; ATSDR, 2020), disminución de la fertilidad del hombre (Flora et al., 2011), incremento de la presión sanguínea, perturbación de la biosíntesis

de hemoglobina y anemia. El Pb puede incluso entrar en el feto a través de la placenta de la madre, por lo que podría generar serios daños al sistema nervioso y al cerebro fetal (Castro-Bedriñana et al., 2013).

El **Se** es un elemento mineral natural esencial, por lo que no es considerado un metal pesado, que se encuentra ampliamente distribuido en la naturaleza en la mayoría de las rocas y del suelo. Este elemento se encuentra en la naturaleza en varias formas, ya sea en forma pura como cristales hexagonales o bien combinado con sulfuro, minerales de plata, cobre plomo y níquel. El Se, es también producido por el hombre en sus actividades, la mayor parte del Se procesada por el hombre es usado en la industria electrónica, en la industria del vidrio, como suplemento nutritivo y como componente de pigmentos de plástico, pinturas, esmaltes, tinturas, medicamentos, alimentos para animales, etc. La vía de exposición más común es a través de la dieta, en dos composiciones: la selenometionina y la selenocisteina, que son las dos formas de este metal que más biodisponibilidad alcanzan. A continuación, es absorbido por el tracto gastrointestinal para unirse al plasma sanguíneo junto a la selenoproteína P, a la selenoproteína W y la albumina, para luego distribuirse en el hígado, el riñón, el páncreas y el músculo. La principal vía de excreción del Se en la orina en su forma metilada o acetilada, a través de las heces, a través de la respiración y, por último, a través de la pérdida del cabello y las células de la piel (Casals et al., 2015).

El Se es un elemento que tiene efectos tanto beneficiosos como perjudiciales para el ser humano, a dosis bajas es un elemento necesario para mantener el organismo, pero las exposiciones breves a altas concentraciones pueden ser perjudiciales, provocando selenosis, cuyos síntomas principales son pérdida del cabello, uñas quebradizas y anomalías neurológicas (por ejemplo, adormecimiento y otras sensaciones extrañas en las extremidades). La EPA ha determinado que una forma específica de selenio, el sulfuro de selenio es probablemente carcinogénica en seres humanos. Sin embargo, desde el punto de vista de la salud pública, este compuesto no supone un gran riesgo, dado que es una forma química muy diferente de los compuestos orgánicos e inorgánicos de selenio que se encuentran en los alimentos y en el ambiente. Existen también estudios en animales que han demostrado que niveles muy altos de selenio, sin identificar formas concretas, pueden afectar la producción de espermatozoides y el ciclo reproductivo de las hembras (ATSDR, 2003).

El **As** es está considerado como uno de los metaloides más tóxicos presentes en el medio ambiente. Su distribución y la contaminación provocada se debe a procesos naturales y antropogénicos, presentando una fácil movilización en el ambiente. Alrededor de un tercio del As que se encuentra en la atmósfera proviene de fuentes naturales como erupciones volcánicas o reacciones ambientales. El resto del As proviene de actividades antropogénicas como la actividad minera, el uso de combustibles fósiles, la aplicación de pesticidas orgánicos, herbicidas y desecantes agrícolas, así como del uso del arsénico como aditivo de alimentos para ganado y aves de corral. La principal ruta de exposición humana es a través del consumo de agua contaminada con arsénico**.** Aunque muchos suelos tienen concentraciones de As de manera natural, en otros aparecen grandes cantidades debido a la contaminación industrial, a la contaminación de productos de explotaciones agropecuarias y por el uso de agroquímicos y/o irrigación con agua contaminada, siendo esta última la fuente de contaminación por As más importante del suelo (Ravenscroft et al., 2009). El As se absorbe en forma de arseniatos y

arsenitos a través de dos vías, la oral y la inhalatoria. Por la vía oral, se absorbe un promedio del 80 % del arsénico ingerido, aunque esto varía según las características del compuesto y factores propios del individuo. Sin embargo, en la vía inhalatoria se absorbe hasta un 50% del As ingerido. (Galvao y Corey, 1987). El As y sus metabolitos son eliminados principalmente a través de la orina. La vida media del As en el ser humano es de 10 h para las formas inorgánicas y 30 h para las formas orgánicas. El As es capaz de afectar a todos los aparatos y sistemas del cuerpo debido a su compleja toxicocinética, ya que se ve involucrado en numerosas reacciones enzimáticas. Los efectos más comunes que se pueden observar tras una exposición a As incluyen hiperpigmentación cutánea o queratosis focal (ATSDR, 2016). Tras la exposición también se producen efectos gastrointestinales, consistiendo la principal lesión en el tracto gastrointestinal en un aumento en la permeabilidad de los vasos sanguíneos pequeños, que lleva a la pérdida de fluidos y a la hipotensión. En ciertos casos se puede presentar incluso una inflamación extensiva y una necrosis en la mucosa del estómago e intestino pudiendo producir una perforación de estos. No solo se observan efectos negativos en la piel y en el estómago, también son conocidos sus efectos sobre los riñones, pudiendo ocasionar una necrosis tubular aguda junto con un fallo renal agudo. También se ha reportado insuficiencia renal crónica por necrosis cortical (ATSDR, 2016).

1.3.1.2 Presencia de metales y metaloides en el medio ambiente.

Actualmente existe un gran problema de contaminación por metales pesados en las aguas, tanto marinas como fluviales, debido a la gran toxicidad que presentan estos elementos en los animales que habitan dichos ecosistemas, llegando a poder ocasionar serios problemas de salud pública (Pabón et al., 2020). Como ya se ha mencionado, el factor antropogénico tiene una gran influencia en la contaminación del ecosistema. Este es el caso, por ejemplo, de las actividades mineras que se encuentran cerca de los ríos, y que ocasionan una a tasa de contaminación del agua que puede rondar los 200 millones de metros cúbicos diarios (Reyes et al., 2016). Para evitar estos problemas se han realizado numerosas investigaciones en los últimos años con el fin de encontrar diferentes métodos para poder retener y extraer dichos metales de las fuentes hídricas (precipitación, oxido-reducción, intercambio iónico, filtración, tratamiento electroquímico, tecnologías de membrana y recuperación por evaporación, adsorción y bioadsorción) y poder así reducir la toxicidad en las mismas, garantizado la preservación tanto de los ecosistemas como de la vida humana (Tejada-Tovar et al., 2015). Estos metales no solo pueden encontrarse en el agua, sino también en los suelos representando una seria amenaza para el medio ambiente, seguridad alimentaria y la salud humana y animal. Al igual que para la eliminación de estos elementos del compartimento acuático, también existen métodos para retirar los contaminantes de los suelos como es la biorremediación microbiana, que consiste en la utilización de microorganismos que tienen la capacidad de secuestrar y transformar estos compuestos (González-Henao y Ghneim-Herrera, 2021). Los metales pesados más comúnmente encontrados en suelos contaminados, en orden de abundancia, son Pb, Cr, As, Zn, Cd, Cu y Hg (USEPA, 1997). El transporte y el destino de un metal pesado va a depender significativamente de su forma química y de su especiación. Una vez que el metal llega al suelo, se adsorbe gracias a reacciones químicas rápidas que pueden durar minutos u horas, para que a continuación se den reacciones de adsorción lenta haciendo que se redistribuyan en diferentes formas químicas con biodisponibilidad y toxicidad variables (Buekers, 2007; Shiowatana et al., 2001). Además, las plantas también pueden ser objeto de contaminación por metales pesados, estos

contaminantes pueden ingresar a través de dos vías, a través de los suelos (Arshad et al., 2008) y a través de la atmósfera (Uzu et al., 2010). Aunque los metales pesados se consideran parte inherente del suelo, pueden llegar a causar severos daños tanto a este como a las plantas, el Pb se encuentra dentro de los contaminantes más comunes que afectan a las plantas. (Cecchi et al., 2008; Grover et al., 2010; Shahid et al., 2011).

En un estudio reciente, Ratiu et al. (2018) investigaron la acumulación de metales pesados y sus diferentes concentraciones en sedimentos alrededor del río Tisza (Rumania) y sus afluentes. Sus hallazgos indicaron que la concentración de Cd y Pb en las diferentes áreas estudiadas oscilaba entre 1,3–21 mg/kg y 38–3630 mg/kg, respectivamente, reportando así unos niveles muy por encima de los límites permitidos (debido a su toxicidad elevada, la concentración máxima permitida de Cd en Rumania es de 0,2 µg/L, mientras que para el Pb la concentración máxima permitida 7,2 µg/L en el agua de los ríos).

En China Zeng et al. (2015) estudiaron la acumulación de metales pesados en tres áreas agrícolas. Los resultados indicaron que Cd y Hg registraron los valores medios más altos entre las tres áreas analizadas (1,40 y 14,9 mg/kg, respectivamente), superando los estándares chinos de calidad ambiental para el suelo. Por lo que respecta a las aves, Bertram et al. (2022) obtuvieron una concentración media de 3,4 mg/kg de Hg en plumas de charrán común (*Sterna hirundo*) que pasaron el invierno en Canarias, en Guinea y en Benguela. Esta concentración reportada se encontraba por debajo del nivel umbral admitido de 5 mg/kg. De hecho, algunos estudios han concluido que concentraciones de Hg en las plumas de aves acuáticas entre 5 y 40 mg/kg pueden tener impactos negativos, identificándose trastornos reproductivos para las aves, cambios en el desarrollo neurológico, alteración comportamental o déficit auditivo. Sin embargo, los niveles son variables, como demuestran los resultados (más altos 4,95mg/kg) obtenidos por Bracey et al. (2021) en la misma especie de ave. Furtado et al. (2019) realizaron un estudio en las Islas Falkland en el sur oeste del océano atlántico donde determinó la concentración de Hg en pluma de varias aves marinas de distintas especies, como son el cormoran imperial (*Leucocarbo atriceps*), el pingüino juanito (*Pygoscelis papua*), el pingüino de penacho amarillo (*Eudyptes chrysocome*), el albatros de la ceja negra (*Thalassarche melanophris*) y el prión pico fino (*Pachyptila belcheri*). En general, los niveles de Hg en las plumas del 96% de las aves marinas muestreadas en este estudio se encontraban por debajo de los 5 mg/kg. El valor más alto registrado en ese estudio fue de 6,65 mg/kg en un albatros de ceja negra. Este dato contrasta con los niveles de Hg en plumas de aves marinas en las mismas Islas Falkland hallados en estudios realizados 30 años antes, donde Thompson et al. (1993) obtuvieron niveles de Hg inferiores (2,68 ± 1,20 mg/kg) en pluma de 30 individuos de albatros de ceja negra.

Otros estudios recientes que se han realizado en aves marinas en el Océano Austral han encontrado, de manera similar, un aumento en niveles de mercurio. Por ejemplo, Becker et al. (2016) han reportado concentraciones de Hg en plumas significativamente superiores a los de estudios de años anteriores de varias especies de aves marinas (albatros negro (*Phoebetria fusca*), petrel de plumaje blando (*Terodroma mollis*), petrel del Atlántico (*Pterodroma incerta*), petrel gigante del sur (*Macronectes giganteus*) y petrel gigante del norte (*Macronectes halli*)). Recientemente, Barrales et al. (2021) han determinado la concentración de Cd y Pb en hígado, riñones y plumas de frailecillo atlántico (*Fratercula ártica*) procedente del noroeste de España.

Los niveles de Cd en hígado fueron 13,7±0,9 mg/kg, en riñón 22,1±1,0 mg/kg y en pluma 0,35±0,04 mg/kg, siendo las concentraciones de Pb halladas en hígado de 0,43±0,04 mg/kg, 0,19±0,011 mg/kg en riñón y 1,31±0,10 mg/kg en pluma. Otros estudios realizados en años anteriores con esta misma ave marina, uno realizado en el mar de Baren (Savinov et al., 2003) y el otro en Oklahoma (Beyer et al., 2004), mostraron resultados de Cd ligeramente inferiores a los obtenidos por Barrales et al. en 2021. Todas estas concentraciones fueron inferiores al nivel umbral estimado para la toxicidad en aves (45 a 75 mg/kg de peso húmedo) (Wayland y Scheuhammer, 2011). Al igual que ocurre con el Hg, en las colonias donde se tomaron muestras de aves marinas durante varios años, se ha visto un incremento sustancial de las concentraciones de Cd con el tiempo en todas las especies. Así, Mallory et al. (2014) informó de que se había producido un aumento sustancial de las concentraciones de Cd, siendo de un 370% en 307 individuos de eider común (*Somateria mollissima*) en el Ártico canadiense entre los años 1997 y 2008. Estos autores sugerían además que las concentraciones de Cd en este lugar estaban aumentando dramáticamente, aunque no pudieron apuntar a unas razones concretas que motivasen dicho aumento. Sin embargo, muy recientemente, Bianchini et al. (2022) aseguran en su estudio que las concentraciones de Cd, en lugar de aumentar, fluctúan con el tiempo, basándose en la variación observada entre años distintos en los niveles aparecidos en tres colonias espacialmente dispares. Se han realizado más estudios en los que se encuentran tendencias fluctuantes, estables o decrecientes en torno a las concentraciones de Cd en la biota del Ártico y en las regiones del norte, según el período de tiempo considerado (Gong y Barrie, 2005; Heidam et al., 2004; Rigét al., 2011; Steinnes y Friedland, 2006).

En los últimos años, se ha visto reducida la presencia de estos metales en el medio debido a que muchos países ha regulado o eliminado su uso en la industria. Este es el caso, por ejemplo, de la regulación del Cd y Hg para la fabricación de pilas, materiales y componentes de vehículos (RD 106/2008, y RD 1383/2002). Otro ejemplo claro y trascendente fue el caso del Pb, que fue eliminado de la gasolina en Estados Unidos a partir del 1973, siendo imitados por numerosos países. En referencia a este mismo metal, otro caso importante fue la prohibición de la fabricación de tuberías de plomo para los hogares españoles, que se reguló en el RD 140/2003 de 7 de febrero, donde en su art.20 se prohíbe explícitamente la presencia del plomo en aguas suministradas a través de una red de distribución pública o privada. Aunque durante tiempos se ha limitado la producción Pb por parte del ser humano, hay que mencionar que una fuente importante de contaminación respecto a este metal pesado son las balas fabricadas con este metal que se han utilizado durante siglos para la caza. Se estima que entre 600 y 700 millones de cartuchos que contienen balas de plomo (18.000–21.000 toneladas de plomo) se utilizan anualmente en Europa para la caza. El uso de perdigones y municiones de Pb para la caza varía entre los miembros de la UE, en función de su legislación nacional/regional (Mateo and Kanstrup, 2019). Parte de esta munición se dirige a los animales disparados, pero no todos ellos son abatidos, algunos animales quedan heridos. Las vísceras de los animales heridos que finalmente mueren de algunas especies, como por ejemplo los ciervos, hace que el Pb se disperse en el medio ambiente. Y hay que añadir que una pequeña proporción de los disparos llegan a golpear a sus objetivos, por lo que la munición gastada restante se dispersa ampliamente en el medio ambiente (ECHA, 2017). Mariussen et al. (2017) han estudiado la acumulación Pb en trucha marrón (*Salmo trutta*) del lago Kyrtjønn dentro de un campo de tiro abandonado en Noruega, comparándolo con otro sitio cercano y lo que obtuvieron fue que la

trucha del lago Kyrtjønn del campo de tiro tenía niveles de Pb significativamente superiores en hueso y otros tejidos. Runia y Solem (2016) encontraron niveles por ingestión de disparos de Pb en 660 faisanes de cuello anillado (*Phasianus colchicus*) capturados en cotos de caza en Dakota del Sur cinco veces superior que 1301 aves de áreas fuera de los cotos donde hay disponibilidad de balas de plomo presumiblemente era más bajo. Estudios europeos recientes en Grecia (Aloupi et al., 2015) y en Bulgaria (Mateo et al., 2016) han informado poca o ninguna evidencia de ingestión de disparos en gansos que se alimentan en áreas sin caza o con bajas densidades de disparos. Por lo que parece que la munición es un problema serio en la dispersión del Pb en el medio ambiente. Aunque son numerosos estudios en los que se siguen encontrando concentraciones de Pb en diversos animales. Otero et al. (2018) determinaron niveles de Hg, Cd, Cu, Co, Zn, Mo, Pb, Se y As en biomateriales (plumas, material fecal, cascara de huevo) generados por la gaviota patiaamarilla (*Larus michahelli*). La mayoría de los elementos que estudiaron estuvieron dentro de los valores reportados para biomateriales generados por otras aves marinas. Los niveles de Pb en pluma reportados por Otero et al. (2018) fueron 6,70±15,02 mg/kg y de As 2,48±6,12 mg/kg mientras que varios estudios en aves marinas de años anteriores las concentraciones medias de Pb fueron 0,83±0,37 mg/kg y de As 0,18±0,10 mg/kg. En algunos estudios se ha demostrado una relación directa entre la administración de Pb y los tejidos. Este es el caso de Choi et al. (2021), quienes afirmaron que existe un aumento lineal positivo de la concentración de Pb en el pelo de animales alimentados con sumplemento de Pb (200 ppm de Pb durante 56 días) en el laboratorio. Incluso en condiciones de campo, se ha observado el efecto de los hábitos alimentarios en la acumulación de Pb en el pelo (Fernandez et al., 2021)

El As también se ha visto regulado en los últimos años, por ejemplo, la EPA estableció el nivel permisible de As en el agua de bebida a 10 ppb (nivel máximo contaminante) (EPA, 2016). También en la alimentación, oscilando entre los 0,5 ppm en huevos y en los tejidos comestibles crudos de pollos y pavos, y los 2 ppm en ciertos subproductos crudos de cerdo (ATSDR, 2016). Un estudio reciente realizado por Ndu et al. (2020) se determinaron las concentraciones de As en pluma de pelicanos pardos orientales (*Pelecanus occidentalis carolinensis*) en el golfo de México, obteniendo valores por debajo de 1 ppm, que es el valor promedio informado para As en plumas obtenidas de aves en ambientes no contaminados, ya que 10 ppm es el valor promedio de As en plumas en sitios contaminados (Sánchez-Virosta et al., 2015). Alexe et al. (2021) evaluaron las concentraciones de As en plumas de pigargo europeo (*Haliaeetus albicilla*) en el delta del Danubio en Rumania, obteniendo concentraciones en individuos adultos de 1,16±0,37 mg/kg y en juveniles de 0,63±0,36 mg/kg, valores muy próximos a ambientes no contaminados.

El Se, a diferencia de los cuatro elementos mencionados anteriormente, no es dañino para los seres vivos, ya que desempeña funciones fisiológicas y bioquímicas importantes y es esencial para la producción de varias enzimas dependientes de este metal (selenoenzimas), que realizan funciones antioxidantes indispensables en el cerebro y el sistema neuroendocrino. La diferencia entre el beneficio del selenio y la toxicidad en los organismos es muy pequeña y va a depender de su especiación (Feroci et al., 2005). El efecto protector del Se contra la toxicidad del Hg ha sido ampliamente reconocido. El Hanafi et al. (2022) han afirmado que la selenoneína (derivado del aminoácido ergotioneína en el que el átomo de selenio reemplaza a un átomo de azufre) juega un papel protector, ya que se conjuga con metilmercurio formando HgSe en el hígado de petrel gigante antártico (*Macronectes giganteus*). Trevizani et al. (2022) han

determinado la concentración media de Se en plumas de petrel gigante antártico en varias localizaciones, obteniendo concentraciones de 5,06±1,82 mg/kg en la Isla Elefante, 5,06±1,82 mg/kg en la isla Rey Jorge y 4,92±1,22 mg/kg en la isla Livingston, todos estos lugares están en la Antártida, por lo que se puede establecer un patrón estable de acumulación en toda la zona.

Aunque la producción por parte del hombre de los elementos mencionados se ha visto restringida debido a las diversas regulaciones y prohibiciones, sigue siendo común y necesario llevar a cabo estudios orientados a la evaluación de residuos en suelos, plantas o tejidos de animales, de cara a poder confirmar que, si bien siguen apareciendo niveles en, por ejemplo, las aves marinas, estos suelen ser bastante inferiores a los umbrales de toxicidad. Los perfiles de los metales están asociados con el nicho de un animal, siendo este un factor a considerar cuando se evalua la distribución interna de los contaminantes ambientales en las especies de aves (García-Salas et al., 2023).

1.3.1.3 Efectos tóxicos de los metales y metaloides en las aves.

Los efectos y la distribución de los metales varían dependiendo del elemento considerado. En las aves, el mercurio de la dieta se incorpora a las plumas proveniente de la sangre y se bioacumula a través de la cadena alimenticia (Nriagu, 1989; Arcos et al., 2002). Las aves estudiadas en el presente trabajo son depredadores y, por tanto, se hayan en lo alto de la cadena trófica, por lo que son particularmente susceptibles a que el metilmercurio se bioacumule y biomagnifique. Concentraciones altas de Hg en tejidos de aves marinas han sido asociadas a consecuencias negativas en sus organismos, como disminución de la aptitud física a través de la alteración endocrina y la infertilidad de los huevos, y efectos indirectos como un mayor número de parásitos helmintos (Wayland et al., 2008; Dietz et al., 2013; Tartu et al., 2015). Se ha descrito que la exposición prolongada a Hg puede tener efectos sobre el sistema nervioso central, provocando pérdida de funcionalidad motora, incoordinación, apatía, temblores, cambios en el comportamiento, eretismo y depresión severa, y en el riñón (proteinuria, enzimuria y necrosis del túbulo proximal) (Heinz y Locke, 1976; Spalding et al., 2000).

El Cd no es un nutriente esencial para los animales, así que una exposición tanto aguda como crónica conlleva a una producción intracelular de metalotioneinas. Altas concentraciones de cadmio pueden causar daño al hígado; sin embargo, es el riñón el órgano crítico ante una toxicidad crónica, donde se provocará nefropatía causando una necrosis de las células del túbulo proximal, proteinuria, glucosuria, incremento de cadmio en la orina y aparición de metalotioneína en plasma (Barregard et al., 2022; Marini et al., 2023). Además, una exposición crónica en la dieta puede producir numerosos problemas como daño intestinal, reducción de la captación de nutrientes, defectos en el esqueleto, anemia, alteración de la reproducción y alteraciones en el comportamiento (Wayland y Scheuhammer, 2011).

El Pb es un elemento altamente tóxico que puede causar una gran mortalidad en aves. Tiene efectos subletales graves a nivel del sistema renal, afecta al sistema circulatorio y genera cambios fisiológicos, bioquímicos y en el comportamiento en las aves, además de tener efectos negativos en la reproducción (Scheuhammer, 1987; Mukherjee et al., 2022). Diversos autores han establecido la concentración límite a partir de la cual este metal puede causar efectos en las aves. A nivel hepático, Pain et al. (1995) establecieron que concentraciones por debajo de 2 ppm (peso seco, dw) podrían ser indicativas de una exposición ambiental segura, sin riesgo

toxicológico, y podría considerarse como valor umbral. Por otro lado, los valores de plomo situados entre 6 y 20 ppm (dw) podrían estar directamente asociados con la exposición masiva al Pb. Años después, Wayland et al. (1999) concluyeron que una concentración de 30 ppm (peso húmedo, ww) en el tejido hepático representaba un nivel potencialmente letal en aves. Entre los efectos negativos se han descrito alteraciones en la actividad reproductiva, como por ejemplo un adelgazamiento de la cascara del huevo (Grandjean, 1976) o una disminución en la producción de huevos (Edens y Garlich, 1983).

Con respecto al Se, se ha comprobado que altos niveles de selenometalotioneina en aves han producido una disminución de los nacimientos y deformidades en los embriones (Spallholz y Hoffman, 2002). A través de la dieta se ha observado que el selenito es ligeramente más tóxico para las ánades reales que el selenio en forma de selenometionina, presentando mayores efectos sobre el crecimiento a 20 ppm y sobre la supervivencia a 40 ppm (Heinz et al., 1988). Se ha establecido que concentraciones hepáticas superiores a 10 ppm (ww) de selenio en aves causan efectos subletales importantes, llegando a estar en peligro la supervivencia de estas cuando dichas concentraciones superan los 20 ppm (Ohlendorf y Heinz 2011).

El As pertenece al grupo de metales inactivos-redox que agotan los principales antioxidantes de las células (Koivula y Eeva, 2010). De hecho, igual que ocurre con el Cd y el Hg, el As induce estrés oxidativo mediante la inhibición de la actividad enzimática SOD o uniéndose a los grupos –SH de las proteínas (Kim et al., 2019). El As también es capaz de producir el anión superóxido (O_2^-), oxígeno simple, el radical peroxilo, óxido nítrico y peróxido de hidrógeno. Se ha sugerido que el O_2^- es la principal especie inducida por As, y con su formación conduce a una cascada de otras especies ROS (como H_2O_2 y OH) (Flora et al., 2008). Existen bastantes estudios relacionados con los efectos del arsénico en aves basados en la reproducción, comportamiento o crecimiento. Camarde et al. (1990) observó los efectos del arsenato sódico en el crecimiento y fisiología de los patos en una dieta que contenía 30, 100 y 300 mg/kg de As durante 10 semanas. Se pudo observar una disminución del aumento de peso, disminución de la ingesta de alimentos especialmente con la dosis más alta, además de observar la acumulación del metal en el hígado y en el cerebro. En otra investigación Albert et al. (2008) concluyó que las aves adultas tratadas durante 14 días con 8, 24 y 72 µg/g de Ácido monometilarsónico (MMA) contenían concentraciones de As total en sangre y tejidos de órganos que aumentaban con el aumento de la dosis. Además, las aves a las que se les suministraron 24 y 72 mg/kg de MMA mostraron una pérdida de masa significativa, pudiendo estar asociada con inflamación gastrointestinal.

1.3.2 Contaminantes orgánicos persistentes.

Los contaminantes orgánicos persistentes (COPs) son unos de los compuestos más contaminantes del medio ambiente debido a que son compuestos resistentes a la degradación fotolítica, biológica y química pudiendo perdurar en el ambiente durante extensos periodos y llegar a almacenarse en los tejidos grasos, sobre todo de peces y mamíferos marinos. Estos contaminantes son el producto de las actividades humanas por lo que hace que sean distribuidos ampliamente por todo mundo con posibles efectos adverso sobre la vida silvestre (Dietz et al., 2019), aunque si bien es cierto que la mayoría de ellos han sido estudiados y que tienen restringido su uso o su producción está prohibida (Ryan et al., 2013; Addison et al., 2014). Dentro de los contaminantes orgánicos persistentes se encuentran los policlorobifenilos (PCBs) y los plaguicidas organoclorados (OCPs). Los PCBs son una familia de 209 compuestos congéneres

que se diferencian en la cantidad de cloro que llevan en sus moléculas y en la conformación química lo cual le permite a cada uno actuar de diferente manera en el medio ambiente y en el ser humano. Algunos compuestos organoclorados (como el DDT) fueron los primeros en ser empleados en fumigaciones masivas para combatir la malaria y han sido prohibidos debido a su capacidad de bioacumulación y a su persistencia medioambiental. La transferencia a través de las cadenas alimentarias acuáticas a través de las relaciones depredador-presa dan como resultado una magnificación trófica que conlleva a fomentar la capacidad de acumularse en depredadores principales, como las aves marinas (Fisk et al., 2001). Se ha demostrado que los PCBs tienen elevadas concentraciones en redes alimentarias marinas y precisamente no se encuentran específicamente vinculados a las actividades del ser humano actual, sino que su presencia en el medio ambiente se debe a un legado de lanzamientos anteriores (McGoldrick et al., 2014). El diclorodifeniltricloroetano (DDT), sintetizado en 1874, demostró su potencia insecticida en 1939 y comenzó a ser utilizado como tal en 1942. El hexaclorociclohexano (HCH), sintetizado en 1825, se usó como arma en la 1ª Guerra Mundial y como insecticida en 1942. A partir de la segunda mitad del siglo XX se acelera la síntesis de productos organofosforados (Ferrer, 2003). Sin embargo, se prohibió la comercialización y la fabricación de los PCBs debido a los graves riesgos sobre el medio ambiente y sobre la salud, ya que una exposición a estos contaminantes puede causar cáncer, alergias, disfunciones neurológicas, lesiones cutáneas fotosensibles, cólicos, debilitamiento, trastornos metabólicos, deformidad de columna, trastornos reproductivos e inmunitarios (WHO, 1990; Ledirac et al., 2005). Estos efectos adversos bien documentados de los OCP han llevado a la prohibición de la mayoría de las especies desde la década de 1970 en más de 100 países firmantes del Convenio de Estocolmo. Sin embargo, debido a amplio uso histórico y persistencia, los OCPs todavía se detectan con frecuencia en medios ambientales.

Fue en la Convención de Estocolmo de las Naciones Unidas de 2001 cuando se creó la lista de COPs, incluyéndose 13 OCPs: aldrín, dieldrín, endrín, clordano, heptacloro, hexaclorobenceno (HCB), hexacloro-ciclohexanos (α-HCH, β-HCH, Δ-HCH γ-HCH), endosulfán (I y II), endosulfán sulfato y diclorodifeniltricloroetano (DDT) (Stockholm Convention, 2021).

1.3.2.1 Características de los contaminantes orgánicos persistentes.

Los PCBs se caracterizan por tener siempre la misma estructura bifenilo, es decir formada por dos anillos de benceno (Fig. 1.4). El lugar que ocupen los átomos de cloro de los que se compongan, así como la posición de los anillos, son críticos en la determinación de la toxicidad de estos compuestos. Los PCBs se dividen en dos categorías distintas: los coplanares y los no coplanares. Los coplanares tienen una estructura bastante rígida, con los dos anillos situados en el mismo plano. Por otra parte, los PCBs no coplanares, con átomos de cloro situados en las posiciones orto, presentan una toxicidad más reducida y no relacionada con la activación del receptor de hidrocarburos de arilo (AhR), sin embargo, suelen encontrarse en mayores concentraciones en el medioambiente (O'Hara y Rice, 1996).

Los PCBs se absorben eficazmente por vía gastrointestinal, por vía inhalatoria y por contacto cutáneo. Para el ser humano la principal fuente de exposición a estos contaminantes es a través de los alimentos, más concretamente los peces y las aguas contaminadas. Menos importante es el aire como fuente de contaminación. La piel puede ser una importante vía de absorción para aquellas personas que se encuentra expuestas en su lugar de trabajo. Los peligros

de los PCBs están asociados a la exposición a estos contaminantes de manera crónica pudiendo llegar a imitar el comportamiento de alguna hormona, como por ejemplo los estrógenos y pudiendo llegar a modificar el sistema endocrino, por lo que son considerados disruptores endocrinos, siendo unos de los mecanismo que han acaparado la atención en la última década, por el riesgo alto que supone incluso a bajas concentraciones de estos contaminantes (Petrovic et al., 2004; Sosa-Ferrera et al., 2013). Los efectos tóxicos de estos contaminantes son bastante difíciles de predecir debido a su compleja naturaleza, estudios toxicológicos han confirmado que los PCBs son potencialmente tóxicos sobre los mamíferos incluido los seres humanos, llegando a producir retrasos en el crecimiento y toxicidad en la reproducción (Berghuis y Roze, 2019), disminución de la atención paterna y efectos neurológicos, hepatotoxicidad (Xie et al., 2019), neurotoxicidad (Pessah et al., 2019) entre otros (Guo et al., 2020). La Agencia Internacional para la Investigación del Cáncer clasificó a los PCB como carcinógenos humanos del Grupo I (IARC, 2016), y la Agencia de Protección Ambiental clasificó a los PCB como compuestos cancerígenos humanos subyacentes (EPA, 2015).

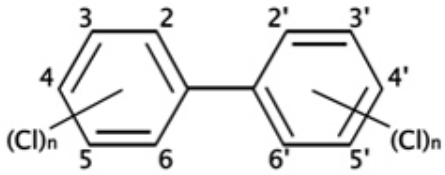


Fig. 1.4. Estructura química básica de los PCBs. Los números 2-6 y 2'-6' representan posibles posiciones del átomo de cloro (Cl) dentro de cada anillo bencénico.

Los PCBs fueron introducidos en el medio ambiente como mezclas comerciales de congéneres (por ejemplo, Aroclor en los Estados Unidos o Clophen en Alemania) (EPA, 1980), por lo que los PCBs suelen entrar en el medio ambiente como mezclas (Barron et al., 1995). En la presente tesis se han estudiado los PCBs 28, 52, 101, 118, 138, 153 y 180, ya que están presentes predominantemente en matrices bióticas y abióticas y han sido reconocidos como compuestos representativos de todo el grupo de PCB por la Agencia de Sustancias Tóxicas y registro de enfermedades (ATSDR, 2000).

Los plaguicidas organoclorados (OCPs) conforman un grupo de sustancias contaminantes utilizadas en diversos ámbitos, principalmente en la agricultura, por su efecto tóxico sobre diferentes organismos. Estos compuestos son sustancias liposolubles capaces de atravesar la barrera hematoencefálica y con capacidad neurotóxica. Estos contaminantes son compuestos orgánicos derivados de hidrocarburos complejos, en los que un cloro sustituye al hidrógeno. Como características físico-químicas principales, presentan una solubilidad baja en agua y muy alta en lípidos y en solventes orgánicos, lo que hace que se acumulen fácilmente en tejidos grasos. Además, son muy estables, siendo difícil su degradación, presentando una baja presión de vapor. En la Tabla 1.2 se pueden observar las características de los 11 plaguicidas objeto del presente estudio.

1.3.2.2 Presencia de los contaminantes orgánicos persistentes en el medio ambiente.

Los contaminantes orgánicos persistentes como hemos indicado anteriormente son sustancias químicas orgánicas de origen sintético que debido a su lenta degradación es posible encontrar en regiones donde nunca se ha producido la síntesis de estos compuestos, debido sobre todo a

su elevada permanencia en el ambiente y a su capacidad de transporte. Este transporte es conocido como efecto saltamontes, dado que estos compuestos químicos se evaporan en lugares cálidos y se dejan arrastrar por el viento introducidos en partículas de polvo para asentarse en lugares templados, evaporarse y seguir, así, desplazándose (MAATE, 2012).

El suelo es un reservorio muy importante para estos contaminantes, su acumulación va a depender de los microorganismos presentes en el propio suelo, la concentración de los contaminantes y de la interacción de ambos. El contenido de materia orgánica que presente el suelo va a ser un parámetro vital que afecta a la concentración de OCP en el suelo, la materia orgánica sólida se clasifica húmica o no húmica (Bleam, 2016). La materia húmica constituye aproximadamente el 80% del total de materia heterogénea de origen biogénico, presentan estabilidad y complejidad, se caracterizan por un color amarillo y alto peso molecular (Albers et al., 2008). Por otro lado, las sustancias no húmicas consisten en moléculas orgánicas simples como péptidos, aminoácidos y azúcares. Todas estas características que posee el suelo afectan a la contaminación por OCP en diferentes grados La materia orgánica solida actúa como un parámetro clave para la bioacumulación de COP (Sweetman et al., 2005; Dalla Valle et al., 2005). Un suelo rico en huminas, con baja proporción de materia orgánica solida va a ser más eficaz a la hora de acumular estos contaminantes que un suelo con una baja cantidad de humina (Andreux, 1996). Mao et al. (2021) encontraron relaciones directamente proporcionales entre los PCBs estudiados y el contenido de materia orgánica del suelo en China. En este mismo estudio, se reportó una gran variación en los niveles hallados de PCBs en muestras de suelo, oscilando las concentraciones totales de los 209 PCBs entre 64,3 y 4358 µg/kg.

Los sedimentos son considerados como una fuente importante de acumulación de OCPs. Aunque en un primer momento puedan ser transportados en el medio ambiente a través de las precipitaciones, posteriormente se depositan en sedimentos y acaban interactuando con la biota del entorno circundante. Los COPs no solo se encuentran en el agua y en los sedimentos, sino que también se acumulan en la cadena alimentaria ya que pueden entrar en contacto con otras partículas en suspensión o bioacumularse en peces y otros organismos acuáticos, dando lugar a efectos secundarios (Lorenzo, 2018). Existen diversos estudios recientes desarrollados en sedimentos en España. Así, Pérez-Fernandez et al. (2019) determinaron concentraciones en sedimentos de la bahía de Santander de Benzo(ghi)perileno (hidrocarburo aromático policíclico) que oscilaban entre 0,26 y 5,44 µg/kg, y una concentración media de 1,09 µg/kg del congénere PCB 118. En otro estudio realizado por Pintado-Herrera et al. (2017) en la Bahía de Cádiz y Ría de Huelva se demostró que los sedimentos superficiales contenían concentraciones de hidrocarburos aromáticos policíclicos de 1098 µg/kg, de fragancias como Octahidrotetrametil acetofenona 133,5 µg/kg, y de filtros UV como el Octocrileno: 72 µg/kg y el nonilfenol 575 µg/kg.

La contaminación de OCP en agua dulce depende de su producción y fabricación, así como de su distribución carga, propiedades químicas, persistencia, uso y demanda, período de regulación, y propiedades fisicoquímicas. Solo el 10% de las aguas residuales generadas por las industrias y fabricas es tratada para la eliminación de los propios residuos, la parte restante de los residuos sin tratar se descarga directa en los arroyos. Esto va a provocar que los contaminantes se introduzcan en las aguas subterráneas, ríos y otros cuerpos de agua que se utilizan para fines domésticos (De Bruyn et al., 2009).

Una vez que los contaminantes alcancen los ecosistemas acuáticos se verán sometidos a diferentes procesos que vendrán determinados tanto por las propias características físico-químicas, la naturaleza y la reactividad de los propios contaminantes como por la naturaleza y características del ambiente abiótico en el que se encuentran y las características propias de los seres vivos que puedan entrar en contacto con los contaminantes (Katsoyiannis y Samara, 2007; Valsaraj y Thibodeaux, 2010). Estos contaminantes llegan a los seres vivos donde hacen su efecto a través de un proceso de varias etapas: en la primera etapa se produce un intercambio de un producto químico entras las partículas y la fase disuelta del medio, para que a continuación se lleve a cabo el transporte de esta sustancia hasta que se encuentre con un ser vivo, donde se asimilará a través de una membrana celular para que circule por el organismo hasta un lugar donde pueda ejercer un efecto tóxico. Por último, se puede producir la ingesta de ese ser vivo por un organismo en un nivel trófico superior por lo que el ciclo de vida del contaminante continuará (Cal Rodriguez, 2015). Las principales vías por las que llegan a los organismos acuáticos son: bioconcentración por absorción directa del agua a través de las branquias o la piel y biomagnificación por consumo de alimentos contaminados que se transfieren del intestino al cuerpo (Van der Oost et al., 1996; Zhou et al., 2007). El transporte de estas moléculas por el medio ambiente se puede se puede observar de manera esquemática en la Fig 1.5.

Igual de importante es determinar los niveles en matrices bióticas que en las abióticas. De hecho, existen numerosos estudios donde se han evaluado los niveles de contaminantes orgánicos persistentes en animales y plantas. Las plantas pueden ayudar en el ciclo de vida de los COPs, dado que pueden absorberlos a través de sus raíces, lo que las convierte en un eficiente sumidero para estos contaminantes tóxicos. Además, la acumulación también puede producirse por la deposición a través del aire sobre las hojas. Este intercambio de contaminantes que se lleva a cabo entre las plantas y el suelo se puede producir a través de un proceso que se llama difusión, por proceso de flujo medio, o incluso una combinación de ambos (Mechlińska et al., 2009). Algunas plantas poseen la capacidad de transportar y acumular COPs sin efectos drásticos en su funcionamiento y por lo tanto pueden actuar como organismos esenciales para eliminar los contaminantes tóxicos de los entornos (Kumar y Shukla, 2022), siendo usadas en tareas de biorremediación.

Por otro lado, los animales son también un reservorio relevante de estos contaminantes, ya que la propia naturaleza lipídica de los COPs hace que se acumulen en los tejidos grasos de los animales, lo que va a producir un aumento de la exposición continua de otros órganos vitales en el cuerpo. Las ovejas, los peces, las águilas, los osos polares y las ballenas forman parte de varias cadenas alimentarias que están contaminadas por COPs; esto reduce sus poblaciones causando varios desórdenes que son perjudiciales para la supervivencia de estos animales (Nuñez et al., 2012). Lippold et al. (2018) realizaron un estudio donde se encontraron concentraciones de algunos contaminantes orgánicos persistentes en plasma de hembra de oso polar recolectadas en el área del mar de Barents durante los años 1997-2017, concretamente PCB-118, PCB-138, PCB-153, PCB-180. En otro estudio, realizado en Bangladesh, Habibullah-Al-Mamun et al. (2022) hallaron concentraciones de OCPs en peces salvajes y en peces de piscifactorías, en ambos casos de la especie *Heteropneustes fossilis*. De los 8 OCPs investigados (Endrin, Hepatoclor, Hepatoclor epoxi, Metoxicloro, Endosulfan, p,p´-DDT, p,p´-DDE y p,p´-DDD), siete se detectaron en todas las muestras de pescado, faltando por detectar el p,p'-DDT (<LOD). La concentración total de OCPs (suma de todos los detectados) varió entre especies, desde 6,3

µg/kg en los peces de piscifactorías hasta 162 µg/kg en los peces salvajes. Cheng et al. (2021) afirmaron que, en la mayoría de los casos, los peces silvestres presentaban mayores niveles de OCP que las mismas especies criadas en piscifactoría, apuntando como posible causa de esta diferencia a la incorporación de estos compuestos a los recursos hídricos naturales a través de escorrentías agrícolas y aguas residuales industriales.

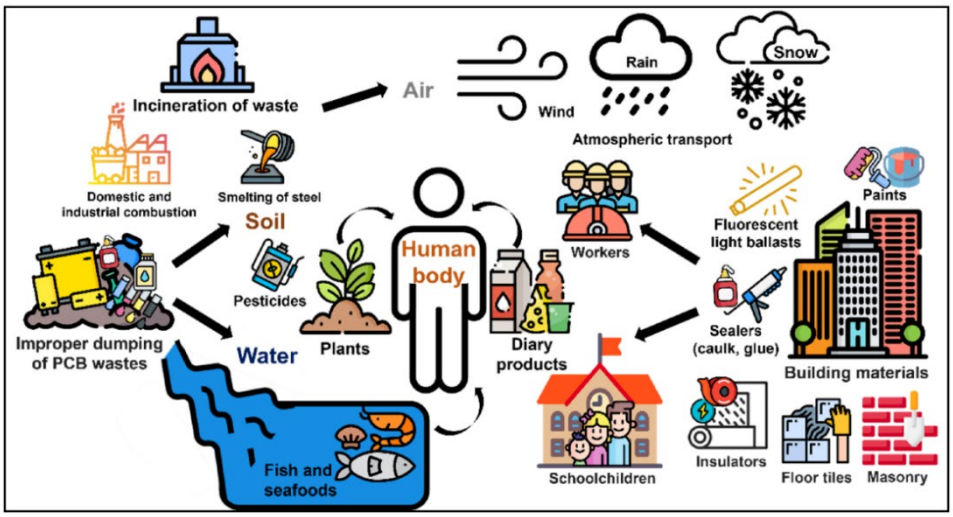


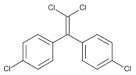
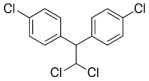
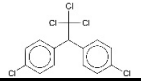
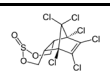
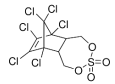
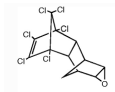
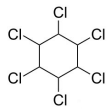
Fig. 1.5. Ilustración esquemática del transporte de PCB en diversas matrices ambientales (Montano et aL., 2022).

1.3.2.3 Efectos tóxicos de los contaminantes orgánicos persistentes en las aves.

La entrada principal de los COPs en las aves es por vía oral, por consumo de alimentos, así como por vía pulmonar. Debido a su liposolubilidad, una vez que se absorben en el organismo, se acumulan en el tejido adiposo, de forma que se producen procesos de biomagnificación a través de la cadena trófica (Hoffman et al., 1996). Son eliminados por la bilis o por las heces, mientras que los metabolitos se eliminan principalmente por orina si presentan una alta polaridad (Smith, 2004). En el caso de las aves, durante la época de puesta, las hembras eliminan estos compuestos a través de la yema de huevo, debido a su elevada afinidad por los lípidos que forman parte de este producto (Ross et al., 2008; Van den Steen et al., 2009). Numerosos estudios, donde las aves han sido utilizadas como bioindicadores de contaminación ambiental, han podido reflejar los efectos de los COPs en estos animales. Los efectos reportados incluyen, por ejemplo, el adelgazamiento y debilitación de la cáscara del huevo de especies de patos domésticos (*Anas platyrhynchos)* (Lundholm, 1997). King et al. (1978) observaron que las cascaras de huevos recogidos en 1980 del cormorán neotropical (*Phalacrocorax brasilianus*) eran más delgadas que las mostradas por los huevos recopilados antes de 1947; sin embargo, este hecho parece no afectar a otras especies como las gaviotas, donde los huevos son relativamente insensibles a los efectos adelgazantes de COPs como el DDT (Hickey y Anderson, 1968). Otros efectos reportados son la feminización de los machos y comportamiento sexual anormal en gaviotas de Californiana (*Larus californicus*) (Fry y Toone, 1981; Iwaniuk et al., 2006), la inhibición de la puesta de huevos y disminución del tamaño de la nidada en cormorán orejudo (*Phalacrocorax auritus*) (Larson et al., 1996), la disminución en el éxito de eclosión (King y Krynitsky, 1986), el incremento en la frecuencia de deformidades en los embriones (Fry y Toone,

1981; Larson et al., 1996) y la reducción del tamaño del cerebro y neurotoxicidad (Iwaniuk et al., 2006). Las especies de aves que principalmente se alimentan de peces presentan una mayor bioacumulación de PCBs que las aves que se alimentan en los niveles tróficos inferiores (Prestt et al., 1970; Gillett y Ram, 1993). Entre las aves afectadas, pueden encontrarse las gaviotas, como ha podido ser demostrado en trabajos realizados con gaviota hiperbórea (*Larus hyperboreus*), donde la exposición a organoclorados provocó cambios de comportamiento durante el cortejo, en la construcción del nido y en el cuidado de los pollos, aumentando el riesgo de ser depredados (Bustnes et al., 2005). Verreault et al. (2004) concluyeron en su estudio con gaviota hiperbórea que existe una asociación entre niveles altos de contaminantes orgánicos halogenados en sangre y una alteración de los niveles de hormona tiroidea (TH) circulantes. En este sentido, hay que aclarar que la TH juega un papel primordial en el inicio y/o la regulación del desarrollo, la reproducción y el comportamiento. El DDE se ha asociado con el adelgazamiento de la cáscara del huevo o con efectos adversos sobre la reproducción en poblaciones silvestres de varias especies de aves (Wiemeyer et al., 1984). También se han establecido correlaciones entre concentraciones de 5 mg/kg de HCB en sangre de aves y efectos a nivel metabólico y alteraciones hormonales (TH) (Blévin et al., 2017).

Tabla 1.2. Características generales de los plaguicidas organoclorados analizados en el presente estudio. DL_{50}= Dosis letal para el 50% de la población, O=Oral, D=Dérmica, R=Ratas, C=Conejos. National Center for Biotechnology Information (2022).

Compuesto		Estructura	Usos	Características	Observaciones	Prohibición	DL_{50}
4,4'-DDE (Diclorodifenildicloroetileno)			Usado en el pasado como insecticida	Aparece como sólido cristalino blanco o polvo blanco	DDE es un producto de descomposición de DDT	1965	880 mg/kg (R) 700 mg/kg (M) >5 gm/kg (H)
4,4'-DDD (Diclorodifenildicloroetano)			Control de insectos en cosechas e insectos portadores de enfermedades (malaria y tifus)	Aparece como un sólido cristalino incoloro. Insoluble en agua y se hunde en el agua.	Es un metabolito del insecticida organoclorado, DDT.		113 mg/kg (R) 600 mg/kg (M) >5 gm/kg (H)
DDT (Diclorodifenil tricloroetano)			Utilizado en plagas agrícolas y para eliminar vectores de enfermedades	Muy resistente. Resistente a la destrucción por la luz y la oxidación. Bioacumulación significativa.	Mezcla de isómeros p,ṕ -DDT	Orden 4 febrero 1994 En 2008 cesa la producción de Dicofol (producto con DDT)	225 mg/kg (O/R)
Dieldrín			Control de plagas de insectos y parásitos	Muy volátil. Cristales blancos inodoros, o en ciertas ocasiones, con un olor suave.	El dieldrín es un metabolito del aldrín	Directiva 76/464 del Consejo, de 4 de mayo de 1976	38,3 mg/kg (R) 38 mg/kg (M)
Endrín			Control de insectos, roedores y pájaros	Sólido cristalino incoloro-color tostado. Bioacumulación considerable.	Epóxido de isodrín. Transformación a aldehído por luz y calor	Orden 4 Febrero 1994	7,5-17,5 mg/kg (O/R) 15mg/kg (D/R)
Endosulfán			Control de insectos en alimentos, cultivos no alimentarios y protector de madera	Sólido cristalino marrón. Estable a la luz solar e inestable a medios alcalinos. No se considera bioacumulable	Mezcla de isómeros I (70%) y II (30%). Se transforma a sulfato (toxicidad similar)	Decisión de la comisión 2005/864/CE	18-43 mg/kg (O/R) 15 mg/kg (D/R)
Endosulfan sulfato			Control de plagas de insectos (moscas blancas, áfidos, langostas, escarabajo de la papa, gusano de la col) en agricultura	Escamas con tendencia a la aglomeración, de color crema a marrón, mayormente cristales de color castaño claro	Pesticida más tóxico en el mercado. mezcla de estereoisómeros, designados "α" y "β," en una relación 7:3	Endosulfán prohibido en: 2009 prohibido en Nueva Zelanda, 2011 Uruguay y Argentina	30 mg/kg (R)
Epóxido de heptacloro			Plaguicida en viviendas, edificios y en cosechas de alimentos	Polvo blanco que huele a alcanfor. La forma de menor pureza es de color blanco	Las bacterias y los animales degradan al heptacloro a epóxido de heptacloro. Más común epóxido.	1998	
HCB (Hexaclorobenceno)			Tratamiento de semillas (trigo), para controlar el crecimiento fúngico	Hidrocarburo clorado cristalino, blanco, estable y no muy soluble en agua	Emite humos muy tóxicos de CO, CO_2, HCL y otros compuestos clorados cuando se calienta hasta la descomposición	Entró en vigor el 17 de mayo de 2004	3500 mg/kg (R) 4000 mg/kg (M)
Hexaclorociclohexano	β-HCH		Subproducto de la producción del insecticida lindano		Mezcla de isómeros: α (60-70%), β (6-8%, casi inactivo),ᾰ (2-5%), γ-HCH (12-15%, mayor actividad insecticida)	Orden 4 Febrero 1994 (HCH) Decisión 200/801/CE (Lindano)	1752,8 mg/kg (R) > 1362,5 mg/kg (C)
	γ-HCH		Lindano. Insecticida, agente contra ectoparásitos en productos veterinarios y farmacéuticos, tratamiento suelos y semillas	Polvo cristalino entre blanco y amarillo. Se acumula moderadamente			

1. 4 Objetivos.

El objetivo general de la presente tesis doctoral es la evaluación de los niveles de metales pesados y contaminantes orgánicos persistentes acumulados en gaviota patiamarilla (*Larus michahellis*), así como de los efectos subletales inducidos por la exposición en el sistema antioxidante.

De esta forma, la tesis se ha dividido en tres partes. La primera parte se compone de una revisión crítica de datos recopilados de la bibliografía sobre niveles de metales pesados y metaloides encontrados en diversas aves marinas (Publicación I). La base de datos generada es de gran utilidad para servir de guía a futuros estudios de biomonitorización.

La segunda parte se centra en la biomonitorización de contaminantes ambientales poniendo especial atención al uso de la gaviota patiamarilla como herramienta de biomonitorización. Para ello, en primer lugar, se realiza un estudio de metales y metaloides acumulados en tejidos internos y en plumas de *Larus michahellis* (Publicación II) y, finalmente, se estudian los niveles de contaminantes orgánicos persistentes (plaguicidas organoclorados y policlorobifenilos) en tejido graso (Publicación III). Los datos generados ayudarán a ampliar la bibliografía disponible en aves marinas.

En la tercera parte de la tesis, se evalúan los posibles efectos subletales que la exposición a los contaminantes analizados puede provocar en el sistema antioxidante de gaviota patiamarilla (Publicación IV). De esta forma, debido a los pocos estudios disponibles en cuanto a niveles de parámetros del sistema antioxidante y efectos que los contaminantes son capaces de producir en biomarcadores de estrés oxidativo, se pretende proporcionar nuevos datos que ayuden a establecer niveles de no efecto en aves.

A continuación, se describen los objetivos específicos de cada publicación:

Parte 1. Generar una base de datos sobre niveles de metales y metaloides detectados en aves marinas.

- ***Publicación I. Mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se), and arsenic (As) in liver, kidney, and feathers of gulls: a review.***

Realizar una revisión crítica sobre el estado actual de los estudios de biomonitorización de contaminantes inorgánicos en el medio ambiente, usando las aves marinas para ello. La presente revisión tiene como objetivo no solo proporcionar datos de referencia globales sobre los niveles de metales y metaloides en las gaviotas y otras aves marinas, sino también comprender sus efectos tóxicos putativos.

Parte 2. Biomonitorizacizacion de contaminantes inorgánicos y orgánicos en gaviota patiamarilla.

- ***Publicación II. Heavy metals and metalloid levels in the tissues of yellow-legged gulls (Larus michahellis) from Spain: sex, age, and geographical location differences.***

El objetivo principal fue determinar la validez de una especie de gaviota como una herramienta útil para la biomonitorización de niveles de metales en el medio ambiente. Para alcanzar este objetivo, se procedió a evaluar la concentración de tres metales pesados (mercurio, cadmio y plomo) y dos metaloides (selenio y arsénico) en hígado, riñón y plumas de *L. michahellis* recolectadas en tres zonas del noroeste de España. Se evaluó la influencia de factores exógenos (localización geográfica y tipo de captura) y endógenos (edad y sexo) sobre los patrones de acumulación.

- ***Publicación III. Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (Larus michahellis) from Spain: role of gender and age.***

El objetivo fue evaluar los niveles de diferentes COPs en tejido adiposo de gaviotas de diferentes regiones de la costa atlántica española, con el fin de determinar si la exposición a contaminantes orgánicos supone una amenaza para el medio ambiente objeto de estudio. Además, con el interés de determinar la idoneidad de esta especie de ave marina como bioindicador, también se investigó las posibles diferencias relacionadas con dos factores endógenos, la edad y el sexo, en los niveles de COPs.

Parte 3. Evaluación de estrés oxidativo en gaviotas patiamarilla expuestas a contaminantes ambientales.

- ***Publicación IV. Biochemical effects of heavy metals and organochlorine compounds accumulated in different tissues of yellow-legged gulls (Larus michahellis).***

Evaluar si los niveles encontrados de metales y metaloides (Pb, Cd, Hg, Se y As) y PCBs en gaviota patiamarilla de Galicia y Asturias generan algún tipo de efecto subletal en los animales, mediante la evaluación de biomarcadores de estrés oxidativo, incluyendo el contenido en glutatión reducido (GSH), la actividad de enzimas antioxidantes (GPx, GR, CAT y GST), y la peroxidación lipídica (MDA).

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2. Mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se), and arsenic (As) in liver, kidney, and feathers of gulls: a review.

Vizuete J., Pérez-López M., Míguez-Santiyán M.P., Hernández-Moreno D.

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2.1 Resumen: Mercurio (Hg), Plomo (Pb), Cadmio (Cd), Selenio (Se), y Arsénico (As) en hígado, riñón, y plumas de gaviota: revisión.

Actualmente algunos metales como el mercurio (Hg), el plomo (Pb), el cadmio (Cd), el selenio (Se) y el arsénico (As) generan una amenaza al medio ambiente llegando a existir una gran preocupación por los posibles efectos sobre los seres vivos que habitan en este. En esta revisión se ha tratado principalmente de recopilar numerosos y diferentes estudios de aves marinas, enfocando la búsqueda principalmente sobre gaviotas, aunque también se estudiado otras especies de estas aves, con el fin de que puedan ser utliles para investigaciones futuras y asuntos regulatorios. En esta búsqueda de artículos se ha tratado de conocer las concentraciones de Hg, Cd, Pb, Se y/o As en hígado, riñón y/o plumas. Los resultados del análisis de esta revisión muestran que el Hg y el Cd se han determinado principalmente en hígado en numerosas investigaciones, sin embargo, los niveles de Pb, Se y As se determinaron en bastantes menos estudios. Además, se ha podido detectar que existe una falta de estudios en los cuales se analicen dos o más metales en más de un tejido, lo que sería de gran utilidad para comprender los posibles efectos después de la exposición desde una perspectiva amplia. Algunos autores han informado que las diferencias interespecíficas en la exposición de elementos están determinadas por múltiples factores, incluidas las propiedades del contaminante, la especie, los hábitos de alimentación, el estado migratorio, el sexo y la edad. En este trabajo se pretende proporcionar una descripción completa y útil de la exposición de los elementos mecionado anteriormente en gaviotas y las otras especies de aves marinas seleccionadas.

2.2 Artículo publicado.

Mercury (Hg), Lead (Pb), Cadmium (Cd), Selenium (Se), and Arsenic (As) in Liver, Kidney, and Feathers of Gulls: A Review

Jorge Vizuete, Marcos Pérez-López, María Prado Míguez-Santiyán, and David Hernández-Moreno

Contents

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1 Introduction

The environment is exposed to different anthropogenic pollutants generated by industrial, domestic, and agricultural activities, which increase level of pollutants derived from natural sources. Altogether makes that, in the last few decades, metals and their impact on the environment have gained attention. Metals such as arsenic (As), mercury (Hg), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), iron (Fe), manganese (Mn), and zinc (Zn) do not degrade. These metals could easily accumulate throughout the trophic chain and persist in nature (Dimari et al. 2008). Some metals are essential to living organisms, but can become highly toxic or toxic when reaching concentrations that adversely affect the organism. Those concentrations, inherent to each specific pollutant, can be reached as a consequence of an acute exposure or by accumulation from low concentration in a long-term exposure. Measuring the levels of metals and assessing their effects on the environment is not easy due to the extreme complexity of issues such as bioavailability and susceptibility. Other complicating factors include measurement tools and detection limits, environmental conditions, the absence of correlations between different metals (Rattner and Heath 1995), and the specifics of the metals themselves (chemical form in which they appear, speciation, etc.). To quantify pollution levels and to evaluate the state of the environment, biomonitoring programs have been established. The aim of biomonitoring is to aid decision-making on ecosystem health problems. Different animals, including fish, birds, and mammals, are potential bioindicators (any species or group of species whose function, population, or status can reveal the qualitative status of the environment) for metal pollution, since the levels of metals in these animals may reflect their concentrations in the surrounding environment. The main tools used to develop biomonitoring programs are biomarkers (substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecules(s) or cell(s) that are measured within a compartment of an organism and can be related to exposure and/or effect) (WHO 2006). Chemicals, metabolites, the products of interactions between chemicals and their target molecules (i.e., inductions in acetylcholinesterase or oxidative stress enzymes activities), and cells can all be used as biomarkers. Monitoring trace metals in the environment is important to understand and to anticipate on the extent of contamination and damage, to evaluate decisions on public health or environmental protection, to determine research priorities, and to serve as scientific basis for regulatory actions (Cid et al. 2009).

Regarding birds, only a few publications provide guidance for establishing the suitability of avian species as biomonitors (Burger et al. 2007). However, Hollamby et al. (2006) presented a list with the ideal requirements for a biomonitoring program using avian species. According to this list, the most relevant characteristics and factors for selection of an avian bioindicator are the following: (1) The population is large enough not to be adversely affected by sampling; (2) Species should be long-lived; (3) Size, age, and sex differences within the species can be documented; (4) Size, age, and sex variation in bioaccumulation of the chemicals that will be

evaluated can be documented; (5) Specimens selected for sampling can be stored until analysis; (6) For bioaccumulation to reflect local environmental contamination, species should be nonmigratory and non-nomadic, at least for that part of their life cycle during which sampling occurs; (7) To study bioaccumulation, the species used as bioindicator has to occupy a high tropic level in the food chain; (8) The chemical's route of exposure should be documented; and (9) Knowledge of the chemicals to be examined, including their local usage and their activity and reactions in the local environment, can be documented.

In this sense, birds are conspicuous animals that can be influenced by climate change and changes in contaminant levels in the environment, existing some evidences citing them as bioindicators of environmental pollution, even if there is not a proper guidance about their use in this line. These animals are considered to be appropriate sentinel organisms, since they can accumulate high levels of contaminants. Unexpected changes in terms of populations, health, or breeding success may indicate a pollution or a food supply problem (Furness and Camphuysen 1997).

Field studies in which metal levels are monitored and in which their effects on bird populations are evaluated are necessary to determine the impact of environmental levels of metals/metalloids in wild bird species. This is not only related to acute exposure; it has also been reported that long-term pollution may induce changes in plant and animal communities, which in turn cause changes in food quantity and quality and induce secondary effects on bird species (Eeva et al. 2005).

Birds incorporate metals and metalloids in their organism by ingestion of prey, sediments, or soils that have accumulated these elements or inhalation. Absorption rates vary among metals and metalloids depending on species' physiology, intrinsic properties, and bioavailability (Burger et al. 2004). Indeed, susceptibility to contaminants in birds often varies with age, reproductive stage, gender, life history strategy, diet, and habitat use (Burger 1994; Peakall and Burger 2003).

In general, seabirds are exposed to a wide range of chemicals because they are top predators and scavengers (Samelius and Alisauskas 1999). One of the main routes of contaminant exposure is the ingestion of food and water (oceans act as a sink for most pollutant) (Burger et al. 2007). As a consequence of it, a phenomenon called biomagnification of pollutants occurs, which involves the accumulation of a substance directly related to the increase in the trophic chain. These animals are therefore useful bioindicators of coastal and marine pollution (Furness and Camphuysen 1997).

Seabirds act as biological vectors of contaminants in three different ways: (1) contaminant collection from the environment, (2) contaminant transport, and (3) contaminant deposition, release, or transfer at receptor sites (Blais et al. 2007).

Gulls fulfill most of the above described criteria for suitable bioindicators. Many species of gulls have been used as bioindicators, including Herring gull (*Larus argentatus*), Glaucous gull (*Larus hyperboreus*), great black-backed gull (*Larus marinus*), and kittiwake (*Rissa tridactyla*). These species have common characteristics: they are considered top predators, they spend a considerable part of their lives in coastal and marine environments, they have a wide ecological adaptability, they are abundant and easily accessible, and developing chicks are usually fed foods from

within a few kilometers of the breeding colony (Drury and Kadlec 1974; Duncan 1981). Over the years many gull species have been exhibiting plastic feeding behavior, learning and adapting to the resources available, and some count interspecific kleptoparasitism among their skills. This can be a successful foraging strategy and demonstrates the gulls' mastery of their habitat and their application of brainpower to problem-solving (Morand-Ferron et al. 2007). It is also important to know the migratory nature of these birds to be able to establish a relationship between these animals and pollution in the surrounding area. It has been shown that the migratory nature of gull differs between species. Olsson (1958) has, for example, shown that *L. argentatus* tend to stay in places where feeding conditions are favorable and only move further away if conditions are unfavorable. Moreover, Spaans (1971) reported that the majority of the *L. argentatus* population remains within 100 km of the ringing location. Indeed, it has been reported that *L. argentatus* inhabit Northwest Europe and the Atlantic coast of Portugal and Spain (Collinson et al. 2008). In contrast, although *R. tridactyla* is not considered a real migratory species, this species usually migrates from Europe to pelagic waters of the Atlantic after breeding season. Furthermore, *L. hyperboreus* behaves as a dispersive species that migrates from the Western Palearctic to the British Isles and the North Sea in winter.

Another important factor in evaluating the suitability of these birds as bioindicator is their feeding habit. For example, *L. glaucoides* and *L. ridibundus* have a diet that predominantly consists of small fish, marine invertebrates, bird eggs, chicks, seeds, and fruits (Hoyo et al. 1996). In contrast, *L. argentatus* and *L hyperboreus* are opportunists and generalist foragers that eat a wide variety of food. The diet of these gulls includes mussels, starfish, fish obtained by direct capture from shoals near the surface, and fish offal scavenged from quays and fishing boats (Watson 1981). In addition to these feeding habits, it is worthy to mention that increases in the human population and a corresponding increase in the volume of garbage produced have allowed this species to find easily accessible food.

The 2015 Substance Priority List, created by the Agency for Toxic Substances and Disease Registry (ATSDR), identifies hazardous substances that are most commonly found and, also, which are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure (ATSDR 2015). According to this list, As, Pb, Hg, Cd, and Se are ranked as the first, second, third, seventh, and 145th compounds, respectively. Taking all of the previously reported information into account, the aim of the present literature review is to collect data on Hg, Pb, Cd, Se, and As concentrations in the liver, kidney, and feathers of gulls and other seabirds, since these are the elements that have been analyzed in the majority of studies that use seabirds as bioindicators of environmental pollution. Also, in this review, an analysis of the abovementioned data is provided in terms of species, location, sample, and method in order to serve as a reference for future environmental biomonitoring studies.

Concentrations of Hg, Pb, Cd, Se, and As and their distributions in the liver, kidney, and feathers in 15 species of gulls and 16 species of other seabirds collected around the world are discussed. The present review aims not only to provide global

baseline data on metal and metalloid levels in gulls and other seabirds but also aims to understand their putative toxic effects.

2 Methods: Data Sources

In this review, an extensive search of the available literature was conducted using different databases, including PubMed, Science Direct, Scopus, Springer, and Web of Science. Different keywords and combinations of terms were used, such as "mercury," "cadmium," "lead," "selenium," "arsenic," "heavy metals," "metalloid," "trace element," "pollution," "environmental contamination," "seabirds," "seagulls," "gull," "*Larus,*" "biomonitoring," "liver," "kidney," "feathers," and "tissues." The reference lists of each paper containing data about Hg, Cd, Pb, Se, and As were consulted. Furthermore, multiple Google searches were performed to retrieve reports and other documents that are not available in the major databases.

3 Mercury (Hg), Lead (Pb), Cadmium (Cd), Selenium (Se), and Arsenic (As) Concentrations in Liver, Kidney, and Feathers of Gulls and Other Seabirds

This review presents a comprehensive overview of these elements' exposure in seabirds, and more particularly in gulls, paying special attention to the most frequently analyzed matrices in seabirds (liver, kidney, and feathers). For this purpose, we have created a database providing the levels of Hg, Cd, Pb, Se, and As in different tissues such as the liver, kidney, and feathers in gulls (Table 1) and other seabirds (Table 2) and compared these data in terms of species, location, sample, and method.

In Table 1, the concentrations of Hg, Cd, Pb, Se, and As in the liver, kidney, and feathers of different species of gulls, as reported by several authors who studied metal pollution in different geographic areas, are shown. The methods used to measure these elements are also shown. The species of gulls included in this review are *Larus argentatus*, *L. atricilla*, *L. audouinii*, *L. canus*, *L. crassirostris*, *L. dominicanus*, *L. glaucescens*, *L. glaucoides*, *L. heuglini*, *L. hyperboreus*, *L. minutus*, *L. pipixcan*, *L. ridibundus*, *L. sabini*, and *Rissa tridactyla.*

In Table 2, the levels of these elements in different types of seabirds are shown, including *Phalacrocorax carbo*, *Uria lomvia*, *Uria aalge*, *Somateria mollissima*, *Morus bassanus*, *Fulmarus glacialis*, *Fratercula arctica*, and different species of albatross (*Thalassarche cauta*, *Thalassarche chlororhynchos*, *Diomedea epomophora*, *Diomedea exulans*, *Thalassarche melanophris*, *Phoebastria immutabilis*, *Phoebetria palpebrata, Thalassarche chrysostoma*, *Phoebastria nigripes*).

Table 1 Concentrations of mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se), and arsenic (As) in the liver, kidney, and feathers of several gull species from different areas

Gull species	Location	Metal concentration µg/g dry weight									Method	Reference
		Hg			Cd			Pb				
		Liver	Kidney	Feathers	Liver	Kidney	Feathers	Liver	Kidney	Feathers		
L. argentatus	German Coast (Germany)	4.54 ± 2.33		5.64 ± 2.72							CVT	(Lewis and Furness 1993)
	Wadden Sea (Germany)			4.87 ± 2.04								(Lewis et al. 1993)
	Toscana (Italy)	8.68 ± 3.37	7.47 ± 3.69		1.91 ± 0.54	4.50 ± 2.11		3.30 ± 1.76	13.25 ± 4.68		AAS	(Leonzio et al. 1986)
		13.08 ± 6.62	10.65 ± 7.82		3.54 ± 1.87	8.26 ± 4.74		3.78 ± 3.46	3.4 ± 1.95			
		13.30 ± 9.25	10.81 ± 7.65		0.73 ± 0.37	3.75 ± 1.39		1.82 ± 2.01	3.09 ± 1.68			
		8.38 ± 3.52	6.73 ± 3.15		0.85 ± 0.46	3.95 ± 0.98		<0.10	<0.10			
	Gull Island (Canada)	1.70 ± 0.69	1.95 ± 0.46		3.6 ± 1.80	34 ± 12		0.69 ± 0.40			AAS, CVT (Hg) GFT (Pb)	(Elliott et al. 1992)
	Manawagonish Island (Canada)	0.69 ± 0.18	1.11 ± 0.22		1.7 ± 0.4	13 ± 4.2		1.3 ± 1.0				
	Kent Island (Canada)	1.5 ± 0.53	1.60 ± 0.35		5.40 ± 5.0	40 ± 28		0.24 ± 0.11				
	Chaun (Siberia)	4.01 ± 2.14	3.02 ± 0.95	6.06 ± 4.60	26.3 ± 16.1	159 ± 30	0.57 ± 0.47				AAS, CVT (Hg)	(Kim et al. 1996a)
	Hornøya (Norway)	1.11 ± 0.67			2.18 ± 0.27						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Isle of May (UK)	4.08 ± 0.58	3.87 ± 0.44		2.01 ± 0.13	13 ± 0.89					AAS, CVT (Hg), NAT (Se)	(Hutton 1981)
	Shinnecock Island			2.59 ± 0.27			0.22 ± 0.12			2.33 ± 0.45	CVT (Hg)GFT (Cd, Pb, Se)	(Burger 1997)
	Captree Western Long Island (USA)			1.40 ± 0.27			0.099 ± 0.028			2.49 ± 0.12		
	Loop Western Long Island (USA)			1.64 ± 0.27			0.37 ± 0.26			1.76 ± 0.24		
	New York-New Jersey Harbor Huckleberry (USA)			2.83 ± 0.27			0.39 ± 0.14			1.77 ± 0.22		
	Prall's Island, New York-New Jersey (USA)			0.88 ± 0.16			0.11 ± 0.02			3.57 ± 0.33		
	Lavallette, New Jersey (USA)			1.76 ± 0.35			0.08 ± 0.01			1.5 ± 0.26		

	Harvey Sedge, New Jersey (USA)			2.58 ± 0.23			0.12 ± 0.05			1.02 ± 0.12		
	Virginia (USA)			0.76 ± 0.11			0.19 ± 0.05			1.55 ± 0.28		
	Gulf of Gdansk (Poland)	0.58 (0.29–1.02	0.72 (0.23–1.57	3.02 (1.25–4.34)							AAS	(Szumiło et al. 2013)
	May Island (Scotland)	2.22 ± 0.74	2.01 ± 0.33		1.86 ± 0.25	13.7 ± 1.78					AAS	(Nicholson 1981)
L. atricilla	New York	0.52 ± 0.06	0.50 ± 0.05	3.66 ± 0.46	0.87 ± 0.13	2.44 ± 0.57	0.19 ± 0.03	0.58 ± 0.09	2.19 ± 0.47	2.82 ± 0.22	CVT (Hg) GFT	(Gochfeld et al. 1996)
L. audouinii	Ebro Delta (Spain)	8.36 (1.46–22.26)	4.33 (0.68–8.06)	1.27 (0.29–5.18)							AFS	(Arcos et al. 2002)
L. cachinnans	Ebro Delta (Spain)	2.98 (0.95–5.91	2.17 (0.82–4.28)	2.10 (0.75–4.36)							AFS	(Arcos et al. 2002)
L. canus	Caspian Sea (Iran)	1.2a		2.1–2.9a							CVT	(Rajaei et al. 2010)
L. crassirostris	North Pacific										HPLC-ICP-MS	(Fujihara et al. 2004)
	Japan	1.9 ± 0.30	1.4 ± 0.50	4.1 ± 0.70	10.3 ± 15.7	99.6 ± 96.10	0.04 ± 0.02	0.02 ± 0.01	0.15 ± 0.03	0.75 ± 0.19	ICP-MS, HGAAS (Hg, Se)	(Agusa et al. 2005)
L. dominicanus	Pájaros Islet (Chile)				9.84 ± 0.125						AAS	(Cortés and Luna-Jorquera 2011)
	La Herradura (Chile)				9.61 ± 0.097							
	Talcahuano (Chile)			1.13 ± 0.13			0.03 ± 0.04			5.97 ± 6.0	AAS	(Sepúlveda and González 2014)
	Florianópolis (Brazil)						0.07 ± 0.02			7.54 ± 1.66	GFT	(Barbieri et al. 2010)
L. glaucescens	Adak Island (Alaska)			2.97 ± 0.57			0.13 ± 0.04			1.05 ± 0.10	CVT (Hg), GFT (Cd, Pb, Se, As)	(Burger et al. 2009)
	Amchitka Island (Alaska)			4.01 ± 0.54			0.05 ± 0.05			0.62 ± 0.15		
	Aleutian Islands (Alaska)			3.68 ± 0.37			0.07 ± 0.01			0.86 ± 0.13		Burger and Gochfeld 2009)

(continued)

Table 1 (continued)

Gull species	Location	Metal concentration µg/g dry weight									Method	Reference
		Hg			Cd			Pb				
		Liver	Kidney	Feathers	Liver	Kidney	Feathers	Liver	Kidney	Feathers		
L. glaucoides	CW Greenland				0.04 ± 0.66[a]	0.08 ± 0.51[a]					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	Newfoundland (Canada)			1.90 ± 1.00							AAS	(Bond and Robertson 2015)
L. heuglini	Southern Iran						1.16			7.04	AAS	(Mansouri et al. 2012)
	Southern Iran	1.87 ± 0.18	1.97 ± 0.21	2.99 ± 0.27							AAS	(Majidi et al. 2015)
	Southern Iran				1.10 ± 0.20	2.20 ± 0.70		5.10 ± 0.80	8.80 ± 2.50		AAS	(Hoshyari et al. 2012)
L. hyperboreus	Northwest Greenland	0.79 ± 0.50[a]	0.51 ± 0.47[a]		3.58 ± 0.61[a]	15.04 ± 0.38[a]					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	CW Greenland				0.86 ± 2.02	3.15 ± 2.68						
	Chaun (Siberia)	3.28	2.65	5.96	3.90	18.70	ND				AAS, CVT (Hg)	(Kim et al. 1996a)
	Bjørnøya (Norway)	1.02 ± 0.57			6.06 ± 3.57						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Guba Chernaya (Russia)	1.38 ± 0.54			2.54 ± 1.59							
	Kolguyev Island	1.21 ± 0.57			1.21 ± 0.46							
	Vaygach Island	0.97 ± 0.20			1.42 ± 0.73							
	Ny-Alesund (Norway)	1.54 ± 1.10			10.3 ± 8.51							
	Akpatok Island (Canada)	9.44			17.4	90.3 ± 46.26		<0.09			CVT(Hg), GFT (Se, Pb), AAS (Cd)	(Braune and Scheuhammer 2008)
	Coats Island (Canada)	6.78			8.51	79.8 ± 66.89		<0.08				
	Svalbard (Norway)	1.17 ± 0.16									CVT	(Jæger et al. 2009)
	Arctic Canada			2.31 ± 1.68							AFS	(Mallory et al. 2015)
L. minutus	Caspian Sea, Iran	3.9a		5.2–7.1a							CVT	(Rajaei et al. 2010)

L. pipixcan	Talcahuano (Chile)			1.15 ± 0.17			0.20 ± 0.15			2.57 ± 5.5	AAS	(Sepúlveda and González 2014)
	Agassiz (USA)			0.66 ± 0.06			0.53 ± 0.08			2.86 ± 0.67	CVT (Hg) GFT (Cd, Pb, Se,)	(Burger 1996)
	Lake Alice (USA)			1.44 ± 0.51			0.36 ± 0.20			3.06 ± 1.50		
	Sand Lake (USA)			1.36 ± 0.51			0.14 ± 0.02			0.89 ± 0.34		
	Benton (USA)			0.96 ± 0.08			0.851 ± 0.56			3.27 ± 1.13		
L. ridibundus	Toscana (Italy)	2.58 ± 1.37	2.49 ± 1.71		4.20 ± 4.22	17.28 ± 9.92		7.46 ± 5.41	30.96 ± 22.81		AAS	(Leonzio et al. 1986)
		3.19 ± 2.75	4.02 ± 2.0		3.62 ± 1.07	12.88 ± 2.97		1.66 ± 0.32	2.5 ± 0.98			
	South-Western (Poland)				5.52 ± 2.95	8.33 ± 3.73	0.47 ± 0.27	8.91 ± 1.87	11.11 ± 4.00	10.40 ± 5.36	AAS	(Orlowski et al. 2007)
	North Italy				4 ± 3.3	32 ± 26					AAS	(Carpenè et al. 1995)
	Caspian Sea, Iran	3.2a		4.2–7.0a							CVT	(Rajaei et al. 2010)
L. sabini	Chaun (Siberia)	3.89 ± 1.80	5.54 ± 0.29	1.70 ± 0.47	16.3 ± 3.0	156 ± 8.0	ND				AAS, CVT (Hg)	(Kim et al. 1996a)
R. tridactyla	Hornøya (Norway)	0.8 ± 0.31			27.8 ± 13.3						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Bjørnøya (Norway)	0.58 ± 0.08			16.2 ± 5.89							
	Ny-Alesund (Norway)	1.95 ± 0.44			48 ± 18.6							
	Hooker Island (Russia)	0.69 ± 0.04			18.5 ± 2.45							
	Prince George Land Island (Russia)	0.79 ± 0.06			45.1 ± 32.7							
	Northbrook Island (Russia)	0.64 ± 0.18			18.1 ± 5.25							
	Kuhn Island Greenland	0.45 ± 0.05			25.7 ± 11.60							
	Guba Chernaya (Kazakhstan)	1.44 ± 0.3			8.14 ± 12.30							
	Vaygach Island (Russia)	0.66 ± 0.37			11 ± 11.90							
	Kharlov Island (Russia)	0.98 ± 0.14			22.3 ± 16.8							

(continued)

Table 1 (continued)

Gull species	Location	Metal concentration µg/g dry weight									Method	Reference
		Hg			Cd			Pb				
		Liver	Kidney	Feathers	Liver	Kidney	Feathers	Liver	Kidney	Feathers		
	Svalbard (Norway)	0.94 ± 0.08									CVT	(Jæger et al. 2009)
	Hornøya (Norway)	2.85 ± 1.02		2.03 ± 0.4	14.37 ± 11.30		0.13 ± 0.09				GFT(Cd, Se) CVT (Hg)	(Wenzel and Gabrielsen 1995)
	Prince George Land Island (Russia)	3.05			24.20	184 ± 110.7		<0.14			CVT (Hg), GFT (Cd, Pb, Se, As), AAS	(Braune and Scheuhammer. 2008)
	Arctic Canada			3.58 ± 0.92							AFS	(Mallory et al. 2015)
	Shoup Bay (Alaska)			0.29 ± 0.18			0.03 ± 0.003			0.71 ± 0.13	CVT (Hg) GFT	(Burger et al. 2008)

Gull species	Location	Metal concentration µg/g dry weight						Method	Reference
		Se			As				
		Liver	Kidney	Feather	Liver	Kidney	Feathers		
L. argentatus	German Coast (Germany)							CVT	(Lewis and Furness 1993)
	Wadden Sea (Germany)								(Lewis et al. 1993)
	Toscana (Italy)	9.97 ± 3.61	11.89 ± 5.83					AAS	(Leonzio et al. 1986)
		15.87 ± 1.68	17.55 ± 4.06						
		17.03 ± 6.81	26.8 ± 9.92						
		13.22 ± 6.40	13.05 ± 70						
	Gull Island (Canada)	3.21 ± 0.84						AAS, CVT (Hg) GFT (Pb)	(Elliott et al. 1992)
	Manawagonish Island (Canada)	3.20 ± 0.67							
	Kent Island (Canada)	3.36 ± 0.62							
	Chaun (Siberia)							AAS, CVT (Hg)	(Kim et al. 1996a)
	Hornøya (Norway)	2.18 ± 0.27			2.59 ± 1.32			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Isle of May (UK)	7.86 ± 0.30	14.1 ± 1.45					AAS, CVT (Hg), NAT (Se)	(Hutton 1981)

	Shinnecock Island			1.14 ± 0.07				CVT (Hg) GFT (Cd, Pb, Se)	(Burger 1997)
	Captree Western Long Island (USA)			1.833 ± 0.11					
	Loop Western Long Island (USA)			1.12 ± 0.06					
	New York-New Jersey Harbor Huckleberry (USA)			1.09 ± 0.05					
	Prall's Island, New York-New Jersey (USA)			1.57 ± 0.12					
	Lavallette, New Jersey (USA)			1.72 ± 0.11					
	Harvey Sedge, New Jersey (USA)			0.96 ± 0.03					
	Virginia (USA)			1.99 ± 0.07					
	Gulf of Gdansk (Poland)							AAS	(Szumiło et al. 2013)
	May Island (Scotland)							AAS	(Nicholson 1981)
L. atricilla	New York	1.59 ± 0.16	1.90 ± 0.08	1.44 ± 0.15				CVT (Hg) GFT	(Gochfeld et al. 1996)
L. audouinii	Ebro Delta (Spain)							AFS	(Arcos et al. 2002)
L. cachinnans	Ebro Delta (Spain)							AFS	(Arcos et al. 2002)
L. canus	Caspian Sea (Iran)							CVT	(Rajaei et al. 2010)
L. crassirostris	North Pacific				1.6 ± 0.8	1.2 ± 0.4		HPLC-ICP-MS	(Fujihara et al. 2004)
	Japan	4.9 ± 1.50	9.0 ± 2.80	1.1 ± 0.10				ICP-MS, HGAAS (Hg, Se)	(Agusa et al. 2005)
L. dominicanus	Pájaros Islet (Chile)							AAS	(Cortés and Luna-Jorquera 2011)
	La Herradura (Chile)								
	Talcahuano (Chile)						0.75 ± 0.21	AAS	(Sepúlveda and González 2014)
	Florianópolis (Brazil)							GFT	(Barbieri et al. 2010)
L. glaucescens	Adak Island (Alaska)			1.05 ± 0.10			0.18 ± 0.09	CVT (Hg), GFT (Cd, Pb, Se, As)	(Burger et al. 2009)
	Amchitka Island (Alaska)			0.98 ± 0.08			0.14 ± 0.02		
	Aleutian Islands (Alaska)			0.99 ± 0.05			0.144 ± 0.03		Burger and Gochfeld 2009)
L. glaucoides	CW Greenland	2.02 ± 1.4[a]	4.60 ± 1.49[a]					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	Newfoundland (Canada)							AAS	(Bond and Robertson 2015)

(continued)

Table 1 (continued)

Gull species	Location	Metal concentration µg/g dry weight						Method	Reference
		Se			As				
		Liver	Kidney	Feather	Liver	Kidney	Feathers		
L. heuglini	Southern Iran							AAS	(Mansouri et al. 2012)
	Southern Iran							AAS	(Majidi et al. 2015)
	Southern Iran							AAS	(Hoshyari et al. 2012)
L. hyperboreus	Northwest Greenland	1.09 ± 0.44[a]	1.38 ± 0.35[a]					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	CW Greenland								
	Chaun (Siberia)							AAS, CVT (Hg)	(Kim et al. 1996a)
	Bjørnøya (Norway)	5.25 ± 0.66			12.8 ± 4.33			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Guba Chernaya (Russia)	6.2 ± 1.55			76.80 ± 8.27				
	Kolguyev Island	6.00 ± 0.96			3.59 ± 1.79				
	Vaygach Island	4.33 ± 0.13			2.91 ± 0.70				
	Ny-Alesund (Norway)	5.54 ± 2.18			1.98 ± 1.61				
	Akpatok Island (Canada)	19.60						CVT(Hg), GFT (Se, Pb), AAS (Cd)	(Braune and Scheuhammer 2008)
	Coats Island (Canada)	9.20							
	Svalbard (Norway)							CVT	(Jæger et al. 2009)
	Arctic Canada							AFS	(Mallory et al. 2015)
L. minutus	Caspian Sea, Iran							CVT	(Rajaei et al. 2010)
L. pipixcan	Talcahuano (Chile)						0.64 ± 0.37	AAS	(Sepúlveda and González 2014)
	Agassiz (USA)			0.84 ± 0.07				CVT (Hg) GFT (Cd, Pb, Se,)	(Burger 1996)
	Lake Alice (USA)			0.92 ± 0.34					
	Sand Lake (USA)			1.15 ± 0.13					
	Benton (USA)			1.30 ± 0.13					
L. ridibundus	Toscana (Italy)	10.49 ± 4.92	15.56 ± 6.84					AAS	(Leonzio et al. 1986)
		16.27 ± 8.52	18.0 ± 8.05						
	South-Western (Poland)							AAS	(Orlowski et al. 2007)
	North Italy							AAS	(Carpenè et al. 1995)
	Caspian Sea, Iran							CVT	(Rajaei et al. 2010)

L. sabini	Chaun (Siberia)							AAS, CVT (Hg)	(Kim et al. 1996a)
R. tridactyla	Hornøya (Norway)	22.4 ± 9.83			1.83 ± 0.42			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Bjørnøya (Norway)	14.3 ± 4.97			0.82 ± 0.26				
	Ny-Alesund (Norway)	15.1 ± 4.17			3.12 ± 1.18				
	Hooker Island (Russia)	8.59 ± 1.15			24.4 ± 8.52				
	Prince George Land Island (Russia)	13.1 ± 3.16			9.05 ± 3.39				
	Northbrook Island (Russia)	14 ± 2.21			3.71 ± 0.10				
	Kuhn Island Greenland	9.36 ± 1.76			6.92 ± 4.84				
	Guba Chernaya (Kazakhstan)	13.7 ± 9.84			98.1 ± 69				
	Vaygach Island (Russia)	9.1 ± 1.80			1.65 ± 1.06				
	Kharlov Island (Russia)	17.7 ± 6.72			2.1 ± 1.64				
	Svalbard (Norway)							CVT	(Jæger et al. 2009)
	Hornøya (Norway)	16.91 ± 9.50		1.75 ± 0.25				GFT(Cd, Se) CVT (Hg)	(Wenzel and Gabrielsen 1995)
	Prince George Land Island (Russia)	36.20						CVT (Hg), GFT (Cd, Pb, Se, As), AAS	(Braune and Scheuhammer. 2008)
	Arctic Canada							AFS	(Mallory et al. 2015)
	Shoup Bay (Alaska)			2.42 ± 0.11			0.16 ± 0.02	CVT (Hg) GFT	(Burger et al. 2008)

Data are expressed in dry weight basis (μg/g dw). *ND* not detected
[a]Approximate values taken from figures

Table 2 Concentrations of mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se), and arsenic (As) in the liver, kidney, and feathers of several seabirds (except gulls) from different areas

Species	Location	Metal concentration µg/g dry weight									Method	Reference
		Hg			Cd			Pb				
		Liver	Kidney	Feathers	Liver	Kidney	Feathers	Liver	Kidney	Feathers		
Albatross												
Diomedea epomophora	Southern Indian Ocean	58.1	14.9	6.8							CVT	(Kim et al. 1996b)
	North Pacific	27.4			8.05			0.071			CVT (Hg) ICP-MS	(Kim et al. 1998)
	Auckland (New Zealand)	449 ± 490	12.90 ± 8.70		11.50 ± 5.60	132.2 ± 84.90					CVT (Hg) AAS	(Stewart et al. 1999)
Diomedea exulans	Bird Island (Georgia)						0.32 ± 0.48				DRC-ICP-MS	(Anderson et al. 2010)
	Auckland (New Zealand)	360 ± 183	23.7 ± 16.3		27.20 ± 21.50	125.4 ± 62.50					CVT (Hg) AAS	(Stewart et al. 1999)
	North Pacific	306 ± 260	37.40 ± 11.80	39.70 ± 11.80							CVT	(Kim et al. 1996c)
	North Pacific										HPLC-ICP-MS	(Fujihara et al. 2004)
	North Pacific	22.7			70.6			0.12			CVT (Hg) ICP-MS	(Kim et al. 1998)
Phoebastria immutabilis	Midway Atoll North Pacific Ocean			3.46 ± 0.33			0.36 ± 0.10			0.80 ± 0.06	CVT (Hg) GFT	(Burger and Gochfeld 2000a)
	North Pacific	38.90 ± 11.20		7.20 ± 2.80							CVT	(Kim et al. 1996b)
Phoebastria nigripes	Midway Atoll North Pacific Ocean			19.60 ± 0.02			0.15 ± 0.03				CVT (Hg) GFT	(Burger and Gochfeld 2000a)
		22.7			70.6			0.12			ICP-MS	(Kim et al. 1998)

Phoebetria palpebrata	Southern Indian Ocean	81.1			23			0.04			CVT (Hg) ICP-MS	(Kim et al. 1998)
Thalassarche cauta	Southern Indian Ocean	33.4			16.1			0.07			CVT (Hg) ICP-MS	(Kim et al. 1998)
	Auckland (New Zealand)	35 ± 17.6	5.10 ± 5.10		7.70 ± 4.10	74.20 ± 23.90					CVT (Hg) AAS	(Stewart et al. 1999)
Thalassarche chlororhynchos	Bird Island (Georgia)						0.20 ± 0.16				DRC-ICP-MS	(Anderson et al. 2010)
	Southern Indian Ocean	57.4			45.1			0.05			CVT (Hg) ICP-MS	(Kim et al. 1998)
Thalassarche chrysostoma	Southern Indian Ocean	31.1			15.6			0.034			CVT (Hg) ICP-MS	(Kim et al. 1998)
Thalassarche melanophris	Midway Atoll North Pacific Ocean			19.60 ± 1.75			0.15 ± 0.03			0.97 ± 0.13	CVT (Hg) GFT	(Burger and Gochfeld 2000a)
	Auckland (New Zealand)	124.60 ± 74.60	7.60 ± 11.50		19.40 ± 7.90	85.70 ± 33.80					CVT (Hg) AAS	(Stewart et al. 1999)
	Patagonian Shelf						0.2			ND	AAS	(Pon et al. 2011)
	Bird Island (Georgia)						0.58 ± 0.25				DRC-ICP-MS	(Anderson et al. 2010)
Cormorants												
Phalacrocorax carbo	Lake Biwa (Japan)	3.39 ± 1.39	4.05 ± 2.18	2.85 ± 0.60	1.25 ± 0.31	6.83 ± 0.64	0.05				CVT (Hg) AAS	(Saeki et al. 2000)
	Ecka Fish Farm (Serbia)	3.18 ± 1.11		2.70 ± 1.49							ICP-OES	(Skoric et al. 2012)
	Czech Republic	42.2 ± 6.28	7.2 ± 1								CVT	(Houserová et al. 2007)
	Lake Biwa (Japan)	12 ± 9	14 ± 9	2.80 ± 2.70	1.15 ± 0.24	3.31 ± 0.36	0.04 ± 0.02	0.05 ± 0.02	0.31 ± 0.38	1.65 ± 0.50	ICP-MS (Cd, Pb) HGT (Se) CVT (Hg)	(Nam et al. 2005)

(continued)

Table 2 (continued)

Species	Location	Metal concentration µg/g dry weight									Method	Reference
		Hg			Cd			Pb				
		Liver	Kidney	Feathers	Liver	Kidney	Feathers	Liver	Kidney	Feathers		
	Vistula Lagoon (Poland)	15.51 ± 17.30	30.2 ± 47.9								CVT	(Misztal-Szkudlinska et al. 2011)
	Caspian Sea	8.32	9.32	4.44							AAS	(Mazloomi et al. 2008)
	Murcia (Spain)				0.22 ± 0.12	0.70 ± 0.23	0.19 ± 0.002	0.15 ± 0.17	0.50 ± 0.06	22.51 ± 20.07	ICP-MS	(Navarro et al. 2010)
	Iran	5.67 ± 0.91	3.59 ± 2.24	8.68 ± 0.80							AAS	(Aazami et al. 2011)
Eider												
Somateria mollissima	Ny-Alesund (Norway)	1.80 ± 0.22			17.20 ± 5.57						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	CW Greenland	0.19 ± 0.55	0.05 ± 0.35		0.92 ± 0.48	3.20 ± 0.45		0.01 ± 0.67	0.02 ± 0.44		GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	S Greenland	0.23 ± 0.58			0.78 ± 0.54	2.76 ± 0.47						
	Aleutian Chain (Alaska)	0.30 ± 1.51		0.84 ± 0.081			0.08 ± 0.004			0.99 ± 0.13	CVT (Hg) GFT (Cd, Pb, Se, As)	(Burger et al. 2007)
	Arctic Canada			0.59 ± 0.21							AFS	(Mallory et al. 2015)
Fulmar												
Fulmarus glacialis	Prince Leopold Is. (Canada)	8.12			39.4	157 ± 49.9		<0.08			GFT (Cd, Pb), HGT (Se, Hg)	(Braune and Scheuhammer 2008)
	North Greenland	0.66 ± 0.47	0.19 ± 0.31		3 ± 0.56	8.53 ± 0.39					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	Bjornoya (Norway)	1.95 ± 1.29			36.60 ± 19.30							(Savinov et al. 2003)

	Ny-Alesund (Norway)	18.80 ± 5.72			109 ± 61.10						AAS, GFT (As, Se) HS (Hg)	
	S-W Barents Sea	2.01 ± 1.42			16.40 ± 17.80							
	St Kilda (UK)	29.40 ± 4.77	13.40 ± 3.19	3.34 ± 1.49	49.0 ± 16.0	228 ± 71.0	<0.351 ± 0.243				AAS	(Osborn et al. 1979)
	North Pacific	2.3			16.2			0.09			CVT (Hg), ICP-MS	(Kim et al. 1998)
	North Pacific	14.2 ± 10	6.70 ± 3	4.80 ± 2.40							CVT	(Kim et al. 1996b)
	North Pacific	14.80 ± 10									ICP-MS (Se), CVT	(Kim et al. 1996c)
	Svalbard (Norway)	2.57 ± 0.17									CVT	(Jæger et al. 2009)
Gannet												
Morus bassanus	Portugal	2.60 ± 0.8	3.42 ± 0.65	5.14 ± 1.44	4.25 ± 0.93	35.20 ± 4.58	0.07 ± 0.02	0.10 ± 0.02	0.26 ± 0.07	1.10 ± 0.23	ICP-MS	(Mendes et al. 2008)
Murre												
Uria aalge	Kuvshin Island (Russia)	1.09 ± 0.28			1.69 ± 0.52						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
		1.08 ± 0.09			1.98 ± 0.56							
	Bay of Fundy (Canada)			0.99 ± 0.36							CVT	(Bond and Diamond 2009)
	Hornøya (Norway)	1.83 ± 0.41	1.46 ± 0.18	0.88 ± 0.19	3.08 ± 1.12	24.06 ± 7.46	0.03 ± 0.01				GFT (Cd, Se) CVT (Hg)	(Wenzel and Gabrielsen 1995)
Uria lomvia	Hornøya (Norway)	0.88 ± 1.17			5.80 ± 1.98						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Ny-Alesund (Norway)	1.61 ± 0.42			14.90 ± 6.16							
	Northbrook Island (Russia)	0.58 ± 0.01			13.10 ± 1.13							
	Guba Chernaya (Russia)	0.79 ± 0.11			3.98 ± 1							

(continued)

Table 2 (continued)

Species	Location	Metal concentration µg/g dry weight									Method	Reference
		Hg			Cd			Pb				
		Liver	Kidney	Feathers	Liver	Kidney	Feathers	Liver	Kidney	Feathers		
	Kharlov Island (Russia)	0.93 ± 0.07			2.55 ± 1.93							
	Prince Leopold Is. (Canada)	3.61			23.5	154 ± 63.10		<0.09			CVT (Hg), GFT (Se, Pb), AAS (Cd)	(Braune and Scheuhammer 2008)
	Hornøya (Norway)	1.11 ± 0.51	1.16 ± 0.21	0.78 ± 0.18	5.51 ± 4.24	46.73 ± 31	0.12 ± 0.08				GFT (Cd, Se) CVT (Hg)	(Wenzel and Gabrielsen 1995)
	Bay of Fundy (Canada)			1.38 ± 0.99							CVT	(Bond and Diamond 2009)
	Arctic Canada			1.94 ± 0.63							AFS+S9	(Mallory et al. 2015)
Puffin												
Fratercula arctica	Kuvshin Island (Russia)	1.17 ± 0.23			2.68 ± 1.30						AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
		1.37 ± 0.12			3.22 ± 1.35							
	Ny-Alesund (Norway)	1.12 ± 0.32			9.77 ± 3.21							
	Hornøya (Norway)	1.22 ± 0.35			2.60 ± 1							
	St Kilda (UK)	4.54 ± 0.42	5.02 ± 0.52	7.94 ± 0.80	19.90 ± 2.85	114 ± 12.30					AAS	(Osborn et al. 1979)
	Gull Island (Canada)	2.60 ± 0.80	2.6 ± 0.17		8.90 ± 2.50	92.0 ± 32		0.17 ± 0.15			AAS, CVT (Hg) GFT (Pb)	(Elliott et al. 1992)
	Ile St. Marie (Canada)	1.40 ± 0.52	1.0 ± 0.55		5.90 ± 5.10	45.0 ± 38.0						
	Bay of Fundy (Canada)			1.8 ± 0.67							CVT	(Bond and Diamond 2009)
	Arctic Canada			2.71 ± 0.72							AFS	(Mallory et al. 2015)

Species	Location	Metal concentration µg/g dry weight						Method	Reference
		Se			As				
		Liver	Kidney	Feather	Liver	Kidney	Feathers		
Albatross									
Diomedea epomophora	Southern Indian Ocean							CVT	(Kim et al. 1996b)
	North Pacific	16.7						CVT (Hg) ICP-MS	(Kim et al. 1998)
	Auckland (New Zealand)							CVT (Hg) AAS	(Stewart et al. 1999)
Diomedea exulans	Bird Island (Georgia)			4.57 ± 0.88			0.09 ± 0.03	DRC-ICP-MS	(Anderson et al. 2010)
	Auckland (New Zealand)							CVT (Hg) AAS	(Stewart et al. 1999)
	North Pacific							CVT	(Kim et al. 1996c)
	North Pacific						16 ± 11	HPLC-ICP-MS	(Fujihara et al. 2004)
							11 ± 6		
	North Pacific	107						CVT (Hg) ICP-MS	(Kim et al. 1998)
Phoebastria immutabilis	Midway Atoll North Pacific Ocean			2.29 ± 0.29			0.11 ± 0.25	CVT (Hg) GFT	(Burger and Gochfeld 2000a)
	North Pacific							CVT	(Kim et al. 1996b)
Phoebastria nigripes	Midway Atoll North Pacific Ocean			0.33 ± 0.32			0.21 ± 0.05	CVT (Hg) GFT	(Burger and Gochfeld 2000a)
					107			ICP-MS	(Kim et al. 1998)
Phoebetria palpebrata	Southern Indian Ocean	71.5						CVT (Hg) ICP-MS	(Kim et al. 1998)
Thalassarche cauta	Southern Indian Ocean	41.4						CVT (Hg) ICP-MS	(Kim et al. 1998)
	Auckland (New Zealand)							CVT (Hg) AAS	(Stewart et al. 1999)
Thalassarche chlororhynchos	Bird Island (Georgia)			5.40 ± 1.47			0.13 ± 0.03	DRC-ICP-MS	(Anderson et al. 2010)
	Southern Indian Ocean	54.1						CVT (Hg) ICP-MS	(Kim et al. 1998)
Thalassarche chrysostoma	Southern Indian Ocean	40						CVT (Hg) ICP-MS	(Kim et al. 1998)
Thalassarche melanophris	Midway Atoll North Pacific Ocean			3.26 ± 0.32			0.21 ± 0.05	CVT (Hg) GFT	(Burger and Gochfeld 2000a)
	Auckland (New Zealand)							CVT (Hg) AAS	(Stewart et al. 1999)
	Patagonian Shelf							AAS	(Pon et al. 2011)
	Bird Island (Georgia)			3.38 ± 0.93			0.12 ± 0.04	DRC-ICP-MS	(Anderson et al. 2010)
Cormorants									
Phalacrocorax carbo	Lake Biwa (Japan)							CVT (Hg) AAS	(Saeki et al. 2000)
	Ecka Fish Farm (Serbia)				1.28 ± 0.25		1.18 ± 0.22	ICP-OES	(Skoric et al. 2012)
	Czech Republic							CVT	(Houserová et al. 2007)
	Lake Biwa (Japan)	8.20 ± 1.70	12 ± 4	1.50 ± 0.20				ICP-MS (Cd, Pb) HGT (Se) CVT (Hg)	(Nam et al. 2005)

(continued)

Table 2 (continued)

Species	Location	Metal concentration µg/g dry weight						Method	Reference
		Se			As				
		Liver	Kidney	Feather	Liver	Kidney	Feathers		
	Vistula Lagoon (Poland)							CVT	(Misztal-Szkudlinska et al. 2011)
	Caspian Sea							AAS	(Mazloomi et al. 2008)
	Murcia (Spain)	7.73 ± 4.94	9.28 ± 6.11	1.16 ± 0.70	6.97 ± 0.84	8.85 ± 0.96	0.51 ± 0.01	ICP-MS	(Navarro et al. 2010)
	Iran							AAS	(Aazami et al. 2011)
Eider									
Somateria mollissima	Ny-Alesund (Norway)	9.21 ± 0.90			0.13 ± 0.10			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	CW Greenland	1.89 ± 0.85	1.50 ± 0.41					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	S Greenland	1.81 ± 0.45	1.25 ± 0.37						
	Aleutian Chain (Alaska)			0.88 ± 0.90			0.14 ± 0.02	CVT (Hg) GFT (Cd, Pb, Se, As)	(Burger et al. 2007)
	Arctic Canada							AFS	(Mallory et al. 2015)
Fulmar									
Fulmarus glacialis	Prince Leopold Is. (Canada)	34.4						GFT (Cd, Pb), HGT (Se, Hg)	(Braune and Scheuhammer 2008)
	North Greenland	2.82 ± 0.38	4.38 ± 0.29					GFT (Cd, Pb), HGT (Se, Hg)	(Dietz et al. 1996)
	Bjornoya (Norway)	10.20 ± 1.22			2.86 ± 2.24			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Ny-Alesund (Norway)	18.80 ± 3.69			3.73 ± 2.60				
	S-W Barents Sea	16.60 ± 5.72			10.20 ± 4.72				
	St Kilda (UK)							AAS	(Osborn et al. 1979)
	North Pacific	29.8						CVT (Hg), ICP-MS	(Kim et al. 1998)
	North Pacific							CVT	(Kim et al. 1996b)
	North Pacific	32.0 ± 11.50						ICP-MS (Se), CVT	(Kim et al. 1996c)
	Svalbard (Norway)							CVT	(Jæger et al. 2009)

Gannet									
Morus bassanus	Portugal	18.06 ± 4.06	26.12 ± 4.32	2.13 ± 0.51	1.47 ± 0.29	1.99 ± 0.65	1.23 ± 9.43	ICP-MS	(Mendes et al. 2008)
Murre									
Uria aalge	Kuvshin Island (Russia)	21.30 ± 3.56			1.03 ± 0.40			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
		18.70 ± 3.94			1.34 ± 0.52				
	Bay of Fundy (Canada)							CVT	(Bond and Diamond 2009)
	Hornøya (Norway)	17.60 ± 5.04	43.74 ± 9.60	2.59 ± 0.33				GFT (Cd, Se) CVT (Hg)	(Wenzel and Gabrielsen 1995)
Uria lomvia	Hornøya (Norway)	7.53 ± 2.01			1.86 ± 0.25			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
	Ny-Alesund (Norway)	5.49 ± 1.89			4.61 ± 2.65				
	Northbrook Island (Russia)	4.26 ± 0.40			5.30 ± 0.35				
	Guba Chernaya (Russia)	6.27 ± 0.90			96.40 ± 70.90				
	Kharlov Island (Russia)	13.94 ± 4.06			1.48 ± 0.72				
	Prince Leopold Is. (Canada)	6.11						CVT (Hg), GFT (Se, Pb), AAS (Cd)	(Braune and Scheuhammer 2008)
	Hornøya (Norway)	7.05 ± 3.48	15.77 ± 5.7	2.75 ± 1.27				GFT (Cd, Se) CVT (Hg)	(Wenzel and Gabrielsen 1995)
	Bay of Fundy (Canada)							CVT	(Bond and Diamond 2009)
	Arctic Canada							AFS+S9	(Mallory et al. 2015)
Puffin									
Fratercula arctica	Kuvshin Island (Russia)	14.0 ± 2.25			1.04 ± 0.47			AAS, GFT (As, Se) HS (Hg)	(Savinov et al. 2003)
		27.10 ± 3.95			0.79 ± 0.30				
	Ny-Alesund (Norway)	11.9 ± 3.48			1.36 ± 0.61				
	Hornøya (Norway)	9.18 ± 1.68			0.79 ± 0.56				
	St Kilda (UK)							AAS	(Osborn et al. 1979)
	Gull Island (Canada)	11.7 ± 1.60						AAS, CVT (Hg) GFT (Pb)	(Elliott et al. 1992)
	Ile St. Marie (Canada)	8.29 ± 3.43							
	Bay of Fundy (Canada)							CVT	(Bond and Diamond 2009)
	Arctic Canada							AFS	(Mallory et al. 2015)

Data are expressed in dry weight basis (μg/g dw)

Several abbreviations for the analytical methods are presented in the tables. The terms used are as follows:

AAS atomic absorption spectrometry, *CVT* cold vapor technique, *GFT* graphite furnace technique, *HS* hydride system, *HGT* hydride generation technique, *ICP-MS* inductively coupled plasma mass spectrometry, *ICP-OES* inductively coupled plasma optical emission spectrometry, *NAT* neutron activation technique, *AFS* atomic fluorescence spectrophotometry, *HPLC-ICP-MS* high-performance liquid chromatography with inductively coupled plasma mass spectrometry, and *DRC-ICP-MS* dynamic reaction cell inductively coupled plasma mass spectroscopy.

3.1 Mercury (Hg)

3.1.1 Hg in the Environment and Its Chemical Forms

Mercury is a heavy, silvery-white metal that at room temperature is an odorless liquid. It appears in the environment in several forms: elemental (without any additional atoms attached to it), organic, and inorganic. These forms are interconvertible and can all induce systemic toxicity (Graeme and Pollack 1998). Hg is emitted to the environment by multiple natural sources including volcanic activity, continental particulate and volatile matter, and fluxes from the marine environment (Nriagu 1989). Other anthropogenic sources of Hg include mining, fossil fuel combustion and incineration, emission from smelters, fungicides, and catalyst activities. Although Hg can volatilize and return to the atmosphere, the vast majority precipitates in the coastal sea due to the very low solubility of these compounds (Chiarelli and Roccheri 2014). Other studies have demonstrated that, due to its high volatility and long residence time, Hg can reach remote areas of the planet through long-range atmospheric transport (Nriagu 1989; Fitzgerald et al. 1998; Lindberg et al. 2007). Atmospheric Hg predominantly exists in its gaseous elemental form (Hg^0), which can be oxidized to Hg^{+2} and then deposited. It is estimated that more than 80% of the Hg deposited in the ocean is re-emitted to the atmosphere as Hg^0, driving the cycle of Hg through biogeochemical reservoirs (Strode et al. 2007). The half-life of atmospheric Hg^0 in polar areas is short (Driscoll et al. 2013) but, once Hg^0 is deposited in aquatic ecosystems, it can be methylated by microorganisms to form methylmercury (MeHg). This is highly toxic and bioaccumulates throughout the food chain almost entirely via dietary uptake, reaching the highest concentrations in organisms at the top of the food chain (Wiener et al. 2007). In fact, MeHg is the most stable form of the metal and has been much more extensively studied than the inorganic forms. The main driver for increased Hg emissions is the expansion of coal-fired electricity generation in the developing world, particularly Asia, and the degree to which control technologies are implemented (Driscoll et al. 2013).

This highly dynamic process of air-sea exchange plays a role in the redistribution of Hg across the earth's surface (Lamborg et al. 1999). Emitted Hg can be deposited anywhere on its hemisphere of origin, but can also be transported between

hemispheres, albeit to a lesser extent (Driscoll et al. 2013). Although inputs in the Northern Hemisphere have declined in recent decades (Mason et al. 2012; Burgess et al. 2013; Driscoll et al. 2013), most industrial activity still occurs in northern regions (UNEP 2013). Hg concentrations are therefore, in general, lower on the Southern Hemisphere (30% in air, Driscoll et al. 2013; Becker et al. 2016). Trends in global emissions of Hg, analyzed between 1990 and 2007, indicate that emissions are increasing in Asia and decreasing in Europe and North America.

Fish-eating birds bioaccumulate significant levels of Hg from their environment, particularly *MeHg*, that is known to exert neurotoxic effects. After ingestion, MeHg is deposited in body tissues, demethylated in the liver or brain, or depurated into feathers or eggs. MeHg is lipid-soluble and can be stored in lipid-rich tissues. This compound has a relatively long biological half-life since, in comparison to other organomercurials, it is metabolized more slowly and has a lower excretion rate (Swensson and Ulfvarson 1968; Stickel et al. 1977; Braune and Gaskin 1987a; Spalding et al. 2000; Monteiro and Furness 2001). This long half-life results in biomagnification through food chains, for example, Cristol et al. (2008) showed Hg biomagnification in biota from a terrestrial habitat adjacent to a Hg-contaminated river in Virginia. Hg concentrations increased in known avian prey items (nearly 50% of the Hg in spiders, which comprised 20–30% of diet in three focal songbird species, was in the form of bioavailable MeHg), concluding that aquatic Hg moved into and through the terrestrial food web, where avian consumption of predatory invertebrates increased the food chain length and caused Hg to biomagnify.

3.1.2 Hg Levels in Birds: Differences Among Tissue Types

As shown in Table 1, average Hg concentrations in the liver of seagull species range from 0.45 ± 0.05 μg/g in *R. tridactyla* in Kuhn Island of Greenland (Savinov et al. 2003) to 13.30 ± 9.25 μg/g of *Larus argentatus* in Italy (Leonzio et al. 1986). Renal levels of Hg range from 0.50 ± 0.05 μg/g in *L. atricilla* in New York (Gochfeld et al. 1996) to 10.81 ± 7.65 μg/g in *L. argentatus* in Italy (Leonzio et al. 1986). In this last study, *L. argentatus* displayed the highest concentrations of Hg in both the liver and kidney, whereas in other tissues (muscle, brain, uropygial gland, and subcutaneous fat), levels were lower. Additionally, Hg levels (determined by AAS) were found to be higher in gulls feeding in coastal areas compared to those feeding on the inland dumps. This suggests that food items from marine areas are responsible for the high levels of Hg, which is consistent with the observed correlation between the amount of fish in the diet and the levels of Hg in tissues (Leonzio et al. 1986). *L. argentatus* may be considered as a consumer that occupies a slightly higher trophic level than *L. ridibundus*. It is defined as a scavenger, food-pirate, and predator of fish and other vertebrates (Cramp and Simmons 1983). Its dietary behavior could explain the high Hg levels in this species (Leonzio et al. 1986).

In most of the studies reviewed in this paper, Hg levels were, in general, found to be higher in the liver compared to the kidney. It has been reported that in response to inorganic Hg, only the kidney accumulates high levels of Hg, being the kidney/liver

ratio >1 under such exposure conditions, whereas if the exposure is to MeHg, the ratio will be closer or lower to unity (Scheuhammer 1987). Therefore, higher levels of Hg found in the liver in our reviewed studies suggest that MeHg is the predominant chemical form of Hg to which most gulls are exposed. In contrast, Hg levels were found to be higher in the kidney compared to the liver in *L. argentatus* in Canada and the USA (Elliot et al. 1992), in *L. argentatus* in Poland (Thompson et al. 1990), in *L. ridibundus* in Italy (Leonzio et al. 1986), and in *L. sabini* in Siberia (Kim et al. 1996a). As shown in Table 1, Hg concentrations in feathers range from 0.29 ± 0.18 μg/g in *R. tridactyla* in Alaska (Burger et al. 2008) to 6.06 ± 4.60 μg/g in *L. argentatus* in Siberia (Kim et al. 1996a). This range is lower than the one described by Burger (1994) in which Hg concentrations in feathers of gulls and terns from 19 studies ranged from 0.45 to 14.9 μg/g, with a median of 1.9 μg/g. However, in other seabirds included in our review, the range of Hg concentrations was found to be higher than described by Burger (1994).

As can be observed in Table 1, only six studies of Hg concentrations were analyzed simultaneously in the liver, kidney, and feathers. In four of these studies, feathers contained the highest Hg concentrations in *L. argentatus* in Siberia and Poland (Kim et al. 1996a; Szumiło et al. 2013), *L. crassirostris* in Japan (Agusa et al. 2005), and *L. hyperboreus* in Siberia (Kim et al. 1996a). However, in *L. cachinnans* in Spain (Arcos et al. 2002) and *L. sabini* in Siberia (Kim et al. 1996a), Hg concentrations in feathers were found to be lower than in the liver and kidney. When monitoring Hg levels in feathers, the time of the determination is of relevance. It should be noted that Hg concentrations in the tissues of birds are greatly influenced by metabolic changes such as molting (Hughes et al. 1997) and, directly associated with, the type of molting cycle (Saeki et al. 2000). The higher Hg concentrations in the body tissue are partly attributable to the differences in the sampling season, since birds are able to eliminate a substantial portion of their mercury body burden via their plumage during the molting period, levels in internal tissues drop as they are sequestered into the feathers (Honda et al. 1986; Braune and Gaskin 1987a; Lewis and Furness 1991). Therefore, feathers replaced first should contain the highest concentrations. Animals captured by Kim et al. (1996a) were at the beginning of the molt, as well as those captured by Agusa et al. (2005), collected in a breeding colony just during the postnuptial molt. This period of time can explain the high levels on feathers. Lewis and Furness (1993) have shown that in *L. ridibundus,* independently of the dose, 49% of Hg administrated to these animals was accumulated in the plumage. In these animals, approximately 65% of the Hg body burden was shown to accumulate in feather growth. Furthermore, Braune and Gaskin (1987a) showed that, although in *L. philadelphia* plumage only forms 10% of total body weight, 93% of the accumulated Hg body burden is present in the plumage. The remaining Hg burden was found to be present in diverse body tissues, including the liver (36%), kidney (6%), muscle (9%), brain (1%), and carcass (48%). Nevertheless, results obtained about Hg levels in feathers should be interpreted with caution, since feathers are less accurate in predicting recent Hg exposure or whole-body burdens, mainly due to atmospheric deposition (Eagles-Smith et al. 2008). Indeed, it has been reported that, depending on sample preparation prior to analysis,

feathers may be used to evaluate external contamination through dust and particulate matter (Ahmadpour et al. 2016), giving information about external concentrations but not leading to good conclusions about bioaccumulation.

The ratio of Hg in feathers, liver, and muscle (fresh weight) is, by some authors, estimated to be 7:3:1. This ratio is often used to estimate the Hg concentration in one tissue based on the concentration measured in one of the other tissues (Appelquist et al. 1985; Berg et al. 1966). According to other authors the ratio is slightly higher, varying among species between 10:3:1 (cormorant) and 23:3:1 (mallard and coot) (Aazami et al. 2011). According to these ratios, the concentration of Hg in feathers should be at least two times higher than the concentration in the liver. However, in all studies included in this review, differences were below twofold (range from 1.2× to 1.8×). Importantly, our data were expressed in dry weight and not fresh weight.

Values of Hg in the liver, kidney, and feathers in other seabirds range from 0.19 to 449, 0.19 to 37.4, and 0.59 to 39.7 μg/g, respectively (Table 2). In general, for these three tissues, low concentrations were found to be present in *S. mollissima* in Alaska, Greenland, and Canada (Burger et al. 2007; Dietz et al. 1996; Mallory et al. 2015). The highest concentrations were observed in *D. epomophora* in New Zealand (liver) and *D. exulans,* an endangered albatross species, in the North Pacific (kidney and feathers) (Kim et al. 1996c; Stewart et al. 1999). Of all data collected in this review, the highest concentrations of Hg were observed in the liver of three species of albatrosses located in New Zealand and the North Pacific (*D. epomophora* (449 μg/g), *D. exulans* (360 μg/g), and *P. nigripes* (306 μg/g)) (Kim et al. 1996c; Stewart et al. 1999).

In wild birds living in environments with little or no industrial activities, Hg levels in the liver should not exceed 10 μg/g (Firmreite 1974). In gulls, Hg liver concentrations only exceeded 10 μg/g in *L. argentatus* in Italy (13.08 ± 6.62 and 13.30 ± 9.25) (Table 1). Hg levels were higher in birds from the coastal environment than those caught on inland dumps (Leonzio et al. 1987). However, in other seabirds, in 44% (21 from 48 total data) of data collected in this review, Hg levels exceeded 10 μg/g. Therefore, it seems that at the ecosystems inhabited by the gulls selected for most of the studies, levels of Hg were not as high as to generate a risk for these animals.

In the kidney and feathers, the highest Hg levels were measured in *P. nigripes* (Kim et al. 1996a). Since birds each year retain a proportion of ingested Hg, high levels of Hg are, at least in part, due to enhanced longevity. Accumulated Hg may be demethylated and stored. Additionally, as described for, the abovementioned wandering, royal, and black-browed albatrosses (*D. exulans, D. epomophora*, and *T. melanophrys*), high Hg levels may be due to slow molt cycles, thereby restricting Hg excretion. Furthermore, high levels of Hg in these albatross species also are due to the large intake of fish and cephalopods (Stewart et al. 1999).

In other seabirds in which all three tissues were analyzed, feathers only contained the highest Hg levels in 3 out of 11 studies. This may be due to different issues: the capacity of these seabirds to demethylate organic Hg in tissues such as the liver and the oxidation of elemental Hg to inorganic Hg produced in the kidneys

(Thompson et al. 1990; Kim et al. 1996a, b; Aschner and Costa 2017) and store a large fraction of their Hg burden in inorganic form in these organs.

3.1.3 Differences in Hg Levels Depending on Diet/Age/Gender

Experimental laboratory (Lewis and Furness 1991, 1993) and field (Monteiro and Furness 1995) studies have shown that the levels of Hg in feather growth and Hg levels in the growing feather are directly related to the dietary intake of MeHg in chicks. The concentrations of Hg in feathers have been correlated with Hg levels in internal tissues (Szumiło et al. 2013). The use of feathers therefore represents a noninvasive approach for monitoring Hg accumulation in seabirds and the marine environment (Appelquist et al. 1985; Furness et al. 1986; Szumiło et al. 2013; Bond and Diamond 2009).

Trace metals are not equally distributed between tissues, being important to select the most appropriate tissue for monitoring studies (Furness et al. 1993). In seabirds, dietary Hg is incorporated in a dose-dependent manner (Lewis and Furness 1991), is accumulated in internal tissues (mainly in the liver and the kidney) between molt periods, and is excreted to the growing feathers during molts (Furness et al. 1986; Honda et al. 1986; Braune and Gaskin 1987a, b; Monteiro and Furness 2001). Hg in blood represents recent dietary exposure before it can be demethylated in the liver or mobilized from body stores before depuration in feathers or guano (Kim et al. 1996a; Monteiro and Furness 2001). Once feathers mature, the vascular connection atrophies, leaving the feather as a record of blood levels at that point in time. The concentrations of metals in mature feathers remain constant until the feathers are shed at the next molt (Braune and Gaskin 1987b).

Since Hg biomagnifies through the food chain, variation in diet among trophic levels in time may change the exposure of bird populations to Hg, as it was reported for murre. Other studies have shown how changes in diet have altered patterns of contaminant exposure in biota (Hebert and Weseloh 2006; Hebert et al. 2009).

Inter- and intraspecific differences of Hg levels in seabirds are influenced by several factors such as diet, age, specific metabolism, body size, molt strategy, migration pattern, physiology, or their combination, being also species-related excretion levels and rates important (Leonzio et al. 1986; Braune and Gaskin 1987a, b; Monteiro and Furness 1995; Monteiro et al. 1998; Bearhop et al. 2000). Arcos et al. (2002) have shown that diet composition may be the main determinant of Hg levels in various seabird species in Ebro Delta (Spain), with Hg levels being higher in those species consuming discards (i.e., demersal fish). Indeed, Hg levels in discards were more than double those found in epipelagic fish, corresponding the highest values to the most strictly benthic species (gobies (Gobiidae), dragonets (Callionymidae), brown comber (*Serranus hepatus*), and flatfish (Pleuronectiformes)). The levels of Hg in these species of fish from the Mediterranean Sea were higher compared with the same species from the Atlantic Ocean (Renzoni et al. 1998). Moreover, some other factors can influence the amount of mercury in the habitat, and, for example, natural inputs, high development of agricultural and industrial activities along the river, and

local pollution in the study area could explain the unusually high concentrations of mercury even for a Mediterranean context. In gulls, the relationship between Hg levels and use of discards was also found to be evident. Audouin's gull (*L. audouinii*), for example, preferentially feeds on discards (Clupeiformes and benthic fish) during the breeding season (Oro et al. 1997), although it also captures epipelagic fish to a variable degree. In this species, high Hg levels were measured in the summer liver samples. Also the yellow-legged gull (*L. cachinnans*) combines discards with foods of terrestrial and freshwater origin (Oro et al. 1995), the latter presenting negligible amounts of Hg compared to marine prey (Leonzio et al. 1986; Thompson 1996). Although Hg levels were found to be high in this species, levels were significantly lower compared to those exhibited by Audouin's gull.

Given the importance of the diet in explaining inter- and intraspecific differences in Hg levels of seabirds, research efforts should be directed at ascertaining the content of Hg both in diet samples and in a wide range of potential preys (Monteiro et al. 1998). In addition to this information, the feeding ecology of the seabird species selected for monitoring studies should be assessed (Monteiro et al. 1999). The diet of marine species has changed during the last century due to increased consumption of marine waste from fisheries (Tasker et al. 2000). Marine waste should therefore be considered as a potential extra source of Hg in marine species. Indeed, seabirds that use discards and offal for their food have access to species that would normally be unavailable to them (Furness et al. 1986). This could expose them to higher levels of Hg than present in their natural prey. This would, for example, be the case if discards are composed of either demersal fish (unavailable to surface-feeding seabirds) or mesopelagic fish (unavailable to both inshore and diurnal species, in the latter case because mesopelagic organisms are subject to diel vertical migrations and are available from the surface only at night) (Whitehead et al. 1986). Demersal fish showed higher levels of Hg than epipelagic fish, being this fact reflected in the high average concentration of this metal in trawler discards (Arcos et al. 2002).

Calle et al. (2015) did not observe statistically significant differences in Hg concentrations between different feathers regions in three species of birds (gentoo penguins (*P. papua*), chinstrap penguins (*P. antarcticus*), and brown skuas (*C. antarctica lonnbergi*)), including the calamus and the tip. However, since a relative increase in Hg levels from the calamus to the rachis tip of the feather has been observed, this may still be biologically significant in terms of tissue deposition and gradual accumulation of this metal in feathers. As previously indicated, molting and inter-molting periods affect the accumulation of Hg in feathers. Large feathers that molted first were reported to exhibit more Hg than feathers that molted later (Furness et al. 1986; Thompson et al. 1998). For future biomonitoring studies on pollutants in feathers, it is therefore important to carefully select the appropriate part of the feather (mainly those regions exhibiting the highest concentrations) and the sampling period.

Some researchers have reported that Hg concentrations are higher in adult birds compared to juveniles (Hoffman and Curnow 1979; Saeki et al. 2000). Dietz et al. (1996) have, for example, shown that Hg increased with age in the liver

(0.05 ± 1.81 μg/g juveniles, 0.78 ± 1.96 μg/g adults) and kidney (0.02 ± 4.52 μg/g juveniles, 0.30 ± 1.51 μg/g adults) of common eider. Also Mendes et al. (2008) have shown that the liver and kidney Hg levels are higher in adult *M. bassanus* compared to young birds. Furthermore, Burger (1994), in a literature review looking for differences in Hg levels in feathers between adults and young, found that adult birds had significantly greater levels in 20 of the 21 studies reviewed. Also Burger (1995) has determined that Hg was accumulated to a higher extent in feathers of adults compared to young animals in various species of seagulls. It is important to mention that newborn chicks usually exhibit a high amount of Hg, which is eliminated during the pre-fledging molt (Hobson and Clark 1992). However, older chicks tend to accumulate more Hg. The high Hg levels in young chicks can be explained by the Hg-containing food of the nest and the Hg deposited by the mother in the egg. The amount of Hg that comes from the mother is usually larger than the amount of Hg in food. During growth of the chicks, Hg tends to accumulate during feather formation due to exposure to this metal. A similar pattern in Hg levels, including a high level of Hg immediately after birth and accumulation after an initial decrease, was observed in chicks of *R. tridactyla* (Wenzel and Gabrielsen 1995).

In terms of gender differences, females of *L. dominicanus* (0.94 ± 0.09 μg/g $n = 25$) had significantly lower Hg levels compared to males (1.33 ± 0.19 μg/g, $n = 29$) (Sepúlveda and González 2014). This difference has also been reported for other avian species (black skimmers) and is likely due to transfer of Hg to eggs (Burger and Gochfeld 1992). Another possible explanation is the smaller size of females, involving their feeding with smaller amount of fish and, thus, acquiring lower concentrations of Hg (Burger and Gochfeld 1992). However, Burger and Gochfeld (2000) found higher Hg levels in birds with smaller size. Contrary to the idea that larger animals that typically live longer and can eat larger food items are expected to have higher levels of pollutants in their bodies, they argued that larger animals may not always eat larger food items. Similar to their finding, Rajaei et al. (2010) found the highest Hg levels in the smallest bird, finding the highest Hg in *L. minutus*, which is the smallest in size among the birds they studied. Differences in metabolic rate can also be attributed to varying Hg body burdens. *L. minutus* has a high metabolic rate, needing high caloric intake, associated with a potential increase in contaminant intake. Also Braune et al. (2007) found highest contamination level in smallest *P. eburnea* and attributed this high level of contamination in a small bird to the bird's high metabolic rate. Differences among findings in these studies pointed out to possible effects of gender in the Hg levels, not being only consequence of the intake.

3.1.4 Effects of Hg on Health of the Animals

Hg can cause significant damage in birds, being usually the endpoint of more direct effects on behavior, neurology, endocrinology, and development (Grandjean et al. 2010), with impacts on reproduction and ultimately reducing their survival capacity. High Hg concentrations in some tissues (e.g., kidney and liver) can also negatively

affect seabird survival by induction of acute poisoning (Wolfe et al. 1998). This element is also associated with deleterious effects, such as decreased food intake, which leads to weight loss, weakness in wings and legs combined with coordination problems, difficulties in flying and walking, paralysis, convulsions, and even death (Scheuhammer 1987).

Nicholson and Osborn (1983) noted kidney lesions and other nephrotoxic effects in *Fratercula arctica,* finding concentrations of Hg in the kidney around 5.02 mg/kg of dry weight. The same authors found 13.4 mg of Hg/kg in *Fulmarus glacialis* (see also Osborn et al. 1979). Pathological features included necrosis and degeneration of the proximal tubular epithelium together with the direct observation of obstruction of the more distal nephron segments by necrotic cellular debris. Also Hg concentrations of 49–125 mg/g in the liver have been reported for free-living birds found dead or dying (Thompson 1996).

In some piscivorous species (*Gavia immer*, *Ardea herodias*, *Casmerodius albus*, *Ajaia ajaja*, *Cathartes aura*, *Mergus merganser*, *Corvus corax*, *Gavia immer*), Hg concentrations of 0.5 μg/g in eggs and 9–20 μg/g in feathers have been shown to correlate with decreased reproductive success (Ochoa-Acuña et al. 2002). High levels of Hg are of concern, since it has been described that Hg induces eggshell thinning and malformations, inhibits egg production, and has embryotoxic effects (Lundholm 1995; Heinz and Hoffman 2003).

Hg levels in feathers exceeding 5,000 ppb (5,000 ng/g=5 μg/g) are thought to associate with adverse reproductive effects in birds, such as lower clutch size and/or egg size, lower hatching rate, and decreased chick survival and other effects (Eisler 1987; Burger and Gochfeld 2000a). Levels of Hg were found to exceed 5 μg/g in *L. argentatus* in Germany (Lewis and Furness 1993) and Chaun (Siberia); *L. sabini* (Kim et al. 1996a) and *Phalacrocorax carbo* in Iran (Aazami et al. 2011)*; Morus bassanus* in Portugal (Mendes et al. 2008); *Diomedea epomophora*, *Diomedea exulans*, and *Phoebastria immutabilis* in the North Pacific (Kim et al. 1996c; Burger and Gochfeld 2000a); and *Fratercula arctica* in St Kilda (UK) (Osborn et al. 1979). This may be considered of high concern and be taken into account when a biomonitoring program is developed, trying to associate metal levels and reproductive disruption. These levels can be used to assess whether there are potential reproductive deficits in populations (Burger 1994). Nonetheless, the adverse effect thresholds based on total Hg concentrations in the liver usually assume that any other form of Hg (different than MeHg or inorganic Hg) present in the tissue is negligible. However, this assumption should be completely wrong, especially for long-lived species for which part of the liver Hg may be present as a nontoxic complex of inorganic Hg associated with Se (Scheuhammer et al. 2015).

3.1.5 Conclusion: Hg

Hg can be especially damaging to the environment being able to accumulate in living organisms. Several studies reported Hg levels exceeding thresholds assumed as deleterious, thus, trying out that Hg concentrations in the aquatic environment are

still of concern. Data from the present review point out to feathers as the main organ to accumulate Hg, being the most useful for the determination of long-term exposition to this metal in seagulls; however, the obtained results should be interpreted with caution due to atmospheric deposition influence. Directly related, different factors, as, for example, the area of the feather and the molting period, have to be considered for biomonitoring purposes. Gulls showed, in general, lower levels of Hg than other seabirds (as albatrosses and cormorants), being these differences related to different behavioral patterns and habitats.

3.2 Cadmium (Cd)

3.2.1 Cd in the Environment and Its Chemical Forms

Cadmium is an abundant, nonessential element of high concern due to its accumulation in the environment. It is widely distributed in the earth's crust at an average concentration of about 0.1 mg/kg, being the highest level of Cd compounds accumulated in sedimentary rocks and marine phosphates (Gesamp 1987). Also this element is produced as a result of industrial and agricultural activity.

Since this metal is not prone to bacterial detoxification, it is eliminated very slowly. Cadmium-associated toxicity is amplified in organisms due the metal's long biological half-life (15–30 years) (Jarup et al. 1998). This metal is accumulated in aquatic animals and can be toxic to those who live in both fresh and salt water. For many predators, Cd is easily accumulated by consuming food, since this metal is present in prey cells. It has been shown that predators assimilate Cd located in the cytosol of prey cells more efficient than Cd associated with insoluble prey components (Dubois and Hare 2009).

The factors controlling metal transfer between prey and predator are important for predicting both trends in metal concentrations along aquatic food chains and the likelihood of toxic effects on animals at upper trophic levels. High bioconcentrations of this trace metal have been found in diverse both aquatic and terrestrial organisms, including invertebrates (earthworms, spiders, beetles) and vertebrates such as fish and insectivorous small mammals (shrew, voles) (Chiarelli and Roccheri 2014). Cephalopods (*Nototodarus gouldi*, *Ommastrephes bartrami*, *Sthenoteuthis oualaniensis*, *Loligo opalescens*) are considered to be a significant Cd source, since they are an important component of gulls' diets (Honda et al. 1983). These levels of Cd in those organisms come mainly from anthropogenic sources, like mining activities (nonferrous metal productions, iron and steel making), fuel combustion, and refuse incineration, that have also been associated with accumulation of this metal in birds. The environmental impact of mining activity has, for example, been studied in a number of marine species (fish, prawns, seaweed, and mussels) from Greenland since the early 1970s (Johansen et al. 1991). Those authors monitored the impact of a lead-zinc mine in the mentioned organisms by means of the evaluation of different metal levels (Cd, Cu, Pb, and Zn).

3.2.2 Cd Levels in Birds: Differences Among Tissue Types

In Table 1 is shown that the Cd levels in the liver of different species of gulls ranged from 0.035 ± 0.66 μg/g in *L. glaucoides* in Greenland (Dietz et al. 1996) to 45.1 ± 32.7 μg/g in *R. tridactyla* in Ny-Alesund (Norway) (Savinov et al. 2003). The Cd concentrations observed in the kidney were higher than in the liver; the range of this metal was from 0.083 ± 0.51 μg/g in *L. glaucoides* (Dietz et al. 1996) to 184 ± 110.7 μg/g in *R. tridactyla* in Prince Leopold Island (Canada) (Braune and Scheuhammer 2008) and 159 ± 30 μg/g in *L. argentatus* in Siberia (Kim et al. 1996a). The concentration of Cd in feathers was found to be much lower than in the kidney and liver. The levels of this metal in feathers ranged from 0.03 ± 0.04 μg/g in *L. dominicanus* in Chile (Sepúlveda and González 2014) and 0.032 ± 0.003 μg/g in *R. tridactyla* in Alaska (Burger et al. 2008) to 0.851 ± 0.558 μg/g in *L. pipixcan* in Benton, USA (Burger 1996).

Although in this review Cd concentrations were found to be higher in the kidney than in the liver and feathers in both gulls (Table 1) and other species of seabirds (Table 2), Scheuhammer (1987) advocated the use of the liver in monitoring biological exposure to Cd. The liver is considered to be a suitable organ for biomonitoring studies, since it accumulates approximately 50% of the total Cd burden, and the Cd content in this tissue appears to be extremely stable. This stability is probably due to the general resistance of the liver to Cd-induced toxicity (Goyer et al. 1984). A higher level of Cd in the kidney compared to the liver usually indicates chronic exposure to low Cd concentrations (Scheuhammer 1987). Approximately 90% of the total Cd body burden is present in the liver and the kidney (Friberg et al. 1974).

Table 2 shows the average Cd concentrations in the liver of several seabirds, ranging from 0.217 ± 0.123 to 109 ± 61.1 μg/g in *P. carbo* in Spain and *F. glacialis* in Ny-Alesund, respectively (Navarro et al. 2010; Savinov et al. 2003). Renal Cd concentrations ranged from 0.704 ± 0.226 μg/g in *P. carbo* in Spain (Navarro et al. 2010) to 228 ± 71 μg/g also in *F. glacialis* in the UK (Osborn et al. 1979). The differences between the low and high Cd concentrations detected in the liver and kidney can be explained by the methods used to determine Cd levels: while the lower concentration was detected using ICP-MS, the higher concentration was detected using AAS. However, ICP-MS and ASS were several times probed as good techniques to analyze Cd, and they should be optimal for Cd evaluation once the levels in the samples are above the limit of detection.

Some authors have used the kidney/liver ratio as an indicator for recent Cd exposure (Honda et al. 1986; Kim et al. 1996a; Navarro et al. 2010), since the Cd resides for longer periods in kidney stores compared to liver stores. In the present review, we have calculated the kidney/liver Cd ratios in those cases where both concentrations were determined. In seabirds, this ratio was found to be low (2.9) in *P. carbo* in Japan (Nam et al. 2005) and high (11.5) in *D. epomophora* in New Zealand (Stewart et al. 1999). In gulls, the ratio was found to be low (1.5) in *L. ridibundus* in Poland (Orlowski et al. 2007) and high (9.6) in *L. savini* in

Siberia (Kim et al. 1996a). The lower ratio in *P. carbo* and *L. ridibundus* suggests that these birds have recently been exposed to Cd. The high Cd concentration (Table 1) in birds from Japan and Poland is probably due to their feeding habits on the breeding ground in these countries. The observed differences between different countries may be due to country-specific differences in prey animals. Cd concentration in the liver and kidney of breeding adult birds can vary by a factor of 4 between different stages in the breeding season as a result of change in tissue physiology (Wals 1990).

In *P. carbo* different kidney/liver Cd ratios have been published: 3.2 in Spain (Navarro et al. 2010) and 2.9–5.5 at Lake Biwa in Japan (Nam et al. 2005; Saeki et al. 2000). The main differences between the two studies in Japan were the periods in which the birds were sampled and the methods used to determine Cd levels. In the study of Nam et al. (2005), ICP-MS was used, while AAS was used in that of Saeki et al. (2000). It has been reported that differences in sensitivity between methods render it impossible to compare studies developed with different methods of analysis. Indeed, the dominant source of error in very low-level analytical measurements is often the systematic bias of the analytical methodology itself. However, in the present years, a variety of robust analytical techniques is available for trace analysis (Brown and Milton 2005), which makes possible to analyze really low concentrations of metals in environmental samples. Therefore, the selection of the methodology will lead to a high or low reliability of the result. Nonetheless, most of the currently used techniques would result in similar concentrations from the analysis of the same sample, being only differences when the amount of metal is close to the limit of detection (LOD). Taking this idea in mind, since AAS and ICP-MS were probed as valid techniques to analyze Cd concentrations, and the concentrations in the kidney and liver were above the LOD in the studies, it can be expected that the mentioned differences in this case may be influenced more by the sampling time.

The lowest levels of Cd were found in feathers of seabirds (Table 2), with average Cd concentrations ranging from 0.026 ± 0.010 to 0.578 ± 0.246 in *Uria lomvia* in Canada (Braune and Scheuhammer 2008) and *Thalassarche melanophris* in Georgia (Anderson et al. 2010), respectively. This tissue was, therefore, confirmed as a no main target for Cd accumulation. As previously mentioned, sample treatment influences the result obtained; in fact levels of Cd, Cu, and Pb have been reported as higher in unwashed feathers compared to washed feathers (Frantz et al. 2016).

3.2.3 Differences in Cd Levels Depending on Diet/Age/Gender

High Cd levels may be due to feeding habits. As mentioned above, Cephalopods are considered to be a significant Cd source for their predators, including seabirds (Muirhead and Furness 1988). Kim et al. (1996a) state that higher Cd levels in *L. argentatus* (Chaun, Siberia) may be due to the localization of their colonies. These colonies are often located in close proximity of mosquitoes and other species of aquatic insects, which all exhibit considerable amounts of Cd and are eaten by these birds. This suggests that terrestrial and marine invertebrates, upon which they

actively prey, are the source of this metal. Indeed, Cd concentrations in the major taxa were, ranked in decreasing order, as follows: Isopoda, Oligochaeta, Lycosidae, Opiliones, Linyphiidae, Collembola, Carabidae, Staphylinidae, Chilopoda, Curculionidae, and Orthoptera (Hunter et al. 1987). Regarding invertebrates, mollusks, arthropods, and echinoderms are known to accumulate high levels of heavy metals in their tissues and yet survive in polluted environments, thus, having a peculiar position in the marine trophic chain, where pelagic larvae are part of the diet of several planktonic and benthic organisms (Chiarelli and Roccheri 2014). Leonzio et al. (1986) have shown that Cd levels are low in fish of the Mediterranean and other seas.

Similar to Hg, the highest Cd levels in both the liver and kidney were expected to be detected in albatross species, due to their specific behavioral patterns and habitats. However, the highest levels were found in *Fulmarus glacialis*. The high Cd levels in this species may be due to its feeding habits. This species usually feeds on fishery offal, squid, polychaetes, crustaceans, and small fish (Mehlum 1990). It is well known that invertebrates (squid and crustaceans) may accumulate considerable amounts of Cd. Cd levels detected in seabirds are the result of two main processes: bioaccumulation (mainly via food) and biodegradation processes in the organs of these birds. *F. glacialis* is one of the longest-lived species among seabirds (Ollason and Dunnet 1988); the bioaccumulation along the lifetime can derive in higher levels detected in their tissues.

Age has been also reported as a factor influencing Cd accumulation in other species (Furness and Hutton 1979; Hutton 1981). Saeki et al. (2000) have shown that Cd levels in the liver and kidney significantly increase during aging from chick to juveniles and adults. In the same line, Orlowski et al. (2007) have reported an extraordinarily high concentration of Cd in the liver and kidneys of adult gulls. Moreover, the high Cd concentration detected in fulmar by Savinov et al. (2003) may be due to Cd bioaccumulation with age. Cd levels exceeding the expected levels, based on the age of the chicks, provide an indication for enhanced accumulation of this metal due to contaminated local food. Contamination of the nestlings is thereby indicative for environmental metal burden of maternal feeding grounds during the breeding period (Furness et al. 1993). However, once the nestlings became older, it seems that Cd levels in feathers may decrease, as showed by Wenzel and Gabrielsen (1995) in *R. tridactyla*. Nonetheless, some other authors disagree regarding the age effect; for example, Nicholson (1981) did not find a correlation between the levels of Cd in any tissue and age of seagulls.

Burger and Gochfeld (1992) have reported significantly higher levels of Pb and Cd in feathers of females compared to males. A possible explanation could be the higher investment in grooming behavior in males; thus, preening with the bill is the most important grooming activity during which birds handle a great number of their feathers every day with their bills. This behavior may efficiently remove some of the external metal contamination, which could then be transferred via the bill to the digestive track and increase the internal level (Frantz et al. 2016). In contrast, Stewart et al. (1999) did not find any gender-related differences in Cd or Hg levels in the liver and kidney of different albatross species.

3.2.4 Effects of Cd on Health of the Animals

Cd is not physiologically present in organisms, and as a toxic metal, is known to induce kidney toxicity, lesions in intestinal tissue, disruption of calcium metabolism, decreased food intake, and thinning of eggshells (Burger et al. 2008). This metal can cause intracellular reduction in the level of the main antioxidant compounds, enhancing the production of free radicals and lipid peroxides (Li et al. 2010) and disrupting the antioxidant systems. Cd-induced ROS (reactive oxygen species) will lead to lipid peroxidation, damage in DNA, and apoptosis. Cd can inactivate enzymes and other antioxidant molecules by interaction with thiol groups in these molecules (glutathione or proteins) (Filipic et al. 2006).

Moreover, Cd is bound within the liver to a group of proteins with a low molecular weight called metallothionein (MTs), a family of universal, small proteins, sharing a high cysteine content and an optimal capacity for metal ion coordination. They take part in a plethora of metal-related events (from detoxification to homeostasis, storage, and delivery), in a wide range of stress responses, and in different pathological processes (tumorigenesis, neurodegeneration, and inflammation) (Atrian and Capdevila 2013). The Cd detoxification in the organism is mainly due to the binding potency of MTs, leading to chemical inactivation before sequestration in lysosomes or in mineral concretions or excretion via body fluids. Their presence has been documented in numerous phyla, including vertebrates, invertebrates, and microorganisms (Amiard et al. 2006; Klaassen et al. 1999).

It has been shown that renal lesions, associated with Cd intoxication, occur if renal Cd concentrations exceed 100–200 μg/g (wet weight, ww) in most avian species (White et al. 1978). Individual toxic concentrations of renal Cd were found to be 183 mg/kg in apparently healthy adult *L. hyperboreus* from Greenland (Nielsen and Dietz 1989) and 148 mg/kg in a wandering albatross (Muirhead and Furness 1988). High Cd levels were detected in *F. arctica* on the Scottish island of St Kilda (114 ± 12.3 μg/g in the kidney). Despite these high levels in the kidney of Cd, these animals were found apparently healthy (Osborn et al. 1979).

There are some data that describe levels of Cd found in some populations of wild birds considered to be healthy: wandering albatross (32 mg/kg in the liver, 137 mg/kg in the kidney) (Muirhead and Furness 1988), sooty albatross (26 mg/kg in the liver, 76 mg/kg in the kidney) (Muirhead and Furness 1988), kittiwake (11 mg/kg in the liver, 76 mg/kg in the kidney) (Nielsen and Dietz 1989), and fulmar (17 mg/kg in the liver and 55 mg/kg in the kidney) (Norheim 1987). Wals (1990) stated that Cd concentration in healthy wild birds varies from 0.1 to 32 mg/kg in the liver and from 0.3 to 137 mg/kg in the kidney. Renal lesions associated with Cd intoxication occurred when renal Cd concentrations exceeded 100–200 μg/g (ww) in most mammalian and avian species studied (Kjellstrom 1986; White et al. 1978).

Wals (1990) has suggested that such high levels of Cd may lead to an evolutionary consequence of long-term exposure to high natural levels of this metal in oceanic food webs, leading marine birds to evolve and be able to regulate the concentration of Cd. However, another possibility to the tolerance to high tissue Cd levels is

thought to be made possible by the cellular production of the metal-binding proteins, MTs (Wals 1990).

3.2.5 Conclusion: Cd

Cadmium is a metal that produces toxic effects on living organisms, even in very small concentrations. Even when the kidney is the major reservoir of Cd, showing this metal the highest concentrations in this organ, the present review pointed out the liver as the preferred tissue to detect this metal. This can be probably due to the liver resistance to injuries provoked by Cd and also to the stability of this metal, which makes the researchers to select this tissue when sampling is performed. On the other hand, for biomonitoring studies, it seems that feathers are not very adequate for determining Cd concentration in seabirds. Accumulation of Cd is age-related, since it is not easy to degrade this metal; however, the sex does not seem to influence the levels of cadmium in seabirds. In general, Cd levels found in the studied species were not high enough to trigger impairment in the health of the animals, as was confirmed since the reported threshold was not exceeded.

3.3 Lead (Pb)

3.3.1 Pb in the Environment and Its Chemical Forms

Lead is a metal that predominantly exists in two main oxidation states, +2 and +4. Pb is one of the most common metals in contaminated ecosystems (Steinnes 2013). Although Pb can be emitted to the environment by multiple natural sources, including volcanic activity and erosion, the majority of Pb is related to anthropogenic sources (industry, gasoline, agriculture). It is a common industrial and urban contaminant, especially at hazardous waste sites (ATSDR 1988; Abadin et al. 2007). Although the elimination of Pb from gasoline resulted in lowering this metal in the environment and humans (ATSDR 1988; Schwartz 1994), Pb poisoning from paint is still a problem in many inner cities and in aquatic environments (Abadin et al. 2007). Another source of Pb is the ammunition; Pb has accumulated in soil at and around shooting ranges from years of lead shot and bullets being fired into fixed "stopbutt" mounds or at moving clay targets.

Tetraethyl lead and tetramethyllead were once used in the USA as gasoline additives to increase octane rating. While large particles drop to the ground immediately and pollute soils or surface waters, smaller particles can travel long distances through air and remain in the atmosphere. These particles, will, in part, fall back on earth when it is raining upon which it accumulates in water and soil organisms. Moreover, Pb is a bio-persistent pollutant that accumulates at the top of the food chain (Chiarelli and Roccheri 2014).

Pb is a highly toxic metal that acts as a nonspecific poison that can affect all body systems (Pain 1987). Once in the bloodstream, Pb is rapidly deposited into soft tissues (liver, brain, and kidney), bones, and growing feathers. Absorption of large amounts of Pb can cause sudden illness and death even in birds that appear to be in good physical condition (Gill and Langelier 1994). A peculiar way of Pb contamination in birds is through ingestion of spent Pb ammunition, which has resulted in mortality in birds worldwide, in particular in waterfowl (Bellrose 1959; Sandersons and Bellrose 1986; Pain 1987).

3.3.2 Pb Levels in Birds: Differences Among Tissue Types

In contrast to Hg and Cd, only a few studies on Pb levels in gulls have been published. These studies revealed that Pb values are higher in the kidney compared to the liver and feathers (Table 1). The Pb concentrations in the liver ranged from <0.1 to 8.91 ± 1.87 μg/g in *L. argentatus* in Italy (Leonzio et al. 1986) and *L. ridibundus* in Poland (Orlowski et al. 2007), respectively. In the kidney, the variation between species was even larger, ranging from <0.1 to 30.96 ± 22.81 μg/g in *L. argentatus* and *L. ridibundus*, respectively, both found in Italy (Leonzio et al. 1986). For feathers, Pb levels ranged from 0.707 ± 0.131 to 10.40 ± 5.36 μg/g in *R. tridactyla* in Alaska (Burger et al. 2008) and *L. ridibundus* in Poland (Orlowski et al. 2007), respectively.

In gulls' feathers, Pb levels ranged from 0.707 ± 0.131 to 10.40 ± 5.36 μg/g in *R. tridactyla* in Alaska (Burger et al. 2008) and *L. ridibundus* in Poland (Orlowski et al. 2007), respectively (Table 1). Burger reviewed levels of Pb in feathers of terns and gulls in general (Burger 1994), of herring gulls (*L. argentatus*) in New York harbor (Burger 1997), and of Franklin's gull (*L. pipixcan*) from the Midwest (Burger 1996). Pb levels in feathers from gulls and terns, as determined in 31 studies, ranged from 0.1 to 4.3 μg/g (median: 1.3 μg/g). In our review, higher Pb levels were detected in feathers from *L. ridibundus*, *L. dominicanus*, and *L. heuglini.* This may be explained by direct atmospheric deposition on the feathers' surface (Kim et al. 1998). Experimental studies with starlings (*Sturnus vulgaris*; Pilastro et al. 1993) and zebra finches (*Taeniopygia guttata*; Dauwe et al. 2002) have revealed that, during preening, lead is deposited onto the surface of the feather. Burger (1994) contended that newer feathers should have lower metal levels than older feathers. The surface contamination of feathers was also probed in urban birds, reporting a decrease of 37% in the Pb concentrations in washed feathers in comparison with unwashed ones (Scheifler et al. 2006). Such evidences suggest that feather Pb concentrations are in part a representation of external contamination, more than only a reflection of diet during feather formation. Therefore, results in feathers should be taken carefully when trying to monitor dietary Pb exposure.

In the livers of other species of seabirds (Table 2), the range of Pb levels was found narrower in comparison to gulls, ranging from 0.034 μg/g in three different species (*T. melanophris*, *T. chlororhyncha*, and *P. palpebrata*) in India (Kim et al. 1998) to 0.17 ± 0.15 μg/g in *F. arctica* in Canada (Elliott et al. 1992). Renal

concentrations ranged from 0.082 ± 1.77 to 0.504 ± 0.615 μg/g in *S. mollissima* in Greenland (Dietz et al. 1996) and *P. carbo* in Spain (Navarro et al. 2010), respectively. In feathers, Pb levels ranged from 0.799 ± 0.058 in *P. nigripes* in Midway Atoll (Burger and Gochfeld 2000a) to 22.508 ± 20.065 μg/g in *P. carbo* in Spain (Navarro et al. 2010).

3.3.3 Differences in Pb Levels Depending on Diet/Age/Gender

Pb levels can be influenced by feeding habits, as documented in gulls feeding on waste dumps and showing high levels of this metal (Leonzio et al. 1986). In waste dumps not only domestic garbage is dumped but also large quantities of industrial residues from the manufacturing of leather, ceramic, and glass. Waste dumps are a source of food; indeed, the mentioned birds eat mollusks and other invertebrates that can be found at these places. However, such high Pb concentrations in the kidneys and liver have been only reported in Mediterranean seabirds that were found dead in a lagoon in Northeastern Italy due to shotgun-induced Pb poisoning (Mute Swans) (Perco et al. 1983). Orlowski et al. (2007) stated that high Pb levels in gulls may be explained by the use of Pb pellets in countries such as Russia, Estonia, Latvia, Lithuania, and Poland. This metal can be also found near dump sites and in products of human industrial activities. Unlike Hg and Cd, Pb is not present in the prey of gulls. Sileo and Fefer (1987) argue that Pb exposure in albatross chicks in Sand Island (USA) is associated with the presence of buildings and related to the ingestion of paint chips, and Finkelstein et al. (2003) referred a similar conclusion in albatross from the Midway Atoll (USA).

Pb levels were found to be higher in the different species of gulls compared to the different species of seabirds. This may be due to the gulls' habitats, since gulls tend to live near man and human industry. It should be noticed that, in comparison to other species such as *P. carbo*, *U. lomvia*, *S. mollissima*, and albatrosses, human garbage comprises a larger fraction of the gulls' diets.

Several studies reported no significant differences in the metal content of the liver, kidneys, and feathers between male and female birds (Zamani-Ahmadmahmoodi et al. 2009; Hutton 1981). In contrast, differences were found in a species with marked sexual size dimorphism, being Pb levels significantly higher in females than males (Burger and Gochfeld 1992).

Regarding age, a study developed with *Puffinus gravis* in the Brazilian coast, adults, which are supposed to be more time far from the coastal line, exhibited higher hepatic Pb levels than juveniles (Barbieri et al. 2007), even if it has been reported that coastal and inshore marine biota would carry the highest Pb burdens. Increasing levels of liver and brain Pb with age may, at least in part, be due to accumulation as toxicologically inactive complexes with MTs, resulting in a long half-life (Zaccaroni et al. 2003). Indeed, Burger (1995) has shown that Pb levels in feathers of adult birds

were higher than Pb levels in young ones, indicating that Pb, similar to Hg and Cd, tends to accumulate in birds with age. However, there are controversial data. Even if accumulation of metals with age could be a natural consequence, when the absorption of the bird exceeds its ability to excrete these inorganic elements, there are several factors that should be considered (species, organ, environment), rendering complicated to evaluate the influence of age on Pb levels.

3.3.4 Effects of Pb on Health of the Animals

Some experimental studies have documented adverse effects of Pb in small birds, leading to modification of feather growth rates (10 ppm in great tits, *Parus major*) and altered immune response (20 ppm in *Taeniopygia guttata*) (Talloen et al. 2008; Snoeijs et al. 2005). In general, it has been shown that adverse effects (negative effects on behavior, thermoregulation, locomotion, and depth perception resulting in lowered nestling survival) in birds occur if Pb levels exceed 4,000 ppb (4 μg/g) in feathers (Custer and Hoffman 1994; Burger and Gochfeld 2000b). However, seabirds are thought to be able to tolerate higher Pb levels (Burger and Gochfeld 2000a). In this review, several gull species exhibited higher Pb levels (Table 1). For example, *L. ridibundus* in Italy (10.40 ± 5.36 μg/g), *L. dominicanus* in Chile and Brazil (5.97 ± 6 and 7.54 ± 1.66 μg/g, respectively), and *L. heuglini* in Southern Iran (7.04 μg/g) exhibited Pb levels in feathers that exceeded 4 μg/g. Nonetheless, for other seabirds, a very high Pb level was only detected in *P. carbo* in Spain (22.51 ± 0.96 μg/g). The differences between these studies may be due to the methods used to determine the metals' concentrations. While in gulls Pb levels were always determined using AAS, in *P. carbo* ICP-MS was used. Since the highest levels of Pb were detected in *P. carbo*, it can be hypothesized that ICP-MS is more sensitive than AAS. However, as mentioned before, for Cd, differences in sensitivity between methods cannot completely explain such variations, since values are above the LOD and these methodologies are probed as valid for Pb detection. Moreover, data reported in the mentioned studies about Pb levels in feathers should be taken and interpreted carefully, since feathers belong to adult animals and, as it was previously mentioned (Sect. 3.3.2), levels in this tissue can be partially due to atmospheric deposition along the time.

Although it is evident that Pb induces poisoning and mortality, little is known about the effects of low Pb levels on neurobehavior. There are only few studies where the behavioral tests were designed to mimic tasks faced by animals in the wild. In this sense, Burger and Gochfeld (2000a), in their study with *Larus argentatus* and *Sterna hirundo*, pointed out that low-level lead affected growth, locomotion, balance, food begging, feeding, thermoregulation, and depth perception in laboratory and wild birds. Since similar effects of Pb have been found in both humans and wildlife, wild animals can be used to understand Pd-induced effects in humans, and vice versa, data on humans can be used to assess effects to wildlife (Porkas and Kneeland 2009). It was documented that Pb induces carcinogenic effects in animals (Sanz-Gallén et al. 1993). In birds, this metal can affect all body

systems, behaving as a nonspecific poison. Upon intoxication, birds usually develop the following symptoms: distension of the proventriculus, green watery feces, anemia, weight loss, and a drooping posture (Franson et al. 1983; Custer et al. 1984; Sandersons and Bellrose 1986; Redig et al. 1991). Sublethal toxic effects have been shown in the nervous system, kidneys, and circulatory system, resulting in biochemical, behavioral, and physiological changes (Scheuhammer 1987). Furthermore, Pb impairs the growth and survival of nestlings; causes hemolytic anemia in wild Pb-poisoned birds; has adverse effects on reproduction, such as decreased plasma calcium and egg production; and causes behavioral impairments (Burger and Gochfeld 1994; Scheuhammer 1987), with disruption of the expression of synaptic neural cell adhesion molecules in young herring gull hatchlings (Dey et al. 2000).

In mallards, it has been shown that death is provoked if Pb levels exceed 200–500 μg/g (dry weight, dw) in the kidney and 100–200 μg/g in the liver (Longcore et al. 1974; Benson et al. 1976). It has been also described that liver concentrations below 2 μg/g (dw) could be indicative of a safe environmental exposure, with no toxicological risk (Pérez-López et al. 2008), which could be considered as threshold value. Indeed, in tissues of adult bird species living in uncontaminated areas, or raised under controlled laboratory conditions, Pb levels were 2–15 μg/g (dw) in the bone, 1–10 μg/g in the kidney, and 0.5–5 μg/g in the liver, close to the mentioned concentration for toxicological risk. It may not be possible to directly compare Pb tissue levels between samples from experimental and field studies, since wild birds with inadequate diets that are exposed to environmental conditions may be more susceptible to Pb than birds maintained under laboratory conditions (Kendall et al. 1983; Custer et al. 1984). From all the reviewed species, only *L. heuglini* from Southern Iran and *L. ridibundus* from Italy or Poland presented liver Pb levels significantly higher than those reported as threshold. The Pb levels found lower than the threshold in the rest of the seabird species should be indicative of a low pollution, in terms of Pb, in the areas where they inhabit.

Apparently healthy adult *L. atricilla* was collected in a contaminated area that had mean liver lead residues of 4 to 5 ppm and kidney residues of 2 ppm ww (Munoz et al. 1976).

3.3.5 Conclusions: Pb

There are few studies of Pb in the seabirds selected for the present review, appearing mainly this metal in the kidney, being in lower extent in the liver and feathers. Feathers may not be a good indicator of dietary exposure to Pb because this metal deposits on their surface, moreover considering that those animals presenting levels above the toxicity threshold were adults, being those levels likely due to atmospheric deposition. It is also probed that, once the metal enters the bloodstream, Pb deposits and accumulates rapidly in internal tissues as the liver and kidney, thus being those organs more suitable for the determination of Pb. Gulls seem to be more exposed to

Pb than other seabird species, as human garbage comprises a relevant fraction of gull's diet.

3.4 Arsenic (As)

3.4.1 As in the Environment and Its Chemical Forms

Arsenic is one of the most important global environmental pollutants and a persistent bioaccumulative carcinogen (Kaur et al. 2011). It is a toxic metalloid that exists in two oxidative states: a trivalent form and a pentavalent form, in the form of arsenous acid (H_3AsO_3) and its salts and arsonic acid (H_3AsO_5) and its salts, respectively. Although, in the natural environment, As is rarely found as a free element. Arsenic compounds can be found in air, water, soil, and all living tissues. The most important anthropogenic As sources are the smelting of Cu, Ni, Pb, and Zn ores and the burning of fossil fuels in households and power plants. Another source of As contamination is the use of arsenical fungicides, insecticides, herbicides, algicides, wood preservatives, and growth stimulants for plants and animals (Matta and Gjyli 2016). Although human activities contribute little to the As increase of the open ocean, they may be important in estuaries and coastal waters receiving As-contaminated drainage from the land (Chappell et al. 1994).

As is considered to be an essential oligoelement for various animals, but many As compounds are highly toxic (Mayer et al. 1993). Organic As is an integral component of nutrients of many organisms in which they may play potentially important biological roles. However, its roles in humans still remain to be determined (in small doses they seem to improve the activity of certain neurotransmitters in the central nervous system). As may be absorbed by ingestion, inhalation, or through permeation of skin and mucous membranes. The mechanisms of As toxicity differ greatly among chemical species, although all appear to cause similar signs of poisoning. High levels of arsenicals were found in marine organisms comprising algae, crustaceans, bivalves, fish, and mammals (Sloth et al. 2005).

Inorganic arsenicals are well absorbed by the oral and inhalation routes. Less is known about the organic arsenicals, but it appears that both monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are well absorbed (WHO 2000). The rate of absorption of As is much higher in soluble forms than in highly insoluble forms. Once absorbed, arsenates are transformed to arsenites and methylated. This process may then be repeated to result in dimethylated As metabolites. However, neither MMA nor DMA is demethylated to yield inorganic As (ATSDR 2007). Detoxication mechanisms mainly occur in the liver and kidneys, especially following ingestion and methylating capacity is highly dependent on species and tissues (Marafante et al. 1985).

3.4.2 As Levels in Birds: Differences Among Tissue Types

Once into the body, As may circulate through it and be deposited in a variety of tissues, depending on the rate and extent of methylation (Vahter and Concha 2001). Most As is promptly excreted in the urine and feces (ATSDR 2007). In birds, As can be sequestered in feathers during molt (Jarus et al. 2001; Geens et al. 2010). Additionally, females can eliminate As by transferring it to their eggs and eggshells during the breeding season (Tsipoura et al. 2008; Orłowski et al. 2010; Ruuskanen et al. 2014). Furthermore, birds can also rid the body of metals by depositing them in the uropygial gland and the salt gland (Burger and Gochfeld 1985; Salibian and Montalti 2009).

In Table 1 the average As concentrations in gulls are shown. Arsenic levels in the liver ranged from 0.82 ± 0.26 to 98.1 ± 69 μg/g both in *R. tridactyla* from Norway and from Guba Chernaya (Russia), respectively (Savinov et al. 2003). In the kidney, As could only be detected in *L. crassirostris* from the North Pacific (Kubota et al. 2002) with a value of 1.2 ± 0.4 μg/g. Similarly, As could only be detected in feathers of a few species of gulls. Levels ranged from 0.144 ± 0.027 μg/g in *L. glaucescens* in Alaska (Burger et al. 2009) to 0.75 ± 0.21 μg/g in *L. dominicanus* in Chile (Sepúlveda and González 2014).

In other seabirds (Table 2) As levels in the liver ranged from 0.13 ± 0.1 μg/g in *S. mollissima* in Ny-Alesund (Savinov et al. 2003) to 107 μg/g in *P. nigripes* in the North Pacific (Kim et al. 1998). Renal averages ranged from 1.99 ± 0.65 μg/g in *M. basanus* in Portugal (Mendes et al. 2008) to 8.85 ± 0.96 μg/g in *P. carbo* in Spain (Navarro et al. 2010). In feathers, As levels ranged from 0.093 ± 0.030 μg/g in *D. exulans* in Georgia (Anderson et al. 2010) to 16 ± 11 μg/g *in P. nigripes* in the North Pacific (Fujihara et al. 2004).

In general, levels of As in healthy living animals are usually found to be below 3 μg/g dw (Braune and Noble 2009). However, in the present review, in many seabirds, As levels in the liver, kidney, and feathers were higher than this threshold value. These high levels were, for example, observed in two species of gulls, *L. hyperboreus* and *R. tridactyla*, from specific islands of Russia and Norway and in other species of seabirds such as *P. carbo* in Murcia (Spain), *P. nigripes* in the North Pacific, and *F. glacialis* in Norway. This suggests that a possible contamination of these areas may have contributed to increased levels of As in these seabirds (Kim et al. 1998; Navarro et al. 2010; Savinov et al. 2003).

3.4.3 Differences in As Levels Depending on Diet/Age/Gender

In general, As is not biomagnified through food chains in marine environments, and lower trophic marine animals exhibit higher levels of As compared to higher trophic animals. Kubota et al. (2001) detected higher As levels in different species of albatrosses. This may be due to a specific mechanism for accumulation of As in these species. These authors also detected high levels of As in other marine animals,

including sea turtles. This high concentration of As is comparable to the levels present in marine organisms at lower trophic levels, confirming the lack of a strong bioaccumulation of this metal.

No significant data about As variations by age and gender have been found, and only a recent study (Burger et al. 2018) confirmed the absence of age-related differences on As content in semipalmated sandpipers (*Calidris pusilla*) sampled in Suriname, South America.

3.4.4 Effects of As on Health of the Animals

Inorganic As is thought to be highly toxic in some seabirds, by acting as an endocrine disruptor. Depending on the concentration, As induces death of an individual, causes sublethal effects, or disrupts reproduction (Eisler 1994; Kunito et al. 2008). Inorganic arsenic can cause muscular incoordination, slowness, falling, hyperactivity, and a number of other symptoms in birds, including destruction of blood vessels, shock, and death (Burger et al. 2018).

As concentrations are usually low (<1 μg/g ww, which approximately represents 3 μg/g dw) in most living organisms (Braune and Noble 2009; Lucia et al. 2010). Some studies have reported that As leads to decrease activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and reduces levels of glutathione. These activities are associated with the production, after exposure to As, of ROS and RNS (reactive nitrogen species), producing the damage of lipid membranes and DNA (Flora et al. 2007; Valko et al. 2005). As presents strong carcinogenic properties and, depending on oxidation state, chemical species, cell type, concentrations, and time exposure, can induce apoptosis. As affects reproduction and growth of birds, reduces adult weight gain and liver weight, delays egg laying, and decreases egg weight and eggshell (Stanley et al. 1994).

Previous studies on metal exposure and related effects on birds have been mainly focused on Pb, Hg, and Cd. As, however, is not a well-documented element when it comes to biota and particularly to birds (Sánchez-Virosta et al. 2015). As can be seen in Tables 1 and 2, in general, As was not included in studies before the year 2000.

3.4.5 Conclusion: As

Arsenic is a nonessential metal that can be found in a wide range of forms in the environment. In general, this metal is not of concern in terms of bioaccumulation up in the trophic chain; however, data collected in the present review confirm the existence of As in levels that can be considered of risk, since they are higher than the minimum concentration reported as toxic for seabirds. Therefore, biomonitoring programs should include this element into the list of chemicals to be analyzed in environmental samples.

3.5 Selenium (Se)

3.5.1 Se in the Environment and Its Chemical Forms

Selenium is a semimetallic trace element that living organisms, including birds, need in small amounts for optimal health. High concentrations of Se in food of wildlife are not limited to areas in which soil is naturally high in Se (Robberecht et al. 1983). Agricultural drain water, sewage sludge, fly ash from coal-fired power plants, and mining of phosphates and metal ores contribute to the Se burden in the aquatic environment (Eisler 1985; Heinz et al. 1988).

Elemental Se is virtually insoluble in water and presents little risk to birds. Although both selenite (O_3Se^{-2}) and selenate (O_4Se^{-2}) are toxic to birds, organic selenides pose the greatest hazard. Among the organic selenides, selenomethionine seems to be the most toxic form for birds and most likely to harm wild birds. Selenomethionine is a major form of Se in wheat (Olson et al. 1970) and soybean protein (Yasumoto et al. 1988) and may be a major form of Se in other plants (Beilstein and Whanger 1987).

3.5.2 Se Levels in Birds: Differences Among Tissue Types

The interaction and co-accumulation mechanisms involving Se and Hg have been several times described, pointing out to a positive significant correlation between Se and Hg levels (see next Sect. 3.4.3). This correlation seems to be confirmed in most of the selected studies when the levels of Se and Hg in the liver are compared. However, this hypothesis was not fulfilled in some species (*L. ridibundus*, *R. tridactyla*, *F. glacialis*, *U. aalge*, *U. lombia*, and *F. arctica*).

Looking at Se concentrations alone, their presence in the liver has been used to evaluate the exposure to and effects of this metal on birds, since accumulation in the liver is dose dependent (Hoffman et al. 1991). Moreover, Se concentrations are quickly built up or declined in this organ when birds are introduced or removed from a selenium-contaminated diet; and it has the ability to interact with other environmental contaminants. All of this complicates the interpretation of critical levels of Se in the presence of elevated levels of other pollutants (Heinz 1993; Hoffman et al. 1991). However, the hepatic Se concentration is not a perfect representation of the health status of birds. Administration of Se as selenomethionine to young and adult mallards resulted in mortality in these animals if hepatic Se concentrations exceeded approximately 20 μg/g (ww). Risks of selenium toxicity have been reported when this metal is present at concentrations exceeding 20–22 μg/g in the livers and kidneys (St Clair et al. 2015), occurring sublethal effects already when Se levels exceed 10 μg/g (Heinz et al. 1983).

The levels of Se in the liver of different species of gulls ranged from 2.02 ± 1.4 μg/g in *L. glaucoides* in Greenland (Dietz et al. 1996) to 2.4 ± 9.8 μg/g in *R. tridactyla* in Norway (Table 1) (Savinov et al. 2003). Se levels in the kidney

ranged from 4.6 ± 1.49 μg/g in *L. glaucoides* in Greenland (Dietz et al. 1996) to 26.8 ± 9.92 μg/g in *L. argentatus* in Italy (Leonzio et al. 1986). In feathers, Se levels ranged from 0.84 ± 0.077 μg/g in *L. pipixcan* in Agassiz (USA) (Burger 1996) to 2.42 ± 0.11 μg/g in *R. tridactyla* in Alaska (Burger et al. 2008). Overall, the levels in feathers were lower than the levels in the other organs of these animals.

In other seabirds, Se levels in the liver ranged from 4.26 ± 0.40 μg/g in *U. lomvia* in Northbrook Island, Franz Josef Land Archipelago (Savinov et al. 2003), to 107 μg/g in *P. nigripes* in the North Pacific (Kim et al. 1998). In the kidney, the levels ranged from 1.25 ± 0.37 μg/g in *S. mollissima* in Greenland (Dietz et al. 1996) to 43.74 ± 9.60 μg/g in *U. lomvia* in Canada (Braune and Scheuhammer 2008). Lower values were measured in feathers, ranging from 0.878 ± 0.088 μg/g in *S. mollissima* in Alaska (Burger et al. 2007) to 5.39 ± 1.47 μg/g in *T. chrysostoma* in Georgia (Anderson et al. 2010).

In 11 studies reviewed by Burger (1994), Se levels in feathers of gulls and terns ranged from 1.2 to 18.7 μg/g, with a median of 3.9 μg/g. These levels were significantly higher than the levels presented in our review (Tables 1 and 2). An explanation for the lower levels found in the present review can be the more recent date when most of the reviewed studies were performed; a reduction in the Se levels in the environment will be followed by a reduction in the accumulation levels in seabirds living in those ecosystems.

In other marine birds, concentrations of Se were found to be similar. In general, the highest Se levels were found in the kidneys, followed by the liver, and finally feathers. This pattern confirmed that Se tends to accumulate to a larger extent in the kidneys compared to the liver.

3.5.3 Differences in Se Levels Depending on Diet/Age/Gender

Trophic position as responsible for Se levels has been studied by means of biomonitoring programs, therefore, trying to discover a relationship between metal levels and diet. However, depending on the species, differences can be found, as happens in Procellariiformes, where no significant relationship could be established between Se levels and trophic position or foraging location. It can therefore be hypothesized that Se does not biomagnify into this family of seabirds (Anderson et al. 2009). On the other hand, Dietz et al. (1996) has previously detected higher concentrations of Se in high trophic levels of different animals in Greenland. A common explanation for this difference can be the interspecific variability that can be expected, which confirm the need to create a big and complete database where environmental metal levels found in almost all the species will be reported.

Se levels are expected to decrease with increasing latitude, since the North Pole is a relatively uncontaminated area (Anderson et al. 2009). However, at the North Pole, higher concentrations of Se have been detected. This may be due to the fact that, in specific regions close to the Arctic continent, the natural sources of Se are elevated.

Proper evaluation of potential toxic effects of Hg in birds not only requires monitoring Hg levels in their tissues but also corresponding Se levels (Scheuhammer

1987). Eagles-Smith et al. (2008) have demonstrated that Se can decrease the toxicity of MeHg. Se may act as a binding site for demethylated Hg and may thus decrease the potential for secondary toxicity, thereby exerting a protective effect on Hg toxicity in animals (Pařízek and Ošťádalová 1967). The co-accumulation of these two elements may signify the presence of an interaction of Se on Hg. Previous studies have provided evidence of a positive significant correlation between Se and Hg levels in marine mammals. The increase of Hg levels above a certain threshold (ca 100 μg Hg/g) would trigger the accumulation of Se in marine mammals on 1:1 molar basis in the liver (Palmisano et al. 1995). However, the levels of Se, on a molar basis, always exceeded Hg levels in birds (Koeman et al. 1973). Kim et al. (1998) also found a positive correlation between Hg and Se in the liver of different birds (black-footed albatross, brown booby, gray petrel, and northern giant petrel). These authors also detected high levels of Se in the liver of *P. nigripes* (107 μg/g) in the Southern Indian Ocean. They suggest that specific accumulation of this element is required to carry out detoxification of toxic elements in seabirds, since a significant positive correlation between Se and Hg/Cd concentrations was found, suggesting that Se has an antagonistic action on the toxic effects of Hg and Cd. In the present review, albatrosses accumulate the highest levels of Se.

Many studies, however, state that Hg and Se are not correlated. Leonzio et al. (1986) and Wenzel and Gabrielsen (1995), for example, did not find any correlation between these two elements in gulls. On the contrary, Burger (1996) showed a significant negative correlation between Hg and Se, enforcing the previously reported idea about having a complete database showing the behavior of metals in a great variety of species.

In the same manner that it was previously reported for Hg, Se may accumulate with age. Indeed, it has previously been described that Se levels are higher in the livers of adult gulls compared to young ones (Wenzel and Gabrielsen 1995). In contrast, Burger (1997) showed that the levels of Se were higher in feathers of young compared to adults *L. argentatus*. Similarly, higher Se levels were detected in young *L. pipixcan* and *L. glaucescens* compared to adults (Burger 1996). This age-related difference may be due to exposure of young birds to higher levels of Se at the breeding grounds and adults at the wintering ground and the effects of Se on detoxification of Cd and Hg (see also Sect. 3.4.4). However, Burger (1994) reported higher levels in feathers of adults than those presented in young animals, in a study developed with *Gymnogyps californianus*, *Bubulcus ibis*, *Calidris alpina*, *Calidris canutus*, *Limosa lapponica*, *Mycteria americana*, and *Sterna dougallii.* With respect to the effect of sex, Kojadinovic et al. (2007) determined that only one of the three considered seabird species of their study showed significant differences on hepatic Se levels between males and females, thus indicating that not only this factor but the age of the animal and its species, the diet, and the health status also appeared to have an impact on elemental levels.

Even when it is not completely clear with low Hg levels, it has been reported that the appearance of Hg-Se complexes derives from the co-accumulation of Hg and Se, and the excess of selenium may reduce the potential threat of Hg poisoning (Bargagli 2005).

3.5.4 Effects of Se on Health of the Animals

Adverse effects of Se in wild aquatic birds have been documented as a consequence of pollution of the aquatic environment by subsurface agricultural drain water and other sources (Hoffman 2002). These effects include mortality, impaired reproduction with teratogenesis, reduced growth, histopathological lesions, and alterations in hepatic glutathione metabolism. Concentrations of Se in the adult bird liver below 10 mg/kg ww (approximately 30 mg/kg=μg/g dw) are considered as "background" (Ohlendorf et al. 1990). Similarly, levels of Se in feathers known to be associated with adverse effects range from 1.8 ppm (sublethal) to 26 ppm (lethal), depending upon species (Burger et al. 2018).

Even if Se is an essential trace element, excess Se is toxic to animals. Heinz (1996) has shown that concentrations exceeding 3 μg/g wet weight (ww) in the liver may impair reproduction, and those levels exceeding 10 μg/g ww (approximately 30 μg/g dw) may be associated with sublethal toxic effects in birds.

Selenomethionine is an important exposure source for wild aquatic birds. At higher concentrations, it becomes toxic to seabirds, for example, mallards. Heinz et al. (1988) exposed mallard ducklings for 6 weeks to dietary selenomethionine, resulting in reduced survival and growth. Moreover, exposure to selenomethionine results in dose-dependent Se accumulation in the liver and hepatotoxicity characterized by oxidative stress (Hoffman et al. 1989). Other studies have quantified and described histopathological lesions of selenomethionine in adult mallards (Albers et al. 1996). At high doses, essential elements, such as copper (Cu), zinc (Zn), and Se, could also have toxic effects on the kidneys and impair reproduction (Heinz et al. 1989).

3.5.5 Conclusions: Se

Unlike the other metals studied, Se is an essential element for living beings; however, hepatic concentrations over 10 μg/g can have toxic effects on birds. Data from the present review show higher renal levels in these animals than those found in liver and feathers, being kidney the best organ to monitor accumulation of Se. Levels of Se are important data in biomonitoring studies, since they can influence the potential toxicity exerted by other metals as Hg.

4 Discussion

Taking into account the amount of data available on elements in seabirds (excluding gulls), and the number of the studies included in this review, the order of metals analyzed from major to minor number of studies found was Hg > Cd > Se > As > Pb.

With respect to the tissues, in studies in which Hg/or Cd levels were monitored, the order was liver > kidney ≥ feathers. In studies in which Pb, Se, or As levels were monitored, the order was different: liver > feathers > kidney.

In studies in gulls in which Hg or Cd levels were monitored, the order was liver > feathers > kidney. This indicates that in gulls, feathers are preferred above the kidney with respect to monitoring the levels of Hg and Cd.

It was not possible to perform a ranking in terms of metal content in birds, neither among tissues, since the number of studies where all the five metals were analyzed was too scarce, and the three tissues for a same metal were used in a small number of articles.

It is difficult to compare the levels of Hg, Cd, Se, As, and Pb in different species of seabirds around the world, since many factors can affect the exposure levels. In addition to intraspecific differences, interspecific factors are also thought to induce species-specific exposure. We have observed (Fig. 1) that the Arctic Ocean is the most-investigated area with respect to monitoring the levels of Hg, Cd, Se, As, and Pb in gulls and other species of seabirds (more than 30 studies selected for the present review were developed close to the Arctic area). But not only the Arctic is an area of concern, numerous studies have been performed in European countries (more than 30 studies reviewed), including Italy, Spain, Germany, Portugal, and Poland. However, in many other places in the world, these type of biomonitoring studies have not been performed, including Australia, Africa, and many countries of South America (except Chile and Brazil, where six studies were identified). To fully

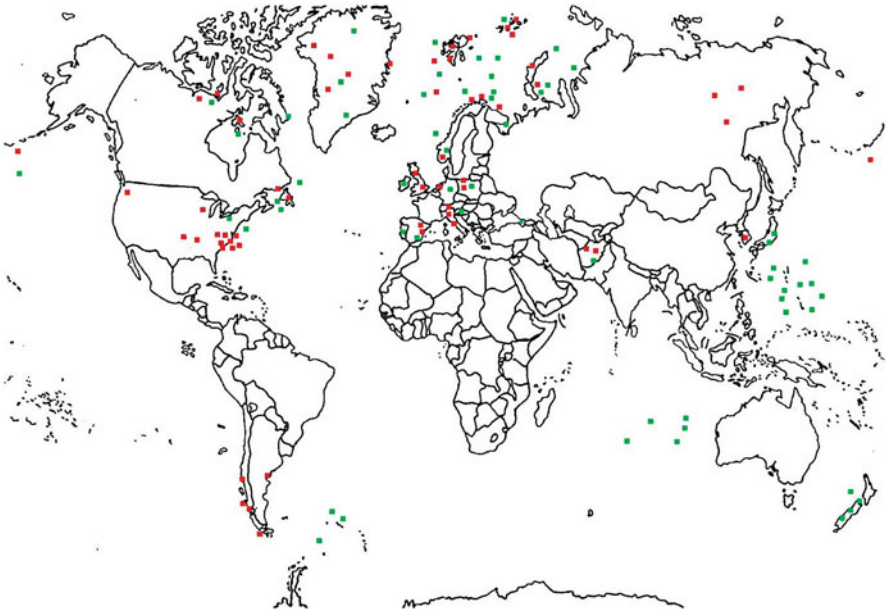

Fig. 1 Map showing the location of the studies included in this review. Red color indicates location of seagulls' studies, and green color studies were performed with other species of seabirds

understand the relationship between the environment and the studied animals, it is important to also perform biomonitoring studies in gulls and other seabirds in these areas, in which contamination levels may be different.

From the 37 evaluations on gull species included in this review, 14 were performed in the twentieth century, while 23 were performed in the twenty-first century. Taking all the species evaluated into account, the difference between the older (47) and the newer (57) studies is the amount of elements that have been included in the individual studies. While in older studies the levels of multiple elements were determined in several organs, in the most recent studies usually the levels of one or two elements are determined in a single organ (48 for Hg and 17 for Cd). In recent years, the use of feathers for biomonitoring studies has gained popularity, especially for Hg (29 in recent studies against 18 in old ones) and Cd (21 in recent and 8 in old studies). This may be due to the fact that this is a noninvasive method.

At the beginning of the 1980s, trace metals started to be determined in different tissues of seagulls, more specifically in the species *L. argentatus*, and from then on more species have been used. However, this species can be the most indicated for the accomplishment of these studies. Indeed, *L. argentatus* has been the most studied due to their ecological characteristics, being the most extended species of seagull and the most adaptable to any environment. Their adaptation to any environment is favored by their ability to feed of any animal and even being able to use human dumpsters like dietary sources.

The metals that have been studied most frequently are Hg, Cd, and Pb. Fifty percent of the references on Hg that were included in this review focused on Hg levels in the liver, followed by the kidney (22%), and feathers (38%). For Cd, the percentages are similar (56% in the liver, 36% in the kidney, and 45% in feathers). For Pb, slightly lower percentages were found (23% in the liver, 14% in the kidney, and 38% in feathers). The number of studies in which Pb levels were determined in feathers exceeded the number of studies in which Pb levels were determined in the liver and kidney. This was unexpected, since as described above, Pb can contaminate the surface of feathers through atmospheric deposition. The levels of Pb in feathers are therefore not only a reflection of the animals' diet but also of atmospheric deposition.

Feathers are useful indicators of metal contamination because of the following: (1) Birds sequester metals in feathers which are composed of sulfur-containing proteins; (2) a relatively high proportion of the body burden of certain metals is stored in the feathers (Burger 1994); and (3) there is a high correlation between levels of contaminants in the diet of seabirds and their feathers, in particular for Hg (Monteiro and Furness 1995). Breast feathers are a useful indicator of whole-body burdens, in particular for Hg (Furness et al. 1986; Janaydeh et al. 2016). Feathers accurately reflect mercury levels in the blood when the feathers are formed, and those concentrations in the feather are stable and inert after formation. However, for most metals, external contamination seems to alter the concentration in the feather after formation, resulting in higher concentration in feathers and in segments of the feather that are exposed most to external conditions (i.e., atmospheric conditions and

preening). Therefore, all factors that may have an influence on the extent of external contamination (i.e., the exposure to external conditions and the age of the feather) have to be taken into account when interpreting heavy metal analysis results of feathers. For other elements the parts in which the feathers are divided may play an important role. The shaft, as long as it is sampled soon after molt, reflects the As, Se, and Pb concentrations deposited in the feather during formation. The vane reflects the exposure to these elements after formation. It should be noted that the feather may only be used under strict conditions: it is essential to know the time when the feather was formed and how long it took, along with the bird's life history after feather formation (migration, etc.) (Goede and Bruin 1984). Therefore, feather is not always a good tissue to determine the concentration of different elements, since external contamination may have especially an important impact on the heavy metal concentrations found in feathers (Burger 1994). This kind of contamination can occur from direct atmospheric deposition or from the deposition of contaminants on feathers during preening. In this sense, the effect of external contamination has been investigated in detail for Hg, whereas for most other metals, few data are available at present on the effect of external contamination (Goede and Bruin 1984; Furness et al. 1986; Monteiro and Furness 2001).

Nine of the studies in gulls carried out with all three tissue types evaluated Hg levels, five of these studies were analyzing Cd, three determined Pb, and two Se. None of these studies analyzed As. In multiple publications, liver and kidney samples have been analyzed simultaneously, being determined Hg in 20, Cd in 25, Pb in 10, Se in 11, and As in 1 study.

We also analyzed which methods were used to analyze the levels of the different element. To determine the levels of Hg, CVP was used most frequently (57%); followed by AAS (17%), AFS (9%), HS (9%), and to a lesser extent HGT (4%); and ICP-MS, ICP-OES, and HG-AAS (<2% each one). For the determination of Cd levels, four methods were used, of which AAS was used most frequently (49%), followed by GFT, ICP-MS, and DRC-ICP-MS (29%, 18%, and 4%, respectively). For the determination of Pb levels, three methods were used. In 47% of the publications, GFT was used, followed by ICP-MS (29%) and AAS (24%). With respect to Se detection, different techniques were used (AAS, GFT, NAT, ICP-MS, DRC-ICP-MS, HGAAS, and HGT). GFT (51%) was used most frequently to determine the levels of Se. Finally, to determine the As levels, six methods were used, namely, GFT (60%), ICP-MS, HPLC-ICP-MS, ICP-OES, HGT, and AAS.

The variation in concentrations of metal residues is often attributed to interspecies differences (Gómez et al. 2004; Taggart et al. 2006). Different levels of these metals may be explained by differences in multiple parameters, including dietary habits, habitat, excretion and/or absorption capacity, or, as specified previously, rate and extent of molt (Elliott and Scheuhammer 1997). Gender-specific differences in metal levels can be expected if male and female animals eat different types of food, different-sized foods, or different proportions of different foods (Burger 1995). Moreover, as previously cited, transferring of metals to the eggs during the offspring is a factor of importance affecting the difference between males and females in terms of metal content. Metallothionein, a metal-binding protein, displays gender-specific

differences. This may, in part, explain the observed differences in metal accumulation between male and female animals (Burger 2007). Additionally, male and female animals may differ in their capacity to produce various metalloproteins, resulting in differences in transport, storage, and excretion of metals (Gochfeld and Burger 1987). To understand metal levels as a function of age, it is necessary to analyze known-aged birds, which requires long-term banding studies. During the review process, it was possible to observe a clear lack of information about differences in metal levels depending on age and gender, being both crucial for biomonitoring programs. Therefore, in order to give a better explanation about accumulation of pollutants in different animals (mammals, birds, etc.), it might be important to focus on new experiments in those endogenous parameters.

5 Conclusions

This extensive review, including articles from different periods of time (twentieth and twenty-first century) about metal levels in seabirds (and specifically in gulls), presents a complete range of concentrations of the most important metals that can be found in the environment in different tissues. In addition to the previous information, putative correlations are shown. This review can be used as a comparative database of metal levels accumulated in the liver, kidney, and feathers of gulls. After reviewing all the studies, some data gaps were detected. It was possible to confirm the lack of evaluations where more than one metal is determined using more than one tissue, which gives a narrow idea about the real effect of pollutant on the studied organism. Moreover, age was confirmed as having a great importance in metals biomonitoring, overall when levels are identified in feathers, being adults more likely to present surface contamination, making results less reliable. Finally, even when all the techniques currently used are validated to determine metal pollution, the comparison of results could be difficult when levels are close to the LOD; therefore, technologies and procedures should be taken into account when data is compared.

6 Summary

There is a high concern about the risk that mercury (Hg), lead (Pb), cadmium (Cd), selenium (Se), and arsenic (As) suppose for the environment and the effects on the animals inhabiting there. The main goal of this review is to summarize data collected from different studies using seabirds, paying special attention to gulls, in order to be helpful for coming studies and regulatory affairs.

The selected articles involved studies where the evaluation of Hg, Cd, Pb, Se, and/or As concentrations was determined in the liver, kidney, and/or feathers in seabirds. Liver was the preferred tissue for determining Hg and Cd. Pb, Se, and As levels were determined to a lesser extent. Moreover, a lack of studies was detected

where two or more metals were analyzed in more than one tissue, which would be helpful to understand possible effects after exposure from a widely view.

Some authors have reported that interspecific differences in the exposure of elements are determined by multiple factors, including properties of the contaminant, species, feeding habits, migratory status, sex, and age.

The present review pretends to be a comprehensive useful overview of the analyzed elements' exposure in different species of seabirds, including gulls.

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3. Heavy metals and metalloid levels in the tissues of yellow-legged gulls (*Larus michahellis*) from Spain: sex, age, and geographical location differences.

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3.1 Resumen: Niveles de metales pesados y metaloides en tejidos de gaviota patiamarilla (*Larus michahellis*) de España: sexo, edad, y diferencias de ubicación geográfica.

En este trabajo se han determinado en hígado, riñón y plumas de gaviotas adultas, juveniles y pollo (*Larus michahellis*) niveles de mercurio (Hg), cadmio (Cd), plomo (Pb), selenio (Se) y arsénico (As) procedentes del noreste de España. La determinación de estos cinco elementos se llevo a cabo mediante la técnica ICP-MS. Para llevar acabo este estudio se han considerado tres variables que son la edad, el sexo y la ubicación geográfica para poder determinar si estas pueden influir en la bioacumulación de los metales mencionados. Las concentraciones medias (peso seco) encontradas en gaviotas fueron 7,01±0,37 mg Hg/kg, 22,82±2,83 mg Cd/kg, 7,36±1,36 mg Pb/kg, 18,64±0,63 mg Se/kg y 10,64±0,59 mg As/kg. De los factores que se han tenido en cuenta el único elemento que mostró diferencias significativas ($p<0,05$) en el sexo fue el Hg, donde las concentraciones fueron superiores en pluma de los machos (1,26 ± 0,12 mg/kg) que en la de las hembras (0,99±0,11 mg/kg). En cuanto la edad de los animales se obtuvo un aumento altamente significativo ($p<0,01$) en los niveles de los animales adultos con respecto a los pollos en el hígado: Hg (adultos (A) 3,33±0,22 mg/kg vs pollos (C) 1,76±0,28 mg/kg), Cd (A 4,74±0,62 mg/kg frente a C 1,79±0,2), Pb (A 0,65±0,12 mg/kg frente a juveniles 0,4± 0,11 mg/kg) y Se (A 7,56±0,43 mg/kg frente a C 5,24±0,53 mg/kg). Ademas se estudiaron posble correlaciones entre los los elementos estudiados donde se encontraron correlaciones positivas entre Cd-Hg y Se-Hg en hígado ($p<0,001$), riñón ($p<0,001$) y plumas ($p<0,05$ y $p<0,001$, respectivamente). Estas correlaciones obtenidas podrían llegar a afirmar una posible interaccion antagónica entre Se y Cd sobre la toxicidad del Hg. Los resultados que se han obtenido en el presente estudio hacen pensar que la gaviota *L. michahellis* puede ser relevante a la hora de poder determinar la contaminación local de los sitios donde se alimente y donde lleve a cabo la reproducción, además puede ser un instrumento de monitoreo muy útil para evaluar la contaminación por metales pesados y las especies centinela de la salud ambiental.

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RESEARCH ARTICLE

Heavy metals and metalloid levels in the tissues of yellow-legged gulls (*Larus michahellis*) from Spain: sex, age, and geographical location differences

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Abstract
In the present study, mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se), and arsenic (As) were measured in liver, kidney, and feathers of adult, juvenile, and chick seagulls (*Larus michahellis*) collected from the northwest of Spain. Age, sex, and the geographical location of samples were considered variables that can influence metal bioaccumulation, for which concentrations were determined by means of ICP-MS. The mean concentrations (dry weight) found in seagulls were 7.01 ± 0.37 mg Hg/kg, 22.82 ± 2.83 mg Cd/kg, 7.36 ± 1.36 mg Pb/kg, 18.64 ± 0.63 mg Se/kg, and 10.64 ± 0.59 mg As/kg. Regarding the different factors analyzed, Hg was the only metal showing sex-related differences, being significantly higher ($p < 0.05$) the concentrations found in feathers of males (1.26 ± 0.12 mg/kg) than those in females (0.99 ± 0.11 mg/kg). A highly significant ($p < 0.01$) increase in levels of some metals was found in liver related to the increase of age: Hg (adults (A) 3.33 ± 0.22 mg/kg vs chicks (C) 1.76 ± 0.28 mg/kg), Cd (A 4.74 ± 0.62 mg/kg vs C 1.79 ± 0.2), Pb (A 0.65 ± 0.12 mg/kg vs juveniles 0.4 ± 0.11 mg/kg), and Se (A 7.56 ± 0.43 mg/kg vs C 5.24 ± 0.53 mg/kg). Positive correlations between Cd-Hg and Se–Hg were found in liver ($p < 0.001$), kidney ($p < 0.001$), and feathers ($p < 0.05$ and $p < 0.001$, respectively). The associations found may reflect antagonistic interactions between Se and Cd on Hg toxicity. The results suggest that *L. michahelli*s can reveal local contamination around the foraging and breeding sites and can be a very useful monitoring instrument for assessing heavy metal contamination and sentinel species of environmental health.

Keywords Metals · Seabird · Bioaccumulation · Sentinel species · Feathers

Introduction

The environment is exposed to different anthropogenic pollutants, such as metals, which can derive from several activities. Metals are natural components in the environment; many of them are essential for the organism (selenium (Se), phosphorus (P), zinc (Zn), copper (Cu)), whereas others are highly toxic or become toxic depending on the exposure concentration. Heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) could come from human activities or natural sources such as erosion of rocks, wind-blowing dusts, volcanic activity, and forest fires. These metals have no biological functions, are not required by organisms, are highly toxic, may induce both acute and chronic toxicological effects, and have important ecotoxicological effects on wildlife and human health. In addition, they biomagnify, accumulate, and persist in the environment and in living organisms, ascending the different trophic levels that make

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up an ecosystem through the food chain (Merian 1991). Therefore, exposure to these chemicals is of particular concern. The transfer of pollutants from the environment to biota is influenced by different environmental and biological parameters (Baker et al. 2003). The list of heavy metals with greater presence in the sediment of Galician estuaries (Spain) includes the following: Hg, Cd, Pb, nickel, Zn, manganese, chromium, Cu, and cobalt (Cobelo-Garcıa and Prego 2003).

Organisms living in wetland systems can bioaccumulate organic and certain inorganic substances over time and are at risk for both lethal and sub-lethal effects, as their body burdens increase (Gochfeld 1997). Studying wildlife populations in such environments provides relevant information about the viability and balance of those ecosystems. The usefulness of studying birds as bioindicators of environmental contamination has been recognized in many research projects because these animals occupy different trophic levels in ecosystems, are widely distributed, and are sensitive to atmospheric changes in the environment (Egwumah et al. 2017). Specifically, waterbirds may serve as sentinel species, since pollutants can enter avian organisms through several routes (dermic, inhalation, ingestion), thus allowing monitoring of the health status of the ecosystem where they live (Zhang and Ma 2011). Seagulls are bird species potentially used as bioindicators (Burger and Gochfeld 2000). Indeed, since 1971, the herring gull (*L. argentatus*) has been used as a sentinel species for monitoring the levels of different elements around the world (Koster et al. 1996; Leonzio et al. 1986; Savinov et al. 2003).

The yellow-legged gulls (*L. michahellis*), a seagull species, represents an abundant population in the Cantabric Sea that has increased by almost 150% during the last three decades (Arizaga et al. 2009). This kind of gull can reach 1.5 kg weight and lives mainly on rocky coastal cliffs and islets in the breeding season, spending the wintertime in bays, estuaries, ports, or even in human buildings (SEO 2018). This species has experienced a dramatic increase in recent decades, due largely to its close link to human activities This can be easily observed along the coasts of islands, and they tend to concentrate in significant quantities in docks, dumps, and solid waste treatment plants (Martín and Lorenzo 2001). Their inhabiting patterns influence their feeding behaviors, which takes place mainly in coastal waters, but also in landfills and ports. Their diet includes predominantly fish, but also invertebrates, chickens and eggs from other birds, as well as carrion and rodents. As any other gull species, they can also feed on dumps and human remains (Watson 1981). Méndez et al. (2020) analyzed the diet of the yellow-legged gull chicks ($n = 101$) in Barcelona during the breeding season of 2018. These authors found that the main prey present in the stomach contents of the chicks consisted of urban birds, mainly rock pigeons and monk parakeets. According to IUCN (BirdLife International 2019, 2021) criteria, this species is considered a kind of "Least Concern" because of its wide distribution and the positive trend of its population (Figure S1). This species has an extremely large range, and hence does not approach the thresholds for vulnerable under the range size criterion. Indeed, mortality of individuals has been verified due to collision with power lines, consumption of toxic substances, etc. However, it is their population increase and predatory behavior that causes conservation problems to pelagic seabirds, especially those of small and medium size (Barone and Lorenzo 2007).

Larus michahellis are considered top predators and have a very wide feeding spectrum as they feed on disparate trophic chains and environments (Ramos et al. 2013). For years, studies evaluating pollutant levels have used accumulation levels in different tissues of seagulls (liver, kidney, blood, fat, bones), as good indicators of contamination levels. The most relevant tissues responsible for storage and detoxification of pollutants are liver and kidney (Lewis and Furness 1991). Birds can also rid their bodies of toxic substances through normal excretion and through deposition in the uropygial gland, salt gland, and in feathers (Burger and Gochfeld 1985, 1993; Braune and Gaskin 1987a, 1987b; Lewis and Furness 1991; Burger et al. 1992). On the other hand, feathers are capable of accumulating metals and organic pollutants from the atmosphere during their formation, giving information of a certain periodic exposure to contaminants (Burger et al. 1992, 2008; Burger 1993, 1996). Females can also sequester heavy metals in eggs and in eggshells (Fimreite 1974; Burger and Gochfeld 1993; Burger 1993; Gochfeld 1997).

Due to the ability of seabirds to travel long distances, their migratory behavior, and their establishment of fixed colonies in certain locations, these animals can serve as convenient indicators of environmental chemical pollution over large areas, in particular areas of inland and coastal waters. Some of the most relevant chemicals included in biomonitoring studies are metals. The appearance of metals in different tissues of animals often has been linked to specific urban areas (Korbecki et al. 2019). In fact, there is a need to carry out ecotoxicological studies to obtain data on specific species over a wider range. Recently, a significant elevation of metal concentrations in birds has been observed related to gross domestic products development. Specifically, the influence of the industrial development was reported as a critical reason for the increase in heavy metal levels in local avian species (Fu et al. 2014). These high concentrations of metals may lead to animal health impairment and a cumulative effect on the body with age, while also differences in tissue metal concentrations between sexes can occur (Barrales et al. 2021).

The main goal of the present study was to determine the validity of a gull species as a useful tool for biomonitoring

metal levels in the environment. To reach this aim, we proceeded to assess the concentration of three heavy metals (mercury, cadmium, and lead) and two metalloids (selenium and arsenic) in the liver, kidney, and feathers of *L. michahellis* collected from three areas of Northwest Spain. These areas are located in Galicia and Asturias, with wide coastal and estuarine perimeters, two regions located along the Spanish coastline that are characterized by touristic, industrial, fishing, dredging and aquaculture activities, and/or suffering several contamination events in recent decades. They present well-preserved natural areas, but there are also some important cities and industrial plants (e.g., close to the cities of Pontevedra, A Coruña, Ferrol, and Gijón). Hg, Cd, and Pb were selected because they are the major contaminants of concern in marine environments (Fowler 1990). As, because of its concern for wildlife in marine and estuarine ecosystems (Neff 1997), and Se are known as a highly toxic element and an essential trace element, which bears a complex relationship with Hg, making it of high importance (Eisler 2000). Birds were collected during population control campaigns, duly authorized by the regional government of Galicia and Asturias, between 2013 and 2014, with no apparent signs or symptoms of injury or diseases or slaughtering in animal recovery centers. Different factors (tissue, sex, age, capture method, and sampling area) were considered during the data analysis to determine their relevance for future biomonitoring programs.

Materials and methods

Reagents

Solvents and reagents were of analytical grade or high purity grade and purchased from Panreac (Moncada i Reixac, Spain) and Merck (Darmstad, Germany). Milli-Q water was used to prepare solutions and dilutions. The internal standard used for elemental analysis by inductively coupled plasma mass spectrometer (ICP-MS) was a solution of yttrium, rhenium, rhodium, and tellurium (10 mg/L) purchased from Perkin Elmer Inc. (Shelton, USA).

Sampling: study areas, species, and methodology

The area of study was located in the northwest of Spain (Fig. 1), which was divided in three zones: Pontevedra (P), A Coruña (C), and Gijón (G). The distance from Pontevedra to A Coruña is 134 kms, and there is 282 km from A Coruña to Gijón.

Samples were collected from two different sources. One group of *L. michahellis* ($n = 43$) was collected in wildlife recovery centers from each area. Gulls were collected during the period of 2014–2016. Samples collected in the wildlife recovery centers were from birds that entered there mainly because of physical injuries, provoked by electrocution and fall from the nest due to inexperience in flying. Only birds held at the rehabilitation center for less than 5 days before dying were used ($n = 43$). The other group of seagulls ($n = 66$) came from population control campaigns in different populations, duly authorized by the regional governments of Galicia and Asturias, with no apparent signs or symptoms of injury or disease. During necropsy, several parameters such as body mass measurements, organ weights, bill development, and physical condition were registered. After adequate euthanasia (pentobarbital, 1 mL/kg, intravenous), the liver, kidney, and feathers (breast feathers) from each animal were collected and stored in individual zip-lock plastic bags, properly labeled and immediately frozen at -20 °C. Proper cleaning practices and replacing of surgical material were performed to avoid metal contamination. For feathers, the cleaning method consisted of washing with tap water, distilled water, Milli-Q water, and acetone.

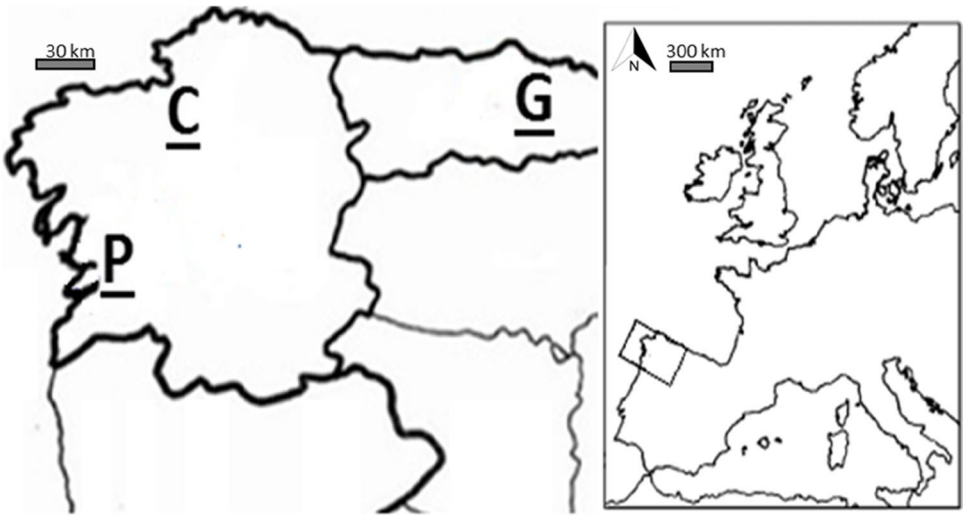

Fig. 1 Geographical location: Pontevedra (P), A Coruña (C), and Gijón (G). NW Spain

Each animal was identified with information of interest (origin, cause of admission, age, sex, etc.). The animals were divided into 3 age groups: adults ($n=63$), juveniles ($n=22$), and chicks ($n=24$) based on the color of plumage. Adult gulls have yellow legs, yellow beak with red spot on tip and yellow eye, their top feathers are grey, and the bottom ones are white. However, juvenile gulls have pink legs, dark beak, and eye of brown color, having plumage of brown color with dark striations. Chicks are attended by both adults for about 45 days, until they become independent and start to fly and are considered juveniles.

Samples were also grouped according to sex (55 males, 54 females). Both sexes are similar in plumage, although males have larger sizes compared to females. Indeed, males presented with wings greater than 465 mm, beaks greater than 62 mm, and tarsi greater than 75 mm. Females had wings shorter than 440 mm, beaks smaller than 54 mm, and tarsi with less than 64 mm. Visual checking of the gonads during the necropsy allowed to differentiate the samples by sex. The remains were hygienically removed by incineration, under current legislation.

Samples were sent to the Veterinary Faculty of Cáceres (Spain) in perfect condition, without breaking the cold chain.

Determination of metals and metalloids

Samples of 3–4 g of liver and kidney were dried in an oven for 72 h at 65 °C. Feathers were washed with deionized water and NaOH 0.25 M to eliminate any possible contamination adhered to the surface. After this, the feathers were dried for 24 h at 40 °C. Finally, the feathers were lyophilized for 48 h and frozen at—80 °C for 24 h.

The metal levels were analyzed at the Elemental and Molecular Analysis Laboratory of the Research Support Service (SAIUEX, accredited by ISO 9001:2008) belonging to the University of Extremadura. A microwave assisted acid digestion procedure was carried out to obtain a suitable sample for metal content evaluation (adapted from Shah et al., 2009). Briefly, two grams of each sample were directly weighed into Teflon PTFE flasks and digested with 6 mL of a freshly prepared mixture of concentrated HNO_3 (69%) and H_2O_2 (30%) (3:1, v/v) (supplied by Fluka) in a microwave digestion system and diluted to 10 mL with deionized water. The flasks were closed and kept for 10 min at room temperature. A blank sample was treated in the same way. All sample solutions were clear. The accuracy of the microwave digestion method was checked with a standard reference material (BCR® certified reference materials—ref. 185R, Community Bureau of Reference, EU). Following digestion, samples were transferred to 25 ml volumetric flasks. Digestion conditions for the microwave system are detailed in Table S1.

The analyses of liver and kidney samples were conducted by means of ICP-MS (Model 7900. Agilent Tech), following the operation conditions detailed in Table S2. This technique was selected because it provides sufficiently low detection limits and allows the simultaneous determination of several metals. For an optimal nebulization of the sample, a Peltier-cooled (2 °C) cyclonic chamber and a low-flow (0.25 mL/min) Meinhard Concentric Nebulizer were employed. Both the collision gas and the argon for the plasma have a purity of 99.999% and have been supplied by Praxair (Madrid, Spain).

Every working day, the ICP-MS was calibrated to obtain the highest values of intensity, indicated by the ratios CeO/Ce < 2.5%, Ce^{2+}/Ce < 3%, and background (220) < 1 cps. Calibrating solutions were prepared daily from a 10 mg/L Multielement Calibration Standard 3 (PerkinElmer, Inc., Shelton, CT) and assayed as the samples. A NIST SRM 1577b bovine liver certified sample was used for quality control of the analytical procedure. Recoveries obtained were between 92% for Hg and 107% for Se, and coefficients of variation for replicate samples ($n=5$) were lower than 6.5%. Metal levels were not adjusted for percent recovery. Limit of detection (LOD) and of quantification (LOQ) were determined according to the ICH-Q2 guideline on method validation (Guideline 2005), after analyzing repeated blanks with the same procedure used for the samples, determining the standard deviation. The final values of both parameters were calculated taking into account the dilution factor and the weight of the samples, being in all cases lower than 0.003 and 0.009 mg/kg for LOD and LOQ, respectively. The coefficients of variation for replicate samples ($n=5$) were lower than 5.3%. Analytical blanks were included in all the run batches of samples.

Statistical analysis

The statistical software Prism 5 version 5.03 for Windows (GraphPad software, Inc., CA) was used to analyze the data. Results were expressed as mean ± standard error of the mean (SEM), and the level for statistical significance was defined as $p<0.05$.

Since data did not show a normal distribution and the variances were not homogeneous, the statistical analyses were performed using a non-parametric Mann Whitney *U*-test, to evaluate the differences related to both sex and age. Data were also compared by pairs applying a one way ANOVA, followed by a Tukey's multiple comparison test, to check for differences between either sex or age related to the sampling area, thus obtaining information about the joint effect that two factors can have. Finally, a Spearman test was performed to determine the correlations among chemical levels. Statistical assessments were limited to those chemicals that could be detected in > 50% of the samples. For statistical testing,

a value of 50% of the limit of detection (LOD) was assigned to samples with metal concentrations below LOD, thus minimizing nominal type I error rates (Clarke 1998).

Results and discussion

Concentration of metals/metalloids in bird's tissues

The present work evaluated the levels of 3 metals and 2 metalloids in the liver, kidney, and feathers. Table 1 shows the results for the selected metals, being expressed as mg/kg dry weight (dw), since dry values are more reliable and consistent compared to wet weight (ww) values (Adrian and Stevens 1979). Data mentioned along the manuscript is related to dw, when data is mentioned as ww it is indicated.

The higher concentrations of Cd in kidney found in the yellow-legged gulls, compared to the liver, have been already shown in other avian species (Bianchi et al. 2008; Abdullah et al. 2015) and reported as a consequence of chronic exposure to low Cd concentrations (Scheuhammer 1987). Higher levels of metals in kidney than in liver were also observed for Se and Pb in the present study. There are not many studies assessing the Pb levels in gulls, but the collected data also revealed that in general Pb values are higher in the kidney compared to liver and feathers (Vizuete et al. 2019). The levels of Hg were similar in the liver and kidney. Arsenic levels were higher in the liver than in the kidney. In feathers, the levels of Pb were the highest of the analyzed elements. This is supported by other recent studies developed with seagulls, as showed by Agusa et al. (2005) in *L. crassirostris* from Japan, or by Mansouri et al. (2012) in *L. heuglini* from Iran.

A recent study developed with yellow-legged gull in the seabird colonies in the Atlantic Islands National Park and reported results of metals content in some biomaterials generated (feathers, fecal material, eggshells, etc.). The present median results of metals in feathers are higher than those reported by Otero et al. (2018), which can be explained by the higher pollution existing in our sampling areas. Moreover, these authors concluded that some biomaterials generated, like the excrement and pellets, contained high concentrations of trace elements. Thus, higher levels of metals may be found in the biomaterials generated by our animals, which will be deposited in their living areas.

Species located on the bottom part of the food chain should have lower tissue levels of heavy metals, because they accumulate less metals through ingestion. Indeed, Sarkka et al. (1978) found that Hg levels increased through the food chain, from water to plants and plankton, and then, to benthic predators. Several studies that have determined the levels of heavy metals in birds and their food have demonstrated that predatory birds have significantly higher levels than their prey, due to the magnification of these contaminants in the food chain (Lindberg and Odsjo 1983). Some studies are oriented to determine basal levels of metals, which provide useful data for biomonitoring purposes. In this sense, in wild birds living in environments with little or no industrial activities, the threshold reported for Hg levels in the liver was 10 mg/kg (Fimreite 1974). Even when some animals surpassed this threshold, our results (mean and median) of Hg in liver and kidney were below the mentioned threshold and were in accordance with those found by Leonzio et al. (1986) in *L. ridibundus* in Italy (2.58 ± 1.37 and 2.49 ± 1.71 mg/kg) and Majidi et al. (2015) in *L. heuglini* in Iran (1.87 ± 0.18 and 1.97 ± 0.21 mg/kg). However, these levels are lower than those found by Leonzio et al. (1986) in liver and kidney of *L. argentatus* in Italy (13.30 ± 9.25 mg/kg in liver; 10.65 ± 7.82 mg/kg in kidney), who suggested that the food source (marine areas) was responsible for the high Hg levels. The authors suggested that feeding patterns in dumps may explain the lower Hg levels in *L. ridibundus*, and this may explain the levels found in birds of the present study (Hg in feathers: 1.13 ± 0.08 mg/kg), which may feed in the human dumps instead of going to the coast. Factors other than food can also influence the Hg levels in feathers, like metabolic changes such as molting during which birds may eliminate a substantial part of Hg through their plumage, thus decreasing the internal tissues levels as they are sequestered in the feathers (Honda et al. 1986; Braune and Gaskin 1987a; Lewis and Furness 1991). Most of the animals were captured during the summer (August), months before the molt, since it is in October where these gulls eliminate the feathers. Nevertheless, our levels agree with those

Table 1 Concentrations of metals (Hg, Cd, Pb, Se, and As) in *L. michahellis* (mg/kg dw) ($N = 109$)

Metal	*Liver*		*Kidney*		*Feather*		*Total*
	Mean + SEM	*Median (range)*	*Mean + SEM*	*Median (range)*	*Mean + SEM*	*Median (range)*	*Mean + SEM*
Hg	2.95 ± 0.21	2.5 (0.34–16.39)	2.94 ± 0.18	2.60 (0.22–11.32)	1.13 ± 0.08	0.87 (0.18–5.92)	7.01 ± 0.37
Cd	4.13 ± 0.59	2.61 (0.11–50.9)	18.56 ± 2.46	9.25 (0.15–149.6)	0.12 ± 0.03	< LOD (< LOD–2.39)	22.82 ± 2.83
Pb	0.55 ± 0.77	0.41 (0.03–7.89)	2.50 ± 0.78	0.95 (0.7–79.81)	4.38 ± 1.09	1.61 (0.22–103.5)	7.36 ± 1.36
Se	7.18 ± 0.33	7.34 (0.31–15.91)	10.92 ± 0.42	10.95 (0.73–23.29)	0.54 ± 0.02	0.57 (< LOD–1.18)	18.64 ± 0.63
As	6.05 ± 0.39	5.34 (0.39–23.56)	4.54 ± 0.27	3.85 (0.45–13.93)	0.04 ± 0.01	< LOD (< LOD–1.46)	10.64 ± 0.59

reported in the literature, with a range of Hg concentrations in feathers of seagulls between 0.29 ± 0.18 µg/g in *R. tridactyla* in Alaska (Burger et al. 2008) and 6.06 ± 4.60 µg/g in *L. argentatus* in Siberia (Kim et al. 1996), as showed in a recent review study (Vizuete et al. 2019).

Another metal evaluated was Cd, an extremely noxious pollutant that can be easily absorbed by seabirds from fish and marine invertebrates, and that accumulates in various tissues, including the kidney and to a lesser extent the liver and muscle (Kojadinovic et al. 2007). In the present study, Cd levels (4.13 ± 0.59 and 18.56 ± 2.46 mg/kg in liver and kidney, respectively) agree with those found by Leonzio et al. (1986) in *L. ridibundus* in Italy (4.20 ± 4.22 mg/kg in liver and 17.28 ± 9.92 mg/kg in kidney). However, 4 to 10 times higher levels in both organs have been reported in other gull species, like *L. ridibundus*, *L. crassirostris*, and *R. tridactyla* (Kim et al. 1996; Agusa et al. 2005; Braune and Scheuhammer 2008). The present Cd concentrations are also lower that those detected by Nielsen and Dietz (1989) in a population of adult Kittiwake considered healthy (76 mg/kg in the liver and 26 mg/kg in the kidney). The high levels of this metal described in gulls may be due to the diet of these animals, mainly consisting of squid, which represents an important source of cadmium for both seagulls and animals high up in the trophic chain (Bustamante et al. 1998). The process of detoxification and storage of non-essential elements in these animals could be confirmed by the high accumulation of Cd in the kidney (Lucia et al. 2008). Levels of Cd higher than 3 ppm in liver have been associated with health impairment and an increase in environmental contamination (Scheuhammer 1987; Burgat 1990). In the present study, the mean concentration of hepatic Cd was found to be slightly higher than this threshold (4.13 ± 0.59 mg/kg), suggesting a future adverse effect for the animal. In general, chronic exposure to low concentrations usually results in a high concentration of Cd at the renal level (Scheuhammer 1987). Some authors have used the kidney/liver ratio as an indicator for recent Cd exposure (Honda et al. 1986; Kim et al. 1996; Navarro et al. 2010). In the present study, the calculated kidney/liver Cd ratio was 4.31, which indicates a recent exposure to Cd. This result could be a good indicator of accumulation related to a specific area, since Cd resides for longer periods in kidney stores compared to liver stores (Honda et al. 1986; Kim et al. 1996; Navarro et al. 2010). These kidney/liver Cd ratios have also been reported by other authors, with levels between 1.5 in *L. ridibundus* in Poland (Orlowski et al. 2007) or 9.6 in *L. savini* in Siberia (Kim et al. 1996). The levels of Cd found in feathers were low (0.12 ± 0.03 mg/kg), something expected, since this tissue has been reported as less significant/a non-main-target for Cd accumulation (Frantz et al. 2016). Our levels are similar to those reported in two other studies developed in the Iberian Peninsula. Navarro et al. (2010) obtained 0.19 ± 0.002 mg/kg in *Phalacrocorax carbo* (Murcia, Spain), Otero et al. (2018) found 0.10 ± 0.07 mg/kg in *Larus michahellis* (Atlantic Islands of Galicia National Park, Spain), and Mendes et al. (2008) obtained 0.07 ± 0.02 mg/kg in feathers of *Morus bassanus* (Portugal).

Poisoning by ingestion of objects that contain lead (Pb) is one of the most common causes of death in birds, especially seabirds. Lead pullets are one of the fastest and most important forms of contamination of this metal in the environment. It can be ingested by animals and moved through the trophic chain or can be disseminated in wetlands, producing pellet ingestion, since these bullets can confuse other animals due to their resemblance to seeds (Bellrose 1959; Sandersons and Bellrose 1986; Pain 1987).

In this study, levels of Pb measured were 0.55 ± 0.077 mg/kg and 2.50 ± 0.78 mg/kg, in the liver and kidney, respectively. These concentrations were also lower than those reported by Leonzio et al. (1986) in other species of seagulls (*L. ridibundus*), who found one of the highest lead concentrations in the kidney (30.96 ± 22.81 mg/kg) and liver (7.65 ± 5.41 mg/kg) recorded in seagulls of Europe. However, higher levels have been reported in birds found dead because of lead poisoning after ingestion of lead bullets (Perco et al. 1983). In general, the concentrations found in the present study for Pb in the liver and kidney can be considered of no toxicological relevance. Indeed, Pb concentrations ranging from 0.5 to 5.0 mg/kg in the liver and from 1.0 to 10.0 mg/kg in the kidney have been reported as background levels for seabirds from uncontaminated areas (Kehrig et al. 2015; Scheuhammer 1987). Agreeing with that, Pain et al. (1995) determined that Pb concentrations below 2 mg/kg in the liver could be safe and considered as a threshold value. Concentrations > 6 mg/kg were indicative of elevated exposure to lead in raptors capable of impairing biological functions, > 20 g/kg indicated lead poisoning, and concentration of > 30 g/kg in hepatic tissue which is considered a potentially lethal level (Pain et al. 1995). The levels of Pb in our samples of feathers were 4.38 ± 1.09 mg/kg, a high level in comparison to the 0.83 ± 0.37 mg/kg in the same species (Otero et al. 2018), or the 0.399 ± 0.048 mg/kg in *Morus bassanus* (Nardiello et al. 2019), both sampled in Galicia (Spain). Similar to that observed in the present study, the highest concentration of Pb was quantified in the feathers of *Morus bassanus* (Nardiello et al. 2019), in comparison with renal and hepatic levels. Pb concentrations of 4 mg/kg in the feathers are known to be the threshold levels of toxicity (Burger and Gochfeld 2000); thus, our recorded levels surpassed this threshold and, therefore, are of concern.

When Se is present as a pollutant in the environment, it can cause numerous harmful effects in birds. Mainly, these effects are mortality, impaired reproduction, and/or alterations in hepatic glutathione metabolism. In birds that survive the last phase of selenium poisoning, bleeding

into the liver is very common. Se is a geogenic pollutant (i.e., it is naturally present in the rock and passes into the water during drilling carried out to collect water (for agricultural drainage waters)) (Hoffman 2002). Heinz (1996) suggested that concentrations > 3 mg/kg ww in the liver may impair reproduction, that sublethal toxic effects in birds can occur when the concentration of Se reaches 10 mg/kg ww (approximately 30 mg/kg dw), and finally that mortality could occur when hepatic concentration of Se reaches ≥ 20 mg/kg on a wet-weight basis (60 mg/kg dw). In the current study, the values of Se obtained in liver were 7.18 ± 0.33 mg/kg and 10.92 ± 0.42 mg/kg in kidney. These values are higher than those obtained by Dietz et al. (1996) in hepatic and renal tissue of *L. glaucoides* in Greenland, where an average concentration of 2.02 ± 1.4 mg/kg in the liver and 4.6 ± 1.49 mg/kg in the kidney were found. We also observed differences with respect to the study carried out on *R. tridactyla* species in Norway, with liver concentrations of 2.4 ± 9.8 mg/kg (Savinov et al. 2003), or the study carried out in Italy by Leonzio et al. (1986), in which mean concentrations at the renal level of 26.8 ± 9.92 mg/kg were detected in *L. argentatus*. These different values suggest the importance of this metalloid in body tissues depending on the geographical area. Indeed, Dietz et al. (1996) detected higher concentrations of Se in high trophic levels of different animals in Greenland, a relatively uncontaminated area compared with urban areas (Anderson et al. 2009).

Levels of Se in feathers are associated with toxic effects, ranging from 1.8 mg/kg (sublethal) to 26 mg/kg (lethality), depending upon the species (Burger 1993). Specifically, the levels of Se in feathers of *L. michahellis* have been reported from 3.0 to 5.8 mg/kg, which suggests potential adverse effects. Higher levels of Se have been reported in shorebird tissues, including feathers (Ohlendorf et al. 1989; Ackerman and Eagles-Smith 2009). However, the Se levels found in feathers in the present study (0.54 ± 0.02 mg/kg) are below the range reported as hazardous.

A potential protective effect has been reported for Se, which can reduce Cd-mediated toxicity in the liver and kidney in animal models and in cell culture studies. In this sense, Se antagonizes the toxicity of Cd mainly through sequestration of this element into biologically inert complexes and/or through the action of Se-dependent antioxidant enzymes (Zwolak 2020). The accumulation of Se found in the present study, therefore, could act as a protective element against the expected impairment generated by the Cd levels (above the hazardous threshold).

Inorganic As has been reported as highly toxic in some seabirds, by acting as an endocrine disruptor (Kunito et al. 2008). Arsenic concentrations are usually low (1 mg/kg ww, which approximately represents 3 mg/kg) in most living organisms (Braune and Noble 2009). Hepatic As values of 0.82 ± 0.26 mg/kg and 98.1 ± 69 mg/kg were found in *R. tridactyla*, in Norway and Russia, respectively (Savinov et al. 2003). In the present study, the concentration of As was 6.05 ± 0.39 mg/kg in the liver and 4.54 ± 0.27 mg/kg for the kidney. The concentrations of As in feathers was below the detection limit in almost all the samples, except for two individuals whose values were relatively low 1.46 and 0.15 mg/kg.

Concentration of metals/metalloids according to sex

Regarding the sex as an influencing factor, there were no significant differences in the liver and kidney concentrations of Hg, Cd, Pb, Se, and As between males and females. This was unexpected, since, for example, female birds should have lower concentrations of Hg than males, because breeding females can depurate methylmercury to their eggs (Ackerman et al. 2016). However, as already mentioned, the selected seagulls did not show sex differences in body burden of Hg, even if males tended to have higher levels of Hg both in liver and kidney. On the other hand, the levels of Hg in feathers were significantly higher ($p < 0.05$) in males than in females (Fig. 2). This fact was reported for Pb in a study of laughing gulls (*L. atricilla*) culled near a major airport, where males had barely significantly ($p < 0.05$) higher levels than females (Gochfeld et al. 1996), but there were not sex-related differences for the other heavy metals. Our levels of Pb were higher in males than in females in both organs, but we did not find significant differences. Only a few studies have suggested differences in levels related to sex, such as Hutton (1981) and Stock et al. (1989) in a study with oystercatchers; however, differences were found in opposite directions, making it difficult to make biologically meaningful conclusions.

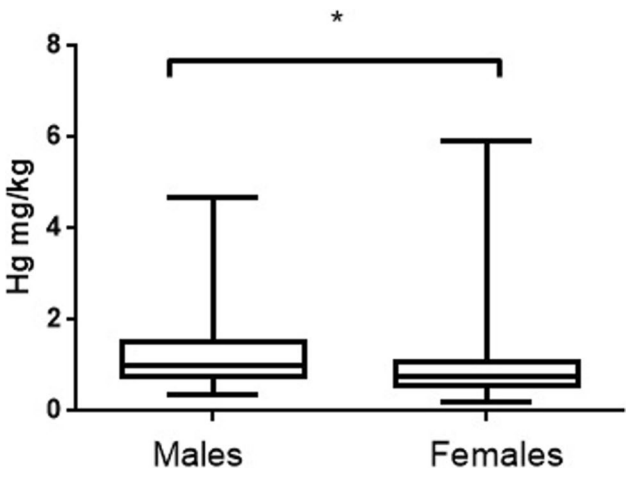

Fig. 2 Hg median levels in feathers of yellow-legged gull according to sex (males, females). Box plots represent median values and 25 to 75% percentile ranges. *$p < 0.05$

A second factor, considered in the present study as a potential reason for different metal content, was the sampling area. The yellow-legged gull (*L. michahellis*) distributes in the circum-Mediterranean region, including the Macaronesian region. It has expanded along the Atlantic coast and to some humid areas of Central Europe (Geroudet 1984). These seagulls present an easy adaptation to their chosen habitat, which comprises of a variety of locations, such as marshes, beaches, and coastal islands. They live also in the vicinity of the coastal population centers, where they can successfully breed in human buildings. These gulls are not selective in terms of feeding, including in their diet fish, amphibians, mollusks, small mammals, and carrion. The two main feeding sources are the dumps and discard produced by fishing activity. There are several reasons for the implementation of culling plans to reduce their populations that include the following: their high reproductive success and ability to adapt to any environment, its pernicious effect on colonies, the negative effects on vegetation of the cliffs and potable water, noise problems, dirt, and damage to buildings (generated by urban colonies). Generally, this species is considered sedentary, as all of them remain close to their breeding colonies; however, some colonies go inland following the courses of the great rivers (SEO 2018).

Concentration of metals/metalloid according to sampling area

Significant differences in liver the (Fig. 3) and kidney (Fig. 4) Hg levels were found among sampling areas. In the liver, there are significant differences between Pontevedra and A Coruña and between Pontevedra and Gijón. Something similar occurs in the kidney, which presents also significant differences between Pontevedra and A Coruña. Hg pollution studies in animals are scarce in Galicia. Beiras et al. (2002) found different Hg concentrations in different areas of Galicia: 73.5 ng/L in seawater, 0.186 mg/kg in sediments, and 0.741 mg/kg in mussels in the Pontevedra Rias. These authors compared Hg concentrations in different coastal areas of Galicia, finding higher metal levels in the Rias of Pontevedra than in those of Vigo, A Coruña and Arousa. The present study also found higher levels in Pontevedra than in the other 2 regions. Galician wild mussels showed Hg contents of 0.05–0.2 mg/kg (Besada et al. 1997). Both studies demonstrate the persistence of local Hg pollution, despite recent efforts undertaken by the chlor-alkali industry aimed at reducing the levels of Hg in their effluent. The levels of Hg found in our gulls (Table 2) in livers were 3.71 ± 0.33 mg/kg, 1.79 ± 0.29 mg/kg, and 2.24 ± 0.21 mg/kg in Pontevedra, A Coruña, and Gijón, respectively. The same pattern was observed in the kidney (3.38 ± 0.31 mg/kg, 2.03 ± 0.18 mg/kg, and 2.72 ± 0.25 mg/kg). Hg enters the marine ecosystems

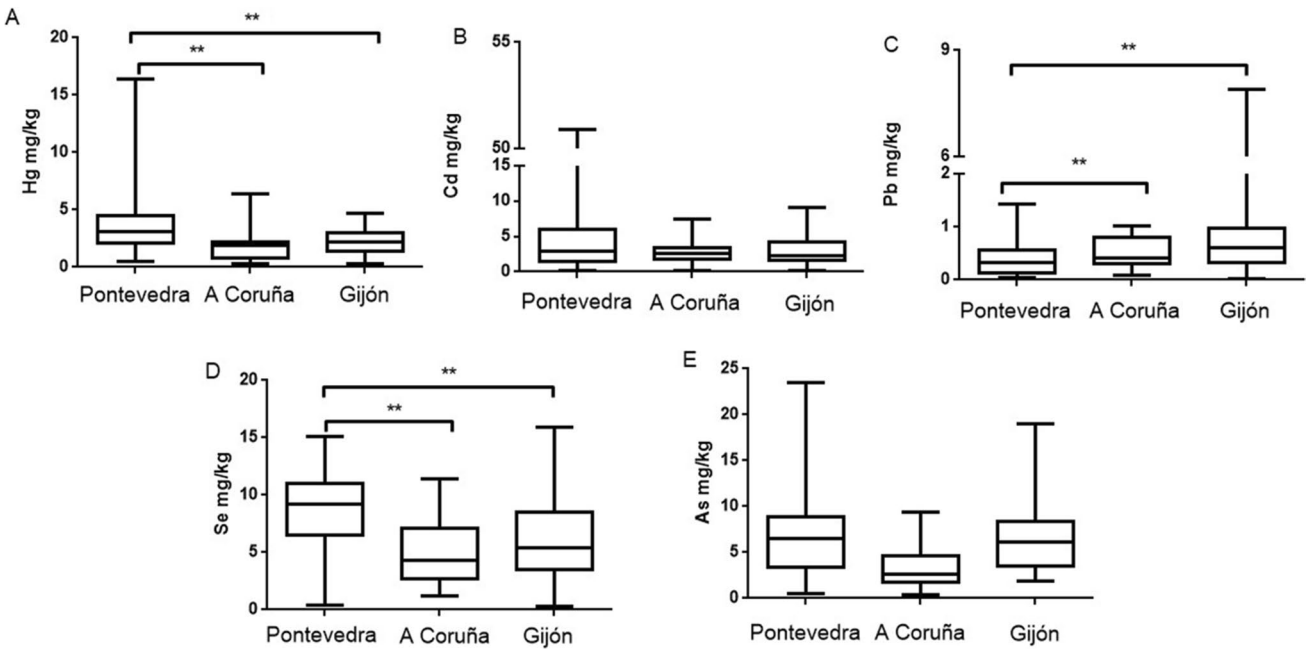

Fig. 3 Hg (**A**), Cd (**B**), Pb (**C**), Se (**D**), and As (**E**) levels in liver of yellow-legged gull according to the three different sampling areas: Pontevedra, A Coruña, and Gijón. Box plots represent median values and 25 to 75% percentile ranges. $^{**}p < 0.01$

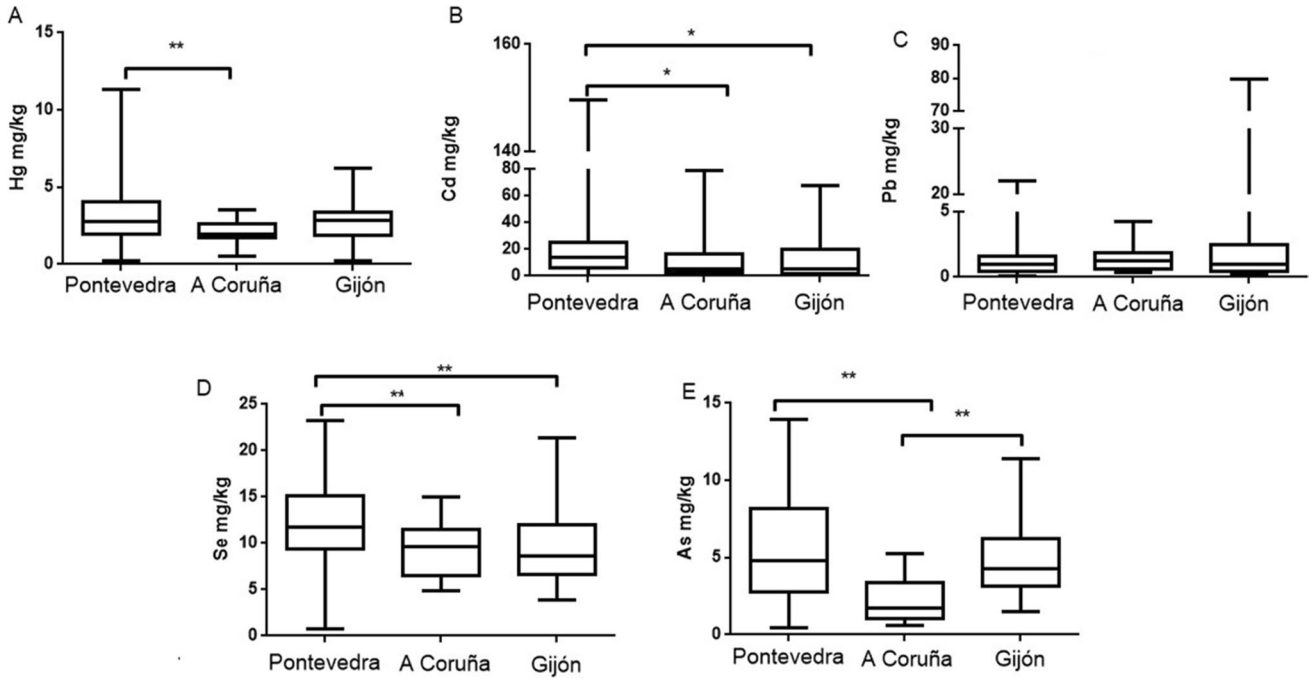

Fig. 4 Hg (**A**), Cd (**B**), Pb (**C**), Se (**D**), and As (**E**) levels in kidney of yellow-legged gull according to the three different sampling areas: Pontevedra, A Coruña, and Gijón. Box plots represent median values and 25 to 75% percentile ranges.*$p < 0.05$, **$p < 0.01$

Table 2 Metal concentrations in *L. michahellis* (mean ± SEM; mg/kg dw) according to sampling area, age and capture method

Metal	Metal concentration in *Larus michahellis* (mean ± SEM; mg/kg) according to							
	Sampling area			*Age*			*Capture method*	
	Pontevedra ($n=58$)	A Coruña ($n=21$)	Gijón ($n=30$)	Adults ($n=63$)	Juveniles ($n=22$)	Chicks ($n=24$)	Control ($n=66$)	Recovery center ($n=43$)
	Liver			*Liver*			*Liver*	
Hg	3.71 ± 0.33	1.79 ± 0.29	2.24 ± 0.21	3.33 ± 0.22	3.13 ± 0.71	1.76 ± 0.28	2.36 ± 0.19	3.85 ± 0.41
Cd	5.11 ± 1.06	2.83 ± 0.38	3.14 ± 0.41	4.74 ± 0.62	4.97 ± 2.27	1.79 ± 0.2	4.32 ± 0.78	3.85 ± 0.90
Pb	0.38 ± 0.4	0.51 ± 0.06	0.88 ± 0.24	0.65 ± 0.12	0.4 ± 0.11	0.42 ± 0.06	0.64 ± 0.12	0.41 ± 0.05
Se	8.60 ± 0.39	4.97 ± 0.6	5.86 ± 0.62	7.56 ± 0.43	8.21 ± 0.72	5.24 ± 0.53	6.29 ± 0.41	8.56 ± 0.46
As	6.71 ± 0.60	3.40 ± 0.51	6.48 ± 0.62	5.81 ± 0.48	8.25 ± 1.21	4.67 ± 0.43	5.82 ± 0.51	6.41 ± 0.63
	Kidney			*Kidney*			*Kidney*	
Hg	3.38 ± 0.31	2.03 ± 0.18	2.72 ± 0.25	3.14 ± 0.22	3.1 ± 0.61	2.29 ± 0.25	2.65 ± 0.18	3.93 ± 0.37
Cd	23.63 ± 3.98	11.86 ± 3.91	13.39 ± 3.32	27.81 ± 3.77	9.07 ± 2.2	2.98 ± 0.46	16.71 ± 2.37	21.41 ± 5.09
Pb	1.58 ± 0.39	1.48 ± 0.26	4.86 ± 2.62	3.50 ± 1.35	1 ± 0.29	1.24 ± 0.22	3.04 ± 1.24	1.67 ± 0.51
Se	12.24 ± 0.61	9.34 ± 0.68	9.47 ± 0.69	11.75 ± 0.55	10.29 ± 1.24	9.13 ± 0.7	10.24 ± 0.46	11.97 ± 5.91
As	5.30 ± 0.41	2.85 ± 0.29	4.72 ± 0.4	4.77 ± 0.37	4.89 ± 0.69	3.65 ± 0.4	3.66 ± 0.30	5.91 ± 0.44
	Feather			*Feather*			*Feather*	
Hg	0.83 ± 0.06	0.98 ± 0.11	1.8 ± 0.23	0.87 ± 0.11	0.87 ± 0.11	0.87 ± 0.05	1.41 ± 0.12	0.69 ± 0.05
Cd	0.09 ± 0.03	0.026 ± 0.01	0.22 ± 0.07	0.15 ± 0.05	0.09 ± 0.03	0.07 ± 0.02	0.17 ± 0.04	0.03 ± 0.01
Pb	2.67 ± 0.46	1.88 ± 0.36	9.27 ± 3.68	4.24 ± 1.65	3.22 ± 0.82	5.83 ± 2.28	6.10 ± 1.76	1.71 ± 0.23
Se	0.42 ± 0.02	0.63 ± 0.05	0.69 ± 0.03	0.43 ± 0.04	0.43 ± 0.04	0.68 ± 0.03	0.60 ± 0.02	0.44 ± 0.03
As	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.01	0.035 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.02	0.01 ± 0.01

through fluvial and atmospheric pathways both leading to the water column where, either dissolved or associated with small particles, it is available for suspension feeders such as marine mussels (Beiras et al. 2002), located in the low zone of the food chain. Regarding Cd levels, there were no significant differences in levels in the liver between the three studied areas; however, differences appeared in kidney tissues between Pontevedra and Gijón and between Pontevedra and A Coruña. These differences may be due to the fact that Cd mainly accumulates in the kidney and differences can be more marked when accumulated levels are higher. In both cases, Cd levels were higher in Pontevedra than in the other two areas. The higher levels of Cd in Pontevedra compared to the other areas may be due to inadequate waste water treatment, which fails to comply with current regulations on wastewater treatment. Even sediments and sludge produced in estuaries containing Cd as the main heavy metal makes Pontevedra one of the most polluted areas. The Court of Justice of the European Union ratified the repeated noncompliance with the treatment of wastewater in the Ria de Vigo (Galicia), where a poor treatment in its sanitation is being caused (https://ec.europa.eu/commission/presscorner; 17 November 2016).

Seagulls from Gijón showed higher levels of Pb, in both organs, compared to the other two regions. However, only significant differences were found in livers between Pontevedra and the other two areas. A factory that uses Pb for the manufacturing of acer is located in Gijón, being a potential source of this metal and a potential explanation for the higher levels of Pb in this area.

Significant differences can be observed in levels of Se between Pontevedra and A Coruña or Gijón, both in liver and kidney tissues. In both organs, Se levels were higher in Pontevedra. Ohlendorf et al. (1988) describes that the ratio liver/kidney for Se is about 1 for freshwater aquatic birds in Se-contaminated areas; our ratios did not reach this value, approaching levels of 0.70, 0.53, and 0.62 in Pontevedra, A Coruña, and Gijón, respectively (from Table 3). Therefore, it was considered that these areas did not present a high Se pollution. Finally, As levels show that gulls from Pontevedra accumulated more As in both organs, showing significant differences in A Coruña when compared to the other two areas.

In general, feathers were the most consistent tissue to differentiate polluted areas. Seagulls from Gijon presented always higher levels of Hg, Cd, Pb, and Se than the other two sampling areas (Fig. 5). Korbecki et al. (2019) also reported the differences in metal levels (Pb) found in feathers depending on the studied area and species. In general, feathers have been reported as a potential non-invasive tool for metal biomonitoring in seabirds (Hernández-Moreno et al. 2021).

Table 3 Correlations between the pairs of the studied elements into the specific organ (liver, kidney, and feathers) and between tissues for a specific metal

	$p<0.05$	$p<0.01$	$p<0.001$
Liver	*Cd-Se (r=0.23)*		*Hg-Cd (r=0.38)*
			Hg-Se (r=0.36)
Kidney	*Hg-As (r=0.20)*		*Hg-Cd (r=0.39)*
			Hg-Se (r=0.33)
			Cd-Se (r=0.44)
Feather	*Hg-As (r=0.23)*	*Hg-Cd (r=0.31)*	*Hg-Se (r=0.50)*
	Cd-As (r=0.24)	*Hg-Pb (r=0.26)*	*Cd–Pb (r=0.51)*
	Pb-Se (r=0.24)	*Pb-As (r=0.25)*	*Se-As (r=0.40)*
Liver-kidney			*Hg (r=0.46)*
			Cd (r=0.68)
			Pb (r=0.46)
			Se (r=0.43)
			As (r=0.53)
Liver-feather			*Pb (r=0.34)*

Concentration of metals/metalloid according to age

The age as factor in the metal(oids) content in the liver, kidney, and feathers is shown in Figs. 6, 7, and 8 and Table 2. With increasing age, adult gulls are exposed to metals through food and water. Once ingested, contaminants can be directly excreted or absorbed into the circulatory system. Age-related differences were expected for most of the studied metals because adults had a longer life-time to accumulate them. This usually occurs in the case of Hg, Cd, and Pb in the liver. However, this increase was clearer in kidney tissues, where levels of Hg, Cd, Pb, and Se were significantly higher in adults than in juveniles and chicks. In agreement with the present results, Friberg et al. (1974) obtained age-related increased concentrations of Cd in several tissues of oystercatcher and great skua. An explanation for this accumulation related to age is the long biological half-time (15–30 years) of the metal, which is thought to be a consequence of the binding of Cd to metallothionein and its subsequent retention in the kidney (Friberg et al. 1974; Kowal et al. 1979). In addition, it is possible to observe greater age-related differences in the kidney than in the liver, mainly because the kidney is the target organ for Cd. On the other hand, Stewart et al. (1994) demonstrated seasonal changes in Cd levels in internal tissues of guillemots and highlighted some kind of regulation mechanism for this metal. Through regulation, Cd would not continue to accumulate with increasing adult age, and it would not result in older birds having higher Cd burdens than younger adult birds. Studies developed during the 1980s showed no age-related Cd accumulation in herring gulls (*L. argentatus*) (Hutton 1981; Nicholson 1981). However, more recent

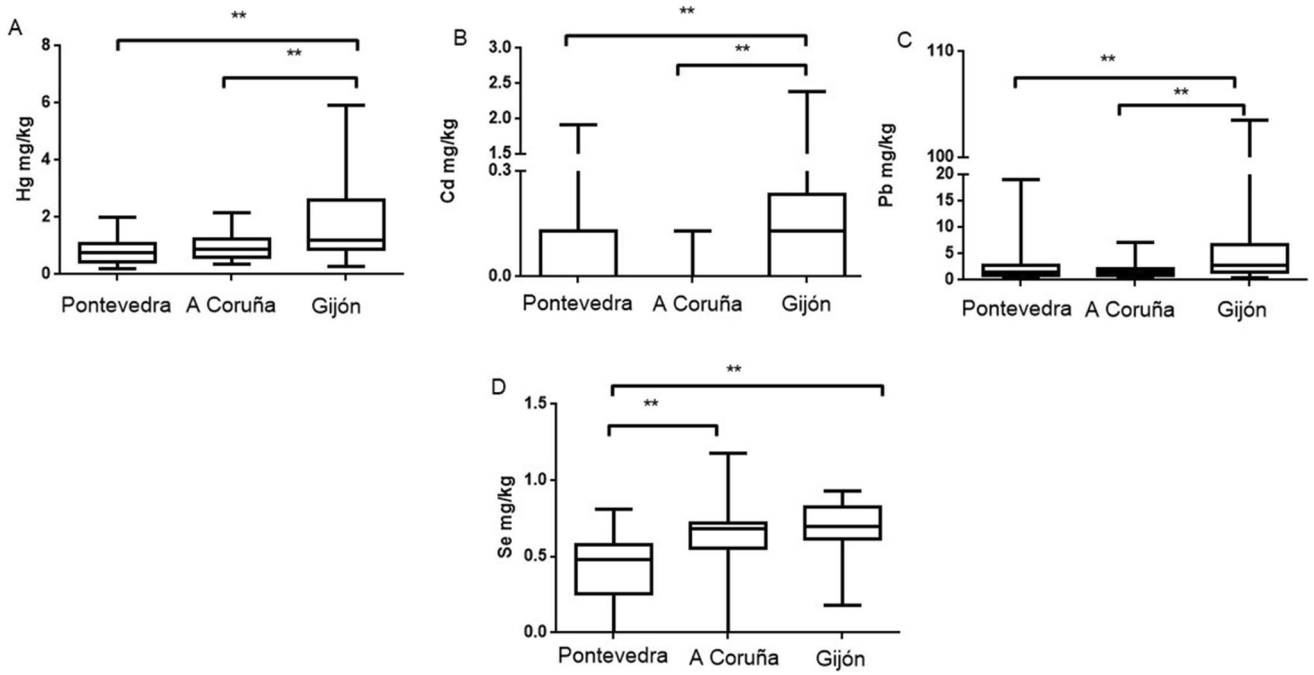

Fig. 5 Hg (**A**), Cd (**B**) Pb (**C**), and Se (**D**) levels in feathers of yellow-legged gull according to the three different sampling areas: Pontevedra, A Coruña, and Gijón. Box plots represent median values and 25 to 75% percentile ranges. $^{**}p < 0.01$. As levels could not be represented because many samples showed levels below the limit of detection

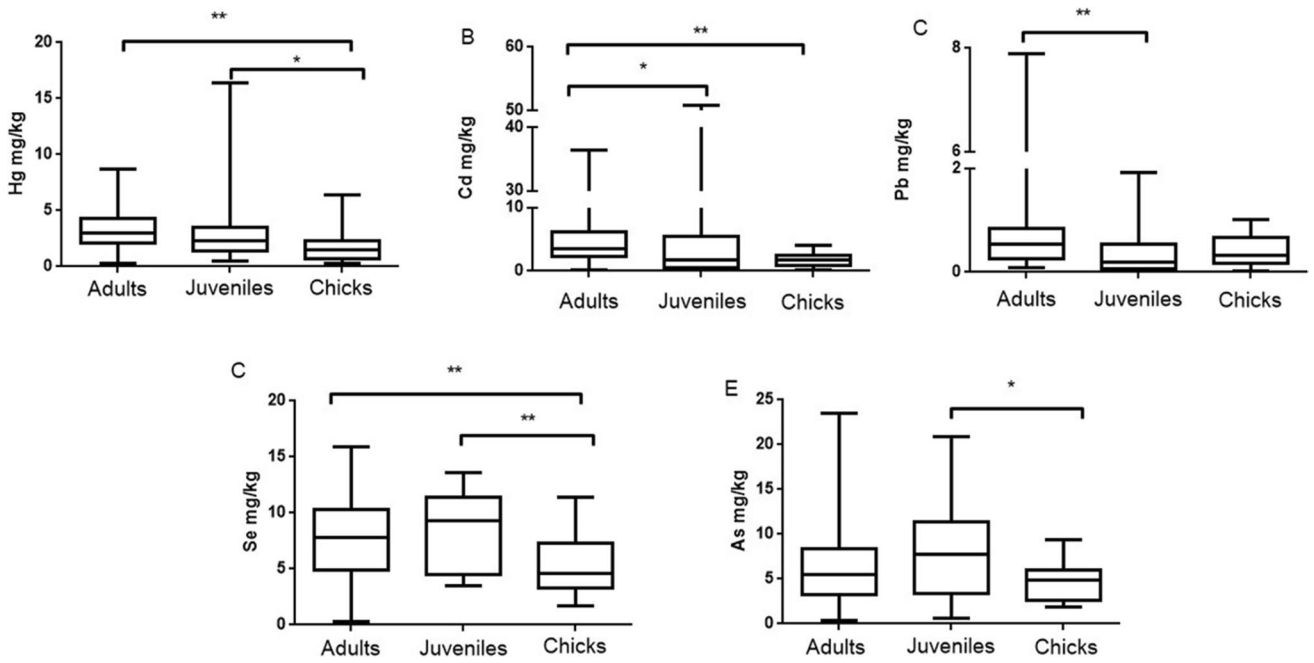

Fig. 6 Hg (**A**), Cd (**B**), Pb (**C**), Se (**D**), and As (**E**) levels in liver of yellow-legged gull according to age: adults, juveniles, and chicks. Box plots represent median values and 25 to 75% percentile ranges. $^{*}p < 0.05$, $^{**}p < 0.01$

studies (Kim and Oh 2017) reported that adults of *L. crassirostris* presented higher levels of Cd than nestlings. Some other authors reported also higher liver concentrations of Cd in adult birds than in nestlings or juveniles for black-headed gulls (*L. ridibundus*) (Orlowski et al. 2007) and glaucous gulls (*L. hyperboreus*) (Migula et al. 2000).

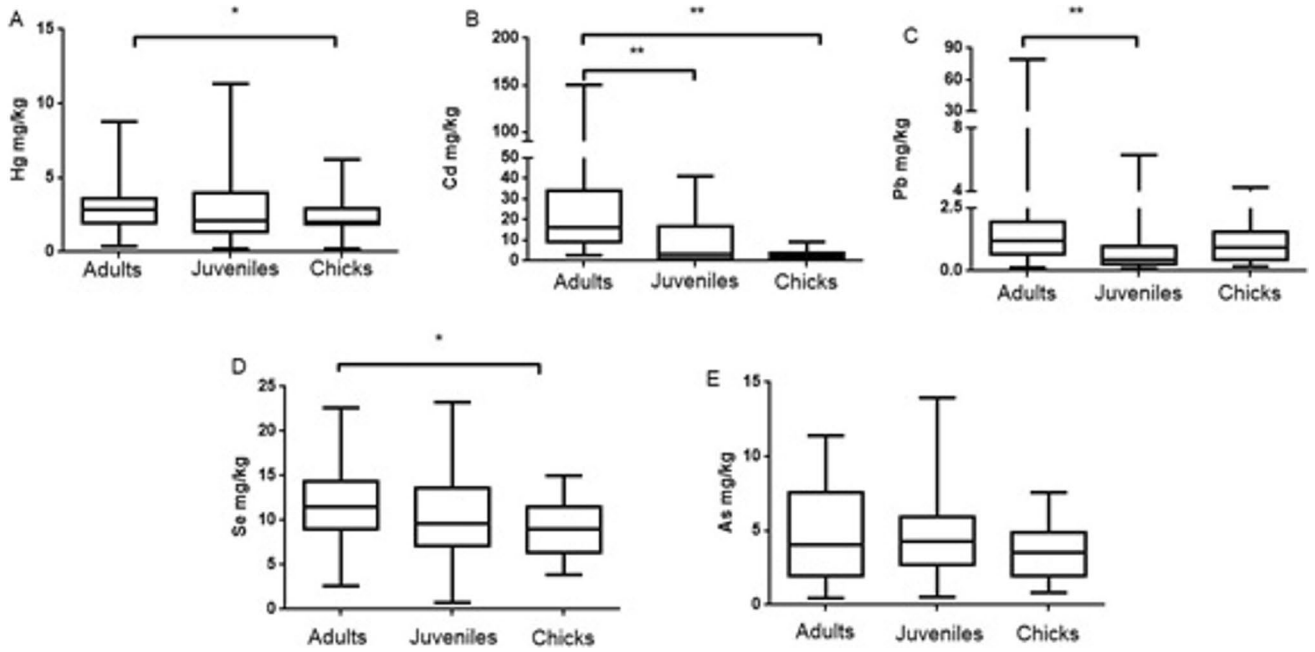

Fig. 7 Hg (**A**), Cd (**B**), Pb (**C**), Se (**D**), and As (**E**) levels in kidney of yellow-legged gull according to age: adults, juveniles, and chicks. Box plots represent median values and 25 to 75% percentile ranges. $*p < 0.05$, $**p < 0.01$

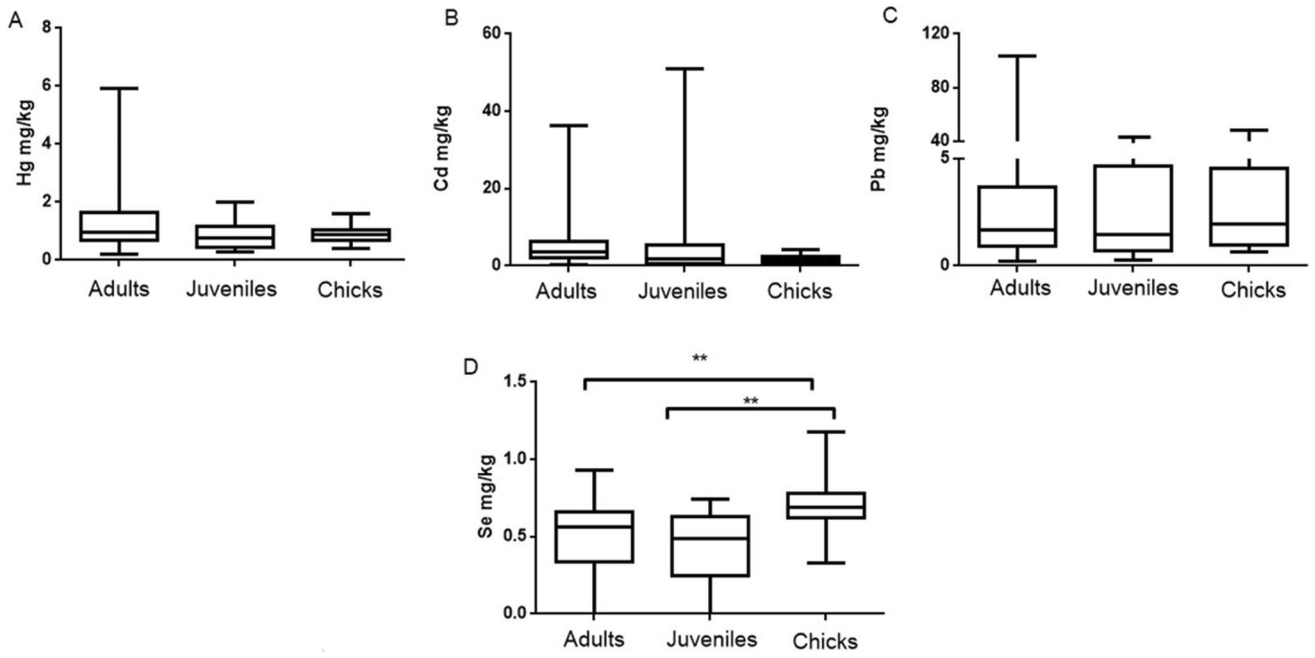

Fig. 8 Hg (**A**), Cd (**B**), Pb (**C**), and Se (**D**) levels in feathers of yellow-legged gull according to age: Adults, Juveniles and Chicks. Box plots represent median values and 25 to 75% percentile ranges. $**p < 0.01$. As levels could not be represented because many samples showed levels below the limit of detection

Regarding Pb, levels were higher in adults, followed by chicks (without significance) and, lastly, the juveniles. The higher values found in adults may be due to the accumulation of this metal associated with age and their wide mobility to find food. The high levels found in chicks could be related to those in adults by direct transmission, once all of them are in the nest. Finally, the lower levels in juveniles may be explained by the metal elimination together with the

feathers during molting. Pb concentrations are increased in those areas with high population, showing the importance of establishing monitoring programs in areas with a high density of buildings. Stripping paint off buildings without proper containment control of chips appears to increase the availability of Pb to chicks and compounds the problem (Work and Smith 1996).

Moreover, the juveniles of the present study have higher levels of Se and As in liver than the other two groups. The diet of these animals may explain the differences found between adults and fledglings. Changes in the diet of this species can take place during the breeding period, leading to a different nutrition between adults and young. In fact, the main diet of seagulls consist of invertebrates rather than fish, especially in chicks. However, when breeding period occurs, the adult gulls base their diet mainly on larger fish (Burger, unpublished data). On the other hand, Se and As show the normal pattern in the kidney, with higher levels in adults compared to juveniles and chicks.

In feathers, statistically significant differences for Se were found between chicks and adults or juveniles, with in both cases higher levels of Se found in chicks (Fig. 8).

Concentration of metals/metalloid according to capture method

The last factor considered in this study was the influence of the capture method. Results showed different values depending on the tissue (Table 2). In the liver, Hg, Se, and As presented lower levels in birds captured during control campaigns than birds which died in recovery centers, with significant differences in Hg and Se levels. In the kidney, the levels of Hg, Cd, Se, and As were higher in animals from recovery centers in (levels of As were significantly different). However, the metal content in feathers was always higher in the animals captured in control campaigns, finding statistical differences ($p < 0.001$) for Hg, Cd, Pb, and Se.

When the comparisons were performed classifying samples by sex or age related to the specific location, some differences were found by pairs. By sex, differences were found mostly in feather content of Hg, Cd, Pb, and Se. In these cases, females from A Coruña (Hg ($p < 0.05$), Cd ($p < 0.05$ and 0.01), and Pb ($p < 0.0001$)) and Gijón (Hg ($p < 0.05$)) showed higher values than females and males from other areas. Regarding Se, males from Gijón and A Coruña presented the lowest values in feathers ($p < 0.0001$), whereas males from Pontevedra showed higher values of this metal in liver ($p < 0.01$). According to age, adults from Asturias showed higher values ($p < 0.0001$) of Hg in feathers than adults from Pontevedra, as well as from juveniles and chickens. For Cd, there was a clear higher value found in kidney from chickens of Asturias when compared to the other groups ($p < 0.01$ and 0.05). In A Coruña, levels of Pb were significantly higher in feathers of adult animals respect to the other ages and areas ($p < 0.0001$). Regarding Se, the previously reported lower values in feathers of juveniles in comparison to adults and chickens was confirmed, but no clear differences were found related to the sampling area.

Correlations study

Regarding the correlations study, weak to moderate positive correlations were found between pairs of various studied metals ($r = 0.3$–0.5), as shown in Table 3. The liver and kidney showed correlations between Cd-Se, Hg-Cd, and Hg-Se, with the latter found also in feathers. The Hg-Se correlation in the three tissues might indicate a possible implication of the Se in the detoxification of metals, like Hg (Blanco et al. 2003). An analogous relationship between Hg and Se has not been as clearly demonstrated in marine birds, although there are many toxicity studies providing evidence of such a mechanism in the significant correlation of Se–Hg levels (Marier and Jaworski 1983). Many authors suggest that the protective effect of selenite on the toxicity of Hg^{2+} is mainly due to the formation of mercuric selenide (HgSe). This represents a stable and biologically inert complex (Koeman et al. 1973; Das et al. 2003; Eagles-Smith et al. 2008; Yang et al. 2008) that produces a decrease in the toxicity of Hg. A significant positive correlation between hepatic Hg and Se in gulls has been reported (Hutton 1981). Moreover, it has been suggested that both metals (Se and Hg) ameliorate the toxicity of the other (Eisler 2000).

Regarding other elements, the biological significance of the correlations between Cd and Se in seabirds is unclear. Pařízek et al. (1971) stated that this association may suggest a protective influence in the organisms due to the potential antioxidant activity of Se. In the present study, this correlation was found in liver and kidney.

However, no correlations were found between Pb and Se either in the liver or in kidney, showing a weak correlation in feathers ($r < 0.3$). The low levels of Pb may explain the lack of relationship, since Pb levels may not be high enough to increase the levels of metal-binding proteins such as protoporphyrins and metallothioneins (Elliott et al. 1992; Stewart et al. 1996). Nevertheless, a moderate to strong correlation was found in feathers between Pb and Cd ($r > 0.5$). This correlation has been already reported, as well as the lack of correlation of Hg with these two metals in feathers. The authors pointed out as a possible explanation the same origin of Cd and Pb, being different from that of Hg (Bianchi et al. 2008). In the present study, it was possible to establish a correlation between these metals in feathers (Hg-Cd and Hg-Pb) but with a weak r value.

Finally, strong positive correlations between the liver and kidney in levels of Hg, Pb, Se (weak to moderate), Cd, and As (moderate to strong) were found, as tissues representing

major reservoirs and metabolization organs. There was also a positive correlation found between liver and feathers, pointing to a possible release of metal through this external tissue.

Conclusions

In the present study, the results from accumulation assessment of Hg, Cd, Pb, Se, and As in the liver, kidney, and feathers of *L. michahellis* from 3 different areas (Pontevedra, A Coruña, and Gijón) provide evidence that the three tissues are adequate samples to understand heavy metals pollution levels and bioaccumulation along the wetland food chain. Metal concentrations detected during the present study were within or below the range of levels found for other seagulls. Although internalized metal levels will not provoke lethality to the selected animals, their identification in tissues denotes exposure through the environment. Indeed, it was confirmed that the liver, kidney, and feathers of *L. michahelli*s can reveal local contamination around the foraging and breeding sites. This makes the selected species of high interest as an instrument for assessing heavy metal contamination not only following a disaster but also for routine monitoring studies, both in coastal and inland areas. Therefore, *L. michahellis* can be a useful sentinel species of environmental health, providing an early warning of possible exposure for humans inhabiting the same ecosystems and often eating the same foods.

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Author contribution J.V.: formal analysis, investigation, writing—original draft. D.H.M.: conceptualization, formal analysis, supervision, writing—original draft. A.L.B. and L.E.F.: resources, writing—review and editing. F.S.: writing—review and editing. M.P.L.: conceptualization, formal analysis, resources, writing—review and editing. M.P.M.S.: conceptualization, funding acquisition, supervision, writing—original draft.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate There was no need for reviewing and approval by an appropriately constituted committee for animal research, because the sampled animals were already dead.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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3.3 Material complementario.

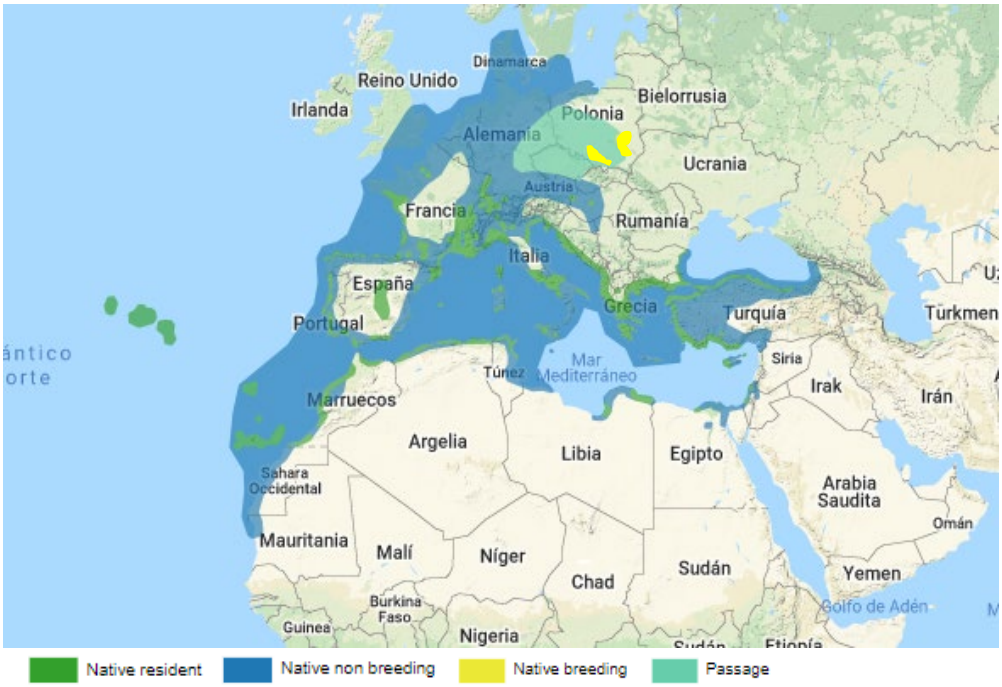

Figure S1. Distribution map of L. michahellis. (Birdlife International, 2021)

Table S1. General conditions for the microwave digestion.

Time (min)	Temperature (ºC)	Potency (W)	Pressure (bar)
5	100	1200	70
7	130	1300	100
8	170	1300	120
10	200	1500	120
15	200	1400	120

Table S2. Operation conditions for the ICP-MS analysis.

Potency RF (W)	1550
Plasma Mode	General purpose
Omega Bias (V)	-120
Omega lens (V)	9.3
Extract 2 (V)	-245
Deflect Lens (V)	1.0
Energy discrimination (V)	5
Collision gas (ml/min)	5
Cell Entrance (V)	-40
Cell Exit (V)	-60

4. Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (*Larus michahellis*) from Spain: Role of gender and age.

Vizuete J., Hernández-Moreno D., Fidalgo L.E., Bertini S., Andreini R., Soler F., Míguez-Santiyán M.P., López-Beceiro A., Pérez-López M.

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4.1 Resumen: Concentraciones de contaminantes clorados en tejido adiposo de gaviota patiamarilla (*Larus michahellis*) procedentes de España: Papel del sexo y la edad.

En el presente estudio se han determinado las concentraciones de 7 congéneres de bifenilos policlorados (PCB) diferentes y once plaguicidas organoclorados (OCP) y metabolitos, incluidos DDT (diclorodifeniltricloroetano), HCH (isómeros de hexaclorociclohexano), endosulfán, sulfato de endosulfán, endrina, dieldrina y HCB (hexaclorobenceno) en tejido adiposo de 57 gaviotas (*L. michahellis*) procedentes del norteoeste de España. En este estudio se han considerado dos factores endógenos que son la edad y el sexo con el fin de encontrar alguna diferencia significativa en ellos. La totalidad de los PCB que se analizaron se encontraron en mas del 66% de las muestras, con niveles de 291,9 (PCB 180), 34,5 (PCB 118), 0,7 (PCB 28), 432,6 (PCB 153), 225,5 (PCB 138), 1,3 (PCB 101) y 0,4 (PCB 52) µg/kg de tejido adiposo. Por otro lado, solamente se detectaron 4,4'-DDE y HCB en más del 50% de las muestras, con medias de 360,6 y 2,5 µg/kg respectivamente. De todas las muestras analizadas solamente se encontraron diferencias significativas ($p<0,01$) en el sexo en los niveles de 4,4'-DDE, siendo las concentraciones superiores en hembras que en machos. Con respecto a la edad se encontraron un mayor numero de diferencias significativas ($p<0,001$), ya que se encontraron tales diferencias en los niveles de PCBs 180, 138, 101, 28 y 153 y asi como 4,4'-DDE siendo superiores los niveles en adultos con respecto a los jóvenes. Los resultados obtenidos en este estudio permiten constituir una línea de base para poder evaluar mejor los impactos ambientales provocados por estos contaminantes (PCB y OCP) en cualquier lugar donde habite esta especie, con especial énfasis en las diferenias relacionadas con la edad y el sexo.

4.2 Artículo publicado.

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Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (*Larus michahellis*) from Spain: Role of gender and age

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ABSTRACT

Concentrations of 7 different polychlorinated biphenyl (PCB) congeners, and eleven organochlorine pesticides (OCPs) and metabolites, including DDTs (dichlorodiphenyltrichloroethane), HCHs (hexachlorocyclohexane isomers), Endosulfan, Endosulfan sulfate, Endrin, Dieldrin and HCB (hexachlorobenzene), were determined in adipose tissue of 57 yellow-legged gulls collected from NW and N Spain. Furthermore, the possible differences due to two endogenous factors, age and gender, were determined. All the analyzed PCBs were detected in over 66% of the samples, with levels of 291.9 (PCB 180), 34.5 (PCB 118), 0.7 (PCB 28), 432.6 (PCB 153), 225.5 (PCB 138), 1.3 (PCB 101) and 0.4 (PCB 52) μg/kg of adipose tissue. With respect to the OCPs and metabolites, only 4,4′-DDE and HCB were detected in more than 50% of the samples, with means of 360.6 and 2.5 μg/kg of adipose tissue, respectively. From all the considered contaminants, only 4,4′-DDE levels presented significant differences depending on the gender, with females showing higher values than males ($p < 0.01$). Significant differences ($p < 0.001$) were also found related to age for the levels of PCBs 180, 138, 101, 28 and 153, as well as 4,4′-DDE, with adult levels being higher than those in young birds. The results of the present study constitute a baseline to better assess the environmental impacts of PCB and OCP contamination at other coastal sites for future biomonitoring studies, with particular emphasis on gender- and age-related differences.

1. Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are included within the group of persistent organic pollutants (POPs). POPs are widely used chemical compounds of environmental concern, and feature long-range transport, resistance to metabolism and potential toxicity (Ashraf, 2017). The presence of these compounds are generally a result of industrial, commercial and agricultural activities. Moreover, they have become ubiquitous in the environment where, in recent decades, they have been found to cause adverse effects on humans and wildlife. Several of these compounds have been implicated, for example, in decreased reproductive success in fish-eating water-bird populations in contaminated areas (Choi et al., 2001a) and it has been shown that they can affect oxidative stress levels (Fenstad et al., 2016). These contaminants are fat soluble and not readily degradable in the environment. Furthermore, they have the potential to biomagnify and to accumulate in high concentrations in animals at the top of the food chain, considered at risk. Aquatic organisms, and those exploiting aquatic resources, are particularly exposed to increasing levels of pollutants since aquatic systems are usually the ultimate pollutant sink, either due to diffuse sources, or direct discharges from the environment (Ramos et al., 2013). Both OCPs and PCBs are a cause for concern for nearshore marine ecosystems already threatened by a variety of human activities and pressures (Good et al., 2014).

Coastal and estuarine areas from Galicia (29,575 km^2) and Asturias (10,603.57 km^2), two regions located along the northern

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Spanish coastline, are characterized by touristic, industrial, fishing, shipping, dredging and aquaculture activities, and/or contamination events. Even if there are well-preserved natural areas in both regions, there are also several important cities and industrial plants (for example, close to the cities of Vigo, A Coruña, Ferrol and Gijón). The NW/N Atlantic coast of Iberia is known to host over 80,000 yellow-legged gull (*Larus michahellis*) breeding pairs, as well as a wintering population comprising several gull species, most of which are yellow-legged gulls travelling from the Mediterranean (Arizaga et al., 2013). These seabirds, considered an integral part of aquatic ecosystems, have become sensitive biomonitors of the changes occurring due to both natural and anthropogenic factors in similar areas of Europe (Morales et al., 2016). Indeed, since the condition and reproductive success of seabirds are influenced both by the conditions of breeding areas and in remote places where they live outside the breeding season, they can be used as proxies to assess the impact of many variables affecting their environment in temporal and spatial terms (Falkowska et al., 2016). Seabirds are generally high consumers and subject to accumulation of marine pollution, and are commonly used as sentinel species for exposure to persistent contaminants. In addition, *Larus michahellis* is markedly adaptable when choosing habitats, often in the vicinity of coastal population centers. In those habitats, they can breed successfully in buildings, feeding on residues from both dumps and discards from local fishing activities. Fledglings, which have not yet been subjected to pollutant bioaccumulation, are useful for monitoring temporal and spatial changes in pollution levels around the breeding area (Abdennadher et al., 2010). Thus evaluation and measure of the effects of contaminants in living organisms and their environment are also influenced by endogenous factors such as gender and age (Burger, 2007). However, many endogenous factors have received considerable attention in wildlife, being gender one of them. The sex of a bird can affect exposure and accumulation of pollutants. One conventional explanation for differences in chemical burden suggests their transfer from breeding females to the eggs. However, results from studies on the effect of gender on toxic burden in birds are not consistent nor established for every chemical.

The aim of the present study was to evaluate the levels of different POPs in adipose tissue of gulls from different regions of the Atlantic coast of Spain, in order to determine whether organic contaminant exposure poses a threat to the environment under study. In addition, with the interest of determining the suitability of this seabird species as a bioindicator, the possible differences related to two endogenous factors, age and gender, on POPs levels was also investigated.

2. Material and methods

2.1. Study areas and sampling

Gulls were collected during the period of 2014–2016 in the regions of Galicia and Asturias (respectively situated in the NW and N of Spain) (Fig. 1). Collected animals were found dead or had died after being injured and referred to the Wildlife Recovery Centers situated in the study areas. Recovered birds suffered mainly from physical injuries, including electrocution, fall from the nest due to inexperience in flying, and others of unknown origin. Injured birds included in the study were those that had not been held at the Recovery Centre for more than 5 days before dying (the average stay was approximately 2 days). Diet during recovery was likely free of environmental contaminants. Different species of fresh fish were bought in the fish market and was for human consumption. The species were chosen depending on the size (preferable small), the protein/fat ratio, and the price.

During necropsy, several parameters such as mass measurements (g), organ weights (g), bill development and physical condition were registered. Age was determined based on the color plumage, as there is a significant color range to pure white adults, and 1-year-old juvenile gulls can easily be discerned from adult conspecifics using plumage characteristics (Grant, 1986). Gender was determined through observation of the gonads during necropsy. Twelve female juveniles, 16 female adults, 13 male juveniles and 16 male adults were identified. After sampling, the remains were destroyed hygienically by incineration, under current European legislation.

After necropsy, all specimens ($n = 57$) were immediately frozen and stored at −20 °C until samples were prepared for analysis. From each corpse, a portion of approximately 3 g of subcutaneous adipose tissue was taken, placed individually in plastic bags, and stored at −20 °C. The complete data set included 25 juveniles and 32 adults. In terms of gender, there were 29 males and 28 females.

2.2. Reagents and quantification of chlorinated compounds by GC/ MS analysis

POPs were analyzed in adipose tissue. Eleven OCPs (including metabolites) were assayed: isomer mixture of hexachlorocyclohexane (HCH) consisting of β and γ-HCH; DDT and its metabolites (namely 4,4′-DDD and 4,4′-DDE); hexachlorobenzene (HCB); and the cyclodiene insecticides heptachlor epoxide, dieldrin, endrin, endosulfan, and endosulfan sulfate. Similarly, 7 indicator PCBs (CBs 28, 52, 101, 118, 138, 153, and 180) were targeted, as they are predominantly present in biotic and abiotic matrices and have been recognized as compounds representative of the whole group of PCBs by the Agency for Toxic Substances and Disease Registry (ATSDR, 2000). Reference materials supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) with a purity of 97–99.7% were used for OCPs standard preparation, with concentrations ranging from 10 ppb to 10 ppm. Similarly, a commercial mix of 7 PCBs from SpexCertiPrep (Stanmore, UK) (10 μg/ml in isooctane) was used for single quantification of PCB congeners IUPAC 28, 52, 101, 118, 138, 153, and 180. Stock solutions (500 μg/ml) were prepared by dissolving reference standards in acetone (Panreac) and stored at −20 °C. Working solutions for sample fortification and for injection in the GC systems were prepared by diluting stock solutions in n-hexane (Panreac®).

The protocol followed to perform the PCB and OCP extraction was adapted from a procedure used by Mateo et al. (2012). Briefly, samples were thawed at room temperature and 0.7 g of the tissue was chopped and mixed with 7 ml of n-hexane. The mixture was homogenized and frozen overnight, allowing the fat to precipitate. Five ml of the supernatant were added with 2 ml of H_2SO_4, the tubes were subsequently shaken in an orbital shaker for 10 min, sonicated for 5 min and centrifuged at 1000 × *g* for 5 min, and the acid-containing phase discarded. The above procedure was repeated until the acidic phase was completely clear. The resulted extract was evaporated, re-suspended in 200 μl n-hexane and then used for OCPs and PCBs concentration measurements.

A Bruker Scion 456 triple quadrupole gas chromatograph mass spectrometer was used to analyze the samples. Analyte separation was achieved on an Rxi-5 Sil MS column (30 m x 0.25 mm, i.d. x 0.25 film thickness). The results were analyzed using specific GCMS software. The multiple-ramp temperature program used involved a first step of 3.5 min at 70 °C, then the temperature was raised to 180 °C at a rate of 25 °C/min. This was followed by an increase to 300 °C at a rate of 15 °C/min and a final increase to 325 °C at a rate of 50 °C/min, and maintained for 5 min. The vaporized samples were injected in splitless mode at a column flow rate of 1.20 ml/min. The temperatures of the injection port, detector and interface were 280 °C, 280 °C and 300 °C, respectively. PCB and OCP residues were quantitatively evaluated carrying out the internal standard method (with 25 μg/l of PCB180 added at the beginning of the extraction process). The calibration curves were obtained by determining the relationship between the peak area and the concentration of the different standards. Solvent blanks (consisting of 500 μl n-hexane instead of tissue) were processed in parallel to the samples to assure the quality of the analyses.

To verify the suitability and performance of the procedure, the

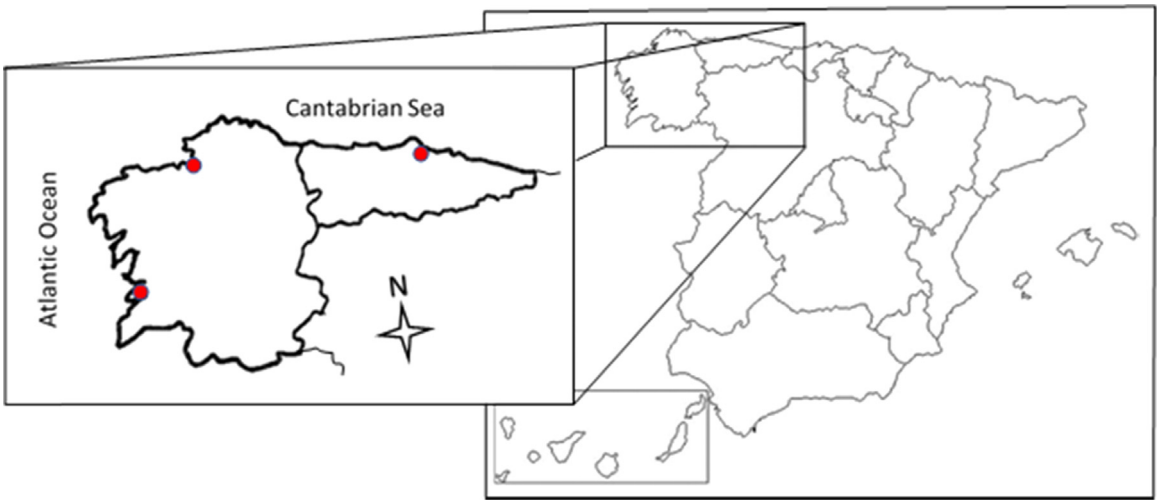

Fig. 1. Sampling area, located in the coastal regions of Galicia and Asturias (from West to East), in NW-N Spain. The red points indicate the Wildlife Rehabilitation Centers where animals/samples were submitted.

Table 1
Concentration of PCBs and OCPs (expressed in μg/kg l.w.) in adipose tissue samples of *Larus michahellis* (n = 57).

Concentration	%	Mean	SEM	Median	Minimum	Maximum
PCBs						
PCB 28	100	0.7	0.2	0.2	0.1	7.2
PCB 52	66.7	0.4	0.1	0.2	< LOD	2.7
PCB 101	98.3	1.3	0.3	0.6	< LOD	11.5
PCB 118	100	34.5	7.7	8.4	0.9	241.6
PCB 138	100	225.3	48.7	50.1	8.5	1280
PCB 153	100	432.6	94.0	107.7	13.6	2856
PCB 180	100	291.9	65.8	68.3	7.2	2009
OCPs and metabolites						
4,4′-DDE	100	360.6	121.3	74.7	5.7	6970
HCB	70.2	2.5	1.1	1.1	< LOD	9.2
Heptachlor epoxide	40.4	1.0	0.3	0.3	< LOD	16.4
4,4′-DDD	35.1	124.3	37.7	36.4	< LOD	1233
Endrin	28.1	0.8	0.3	0.1	< LOD	3.5
Endosulfan	15.8	0.6	0.1	0.4	< LOD	1.2
γ-HCH	12.3	0.8	0.2	0.4	< LOD	11.3
Endosulfan sulfate	5.3	0.7	0.2	0.2	< LOD	2.9
Dieldrin	3.6	0.5	0.2	0.4	< LOD	1.1

% = represents the % of the total amount of samples that contained the specific chemical (samples where the chemical was detected). SEM: standard error mean.
LOD (limit of detection) = PCB 101: 0.028, PCB52: 0.073, HCB: 0.214, heptachlor epoxide: 0.258, 4,4′-DDD: 0.572, endrin: 0.080, endosulfan: 0.324, γ-HCH: 0.379, endosulfan sulfate: 0.167, and dieldrin: 0.231.

accuracy was estimated by means of recovery experiments, analyzing blank adipose tissue samples (n = 10) spiked at five concentrations levels of PCB and OCP mixtures. Previously, the blank sample was analyzed to determine the content of analytes in triplicate. Recoveries were obtained as the ratio (in %) between the calculated concentration of spiked samples and the theoretical concentration added. The recovery percentages for PCB spiked samples were found between 89% and 109% (CV < 20%), while the recovery percentages for OCPs were between 80% and 128% (CV < 20%). The limit of quantification (LOQ) was established as the lowest concentration level validated with satisfactory values of recovery (70–110%) and precision (RSD < 20%). The limit of detection (LOD) was estimated as the analyte concentration that produced a peak signal of three times the background noise in the chromatogram at the lowest fortification level studied for each compound. The LODs of those analytes present in the blank tissue sample were estimated from the chromatograms corresponding to the analyzed blank sample (Hernández et al., 2005). The LODs for PCBs and OCPs ranged between 0.006 and 0.079 μg/kg and 0.070–1.124 μg/kg lipid weight (l.w.), respectively.

2.3. Statistical analysis

Data were analyzed using statistical software Prism 5 version 5.03 for Windows (GraphPad software, Inc., CA). Normality and homoscedasticity of data were assessed. Since data did not show a normal distribution and the variances were not homogeneous, the statistical analyses were performed using a non-parametric Mann Whitney *U*-test, to evaluate the differences related to both gender and age. Finally, a Spearman test was performed to determine the correlations among chemical levels. Results were expressed as mean ± SEM and range, and the level for statistical significance was defined as $P < 0.05$. Statistical assessments were limited to those chemicals that could be detected in > 50% of the samples. A value of 50% of the LOD was assigned to samples with an undetectable contaminant concentration. These values were included in the data-set for statistical testing, a technique that minimizes nominal type I error rates (Clarke, 1998).

3. Results and discussion

3.1. Levels of chlorinated contaminants

Levels of PCBs and OCPs, including metabolites, were determined in adipose tissues from 57 *Larus michahellis* from different locations along the coast line of NW and N Spain. The concentrations (given as mean and standard error) of individual PCBs and OCPs are presented in Table 1, and reported on a lipid weight basis (μg/kg l.w.). Concentrations of both groups of contaminants are known to be higher in adipose tissue and provide a more representative evaluation of the cumulative internal exposure than those found in different tissues. This is due to the fact that measurement in other tissues (for example blood) will fluctuate during lipid mobilization (i.e., body weight loss, or breeding), rendering adipose tissue samples as preferable bioindicators of body burden, when available (Achour et al., 2017).

PCBs were the dominant compounds among various organochlorine compounds (OC) analyzed in terms of percentage of positive samples, although the concentration of both congeners 101 and 52 were below the limit of detection in 1 and 19 samples, respectively. On the other hand, the five remaining congeners were detected in 100% of the analyzed samples, with highest mean and maximum concentrations corresponding to PCBs 153 and 180.

Regarding OCPs and their metabolites, and in agreement with

similar studies (Hela et al., 2006), 4,4′-DDE was the most frequently detected compound (100% of the samples). This is likely due to the high chemical stability and persistence of 4,4′-DDE in the environment and in living organisms. The high frequencies and concentrations of 4,4′-DDE and 4,4′-DDD measured in gulls and the complete absence of 4,4′-DDT may be interpreted as the result of a non-recent contamination (Naso et al., 2003). β-HCH was not detected in any of the analyzed samples, and γ-HCH was only found in a small number of individuals. These compounds have high elimination rates and are considered less persistent in marine birds than other OCPs such as DDTs (Colabuono et al., 2012). Moreover, it must be noted that the ban on the use of lindane by the European Union, through the Decision 2000/801/EC, has led to a general decrease in their levels in different bird species, and the concentrations currently detected in birds in Europe have decreased until below the levels thought to cause adverse effects (Martínez-López et al., 2009).

Similarly, endosulfan is a relatively water-soluble compound and tends not to accumulate in tissues. Generally, the limited presence of this compound may be explained by its non-assimilation by the birds or its rapid biotransformation within the organism (Colabuono et al., 2012). Endosulfan was banned in Spain in 2007, following the Decision 2005/864/EC. However, an amendment to Annex A of the Stockholm Convention on Persistent Organic Pollutants was adopted in 2011, in which technical endosulfan was given specific exemption. This could explain the relatively high number of gulls in which endosulfan was detected (15.8% of the samples, i.e., 9/57 gulls). There is limited information on residue levels of endosulfan sulfate in avian species and in wildlife in general. In the present study, only 5.3% of the 57 adipose tissue samples were found to be positive for this compound. Endosulfan does not persist in warm-blooded organisms and can be converted to dialcohol by hydrolysis and to endosulfan sulfate by oxidation. It has been suggested that the presence of endosulfan sulfate in birds could indicate recent exposure to endosulfan (Martínez-López et al., 2009).

Dieldrin is a commonly detected environmental contaminant because it is stable and relatively easy to measure. Dieldrin is accumulated in wildlife species, and its ubiquity and long-term existence as an environmental pollutant has been reported (Gómez et al., 2011). It is therefore a useful indicator of global contamination by organochlorines. This pesticide was detected in only 3.6% of the gull adipose tissue samples (2/57). Moreover, despite having high environmental stability, this pesticide was detected at relatively low levels (means of 0.5 μg/kg adipose tissue). Endrin is an isomer of dieldrin, may be metabolized by animals and has a low degree of accumulation in lipids. This compound was only detected in 16 of the studied specimens, but although it has been shown that the effects of endrin are harmful to bird species at the top of food chain, such as *Accipiters* and other birds of prey (Blus et al., 1983), information on the effects of endrin on *Laridae* and similar seabird species is scarce. Nevertheless, the levels in gull adipose tissue appear to be too low to pose any immediate threats to the birds.

Finally, HCB, formerly used as a fungicide, but currently considered to be an industrial chemical that is highly toxic and carcinogenic, was detected in 70.2% of the adipose tissue samples of gulls, thus representing 40/57 of the studied animals.

The accumulative nature of organochlorines is responsible for their presence in the environment; moreover, due to their specific physical-chemical characteristics, the biota accumulates greater concentrations of POPs relative to concentrations of these substances in non-biotic portions of the environment (Blankenship et al., 2005), thus their detection in the animals sampled for the present study was to be expected. Levels of POPs in organisms are dependent not only on their position in the trophic chain and other factors (habitat, gender, age, and dietary habits, for example), but also on local environmental contamination (Tomza-Marciniak et al., 2014). Seawater pollution, distance of the colony to land, industrial activities, feeding ecology and ability to biotransform contaminants all play an important role in the accumulation of POPs (Morales et al., 2016). Different studies on seabird species have confirmed that differences in POP profiles are a result of the transfer of chemical substances from one environmental medium to another, as well as to interspecies differences related to physiological and biochemical processes responsible for the absorption, distribution, and elimination of these xenobiotics. Common eider (*Somateria mollissima*), which feed on benthic organisms, and little auk (*Alle alle*), which mainly feed on copepods, had low POP levels (0.5–1.0 μg/g) (Savinova et al., 1995). However, in fish-eating species such as black-legged kittiwakes (*Rissa tridactyla*), common and Brünnich's guillemots (*Uria aalge* and *Uria lomvia*) and puffins (*Fratercula arctica*), the POP levels were somewhat higher (1.0–5.0 μg/g). The highest POP levels (1.0–40 μg/g) were found in different gull species (*Larus argentatus* and *Larus marinus,* for example), with levels 5–10 times higher than in the other seabird species sampled in the same area (Gabrielsen, 2007).

Comparison of PCB and OCP residues among different studies is complex due to variation caused by confounding factors such as sex, age, condition and reproductive cycle, and by the scarce number of studies focused on seabird adipose tissue. The concentrations observed in the present study were compared with corresponding levels in similar species from earlier studies on a lipid weight basis. In agreement with a study of adipose tissue from Arctic gulls (Buckman et al., 2004), 4,4′-DDE had the highest concentrations of any individual OC compound in birds studied here. The same study reported that ΣPCB were the highest among the OC groups, PCB 153 being the most abundant, followed by PCBs 138, 180 and 118, similar to our results. It was also noteworthy to remark that OC concentrations were greater in fat than liver for all OC groups across all seabird species. This confirms the reliability of adipose tissue for biomonitoring POPs, related to the higher percentage of triglycerides in fat and the affinity of POPs for this type of lipid (Espín et al., 2010).

It is important to remember that a bird's capacity to metabolize PCBs decreases with an increasing degree of chlorination (Maervoet et al., 2004). Therefore, more chlorinated compounds (e.g. PCBs 118, 138, 153 and 180) tend to be accumulated, while CBs 52 or 101 are more easily metabolized (Fromant et al., 2016), as seen in the present study. Choi et al. (2001a, 2001b) determined the levels of POPs in fat from different species of gulls (Korea and Japan), and reported that PCB 138, 153, and 180 contributed to over 30% of the total PCBs in all samples. Among OCPs, p,p′-DDE and β-HCH were predominant. Again, these authors found higher amounts of total PCBs in adipose tissue than those quantified in the present study with European gulls. Moreover, given the fact that the use of organochlorine pesticides was restricted in the 1970s in Japan as well as in Europe, the specific presence of DDT in gulls from Japan suggests origination of this compound in Southeast Asia or China by atmospheric transport on a global scale, and similar global movements could be associated to its presence in European gulls.

The particularly strong occurrence of 4,4′-DDE to OCPs in seabirds may be due to both dietary accumulation and DDT metabolism (Fromant et al., 2016), with 4,4′-DDE dominating the OCP pattern in Arctic and Antarctic seabirds (Carravieri et al., 2014). The regions that seabirds inhabit is a key point when the exposure and bioaccumulation of persistent pollutants are evaluated, and in this sense 4,4′-DDE and HCB concentrations found in Antarctic seabirds (Inomata et al., 2004; Taniguchi et al., 2009) were markedly higher than those quantified on the present study.

In general, a decrease on the levels of POPs in seabird samples is currently observed, thus corroborating the general decline in PCBs and OCPs, at least in the Northern hemisphere (Sagerup et al., 2009).

In the present study, the correlations between the different xenobiotics in adipose tissue of gulls have been determined. There is a markedly high incidence of statistically significant correlations for many of the analyzed contaminants, particularly PCBs 180, 101, 118, 153 and 138 with all the different congeners (correlation coefficients ranging between 0.6 and 1.0, and p-values < 0.001).

Many positive correlations were observed between contaminants in

gull adipose tissue, in agreement with the few previous investigations in seabirds (Espín et al., 2010; Fromant et al., 2016). Correlations between POPs of different chemical families have previously been documented in seabird plasma, and strongly suggest that contaminant exposure happens by feeding on prey containing similar relative amounts of both PCBs and DDTs (Finkelstein et al., 2006). In the present study, both contaminant groups (PCBs and OCPs) were positively correlated in males (correlation coefficient: 0.55, and p-value = 0.002) and females (correlation coefficient: 0.6, and p-value = 0.001). It is important to note that correlation studies are important with respect to exposure assessment and determination of how well a measure of one specific contaminant can reflect that from another xenobiotic.

3.2. Gender and age-related differences

Comparisons among sub-groups of samples were performed (juvenile males, juvenile females, adult males and adult females). Results for male-female and juvenile-adult comparisons are shown here. It would appear that the uptake, biokinetics and response to contaminants differs significantly between male and female individuals. This may be due to differences in metabolic rates, hormonal or reproductive states and size (Burger, 2007). As observed in Fig. 2, only the levels of 4,4′-DDE statistically differed according to gender, with females showing a higher mean level than males (606.2 and 115.6 μg/kg adipose tissue, respectively). Conversely, levels of PCBs 180, 138, 101, 28 and 153 were slightly higher in males than in females; however, those differences were not statistically significant.

As shown in Fig. 3, PCBs 180, 138, 101 28 and 153, as well as the pesticide 4,4′-DDE presented significant differences associated with age ($p < 0.001$with higher levels in adult animals compared to young ones. The mean highest concentrations for adult animals corresponded to PCB 153, 4,4′-DDE, PCB 180 and PCB 138 (871.2, 770.6, 588.3 and 457.1 μg/kg adipose tissue, respectively) whereas these same mean values corresponding to young animals were markedly lower (89.9, 40.2, 60.4 and 44.2 μg/kg adipose tissue, respectively).

Significant gender differences in lipid-corrected concentrations have been observed only for DDT in liver of different Arctic seabird species. The present study showed similar results for adipose tissue, suggesting that reproduction (and more specifically egg laying) did not have a lasting effect on OC concentrations (Buckman et al., 2004). In the same way, gender was not found to have significant relevance on the concentrations of different POPs in liver of glaucous gulls sampled in Greenland (Cleemann et al., 2000), even if the concentrations found in adult males were in general higher than those quantified in adult females, and a similar result was obtained with razorbill samples from SW Spain (Espín et al., 2010). However, other studies have confirmed a gender-related influence, associated with the fact that breeding females transfer some of their tissue contaminant burden to their eggs, also suggesting that maternal transfer favors the less persistent congeners

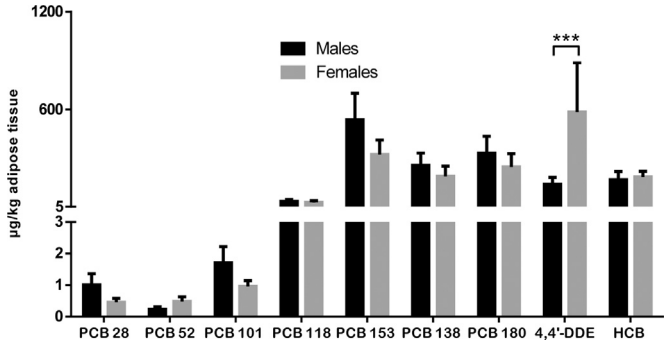

Fig. 2. Levels of chlorinated contaminants (mean ± SEM) according to gender (male vs. female). ***differences were statistically significant ($p < 0.001$) between males (n = 29) and females (n = 28).

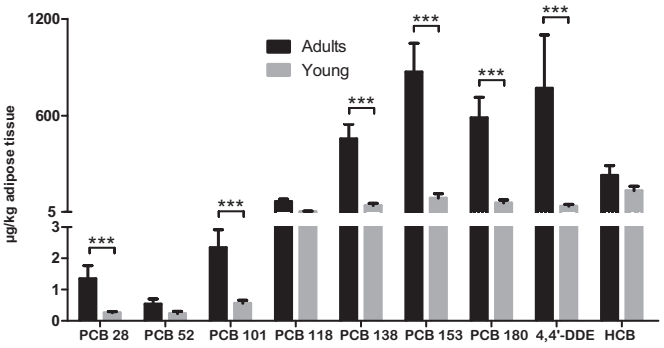

Fig. 3. Levels of chlorinated contaminants (mean ± SEM) according to age (young vs. adults). *** differences were statistically significant ($p < 0.001$) between adults (n = 32) and young (n = 25).

(Bustnes et al., 2008). This statement could not be confirmed with the results found in the present study. Although gender can clearly affect animal exposure, toxicokinetics and levels of contaminants, the effects of gender on POP bioaccumulation do seem to differ among species, populations, organs, and specific contaminant, rendering the study of gender-related differences of extreme interest. More specifically, one of the most challenging aspects of the study of gender-related effects will be in the understanding of mixtures, since living organisms in nature are not exposed to only one, two, or even three contaminants. Information from field studies, like the present study, could offer information about the similar patterns followed by different contaminant groups, which can show correlation in their accumulation. Furthermore, the time course of exposure varies, and the order of exposure may differentially affect outcomes (Burger, 2007). Erikstad et al. (2013) suggested that pollution stress might produce different hormonal responses in males and females, leading to sex-dependent differences in breeding effort. In this sense, males could redirect their behaviour towards their own survival by reducing their breeding investment, due to the higher levels of corticosterone in males than in females provoked by pollution stress. OCs may act as endocrine disrupters by mimicking steroids and binding to hormone receptors, and different hormonal responses related to breeding effort among male and female in gulls seem possible. These data suggest that further study on gender-related evaluation would be of great relevance for future biomonitoring.

When determining the effect of age, it must be considered that bioaccumulation is a key feature in any study on persistent pollutants and is directly correlated with the age of the bird. Based on bioaccumulation, it is reasonable to suppose that older birds exhibit higher concentrations of contaminants in similar diets and geographical ranges (Cipro et al., 2013). This is in accordance to the findings of the present study, where statistical differences ($p < 0.001$) were found between adults (ΣPCBs = 1990 ± 403 μg/kg, ΣOCPs = 1002 ± 340 μg/kg) and young (ΣPCBs = 139 ± 17 μg/kg, ΣOCPs = 171 ± 33 μg/kg). In fact, the majority of studies show a positive association between age and concentrations of both DDT/DDE and PCBs. The few studies that do not show a relationship generally had small sample sizes, a limited age range to allow detection of an age difference, or a low exposure population. Age may also be a marker for cohort-related changes in exposure levels, with older individuals being exposed to higher levels in the past, as well as a marker for age-related shifts in weight and metabolism. Significant differences between adult and young glaucous gulls were found in a study carried out in Greenland (Cleemann et al., 2000); all compounds analyzed, especially PCBs and DDTs, were accumulated with age, and the PCB burden in adults comprised a higher proportion of the higher chlorinated PCBs than that found in young gulls. Similarly, a significant positive relationship was found between age and OC levels in adipose tissues of razorbills from Spain, with the highest levels in adults (Espín et al., 2010). In Gyrfalcons (*Falco*

rusticolus) (Ólafsdóttir et al., 1995), the OC levels were markedly related to age, so that the PCB levels had increased about 100-fold from newly hatched chicks to an 18-months old bird. Phillips et al. (2003) showed that the total PCB concentrations and total PCB body burdens in the adult albatross were higher than those in the chicks, and similarly, there was a general increase in PCB concentrations with increasing age observed in common cormorants from Japan (Guruge et al., 2000). However, contrasting results have been reported in two different seabird species, black guillemots (*Cepphus grille*) in Iceland (Ólafsdóttir et al., 2005) and thickbilled murres *(Uria lomvia)* in Canada (Donaldson et al., 1997), with no evidence of accumulation of PCBs and DDT with age (1–9 years). Moreover, some studies have suggested that steady-state levels are reached at different ages for different chemical compounds. For OC, it was suggested that intake equals the elimination at a yearly basis and that the steady-state equilibrium is reached around the age of reproduction in birds (Erikstad et al., 2013). This is the case for PCBs, which can reach an equilibrium more rapidly than DDE. This may be related to the individual diet in seabirds, which can overshadow the effect of age, perhaps rendering the trophic level at which those animals feed of great relevance (Bustnes et al., 2003).

4. Conclusion

Results from the present study constitute a baseline to better know the environmental levels of PCB and OCP contamination, filling a gap of knowledge related to the specific accumulation of OC in adipose tissue from seabirds. Results will be useful for further environmental biomonitoring studies developed at other coastal sites. Due to the variability in results depending on the species, feeding, pollutant exposure and other conditioners, differences related to gender and age, as well as other directly related factors, must undoubtedly be considered for further biomonitoring studies.

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5. Biochemical effects of heavy metals and organochlorine compounds accumulated in different tissues of yellow-legged gulls (*Larus michahellis*).

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5.1 Resumen: Efectos bioquímicos de metals pesados y compuestos organoclororados en diferentes tejidos de gaviotas patiamarillas (*Larus michahellis).*

En el presente estudio se han determinado concentraciones de metales pesados/metaloides (Hg, Cd, Pb, Se y As) contaminantes organicos persistentes (7 PCB y 11 plaguicidas organoclorados) y biomarcadores de estrés oxidativo (CAT, GPx, GR, GSH, GST, MDA) con el fin de establecer una posible relación entre los contaminantes y los biomarcadores de estrés oxidativo en la especie de gaviota *L. michahellis*. Tanto los metales como los biomarcaodores fueron determinados en hígado y riñón, mientas que los contaminantes orgánicos persistentes fueron determinados en tejido adiposo de esta especie de gaviota. Se han estudiado tres posibles variables influyentes: edad, sexo y zona de muestreo. Unicamente como resultado se encontraron diferencias estadísticamente significativas ($P<0,05$, $P<0,01$) en la zona de muestreo, encontrándose diferencias entre las tres áreas estudiadas (A Coruña, Pontevedra y Gijón) en ambos órganos (hígado y riñón). Ademas se estudiaron posibles correlaciones signfiicativas donde se encontraron correlaciones positivas significativas ($P<0.01$) en hígado (Hg vs GST; Se vs MDA) y en riñón (As vs GR; As vs GPx; PCB52 vs CAT; PCB138 vs CAT). La escasez de correlaciones en el presente estudio sugiere que los niveles de contaminantes encontrados en los animales no eran lo suficientemente elevados como para desencadenar un efecto a nivel oxidativo.

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Biochemical Effects of Heavy Metals and Organochlorine Compounds Accumulated in Different Tissues of Yellow-Legged Gulls (*Larus Michahellis*)

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Abstract

In the present study, livers, kidneys and adipose tissue of Yellow-legged Gull (*Larus michahellis*) were collected. Samples were used to determine relationships between heavy metals/metalloids in liver and kidneys (Hg, Cd, Pb, Se and As) or persistent organic pollutants in adipose tissue (7 PCBs and 11 organochlorine pesticides) with biomarkers of oxidative stress (CAT, GPx, GR, GSH, GST, MDA) analysed in both internal organs. Three possible influencing variables have been studied: age, sex and sampling area. As a result, statistically significant differences ($P < 0.05$, $P < 0.01$) were only found according to the sampling area, with differences among the three studied areas found in both organs. Significant positive correlations ($P < 0.01$) were found in liver (Hg vs. GST; Se vs. MDA) and in kidney (As vs. GR; As vs. GPx; PCB52 vs. CAT; PCB138 vs. CAT). The scarcity in correlations suggests that the levels of pollutants found in animals were not high enough to trigger an effect at the oxidative level.

Keywords Gull · Bioaccumulation · Oxidative stress · Environmental pollution · PCB

Introduction

Metals of anthropogenic origin are very difficult to degrade and can become very toxic for living organisms (Duffus 2001**)**. Not only metals but there are other contaminants produced by human activities, such as the polychlorinated biphenyls (PCBs) which are included within the group of persistent organic pollutants (POPs). POPs are widely used chemicals of environmental concern, because of their resistance to be metabolized and potential toxicity (Ashraf 2017**)**. Both, metals and POPs, have the ability to produce reactive oxygen species (ROS), leading to oxidative stress responses. After an exposure to chemicals, in the organism it can be triggered an imbalance between the production of ROS and the antioxidant defence, leading finally to oxidative damage to biomolecules (Halliwell and Gutteridge 2007). The antioxidant defence represents an important mechanism of action to prevent, neutralize and remove the toxicants from the body (Koivula and Eeva 2010). The antioxidant machinery is primary formed by antioxidant enzymes, as endogenous molecules, that are intended to repair systems (Pamplona and Costantini 2011), and which levels have been probed as useful biomarkers in birds (Koivula and Eeva 2010). The importance of birds as bio-indicators of pollution resides in their ability to modulate their enzyme activities and detoxification systems depending on the pollution levels, and thus adapt for survival in polluted areas (Fossi et al. 1991). The alteration of the antioxidant enzymes levels in several tissues of seabirds can be used as indicative of oxidative stress, since their main function

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is catalyzing the breakdown of free radicals (Congiu et al. 2000; Ercal et al. 2001; Pinto et al. 2003; Berglund et al. 2007).

The main aim of this study is to assess the oxidative stress related to metals and PCBs in the seagull *Larus michaellis*. The origin of the animals was considered, since some samples came from population control campaigns and others were slaughtered in recovery centres (animals with no possibilities of survival). The concentrations of 5 metals (Hg, Cd, Pb, Se and As) in liver and kidney samples, and the concentrations of 18 persistent organic compounds (7 PCB congeners: PCB180, PCB52, PCB101, PCB118, PCB28, PCB153 and PCB138; and 11 organochlorine pesticides (OCP): 4,4'-DDE, 4,4'DDD, DDT, Hexachlorobenzene, Heptachlor epoxide, Endrin, Endosulfan, β-HCH, γ-HCH, endosulfan sulfate and Dieldrin) in adipose tissue were evaluated. A battery of biomarkers of oxidative stress were analysed, including antioxidant enzymes activities (GPx, GR, CAT, and GST) and products (lipid peroxidation measured as MDA and GSH), in order to establish some correlations between the pollutants concentrations and oxidative stress levels in *L. michahellis*.

Materials and Methods

Study Areas and Species

The study area is located in the north-west of Spain, in three zones, A Coruña (Galicia), Pontevedra (Galicia) and Gijón (Asturias). Two of these areas are already known by having potential problem of pollution. In Pontevedra, some studies have demonstrated the persistence of local Hg pollution (Besada et al. 1997; Beiras et al. 2002). Authors compared Hg concentrations in different coastal areas of Galicia, finding higher metal levels in the Rias of Pontevedra. Moreover, sediments and sludge produced in estuaries containing Cd as the main heavy metal makes Pontevedra one of the most polluted areas (Vizuete et al. 2022). Regarding Gijón, there is a factory that uses Pb for the manufacturing of acer, being a potential source of this metal. On the other hand, the potential contamination in A Coruña should also be considered since it houses an important fishing port.

Animals were divided into groups depending on the age: adults (n = 63), juveniles (n = 22), and chicks (n = 24) based on the colour of plumage and other physical characteristics (i.e.: adult gulls have yellow legs, yellow beak with red spot-on tip and yellow eye), while juvenile gulls have pink legs, dark beak, and eye of brown colour). Gulls were also grouped according to sex (55 males, 54 females). Both sexes are similar in plumage, although males have larger sizes compared to females. *L. michahellis* is found throughout Spain and much of Europe. This species shows adaptability in its chosen habitat. In general, it can reside in a variety of locations, such as marshes, beaches and coastal inlands. These gulls have a non-selective feeding, their diet includes fish, amphibians, molluscs, small mammals, carrion…etc. There are two main food sources: the dumps and discarded waste produced by fishing activity. Gulls have a pernicious effect on other bird colonies, a negative effect on vegetation of the cliffs and the water quality, and also generate noise problems, dirt and damage buildings. This species can be considered sedentary in most of the regions they inhabit, because they remain close to their breeding colonies over the whole year, while in other areas they trace the courses of the great rivers to enter inland (SEO 2018).

Sampling Method

Gulls were collected during the period of 2014–2016 in two regions of the north-west of Spain (Galicia and Asturias). The samples obtained have two different origins: the samples of the one group were collected in the wildlife recovery centers and they were from birds that entered there mainly because of physical injuries, provoked by electrocution or fall from the nest due to inexperience in flying. Only birds held at the rehabilitation center for less than 5 days before dying were used. The second group consisted of animals from population control campaigns duly authorized in Galician and Asturian cities, with no apparent signs or symptoms of injury or disease. During necropsy, several parameters such as mass measurements (g), organ weights (g), bill development and physical condition were registered. Age was determined based on the colour of the plumage, as there is a significant colour range to pure white adults, and 1-year-old juvenile gulls can be discerned from adult using plumage characteristics (Grant 1986). Sex was determined through observation of the gonads during necropsy. After sampling, the remains were destroyed hygienically by incineration, under current European legislation. After necropsy, all samples (from 109 animals) were immediately frozen and stored at − 20 °C until their preparation for analysis of metals and POPs, or -80 °C when tissues were used for biomarkers evaluation. Metals and biomarkers were analysed in all the livers and the kidneys (109 animals: A Coruña n = 21, Pontevedra n = 58, Gijón n = 30). OCPs and PCBs were evaluated in the adipose tissue (n = 31: A Coruña n = 10, Pontevedra n = 11, Gijón n = 10).

Liver and Kidney Metals Analysis

Hg, Cd, Pb, Se and As levels were analysed in the liver and kidneys of gulls. Briefly, 3–4 g of tissues were dried in an oven for 72 h at 65 °C. The metal levels were analyzed at

the Elemental and Molecular Analysis Laboratory of the Research Support Service (SAIUEX, accredited by ISO 9001:2008; University of Extremadura), by means of ICP-MS (Model 7900. Agilent Tech). Limit of detection (LOD) and of quantification (LOQ) were determined according to the ICH-Q2 guideline on method validation (Guideline 2005), after analyzing repeated blanks with the same procedure used for the samples, determining the standard deviation. The dilution factor and the weight of the samples were considered to calculate the final values of both parameters (LOD < 0.003 mg/kg and LOQ < 0.009 mg/kg). The coefficient of variation for replicate samples (n = 5) were lower than 5.3%. Analytical blanks were included in all the run batches of samples (Vizuete et al. 2022).

OCPs and PCBs Analysis

POPs were analysed in adipose tissue. Briefly, 0.7 g of the tissue was chopped, mixed with 7 ml of n-hexane, homogenized and frozen overnight, allowing the fat to precipitate. The supernatant was added with H_2SO_4, shaken in an orbital shaker, sonicated and centrifuged, and the acid-containing phase discarded. The resulted extract was evaporated, re-suspended in n-hexane and then used for OCPs and PCBs concentration measurements. A Bruker Scion 456 triple quadrupole gas chromatograph mass spectrometer was used to analyze the samples. To verify the suitability and performance of the procedure, the accuracy was estimated by means of recovery experiments, analyzing blank adipose tissue samples spiked at five concentrations levels of PCB and OCP mixtures. The LODs for PCBs and OCPs ranged between 0.006 and 0.079 µg/kg and 0.070–1.124 µg/kg lipid weight (lw), respectively. The LOQs were 0.159 µg/kg for PCBs and 3.2 µg/kg for OCPs. It was not possible to observe any correlation between OCPs and the assessed biomarkers, therefore, the study focused on the PCBs (CBs 28, 52, 101, 118, 138, 153, and 180). More information about can be found in Vizuete et al. (2018).

Biomarker Analyses in Liver and Kidney

Oxidative stress biomarkers (malondialdehyde (MDA), glutathione-S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT)) were analysed in the spectrophotometer (BioTek) and reduced glutathione (GSH) was analysed in the fluorometric (Synergy™ HT).

Approximately 0.5 g from each sample of liver or kidney were weighted and placed in a tube. Then, 3 ml of phosphate buffer (PBS; 0.1 M pH = 7.4) were added to carry out the homogenization. Samples were kept on ice during the process, to allow slow thawing. The homogenization was performed with a homogenizer 20HS rod (PCU Kinematica). Finally, the samples were centrifuged at 4000 rpm for 5 min (Centronic S-577). The supernatant obtained was divided into two aliquots, the first one was added with 0.1 mL of PCA at 70% and centrifuged (4000 rpm, 15 min, 4 ºC (DIGICEN 21R)) to determine the concentration of MDA and GSH. The second one was centrifuged at 12,000 rpm for 20 min, at 4 ºC to determinate the rest of the oxidative stress biomarkers. Lipid peroxidation, estimated as thiobarbituric acid-reactive substances (TBARS), was determined by the methodology described by Recknagel et al. (1982). GSH levels were evaluated following the fluorometric method reported by Hissin and Hilf (1976).

CAT activity was evaluated following the methodology described by Clairbone (1985). The GST activity was determined using the method described by Habig et al. (1974). GPx activity was evaluated following the protocol, reported by Mahondas et al. (1984) with modifications. An adaptation of the method of Cribb et al. (1989) was used to measure GR activity. Total protein contents were measured in the tissue homogenates following the Bradford (1976) method. Activity/levels were expressed in relation to grams of protein in the homogenates.

Statistical Analyses

Data was analysed using statistical software Prism 5 version 5.03 for Windows (GraphPad software, Inc., CA). Results were expressed as mean ± SEM, and the level for statistical significance was defined as $p < 0.05$. Data did not show a normal distribution and the variances were not homogeneous, thus a non-parametric Kruskal-Wallis test was applied (Zar 1984). Differences among colonies were determined with the Dunn´s test. Correlations (metals-biomarkers, PCBs-biomarkers) were evaluated by a Spearman test.

Results and Discussion

Table 1 shows metal concentrations, enzyme activities, reduced glutathione and lipid peroxidation levels in liver and kidney of *L. michahellis*. Table 2 shows the concentrations of PCBs and 4,4-DDE found in adipose tissue (compound showing concentrations < LOQ are not shown). The distribution of Cd, Pb and Se in liver and kidney were higher in kidney than in liver. This pattern has been already reported for Cd (Bianchi et al. 2008; Abdullah et al. 2015). The levels of Hg were similar in liver and in kidneys, albeit slightly higher in kidneys. Regarding the biomarkers, higher levels were found in liver than in kidneys for GST, CAT and GPx. On the other hand, levels of MDA, GSH and GR were higher in kidneys. No sex or age-related differences

Table 1 Metal concentration (mg/kg dw), lipid peroxidation levels (nmol/mg protein), reduced glutathione (nmol/mg protein), enzyme activities (nmol/min/mg protein), in liver and kidney samples of Larus michahellis

		Liver		Kidney	
Metal	N	Mean ± SEM	Median (range)	Mean ± SEM	Median (range)
Hg	109	2.95 ± 0.21	2.5 (16.39–0.39)	2.98 ± 0.18	2.62 (0.22–11.32)
Cd	109	4.13 ± 0.59	2.61 (0.11–50.9)	18.56 ± 2.46	9.25 (0.15–149.6)
Pb	109	0.55 ± 0.07	0.41 (0.03 ± 7.89)	2.50 ± 0.78	0.95 (0.07–79.81)
Se	109	7.18 ± 0.32	7.34 (0.31 ± 15.91)	10.91 ± 0.41	10.95 (0.73–23.29)
As	109	6.05 ± 0.39	5.34 (0.39 ± 23.56)	5.28 ± 0.75	3.85 (0.45-81)
Biomarker	N	Mean ± SEM	Median (range)	Mean ± SEM	Median (range)
MDA	109	1.25 ± 0.09	1 (0.1–6.29)	1.82 ± 0.12	1.41 (0.25–8.29)
GSH	109	1.13 ± 0.07	1.1 (0.02–3.83)	1.34 ± 0.11	1.03 (0.09–7.76)
GST	109	219.6 ± 10.46	200 (34.17–551.3)	199.3 ± 10.74	174 (39.44–528.9)
CAT	109	1.05 ± 0.1	0.82 (0.8–7.6)	0.35 ± 0.24	0.31 (0.01–1.28)
GR	109	0.03 ± 0.001	0.03 (0.01–0.08)	0.06 ± 0.003	0.06 (0.02 ± 0.27)
GPx	109	0.22 ± 0.02	0.2 (0.02–0.98)	0.21 ± 0.01	0.19 (0.04–0.85)

Table 2 Concentration of PCBs and OCPs (expressed in µg/kg lw) in adipose tissue samples of L. michahellis (n = 31)

PCB	N	Mean ± SEM	Median (range)
PCB 180	31	297.8 ± 91.63	78.80 (7.17–1824)
PCB 52	31	0.33 ± 0.09	0.16 (0-2.73)
PCB 101	31	1.69 ± 0.46	0.83 (0.16–11.52)
PCB 118	31	35.09 ± 10.62	9.25 (1.23–241.6)
PCB 28	31	1.12 ± 0.34	0.29 (0.07–7.16)
PCB153	31	446.8 ± 139	129.6 (14.92–2865)
PCB 138	31	209.2 ± 61.39	51.02 (8.47–1253)
4,4-DDE	31	178.1 ± 51.80	46.94 (5.7–1230)

in the biomarkers were found when they were treated in global for the 109 animals. However, some differences were found related to the sampling area. Results are shown and discussed below for each biomarker.

Malondialdehyde (MDA)

Malondialdehyde (MDA) is a byproduct derived from lipid peroxidation that gives information about oxidative impairment through the measure of TBARS (thiobarbituric acid reacting substance) levels (Pinto et al. 2003). MDA levels were lower in liver (1.25 ± 0.09 nmol/mg protein) than in kidneys (1.82 ± 0.12 nmol/mg protein). Statistically significant differences were found related to the sampling area (liver: Pontevedra > Gijón > A Coruña (Fig. 1); kidneys: Pontevedra > Gijón = A Coruña (Fig. 2)). Regarding the correlation analysis, MDA was only correlated to Se levels in the livers of gulls. This result agrees with the positive correlation found in willets (*Catoptrophorus semipalmatus*) from the San Diego, CA, USA, between hepatic Se concentration and MDA (Hoffman 2002). After a dietary exposure study in mallards, it was reported that 2.8 mg/kg of Se in liver decreased survival and growth and increased MDA concentrations (Hoffman et al. 1989). These levels of Se in the liver are lower than those found in the present study (7.18 ± 0.32 mg/kg) in *L. michahellis*. This difference could justify the positive correlation between MDA and Se (Table S1), and possible harmful effects of this element in the sampled seagulls. The relationship between metals and MDA levels has also been found for other metals. For example, Osičková et al. (2014) found a correlation between lead (Pb) and MDA in the liver of *Coturnix coturnix japonica* when they were exposed to Pb (through insertion of Pb shots (1.5 g)). In the present study, Pb levels were much lower (0.55 mg/kg), which can explain the lack of correlations and liver damage in gulls. MDA-Pb correlations were not found in kidney neither for mallards nor in the present study. The liver is a major detoxifying organ and the main source of ROS generation in birds, being the first organ showing damage (Paskova et al. 2011; Vitula et al. 2011).

Reduced Glutathione (GSH)

In metal-induced oxidative stress, glutathione metabolism has an essential role because the functional thiol group of glutathione serves as a binding site for many metals (Pinto et al. 2003). The levels of GSH (Table 1) in the liver were lower than in kidneys (1.13 ± 0.07 and 1.34 ± 0.11 nmol/mg protein, respectively). There is a diversity of results found in literature regarding this specific biomarker. In this sense, the exposure to metals has been associated to tGSH increased levels in different species of birds, such as Shaoxing ducks, mallards and starlings, whereas other studies did not find any difference on tGSH levels in birds inhabiting a contaminated area respect to the same species of a selected reference area (Koivula and Eeva 2010). In this sense, the samples of the present study did not show any correlation

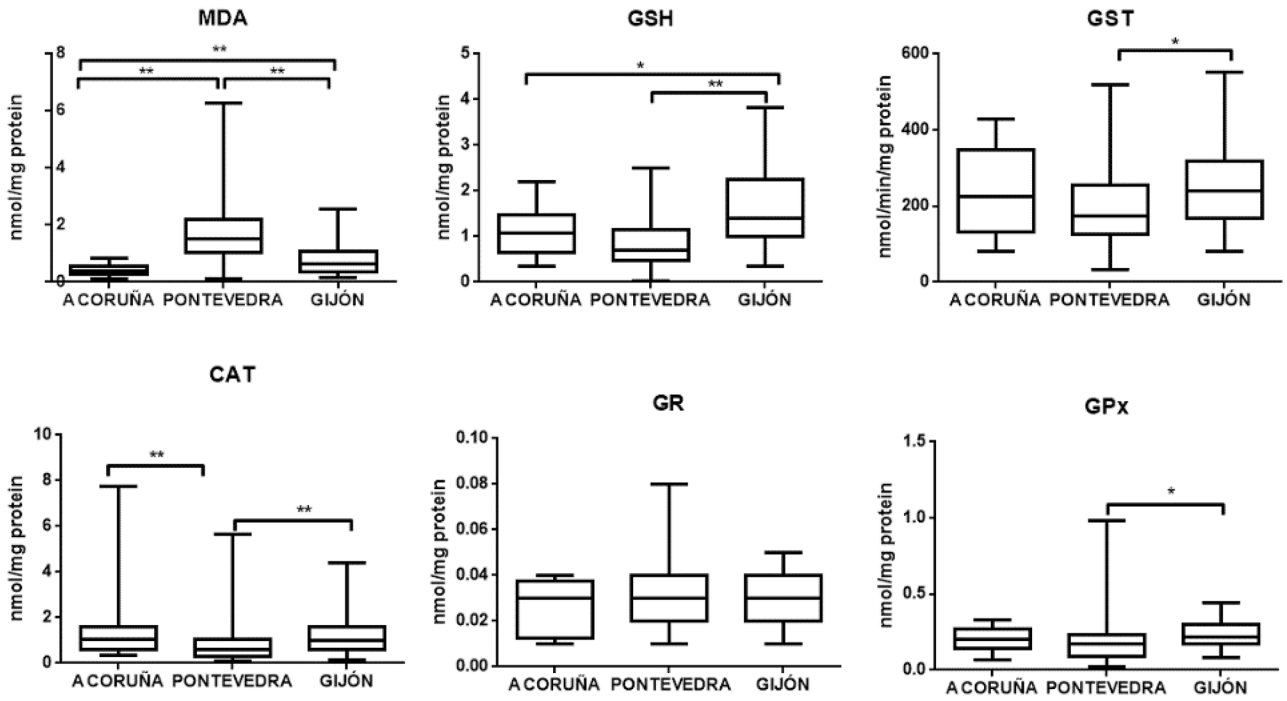

Fig. 1 MDA, GSH, GST, CAT, GR and GPx levels in liver of Yellow-legged gull from three different zones: Pontevedra (n = 21), A Coruña (n = 58) and Gijón (n = 30). Statistical significance *: $p < 0.05$; **: $p < 0.01$

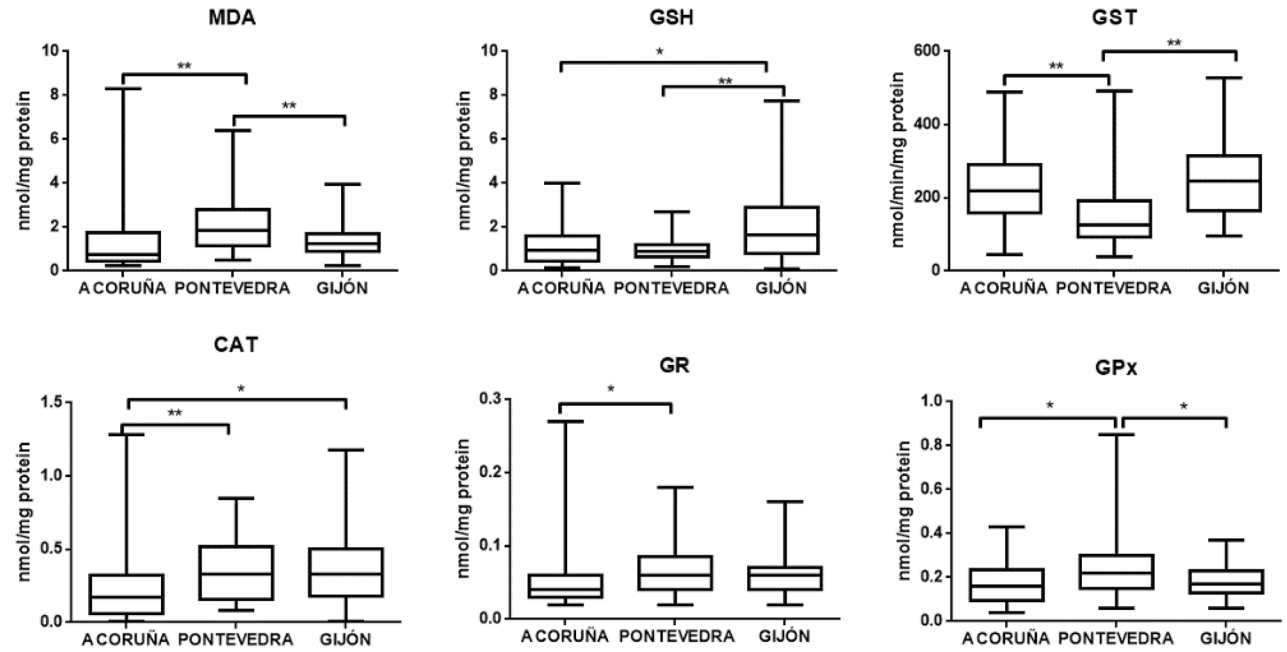

Fig. 2 MDA, GSH, GST, CAT, GR and GPx levels in kidney of Yellow-legged gulls from three different zones: Pontevedra (n = 21), A Coruña (n = 58) and Gijón (n = 30). Statistical significance *: $p < 0.05$; **: $p < 0.01$

between GSH and the studied contaminants. There were statistically significant differences in both livers and kidneys between samples from Gijón and A Coruña ($p < 0.05$) or Pontevedra ($p < 0.01$) (Figs. 1 and 2). Samples from Gijón always showed higher concentrations of metals, followed by A Coruña and, finally, Pontevedra. It was not possible to find any correlation between GSH and any of the five metals studied, neither with any of the PCBs. Moreover, Isaksson et al. (2005) did not find great variation in GSH levels between rural and urban adults of great tit (*Parus major*). They suggested as a possible explanation that the basic levels of GSH in adult birds were already high enough to accommodate the increased antioxidant defence, that is, without increasing the supply of GSH in the plasma, or simply that there is some environmental or physiological limitation in the levels of GSH that adults reach in the habitats.

As previously mentioned, in the present study it was not possible to find any correlation between GSH and any metal, however Hoffman (2002) found a positive strong correlation between Se concentration and GSH peroxidase activity in the livers of diving ducks from the San Francisco Bay, CA, USA ($r = 0.63$, $p < 0.05$), finding a negative strong correlation between hepatic Se and GSH ($r = -0.740$, $p < 0.05$). Accordingly, the present study showed a negative (not significant) correlation between GSH and Se (r=-0.16, $p = 0.09$) (Table S1).

Glutathione-S-transferase (GST). In the present study, the activity of GST was very similar in both organs (Table 1), although the levels in liver were slightly higher (219 ± 10.46 nmol/min/mg protein) than in kidneys (199.3 ± 10.74 nmol/min/mg protein). The GST activity was different depending on the sampling area. Indeed, significant differences were found in the livers and kidneys between Pontevedra and Gijón, and also in kidneys between A Coruña and Pontevedra. When the relationship between GST and the selected contaminants was studied (Table S1), only one negative correlation was found between the enzymatic activity and Hg ($p = 0.02$). Other authors have reported correlations between different enzymatic activities (GR, CAT and GST) and metals in nestling pied flycatchers living near a sulphide ore smelter in Sweden (Berglund et al. 2007). They found an increase in hepatic GR and CAT, specifically influenced by Pb and Fe, suggesting increased oxidative stress as a consequence of the polluted area, but not significantly elevated lipid peroxidation and GST. However, Mateo et al. (2003) found that mallards exposed to Pb showed increased oxidative stress by having decreased GST activity. Altogether shows a species-dependent enzymatic activity.

Catalase (CAT)

The activity of catalase was 1.05 ± 0.10 µmol/min/mg protein in livers and 0.35 ± 0.24 µmol/min/mg in kidneys, which are generally considered as low levels. However, it should be considered that GPx is the main enzyme used by *L. michahellis* and other birds to catalyse H_2O_2 (Hernández-García 2010; Koivula et al. 2011). Cd exposure has been shown to increase H_2O_2 levels in rat pituitary membrane (Pillai et al. 2002). This relationship could not be proven in the present study, since no correlations were found, maybe because Cd levels were not high enough to provoke an effect at the oxidative stress level. Only a slight negative correlation was stablished in kidneys between CAT and PCB52, with a positive correlation found with PCB138 (Table S1). These correlations did not suggest a strong impact of PCB on the oxidative stress, as was shown by Elia et al. (2005), who mentioned that variations in antioxidant response of carp seem to be linked more to biological status than to the presence of PCBs congeners in the liver. The presence of POPs in the organism is usually related to the adipose tissue, being only released in the blood torrent in specific circumstances (as starving periods or weight loss) (La Merrill et al. 2013). In the present study, the physical status of animals did not suggest this impairment and, in general, levels of POPs found in adipose tissue were not high. Thus, these can be the reasons for the lack of effects on the tested biomarkers and their correlation related to PCBs. Statistically significant sampling area-related differences were found (livers (Fig. 1): A Coruña = Gijón > Pontevedra ($p < 0.01$); kidneys (Fig. 2): Pontevedra = Gijón > A Coruña).

Glutathione Reductase (GR)

The GR activity levels obtained in the present study were 0.03 ± 0.0001 nmol/min/mg protein and 0.06 ± 0.003 nmol/min/mg protein in livers and kidneys, respectively. Hoffman et al. (2000) reported increased GR activities in goslings fed with 48%-Pb contaminated sediment (mean hepatic concentration of 6.57 ppm Pb) and they suffered from lipid peroxidation. On the contrary, Mateo and Hoffman (2001) found that young mallards and Canada geese exposed to Pb-contaminated sediments showed increased lipid peroxidation and GSH levels but there were no visible effects on GR activity. In the present study, a slight correlation was found between GR and As in kidneys (Table S1). Regarding the differences among sampling areas, for this enzymatic activity it was only observed for the kidneys between animals sampled in A Coruña and Pontevedra ($p < 0.05$).

Glutathione Peroxidase (GPx)

Both liver and kidney samples showed similar GPx activities, 0.22 ± 0.02 nmol/min/mg protein and 0.21 ± 0.01 nmol/min/mg protein, respectively. The reaction pathway followed by this enzyme involves the use of H_2O_2 as a substrate. The fact that Cd exposure increases H_20_2 levels (Pillai et al. 2002), extrapolated to birds, could explain a possible correlation between GPx activity and Cd concentration in these animals. Indeed, Espín et al. (2014) found this significant correlation in vultures in Alcoy, Spain. However, it was not possible to observe a relationship in the present study. In liver, results showed a significant difference between Pontevedra and Gijón ($p < 0.05$). In kidneys, the significant differences were found in Pontevedra with respect to Gijón and A Coruña ($p < 0.05$). As for GR levels, a slight correlation was found between *As* and GPx in the kidneys ($p < 0.05$) (Table S1).

Correlation Study Applied to Biomarkers

When the Spearman correlation test was conducted among the oxidative stress biomarkers (Table S2), some positive correlations among several biomarkers were found. In the liver, positive significant correlations were found between GSH-GST ($r = 0.41$; $p = 0.02$), GSH-CAT ($r = 0.42$; $p = 0.02$), GST-CAT ($r = 0.53$; $p = 0.002$), GST-GR ($r = 0.38$; $p = 0.03$). However, stronger correlations were found in the kidneys: MDA-GSH ($r = 0.37$; $p = 0.03$), MDA-GR ($r = 0.37$; $p = 0.04$), GSH-CAT ($r = 0.57$; $p = 0.0009$), GSH-GR ($r = 0.7$; $p = 0.00001$), GSH-GPx ($r = 0.58$; $p = 0.0007$), GST-GR ($r = 0.51$; $p = 0.0035$), GST-GPx ($r = 0.38$; $p = 0.04$), CAT-GR ($r = 0.52$; $p = 0.0026$), CAT-GPx ($r = 0.53$; $p = 0.002$), GR-GPx ($r = 0.77$; $p = 0.0000005$). These associations were expected, due to the interconnections existing among the biomarkers belonging to the oxidative stress system.

Relationships Between Factors

Once the results from all the animals were considered as a whole, they were grouped according to pairs of factors: geographical location-sex or geographical location-age. Then we obtained data for: males or females of A Coruña, Pontevedra or Gijón, and adults, juveniles, or chicks of A Coruña, Pontevedra or Gijón. Due to the low number of animals in some groups, it was not possible to apply a Principal Components Analysis, but an ANOVA was performed for each one of the biomarkers and contaminants and, furthermore, compared among them.

For the 109 samples it was possible to observe statistical differences between females and males of Pontevedra in comparison to females and males from the other two areas. In this sense, animals from Pontevedra showed higher levels of MDA and lower levels of GSH and GST (Table S3). The same results were observed when the comparison was performed between adults and juveniles of Pontevedra and adults and chicks from Gijón. The decrease in lipid peroxidation has generally been attributed to the increase in GSH, since this is the substrate for all defense mechanisms against lipid peroxidation. The increase in GSH is related to the stimulation of the detoxification mechanism (such as GST activity) (Ookhtens and Kaplowitz 1998). As mentioned, these relationships can be observed in the present study (Table S3), where the animals from Pontevedra showed higher levels of MDA (lipoperoxidation), with decreased levels of GSH and GST in relation to what was observed in the Gijón seagulls. These results could be related to a greater and more efficient detoxification mechanism in Asturian gulls than in those from Pontevedra. The differences were observed in both liver and kidney, obtaining similar results when the statistical analysis was performed on the geographic location-sex pair than when it was done taking into account the geographic location-age pair. These pairs were also applied to the metals and PCBs results, but no differences were found, so that it was not possible to establish a relationship between contaminants and the effect at the oxidative level.

Conclusion

The potential effect of pollutants in *L. michahellis* was evaluated through the analysis of several biomarkers of effect (MDA, GSH, GST, CAT, GPx and GR), considering endogenous and exogenous factors. It was observed that the sample collection area may indeed be relevant at the time of future biomonitoring studies, according to the differences found in the biomarkers analysed depending on the location where animals were sampled (Gijón, Pontevedra and A Coruña). However, in terms of age and sex, no significant differences were found in any of the biomarkers studied. In addition, after studying the possible correlations with metals and PCBs, there were few cases where statistically significant correlations were found. Thus, it was concluded that the concentration of metals and PCBs were not high enough to provoke the activation of the antioxidant system. The results of the present study highlight the need for ecotoxicological studies to obtain data on specific species over a broader range. Higher levels of metals could represent a risk to animal health, especially Hg, which was positively correlated with GST, or As that was correlated with GPx and GR.

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Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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5.3 Material complementario.

*Table S1. Correlation study between metals or PCBs and oxidative stress biomarkers. Statistical significance *: P<0.05.*

Liver		Hg	Cd	Pb	Se	As
MDA	*r*	0.13	0.1	-0.14	0.22	-0.03
	p values	0.19	0.31	0.14	0.02*	0.77
GSH	*r*	-0.13	0.09	0.08	-0.16	-0.13
	p values	0.18	0.36	0.38	0.09	0.17
GST	*r*	-0.22	0.13	-0.05	2.60E-03	0.04
	p values	0.02*	0.17	0.6	0.98	0.67
CAT	*r*	-0.17	-0.05	-0.03	-0.13	-0.18
	p values	0.08	0.6	0.8	0.18	0.06
GR	*r*	0.05	-0.01	-0.14	0.07	0.05
	p values	0.62	0.93	0.14	0.49	0.64
GPx	*r*	-0.03	-0.05	-0.06	0.11	-0.11
	p values	0.75	0.62	0.55	0.24	0.25

Kidney		Hg	Cd	Pb	Se	As
MDA	*r*	0.03	-0.05	-0.08	-0.01	0.11
	p values	0.78	0.58	0.42	0.95	0.26
GSH	*r*	-0.12	-0.17	-0.06	-0.14	0
	p values	0.2	0.08	0.54	0.14	1.00
GST	*r*	0.02	-0.16	0.05	-0.16	0.06
	p values	0.81	0.1	0.62	0.09	0.55
CAT	*r*	0.17	0.04	-0.03	-0.04	-0.02
	p values	0.08	0.64	0.77	0.67	0.82
GR	*r*	0.11	-0.09	-0.11	-0.05	-0.19
	p values	0.25	0.34	0.25	0.58	0.049*
GPx	*r*	0.09	0.03	-0.07	0.07	0.19
	p values	0.36	0.72	-0.48	0.45	0.048*

Liver		PCB180	PCB52	PCB101	PCB118	PCB28	PCB153	PCB138
MDA	*r*	0.17	-0.15	0.02	0.11	-0.08	0.15	0.22
	p values	0.37	0.41	0.94	0.55	0.66	0.41	0.23
GSH	*r*	0.21	0.08	0.25	0.32	0.32	0.3	0.3
	p values	0.26	0.65	0.18	0.07	0.08	0.1	0.1
GST	*r*	-0.15	0.13	-0.13	-0.2	-0.25	-0.17	-0.2
	p values	0.42	0.49	0.49	0.27	0.17	0.35	0.28
CAT	*r*	-0.15	0.22	0.02	-0.2	-0.11	-0.16	-0.14
	p values	0.42	0.24	0.93	0.27	0.56	0.4	0.46
GR	*r*	-0.06	0.21	0.07	-0.11	0.02	-0.07	-0.09
	p values	0.74	0.25	0.69	0.54	0.93	0.7	0.62
GPx	*r*	0.02	0.18	0.21	0.1	0.1	-0.01	0.01
	p values	0.93	0.32	0.27	0.6	0.59	0.94	0.96

Kidney		PCB180	PCB52	PCB101	PCB118	PCB28	PCB153	PCB138
MDA	*r*	0.19	0.06	0.12	0.17	0.17	0.19	0.22
	p values	0.29	0.73	0.51	0.36	0.37	0.31	0.24
GSH	*r*	0.19	-0.05	0.15	0.12	-0.12	0.14	0.17
	p values	0.3	0.79	0.44	0.74	0.53	0.46	0.36
GST	*r*	0.04	-0.2	-0.13	-0.01	-0.22	-0.04	-0.08
	p values	0.81	0.29	0.48	0.95	0.23	0.82	0.68
CAT	*r*	0.32	-0.1	0.15	0.2	-0.15	0.3	0.34
	p values	0.08	0.01*	0.42	0.28	0.41	0.1	0.01*
GR	*r*	0.07	-0.08	-0.02	-0.02	-0.14	0.05	0
	p values	0.71	0.66	0.93	0.9	0.45	0.8	0.99
GPx	*r*	0.13	-0.28	-0.15	0.02	-0.26	0.08	0.06
	p values	0.48	0.12	0.43	0.9	0.16	0.67	0.74

*Table S2. Correlation study applied to the selected biomarkers of oxidative stress in liver and kidney. Statistical significance *: p<0.05; **: p<0.01; ***: p<0.001.*

	Liver	GSH	GST	CAT	GR	GPx
MDA	***r***	0.16	0.2	0.08	0.16	0.19
	p values	0.39	0.26	0.68	0.36	0.3
GSH	***r***		0.41	0.42	0.32	0.24
	p values		0.02*	0.02*	0.08	0.19
GST	***r***			0.53	0.38	0.21
	p values			1.99E-03**	0.03*	0.25
CAT	***r***				0.06	0.28
	p values				0.74	0.13
GR	***r***					0.22
	p values					0.22

	Kidney	GSH	GST	CAT	GR	GPx
MDA	***r***	0.37	0.31	0.03	0.37	0.27
	p values	0.03*	0.09	0.86	0.04*	0.13
GSH	***r***		0.34	0.57	0.7	0.58
	p values		0.06	8.87E-04***	1.31E-05***	6.57E-04***
GST	***r***			0.15	0.51	0.38
	p values			0.41	3.51E-03**	0.04*
CAT	***r***				0.52	0.53
	p values				2.55E-03**	1.95E-03**
GR	***r***					0.77
	p values					4.74E-07***

*Table S3. Significant differences found in the selected biomarkers of oxidative stress in liver and kidney, when samples were grouped by geographical location-sex or geographical location-age. Numbers in brackets show the Mean±SEM. Statistical significance *: p<0.05; **: p<0.01; ***: p<0.001.*

Geographical location-Sex. M=Males, F= Females, AC=A Coruña, PO=Pontevedra, GI= Gijón

		Liver	
MDA (nmol/mg protein)		F-PO (1.80±0.19)	M-PO (1.72±0.21)
	F-AC (0.46±0.05)	***	**
	M-AC (0.53±0.14)	**	**
	M-GI (0.77±0.16)	**	**
GSH (nmol/mg protein)		F-PO (0.90±0.11)	M-PO (0.80±0.11)
	F-GI (2.02±0.29)	**	***
GST (nmol/min/mg protein)		F-PO (183.89±15.06)	M-PO (207.16±21.56)
	F-GI (309.25±28.14)	*	*
CAT (µmol/min/mg protein)		F-PO (0.92±0.19)	M-PO (0.68±0.09)
	M-AC (2.02±0.80)	*	**
		Kidney	
GSH (nmol/mg protein)		**F-PO (0.88±0.08)**	**M-PO (1.08±0.11)**
	M-GI (2.03±0.32)	**	
GST (nmol/min/mg protein)		**F-PO (132.21±12.89)**	**M-PO (179.10±21.30)**
	F-GI (235.90±33.00)	*	
	M-GI (283.23±29.34)	***	*

Geographical location-Age. A=Adults, J=Juveniles, C=Chicks, AC=A Coruña, PO=Pontevedra, GI= Gijón

		Liver	
MDA (nmol/mg protein)		**A-PO (1.82±0.20)**	**J-PO (1.71±0.15)**
	A-AC (0.51±0.12)	***	***
	C-AC (0.49±0.07)	***	***
	A-GI (0.87±0.18)	*	*
	C-GI (0.82±0.15)		*
GSH (nmol/mg protein)		**A-GI (1.75±0.26)**	
	J-PO (0.70±0.11)	**	
		Kidney	
GST (nmol/min/mg protein)		**A-GI (301.70±26.43)**	
	A-PO (155.94±13.57)	***	
	J-PO (160.66±29.86)	**	

6. Discusión general.

A lo largo de esta tesis se ha pretendido evaluar el estado de contaminación, actual y pasado, del ecosistema en el que habita la gaviota patiamarilla en el noroeste español. Se ha podido comprobar la existencia de niveles de contaminantes ambientales tanto de metales pesados y metaloides como de compuestos orgánicos persistentes en las aves objeto de estudio. Los niveles de contaminantes hallados en aves no son un hecho aislado, como demuestran diversos estudios que han reportado acumulación de estas sustancias en tejidos de otras especies de animales, de plantas y muestras abióticas (suelos, agua y aire) (Zeng et al., 2015; Ratiu et al., 2018; Bracey et al., 2021; Bertram et al., 2022). La preocupación por la aparición de ciertos compuestos químicos se acrecienta cuando se analiza el estado regulatorio para muchos de ellos, cuya producción y uso están prohibidos desde hace décadas. Sin embargo, la comparación de los resultados obtenidos en el presente trabajo (y otros actuales citados en las diferentes publicaciones de esta tesis) con estudios realizados hace décadas, sugieren en la mayoría de los casos una disminución de las concentraciones ambientales, aunque no su completa desaparición. Por todo ello, sigue siendo relevante y necesaria la realización de estudios de monitorización de este tipo de sustancias.

Como se comentó en la introducción, la tesis se presenta como compendio de artículos científicos. El objetivo propuesto en el primero de ellos, titulado **"Mercury (Hg), Lead (Pb), Cadmium (Cd), Selenium (Se), and Arsenic (As) in Liver, Kidney, and Feathers of Gulls: A Review"**, fue la compilación de datos procedentes de la bibliografía sobre niveles de metales y metaloides en aves marinas, fijando unos criterios que permitiesen obtener datos fiables y precisos. La recopilación de datos ha permitido realizar un análisis crítico de los mismos, y la elaboración de una fuente única de resultados de utilidad para futuros estudios de contaminación ambiental. Los estudios seleccionados dan información, no solo de la acumulación de metales en aves, sino de la evolución seguida por los estudios de biomonitorización a lo largo de los años, así como de la importancia que poseen y papel que juegan unos tejidos u otros en la acumulación y persistencia de estos contaminantes. Con esta recopilación se ha podido establecer que la mayor parte de los artículos están enfocados a la evaluación de niveles de Hg y Cd, lo que puede ser explicado por la relevancia de ambos metales desde el punto de vista toxicológico, tanto por el peligro que suponen como por la exposición a la que pueden estar sometidos los organismos vivos que habitan los entornos marinos. Las aves que se alimentan de pescado bioacumulan niveles significativos de Hg de su entorno, más concretamente MeHg. Después de la ingestión, el MeHg se deposita en los tejidos corporales, se desmetila en el hígado o el cerebro, o se depura en plumas o huevos. Este compuesto es liposoluble y puede almacenarse en tejidos ricos en lípidos (Swensson and Ulfvarson, 1968; Spalding et al., 2000; Monteiro and Furness, 2001). Al tener una larga vida media da como resultado biomagnificación a través de cadenas alimentarias (Cristol et al., 2008). Con respecto al Cd, altas concentraciones de este metal se han encontrado en diversos organismos, tanto acuáticos como terrestres, desde invertebrados (lombrices de tierra, arañas) a vertebrados (peces, mamíferos) (Chiarelli and Roccheri, 2014). Los cefalópodos son una fuente significativa de Cd, ya que son un componente relevante en la alimentación de las aves marinas (Oesterwind et al., 2023). Algo similar ocurre con los estudios donde se ha evaluado el Se, algo lógico dada la relevancia que tiene su concurrencia con el Hg. Una evaluación adecuada de los posibles efectos tóxicos del Hg en las aves no solo requiere la monitorización de los niveles de Hg en sus tejidos,

sino también los correspondientes niveles de Se (Scheuhammer, 1987). Esto es debido a que el Hg se elimina del organismo, habitualmente, gracias a la acción del Se (Manceau et al., 2021).

A pesar de la gran cantidad de estudios realizados en aves para evaluar niveles de metales, se ha constatado la escasez de aquellos en los que los 5 elementos seleccionados para el presente trabajo han sido evaluados en conjunto. Este hecho ha imposibilitado la realización de un estudio estadístico en el que se pudieran establecer correlaciones entre los diferentes metales en función del tejido analizado con suficiente fuerza como para ser extrapolable a las aves en general. Un dato interesante es la zona de muestreo, tenida en cuenta como localización geográfica. El análisis de los artículos científicos ha permitido mostrar al océano Ártico como la zona en la que más estudios se han realizado, tanto en gaviotas como en otras aves marinas. Otra zona preferencial a la hora de biomonitorizar metales en aves es el continente europeo, con numerosos estudios desarrollados en España, Italia, Polonia, Alemania o Portugal, entre otros. Sin embargo, en otros muchos lugares del mundo este tipo de estudios han sido escaso, como Australia, África y muchos países del sur del continente americano (excepto Chile y Brasil, donde se identificaron seis estudios).

La evolución en el estudio de metales ha ido acompañada de un progresivo cambio en las muestras recogidas y analizadas. En este sentido, se ha podido observar cómo, en cumplimiento de los requisitos fijados en diversas regulaciones sobre bienestar animal y uso de animales en experimentación, la captura de animales y uso de órganos internos ha cedido el paso a la toma de muestras no invasivas o no destructivas, donde se procura infligir el menor daño posible a los animales (ej. sangre o plumas). En este sentido, de todos los estudios en los que se ha utilizado la gaviota como bioindicador (37 estudios recogidos en la revisión bibliográfica), 14 fueron realizados en el siglo XX y 23 en el siglo XXI. Además, como ya se ha comentado, en los últimos años el uso de las plumas como tejido ha ido cogiendo relevancia especialmente para el Hg (29 estudios recientes frente a 18 antiguos) y Cd (21 estudios recientes y 8 estudios antiguos). Este hecho puede ser debido a que la pluma es una muestra no invasiva y ha mostrado ser idónea para la determinación tanto de Hg como de Cd. La capacidad de secuestrar metales en las plumas puede ser explicada principalmente por la composición de las mismas, formada por proteínas que llevan azufre, el cual facilita la unión del Hg y del Cd a estas proteínas (Burger 1994).

Las variaciones interespecies denotan la influencia de múltiples parámetros en el patrón de acumulación de elementos inorgánicos, como los hábitos alimenticios, el hábitat, la muda de la pluma (tasa y extensión de la muda), capacidad de excreción y/o absorción. Además, existen ciertos factores endógenos que pueden afectar también a los niveles acumulados en los tejidos, como son el sexo o la edad. En este sentido, la transferencia de metales a los huevos durante la descendencia es un factor de vital importancia que afecta la diferencia entre machos y hembras en términos de contenido de Hg (Burger and Gochfeld 1992; Burger 2007). Para entender como el factor edad puede influir en los niveles de estos contaminantes es necesario trabajar con aves de edad exactamente conocida, lo que requiere estudios de anillado a largo plazo. Durante la realización de esta revisión bibliográfica se observó una clara falta de información acerca de las diferencias en niveles de metales en función de la edad y el sexo, siendo ambos cruciales para los programas de biomonitorización.

Ante dicha falta de estudios, en los que se buscase una influencia del sexo y de la edad en patrones de acumulación, en el la segunda publicación de la presente tesis, titulado **"Heavy metals and metalloid levels in the tissues of yellow-legged gulls (*Larus michahellis*) from Spain: sex, age, and geographical location diferences"**, se determinó la concentración de Hg, Cd, Pb, Se y As en hígado, riñones y plumas de la especie de gaviota *L. michahellis*, teniendo en consideración cuatro factores que podían generar diferencias entre los grupos de aves evaluados. Dos de estos factores eran endógenos: la edad y el sexo, y otros dos exógenos: la zona de muestreo y el método de captura. En cuanto al sexo como factor influyente, en este artículo no se encontraron diferencias notables en las concentraciones hepáticas y renales de Hg, Cd, Pb, Se y As. Sin embargo, sí se confirmó la mayor acumulación de Hg en plumas de machos que en el mismo tejido en hembras. Como ha sido ya mencionado, los niveles de Hg suelen ser significativamente superiores en machos, debido a que las hembras reproductoras tienen la capacidad de depurar el Hg hacia sus huevos.

El otro factor endógeno, la edad, sí pareció influir significativamente en los individuos estudiados. Lo habitual en estos animales es que, con el paso de los años, los individuos adultos se encuentren más expuestos a estos contaminantes, obteniéndolos a través de la dieta (comida y agua), siendo por tanto mayor la acumulación de los mismos. Este hecho es frecuente en el caso del Hg, Cd y Pb en el hígado (Saeki et al., 2000; Orlowski et al., 2007; Barbieri et al., 2007; Mendes et al., 2008); sin embargo, en este artículo, la relación entre el aumento de concentraciones de metales y el incremento de edad del individuo se ha observado únicamente a nivel renal, para Hg, Cd, Pb y Se, siendo significativamente mayor en adultos que en juveniles y en pollos. Por otro lado, los individuos juveniles presentaron mayores concentraciones de Se y As en hígado que los adultos y los pollos. Las diferencias encontradas en los niveles de metales, normalmente superiores en adultos, puede ser explicada por cambios de dieta que ocurren durante la reproducción, existiendo una alimentación diferente entre adultos y jóvenes/pollos. En este sentido, la dieta principal de las gaviotas está formada principalmente por invertebrados en lugar de pescado, especialmente en pollos. Sin embargo, durante la época de cría la gaviota adulta cambia su alimentación, estando constituida principalmente por peces de mayor tamaño.

Los animales seleccionados para el presente estudio fueron muestreados en tres zonas geográficas diferentes, de cara a poder establecer si el medio específico en el que habitan las aves tiene influencia en los niveles de metales acumulados. En el caso del Hg, pudo observarse una mayor acumulación en las gaviotas procedentes de Pontevedra, frente a aquellas que habitaron en A Coruña o en Asturias. Estudios recientes desarrollados en la misma zona (Beiras et al., 2002) han mostrado también niveles de Hg superiores en la ría de Pontevedra al ser comparados con otras zonas de Galicia, siendo un estudio relevante, dado que el metal fue evaluado en sedimentos de distintas rías gallegas. Los niveles de Se mostraron el mismo comportamiento que el Hg, siendo los niveles superiores en los animales que procedieron de Pontevedra. Este patrón fue también observado en el caso del Cd acumulado en riñón. El hecho de que existan diferencias en el riñón y no el hígado puede deberse la preferente acumulación del metal en dicho órgano. Sin embargo, en Gijón los niveles de Pb fueron más elevados que en las otras dos zonas (A Coruña, Pontevedra), hecho que puede ser debido a que en dicha zona se encuentra una fábrica que utiliza Pb para la fabricación del acero.

Por último, tras estudiar el método de captura como potencial factor influyente en la acumulación de metales, los resultados mostraron diferentes valores dependiendo del tejido. En el hígado, se observaron niveles más bajos de Hg y Se en las aves capturadas durante las campañas de control de poblaciones que en las aves que murieron en los centros de recuperación. En el riñón, únicamente se encontraron diferencias en la concentración de As, siendo mayor en los animales de los centros de recuperación. Sin embargo, el contenido de metal en las plumas siempre fue mayor en los animales capturados en campañas de control, encontrando diferencias estadísticamente significativas en cuatro de los cinco elementos estudiados (Hg, Cd, Pb and Se). El mayor tiempo pasado en libertad puede haber sido el motivo para estos mayores niveles de metales acumulados en plumas.

De igual forma que se hizo para los metales y metaloides, en la publicación 3 de la presente tesis, titulado **"Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (*Larus michahellis*) from Spain: role of gender and age"**, se determinaron las concentraciones de contaminantes orgánicos persistentes acumulados en el tejido adiposo de las gaviotas, incluyendo en este grupo tanto policlorobifenilos (PCBs) como pesticidas organoclorados. Los factores sexo y edad fueron tenidos en consideración al analizar los datos. Los resultados obtenidos pusieron de manifiesto la persistencia de todos los compuestos analizados, dado que todos ellos fueron identificados y cuantificados.

En referencia al sexo, tan solo para el 4,4'-DDE se identificó una diferencia estadísticamente significativa, siendo superiores los niveles encontrados en hembras. Hay estudios que confirman el hecho de que las hembras reproductoras transfieren parte de su carga contaminante del tejido a sus huevos (Bustnes et al., 2008), lo que también sugiere que la transferencia materna favorece a los congéneres menos persistentes, sin embargo, este hecho no pudo confirmarse con los datos obtenidos en el presente estudio.

Por otro lado, como era de esperar, la edad sí fue un factor determinante en la acumulación de este tipo de sustancias. De hecho, los niveles del PCB 180, 138, 101 28 y 153, así como el plaguicida 4,4'-DDE presentaron diferencias significativas asociadas a edad, con niveles más altos en animales adultos en comparación con animales jóvenes. Esto se debe a que la bioacumulación de los contaminantes se encuentra directamente relaciones con la edad de los individuos estudiados, es razonable suponer que aves mayores exhiben concentraciones más altas de los contaminantes siempre que la dieta y el hábitat sean el mismo.

Una vez identificados los diferentes contaminantes acumulados en las gaviotas, fue el momento de evaluar posibles efectos a nivel subletal, dado que en ningún caso se pudo asociar la muerte de los animales con los niveles de contaminantes internalizados. Por ello, en la cuarta publicación, titulado **"Biochemical effects of heavy metals and organochlorine compounds accumulated in different tissues of yellow-legged gulls (*Larus michahellis*)"**, se evaluaron diversos biomarcadores de efecto a nivel de estrés oxidativo. Los resultados fueron comparados con los niveles de contaminantes obtenidos previamente (publicación 2 y publicación 3). Al aplicar el estudio estadístico de correlaciones entre los biomarcadores (resultados en hígado y en riñón) con las concentraciones de metales y metaloides, fueron pocas las asociaciones que pudieron establecerse. De hecho, tan solo se observaron las siguientes correlaciones: en el hígado se observaron una correlación negativa entre el Hg y GST y una correlación positiva entre

MDA y Se; mientras que en el riñón se encontraron dos correlaciones positivas significativas (As vs GR y As vs GPx).

En cuanto las correlaciones con los biomarcadores y los contaminantes orgánicos persistentes tras realizar el estudio de correlaciones solamente se encontró una correlación estadísticamente significativa entre la concentración de la enzima Catalasa en el riñón con el congénere PCB 52.

La escasez de correlaciones entre las enzimas de estrés oxidativo y las contaminantes estudiados hace pensar que los niveles de estos contaminantes no son lo suficientemente elevados como para haber podido producir daño oxidativo en las muestras analizadas. Sin embargo, en un estudio reciente llevado a cabo por Bjedov et al. (2023) en sangre de polluelos de cigüeña blanca en Croacia, donde se analizaron metales pesados y metaloides y su posible interacción con biomarcadores, sí se pudieron observar cambios en la respuesta de multibiomarcadores con respecto a diferentes áreas contaminadas. Es por ello necesario seguir investigando en diferentes especies y tejidos/tipos de muestras la bioacumulación de contaminantes y los potenciales efectos subletales de las concentraciones internalizadas en los animales, teniendo en consideración los hábitats donde estas especies habiten y/o se reproduzcan.

En general, al considerar los diferentes factores potencialmente asociables a la acumulación de contaminantes, se ha constatado que el sexo no influye a este nivel. Sin embargo, el factor edad y la zona de muestreo sí han mostrado una relación con los niveles encontrados de contaminantes. En ambos casos, esta relación está justificada. Por un lado, una mayor longevidad está asociada a una exposición más prolongada a los contaminantes, ya sea a través de la dieta, o por contacto con los diferentes compartimentos (suelo, agua o aire). Íntimamente ligado a lo anterior, los niveles de contaminantes hallados en el ecosistema (entendido como porciones abióticas) darán lugar a una mayor o menor exposición por parte de los animales que en ellos habitan.

Por todo ello, la presente tesis, además de corroborar que los estudios de biomonitorización siguen siendo relevantes, confirma la necesidad de incluir diversos factores, tanto endógenos como exógenos, cuando se analicen los datos obtenidos de los contaminantes.

Además, parece que los sistemas regulatorios, que a lo largo de los últimos años han ido generando prohibiciones en referencia al uso de múltiples contaminantes, han surtido efecto, y los niveles que se van encontrando en el medio ambiente, aunque existentes, parecen no tener un efecto nocivo sobre la salud de los animales. No obstante, aún sigue siendo necesario complementar estos estudios con otros relacionados más directamente con la salud de los ecosistemas, en los que se analicen muestras de otras especies animales e incluso en tejidos de seres humanos.

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7. Conclusiones generales.

1. La revisión bibliográfica realizada representa una base de datos completa de los metales más importantes que se pueden encontrar en el medio ambiente en diferentes tejidos de aves acuáticas y las concentraciones a las que han sido hallados en diferentes lugares del mundo. Esta revisión supone un estudio comparativo de los niveles de metales acumulados en el hígado, los riñones y las plumas de las gaviotas y de una gran variedad de especies de aves marinas. En ella se ha comprobado que las áreas mayormente estudiadas comprenden el Océano Ártico y Europa, mientras que Australia y África son áreas muy poco biomonitorizadas. Además, se ha detectado una falta de estudios en los que se determine más de un metal utilizando más de un tejido, lo que dificulta una visión global sobre el efecto real de la contaminación ambiental en el organismo estudiado.

2. Los resultados de la evaluación de la acumulación de Hg, Cd, Pb, Se y As en el hígado, riñón y plumas de *Larus michahellis*, procedentes de 3 áreas geográficas diferentes (Pontevedra, A Coruña y Gijón), evidencian que los tres tejidos son muestras adecuadas para obtener información sobre los niveles de contaminación por metales pesados y su bioacumulación a través de la cadena alimentaria en zonas próximas a la costa.

3. Las concentraciones de metales detectadas durante el presente estudio se encuentran dentro o por debajo del rango de niveles encontrados para otras gaviotas. Si bien, los niveles de metales internalizados no provocaron la letalidad de los animales seleccionados, su identificación en los tejidos denota exposición a través del medio ambiente. De hecho, se confirmó que el hígado, los riñones y las plumas de *L. michahellis* pueden revelar contaminación local alrededor de los sitios de alimentación y reproducción.

4. Los resultados obtenidos tras la determinación de los contaminantes orgánicos persistentes permiten conocer con más profundidad los niveles ambientales de contaminación por PCBs y OCPs, contribuyendo a llenar un vacío de conocimiento relacionado con la acumulación específica de OCs en el tejido adiposo de las aves marinas.

5. El efecto potencial de los contaminantes en *L. michahellis* se evaluó mediante el análisis de varios biomarcadores de efecto (MDA, GSH, GST, CAT, GPx y GR), considerando factores endógenos y exógenos. Atendiendo a las diferencias encontradas en los biomarcadores analizados en función de la localidad donde se muestrearon los animales (Pontevedra, A Coruña y Gijón), se observó que la zona de recogida de muestras puede ser relevante a la hora de realizar futuros estudios de biomonitorización.

6. Tras estudiar las posibles correlaciones de los biomarcadores analizados con los metales y los PCBs hallados en los animales, hubo pocos casos en los que se encontraron correlaciones estadísticamente significativas. Por lo tanto, se concluye que las concentraciones de metales y PCBs acumulados en las gaviotas no eran lo suficientemente altas como para provocar la activación del sistema antioxidante.

7. Los resultados obtenidos en la presente tesis doctoral, en conjunto, sugieren que la especie estudiada es de gran interés como instrumento para evaluar la contaminación por metales pesados y contaminantes orgánicos persistentes, no solo después de un desastre medioambiental puntual, sino también para estudios de monitorización de rutina, tanto en áreas costeras como continentales. Por lo tanto, *Larus michahellis* se confirma como una especie centinela útil para el control de la salud ambiental, proporcionando una advertencia temprana de una posible exposición para los humanos y otros animales que habitan en los mismos ecosistemas y, a menudo, ingieren los mismos alimentos.

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