Universidade de Aveiro
2021

TELMO ALEXANDRE FERREIRA CORREIA

ELIMINAÇÃO DE MERCÚRIO POR AVES LIMÍCOLAS EM ZONAS HÚMIDAS TEMPERADAS E TROPICAIS

MERCURY ELIMINATION BY SHOREBIRDS USING TEMPERATE AND TROPICAL WETLANDS

Universidade de Aveiro
2021

TELMO ALEXANDRE FERREIRA CORREIA

ELIMINAÇÃO DE MERCÚRIO POR AVES LIMÍCOLAS EM ZONAS HÚMIDAS TEMPERADAS E TROPICAIS

MERCURY ELIMINATION BY SHOREBIRDS USING TEMPERATE AND TROPICAL WETLANDS

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor José Augusto Alves, Investigador Auxiliar do Departamento de Biologia e CESAM da Universidade de Aveiro, e coorientação da Professora Doutora Susana Patrícia Mendes Loureiro, Professora Auxiliar com Agregação do Departamento de Biologia e CESAM da Universidade de Aveiro.

o júri

Presidente	Professora Doutora Maria Helena Abreu Silva Professora auxiliar da Universidade de Aveiro
Arguente	Doutor Pedro Miguel Araújo Investigador da Faculdade de Ciências da Universidade de Coimbra
Orientador	Doutor José Augusto Alves Investigador Auxiliar da Universidade de Aveiro

agradecimentos

Quero agradecer antes demais aos meus avós que possibilitaram que eu prosseguisse os estudos até hoje, por me incentivarem sempre a dar o melhor de mim de forma a alcançar os meus objetivos e acreditar em mim mesmo. Quero agradecer também à restante família que embora não esteja presente em Portugal sempre me apoiou. Vocês são tudo para mim!

Um grande obrigado ao Professor José Alves por ter sido o meu orientador na tese, por mostrar-se sempre disponível e sobretudo por ter sido muito paciente e compreensível comigo. Quero que saiba que o admiro muito pelo seu trabalho e agradeço todas oportunidades que foi oferecendo e pelo conhecimento que transmitiu.

Obrigado Professora Susana Loureiro por ser coorientadora desta tese, me ter integrado no laboratório e por estar sempre disponível a resolver qualquer problema.

Quero agradecer também à Cátia Santos por me ter ensinado a utilizar o leitor de mercúrio bem como outras técnicas laboratoriais e por me ter aturado quando surgiam problemas com o leitor.

Ritinha, minha companheira de comboio, de laboratório, do funk. Obrigado pelos bons momentos que proporcionaste e pela companhia no laboratório durante toda a semana inclusive aos fins de semana. Vou ter saudades!

Quero agradecer também ao Eduardo pela companhia e ajuda durante o trabalho de campo.

Um obrigado muito especial ao Bruno por me ter sido um pilar ao longo de todo o meu percurso académico e por me ter chamado à atenção inúmeras vezes quando mais precisei.

Um grande obrigado a todos os meus amigos desde os Tropeteiros aos Amados assim como os Hakuna Matata com quem convivo desde o início da licenciatura ou inclusive antes. Posso dizer que tenho os melhores amigos. Apoiaram-me sempre, especialmente numa das fases mais difíceis, e fizeram com que eu acreditasse em mim mesmo e não desistisse.

Não posso deixar de agradecer a todas as pessoas que estiveram envolvidas nas diversas reparações do leitor de mercúrio que foram feitas ao longo do ano. Foi um pesadelo e dos grandes!

Por fim, mas não menos importante, obrigado ao Grupo de Anilhagem do Estuário do Tejo,ao Afonso Rocha e todas as outras pessoas que recolheram amostras provenientes do Arquipélago dos Bijagós e do Estuário do Tejo.

palavras-chave Poluição, Bioacumulação, Penas, Cascas de Ovos, Fezes, Estuário do Tejo, Arquipélago dos Bijagós, Salinas.

resumo

A poluição por mercúrio é um problema global devido ao transporte atmosférico e á sua bioacumulação pelos organismos, resultando em efeitos adversos na vida selvagem. As aves limícolas migratórias exploram diversas zonas húmidas em diferentes latitudes onde a poluição por mercúrio pode ser distinta. Pouco se sabe acerca dos níveis de mercúrio nas aves limícolas em regiões tropicais. Neste estudo determinaram-se os níveis de mercúrio em penas recém desenvolvidas de aves limícolas invernantes em duas importantes zonas húmidas integradas na Rota Migratória Este Atlântico: Arquipélago dos Bijagós (tropical; *Calidris alba, Calidris canutus, Charadrius hiaticula, Tringa totanus, Actitis hypoleucos, Arenaria interpres, Calidris ferrugínea* and *Pluvialis squatarola*) e Estuário do Tejo (temperado; *Calidris alba, Calidris canutus, Charadrius hiaticula, Tringa totanus, Calidris alpina* and *Limosa limosa*). Avaliaram-se também os níveis de mercúrio nas cascas de ovos de *Charadrius alexandrinus* e *Himantopus himantopus*, nidificantes nas salinas da Ria de Aveiro e do Estuário do Tejo, e nas fezes da última espécie referida recolhidas maioritariamente fora da época de nidificação. Os níveis de mercúrio nas penas e nas cascas de ovos variaram significativamente entre espécies e entre zonas húmidas. Os resultados mostraram que de uma forma geral, as aves limícolas invernantes no Estuário do Tejo apresentaram concentrações de mercúrio mais elevadas (2.106±0.681 – 9.918±3.499 ppm) do que as do Arquipélago dos Bijagós (0.932±0.369 – 2.773±0.784 ppm), sugerindo que a exposição é maior nessa zona húmida temperada. As cascas de ovos de *Charadrius alexandrinus* apresentaram concentrações mais elevadas de mercúrio, em ambas as zonas húmidas portuguesas (0.034±0.006 e 0.037±0.006 ppm), do que as de *Himantopus himantopus* (0.018±0.004 e 0.009±0.002 ppm). As fezes de *Himantopus himantopus* continham mercúrio detetável, mas as concentrações médias (0.040±0.003 – 0.085±0.025 ppm) não variaram ao longo dá época. As variações observadas neste estudo foram provavelmente causadas por diferenças espaciais na poluição por mercúrio entre as zonas húmidas estudadas e também por diferenças interespecíficas nos hábitos tróficos das espécies. As concentrações de mercúrio nas penas estiveram genericamente abaixo do nível de efeito do limiar de toxicidade (5ppm), com duas exceções assinaláveis: *Tringa totanus* e *Calidris alba* do Estuário do Tejo.

keywords

Pollution, Bioaccumulation, Feathers, Eggshells, Faeces, Tagus Estuary, Bijagós Archipelago, Saltpans.

abstract

Mercury pollution is a global issue due to atmospheric transport and its bioaccumulation by organisms, resulting in several adverse effects on wildlife. Migratory shorebirds use several wetlands at different latitudes where mercury pollution is likely to differ. Little is currently known about mercury levels in shorebirds in tropical regions. In this study, we assessed mercury levels in freshly grown feathers of several shorebirds species wintering in two important wetlands of East Atlantic Flyway: Bijagós Archipelago (tropical; *Calidris alba, Calidris canutus, Charadrius hiaticula, Tringa totanus, Actitis hypoleucos, Arenaria interpres, Calidris ferruginea* and *Pluvialis squatarola*) and Tagus Estuary (temperate; *Calidris alba, Calidris canutus, Charadrius hiaticula, Tringa totanus, Calidris alpina* and *Limosa limosa*). We also assess mercury levels of breeding species, by analysing eggshells of *Charadrius alexandrinus* and *Himantopus himantopus*, collected in saltpans of the Ria de Aveiro and of the Tagus Estuary, and in the faeces of the last species collected mostly throughout the non-breeding season. Mercury levels in feathers and eggshells varied significantly among species and wetlands. The results showed that, in general, shorebirds wintering in Tagus Estuary had higher mercury concentrations (2.106±0.681 – 9.918±3.499 ppm) than those from Bijagós Archipelago (0.932±0.369 – 2.773±0.784 ppm), suggesting that mercury exposure is higher in this temperate wetland. *Charadrius alexandrinus* eggshells had higher concentrations in both portuguese wetlands (0.034±0.006 e 0.037±0.006 ppm) than *Himantopus himantopus* ones (0.018±0.004 e 0.009±0.002 ppm). The *Himantopus himantopus* faeces contained detectable mercury but the mean concentrations (0.040±0.003 – 0.085±0.025 ppm) did not vary over the season. The differences observed in this study were likely caused by spatial differences in mercury pollution between wetlands and by interspecific differences on trophic habits of species. Overall, mean mercury levels in feather were below the toxicity threshold effect level (5ppm), with two notable exceptions: *Tringa totanus* and *Calidris alba* from Tagus Estuary.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1) INTRODUCTION

Over the past two centuries, anthropogenic activities have contributed to the increase in emissions of pollutants, such as mercury, threatening the health and state of ecosystems and wildlife (Wolf *et al*., 1998; Driscoll *et al*., 2013). Mercury (Hg) is a toxic heavy metal and a well-known environmental contaminant present in the environment in different oxidation states (Hg (0), Hg (I) and Hg (II)) and across a wide geographical range (Wiener *et al*., 2003). This heavy metal is emitted into the environment essentially through anthropogenic sources (waste incineration, fossil fuel combustion, industrial processes, artisanal and small-scale gold mining), although it can also be released through wildfires, permafrost, activity volcanic, wetlands, among other natural sources (Wiener *et al*., 2003; Driscoll *et al*., 2013). In the atmosphere, elemental mercury, Hg(0), has a high residence time, around 1 year, allowing it to be transported over long distances by atmospheric movements and deposited in remote and distant areas far from its emission source (Driscoll *et al*., 2013).

Wetlands are very productive ecosystems supporting high levels of biodiversity, but they also act as mercury sources and sinks (Tjerngren *et al*., 2012). In these and other aquatic ecosystems, the inorganic mercury deposited in anoxic sediments is converted by sulphate-reducing bacteria into methylmercury, which is the most toxic and bioavailable organic form in the environment (Wiener *et al*., 2003; Tjerngren *et al*., 2012). Methylmercury is a very hazardous substance with very slow environmental degradation. Furthermore, it enters the food chain mainly through the ingestion of food, being highly bioaccumulated across organisms and biomagnified along the trophic chain, with organisms of higher trophic levels usually displaying higher concentrations of mercury than those of lower levels (Wiener *et al*., 2003; Lavoie *et al*., 2013). Given the large accumulation of mercury due to runoff, and the high production of methylmercury in wetlands the organisms that occur in these habitats are very vulnerable to the exposure of mercury and its associated effects.

In birds, mercury accumulation can lead to several adverse and sublethal effects including reduced reproductive success, neurological effects, behavioural changes, physiological effects, and dysfunction of the endocrine and immune systems (Wolf *et al*., 1998; Seewagen, 2010; Whitney and Cristol, 2017). Some of these effects might cause a reduction in the bird's body condition and fitness. Although mercury's impacts on migration are still poorly described, it may interfere in several important aspects of migration, such as navigation (e.g., effect on the brain regions that process geomagnetic information), flight endurance (e.g., reduced haemoglobin production limits mitochondrial energy production), refuelling in stopover (e.g., motor skill impairment reduces foraging efficiency and the inactivation of enzymes involved in lipogenesis reduces fattening rate) and oxidative balance (e.g., high production of reactive oxygen species) (Seewagen, 2018). These can lead to carry-over effects such as reduced body condition, delayed arrival to breeding/wintering grounds as well reduced access to high quality habitats and mates (Seewagen, 2018). Despite being mostly documented in non-migratory species, such effects provide some evidence that the accumulation of this heavy metal may possibly impair migratory performance by compromising its success and even affect the bird's survival (Seewagen, 2018; Seewagen *et al*., 2019).

Shorebirds (also called waders) are very vulnerable to mercury accumulation as they inhabit a wide variety of wetlands, feed on invertebrates that tend to accumulate mercury, and occupy intermediate/high trophic levels in the trophic chain. Furthermore, they are long-distance migrants using several stopovers in wetlands along the migration routes between breeding and wintering areas which, make them very susceptible to experience different levels of mercury pollution (Lucia *et al*., 2014; Perkins *et al*., 2016; Su *et al*., 2020). The high food consuption in preparation for migration, both at winter and stopover site promote the ingestion, and consequently accumulation of large amounts of mercury. Along with other threats specially habitat loss, the exposure to contaminants, including heavy metals, may play an important role in the decline of shorebirds populations (Hargreaves *et al*., 2010; Sutherland *et al*., 2012; Burger *et al*., 2018). Given their conservation status, wide distribution, their known molt and migratory patterns, and also for being sensible to environmental changes, shorebirds have been used as bioindicators in several studies and biomonitoring programs (Burger *et al*., 2018).

As most organisms, shorebirds become exposed to mercury during feeding through the ingestion of food items, water, and sediment particles. Inhalation, direct contact with dust and atmospheric deposition in the bird's body are other possible routes of contamination (Burger and Gochfeld, 2001). Once ingested mercury integrates the bloodstream and is distributed to different internal organs (e.g., kidneys, muscle, and liver), where it accumulates over time. It can also be allocated to other tissues (specifically feathers, beak, claws), throughout which it can be eliminated (Furness, 1993; Agusa *et al*., 2005; Grajewska *et al*., 2019). Several physiological and biological aspects such as age, moult patterns, body size, food habits, and sex can influence the exposure, accumulation of mercury in birds (Honda *et al*., 1986; Eagles-Smith *et al*., 2009; Hartman *et al*., 2017). According to some authors, certain species of Procellariiformes are even capable of demethylating mercury into less toxic forms in the liver (Thompson *et al.,* 1993).

Besides the aforementioned pathways for mercury excretion, females can also eliminate it through eggs. Eggs, feathers, faeces, and other tissues (e.g., blood) are excellent biomonitors of environmental contamination as these can be used as a biological matrix to access mercury levels in the organisms (Burger 1993; Furness 1993). Quantifying mercury levels in these different tissues provides information about exposure at a certain time, i.e., when the tissue was metabolically active, however, for a correct interpretation it is important to understand some aspects of the bird's biology and ecology such as its moulting patterns, diet, habitat use, etc (Burger, 1993; Furness, 1993).

Feathers have been used extensively in the assessment of heavy metals levels in birds. Its use is very advantageous for several reasons: 1) it is a non-invasive method, thus avoiding harming the individual; 2) feathers are easy to collect and store; 3) allow to carry out long-term studies; 4) have a significant and stable amount of mercury and sometimes highly correlated with internal organs (Burger, 1993; Rutkowska, 2018). Feathers are the main pathway of elimination and sequestration of mercury in birds, accumulating 70 to 90% of the bird's body load. This large proportion is essentially due to the great affinity of methylmercury with the sulfhydryl groups of the keratin protein, which is the main constituent of feathers (Honda *et al*., 1986; Braune and Gaskin, 1987). Mercury levels in feathers reflect the exposure and body burden during the period of moult (Burger, 1993; Dauwe *et al*., 2003). If moulting schedule and location of moult are known, feathers will provide information about the contamination in the moulting area. In shorebirds, two moult periods per year occur: a pre-breeding moult (body feathers moult); and a post-breeding moult (body and flight feathers). Both occur mainly in the wintering areas, although some birds may occasionally start moulting before post-nuptial migration (Newton 2009). During moult, the growing feathers are irrigated via functional blood vessels that allow the mobilization of mercury accumulated since last moult, from the blood and internal organs into the feathers (Furness *et al*., 1986; Burger, 1993). As the mercury is transferred to the feathers, the internal loads decrease. When the feather is fully developed, it becomes metabolically inert and endogenous accumulation of contaminants is stopped due to the atrophy of the small vessels and the consequent interruption of the blood flow (Burger, 1993; Dauwe *et al*., 2003). From that moment, mercury concentrations in feathers remain stable thus reflecting blood levels during the period of feather growth. After completing moult any increase in mercury levels that may occur in feathers, is the result of external contamination (e.g., Atmospheric deposition) (Braune and Gaskin, 1987; Dauwe *et al*., 2003). Individual feather concentrations can be influenced by the moulting sequence as the first grown feathers tend to have a greater load and may better reflect the levels accumulated in the internal organs between moults (Furness *et al*., 1986; Lewis and Furness, 1991; Dauwe *et al*., 2003).

Eggs are also an important excretory route of contaminants for females, and both contents and shells of eggs are very useful to assess the maternal mercury burden (Furness, 1993; Agusa *et al*., 2005; Kenney *et al*., 2018). The eggs of migratory shorebirds are formed essentially with nutrients obtained in the nesting territories (Klassen *et al*., 2001; Morrisson and Hobson, 2004). During egg formation, mercury is mobilized to the different components of the egg (i.e., yolk, albumen, eggshells and membranes). Therefore, the concentrations of mercury present in the egg reflect the maternal blood levels and consequently, recent dietary and local exposure days before laying the egg (Furness, 1993; Brasso *et al*., 2012; Peterson *et al*., 2017). Lewis *et al*., 1993 estimated that female might eliminate 20% more mercury through eggs than males, explaining in some cases the differences between sexes, in which males have higher concentrations of mercury than females. The use of eggshells for assessing mercury levels instead of egg content brings some advantages, as it is a non-invasive method that has no impact on reproduction and is easy to collect and store.

Although faeces are used to a much lesser extent than eggshell and feathers, they are also a excretation pathway of heavy metals, including mercury (Lewis and Furness, 1991). Faeces are a very sensitive indicator to environmental contamination as are diretly linked to food sources, beeing therefore a good biomonitor of pollution. Mercury concentrations in faeces reflet essentially the levels in non absorved food items but can also have some mercury previously accumulated in body tissues (Lewis and Furness, 1991; Dauwe *et al*., 2004; Kenney *et al*., 2018).

The assessment of mercury levels in shorebirds and other birds has been carried out in several regions of the globe. However, most were performed in wintering and breeding populations from temperate wetlands and polar regions of the Northern Hemisphere (Burger, 1993; Seewagen, 2010). Little is known about the exposure of shorebirds that winter in tropical wetlands, where there is little environmental supervision, and emissions are thought to have increased (Seewagen, 2010). Tagus Estuary and Bijagós Archipelago are two important temperate and tropical wetlands respectively, for migratory shorebirds as wintering grounds and stopovers. High mercury levels have been recorded in Tagus Estuary, which has a strong anthropogenic influence, and therefore shorebirds might be very vulnerable to mercury contamination (Figueres *et al*., 1985; Cesário *et al*., 2016). Despite of the low human pression and pollution in Bijagós Archipelago there is no information, to our knowledge, about mercury levels in shorebirds wintering in this tropical region. Thus, it is crucial and urgent to investigate the levels of mercury in both wetlands to know the risk that birds and other organisms may be.

The main objectives of this work were to: a) determine and compare the levels of mercury in the freshly grown feathers of shorebirds wintering in the Tagus Estuary and the Bijagós Archipelago; b) investigate and compare the mercury levels eliminated through eggshells of *Himantopus himantopus* and *Charadrius alexandrinus* nesting in saltpans of the Tagus Estuary and the Ria de Aveiro; c) explore the detection capacity and seasonal variation of mercury levels excreted by *H. himantopus* in faeces during winter and breeding season.

Given the high industrial activity, and the consequent high emissions to the environment, in temperate developed zones, we expect shorebirds populations in the Tagus Estuary to show higher mercury concentrations in their feathers than those of the Bijagós Archipelago, in tropical West Africa. We aim to test the null hypothesis that there are no differences on mercury levels in shorebirds between these wetlands. For shorebirds breeding in temperate wetlands, their diet and foraging locations are likely to influence mercury load. Birds that feed more on the aquatic environment and have a diet based on polychaetes, molluscs and other benthonic invertebrates tend to have higher mercury levels than birds that feed on more inland sources and may also feed on plant material. Since there may be interspecific differences in diet and feeding areas, we expect differences in mercury concentrations between shorebirds species and test the null hypothesis that mercury concentrations do not vary among species either for eggshells (breeding season) and feathers (non-breeding season). Birds excrete most mercury during seasonal events such as moult and reproduction through eggs. Thus, we expect seasonal variation in the mercury concentrations in faeces, particularly before moult and egg formation which may have higher levels than during or recently after these periods. Therefore, we tested the null hypothesis that there is no seasonal variation in mercury concentrations in *H. himantopus* faeces.

2) MATERIAL AND METHODS

2.1) Study areas

This study was carried out with samples collected in three wetlands of great importance for shorebirds, along the East Atlantic Flyway: the Ria de Aveiro and the Tagus Estuary - two temperate wetlands located in Portugal; and the Bijagós Archipelago – a tropical wetland located in Guinea-Bissau (Delany *et al*., 2009) (Fig. 1). These temperate and tropical wetlands differ in terms of industrial development, population density, climate, and other factors.

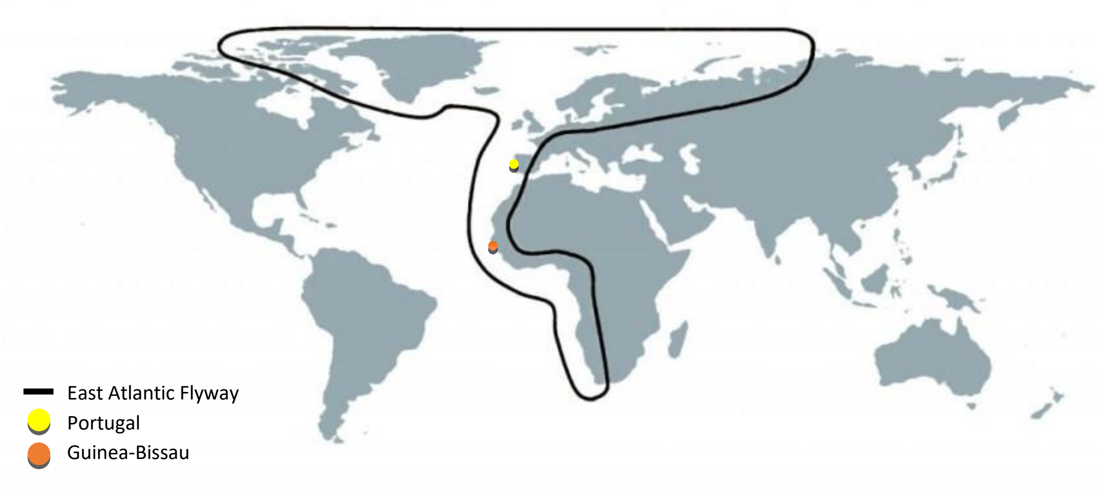

Figure 1: East Atlantic Flyway used by migratory birds including shorebirds. (Image from: https://imrama.eu)

2.1.1) Ria de Aveiro

The Ria de Aveiro, located in northwest Portugal, is a coastal lagoon composed of a complex network of channels and permanently linked to the sea by an artificial channel (Dias *et al*., 1999). Considered as the most extensive lagoon system in Portugal, it extends for 45 km along the coast and comprises a water free area of about 66 and 83 km^2 during high and low tide, respectively. The predominantly semidiurnal tides, with a mean range of 2m, vary between 0.6m during neap and 3.3m during the spring tide (Dias *et al*., 1999). The average depth is 3m, although its larger in some navigation channels, where it reaches 28m (Dias *et al*., 1999; Picado *et al*., 2010). It comprises several habitats such as intertidal mudflats, extensive saltmarshes and reeds, saltpans, among others (Dias *et al*., 1999; Picado *et al*., 2010). With a population of about 300,000 inhabitants in its surroundings, the Ria de Aveiro supports several anthropogenic activities such as agriculture, boats and industrial activities. Many of these contributed to the degradation and heavy metal contamination of the local environment for decades (Dias *et al*., 1999; Silva *et al*., 2010).

The great variety of habitats makes the Ria de Aveiro an important area for waterbirds, frequently hosting about 20,000 individuals with great emphasis on shorebirds. This wetland constitutes one of the main wintering and stopover areas in the Iberian Peninsula for migratory shorebirds, supporting more than 1% of the European wintering populations of some species (Morgado *et al*., 2009). Ria de Aveiro is also an import nesting ground for some species such as *H. himantopus, C. alexandrinus* and *Sterna albifrons* which nest in saltpans (Morgado *et al*., 2009, Múrias *et al*., 2017). Due to its high value for the conservation of waterbirds and critical habitats, the Ria de Aveiro is recognized as a wetland of international importance under the Ramsar Convention and classified as SPA (Special Protection Area – PTZPE0004) by the EU Birds Directive, and as SCI (Site of Community Importance) within the framework of the Natura 2000 network.

2.1.2) Tagus Estuary

The Tagus Estuary, located in the southwest of Portugal, is the largest wetland in the country and one of the largest estuaries on Western Europe. This estuarine system comprises an area of 320 km^2 extending over 40km and is subject to semi-diurnal tides with a tidal range between 1.5 to 3.5m during neap and spring tides respectively (Gutiérrez-Estrada *et al*., 2008). The intertidal area covers approximately 40% of the estuary's total area, and it is composed mainly by extensive mudflats and smaller sandflats, which are surrounded by extensive saltmarshes, beaches, and in some places by urban areas and large agricultural fields (Gutiérrez-Estrada *et al*., 2008). Estuarine margins are very humanized and have a high industrial and urban development, housing several industrial complexes and about 3,5 million people live around this estuary (INE, 2021). Until recently, the Tagus Estuary was the endpoint of industrial and domestic waste discharges increasing the level of pollutants and organic matter in certain zones of the estuary and, in some cases, increasing the densities of tolerant macroinvertebrates with nutrient inputs (Gaudêncio and Cabral, 2007; Alves *et al*., 2012).

It is one of the most important wintering and staging grounds for waterbirds and shorebirds across the East Atlantic Flyway, harbouring about 200,000 shorebirds during winter and more than 1% of the wintering population of several species such as *Calidris alpina*, *Limosa limosa* and *Pluvialis squatarola* (Delany *et al*., 2009; Catry *et al*., 2011). Similarly to Ria de Aveiro, saltpans of Tagus Estuary are important nesting grounds and high-tide roots for several shorebirds. However, during the last three decades, there has been declining trends in shorebirds populations using the high-tide roosts in the estuary (Catry *et al*., 2011). Due to its ecological relevance and the significant numbers of waterbirds supported throughout the year, the Tagus Estuary is classified as a wetland of international importance under to the Ramsar Convention and designated in 1994 as an SPA (Special Protection Area) for birds under the European Union Birds Directive 79/409/EEC. Previously, in 1976, the northern part of the estuary was classified as Natural Reserve with an area of 142 km^2.

2.1.3) Bijagós Archipelago

The Bijagós Archipelago, located on the African Atlantic Coast, is formed by a group of 88 islands and islets relatively close to the coast of Guinea-Bissau and it comprises a total area of 2,624 km^2 (Catry *et al*., 2017). The intertidal area covers over 760 km^2, during low tide, and is mostly composed of extensive intertidal flats, often covered by dense mangrove zones that comprise roughly 430 km^2. Most of the islands are surrounded by these intertidal flats and bordered by mangroves (Catry *et al*., 2017). The archipelago has a population of 30,000 inhabitants dispersed over 26 islands, and its economy is based on seafood harvesting, artisanal fishing and agriculture (Catry *et al*., 2017). The industrial activity and the anthropogenic impacts in the region are very low (Coelho *et al*., 2016). However, its proximity to mainland coast and Geba river estuary make it very vulnerable to pollutants from urban and agricultural areas from Guinea-Bissau and other nearby countries.

Bijagós is the second most important wetland in West African Coast for shorebirds migrating along the East Atlantic Flyway, holding around 700,000 to 900,000 shorebirds, that mostly originate from temperate and arctic regions in Europe and Russia, to spend the winter in this tropical region. For some species, it is the limit of their geographical range while others can travel all the way to South Africa (Salvig *et al*., 1994; Delany *et al*., 2009; Dodman and Sá, 2015). It also supports relevant breeding colonies of some waterbirds such as herons and sterns, highlighting the importance of this area for bird conservation. Its biodiversity, pristine areas and ecological relevance led to the classification in 2014 as a Ramsar site, as Biosphere Reserve by UNESCO in 1996, and the establishment of two National Parks (Orango in 1997, João Vieira in 2000) and of the Community-Based Marine Protected Area at Urok in 2005.

2.2) Sample collection and preparation

2.2.1) Feathers

Feathers were previously collected during the winter season in February 2012-2014 and at the end of winter, just before migration, in April 2019, at the Tagus Estuary and the Bijagós Archipelago respectively, by Tagus Ringing Group and another team in Bijagós. Shorebirds captures were undertaken by mist-netting and cannon-netting and breast feathers were collected and stored in properly identified polyethylene bags until lab treatment for analysis. Following sampling and measuring, all birds were marked with a metal ring and released. Breast feathers were selected because most were already moulted into pre-nuptial plumage reflecting the local exposure. Furthermore breast feathers represent better exposure than other feathers, are not influenced by moulting order and do not affect flight performance and thermoregulation (Furness *et al*., 1986; Burger, 1993).

Before mercury analysis, feathers were subjected to a wash treatment. They were washed manually with tap water to remove gross particles and placed in an ultrasonic bath with deionized water for 5-10 minutes to remove any adherent external contamination. After that, feathers were dried at ambient laboratory temperature and washed for 24 hours in a solution of chloroform – methanol (2:1) to remove oils that could interfere with the mercury concentrations analysis. Feathers were then dried in an oven at 60 ºC for 24 to 48h. After this wash treatment, the feathers of each bird were cut into small pieces of ca. 1-5mm to produce a homogeneous sample. To facilitate cutting procedure, feathers were moistened with ethanol and in the end placed in Eppendorfs with proper identification and then dried in an oven during the night. Each sample was weighed to the nearest 0.001 mg with a micro balance (RADWAG-MYA 2.3Y) and placed in separately in a box with respective identification until mercury analysis.

2.2.2) Eggshells

The eggshells of *H. himantopus* and *C. alexandrinus* were collected in the saltpans of the Ria de Aveiro during the nesting period: April to June of 2020. Eggshells from Tagus Estuary were collected by Tagus Ringing Group. Eggshells from hatched chicks were selected to avoid any impact on breeding performance of *H. himantopus* and *C. alexandrinus*. Once located, nests of these species were marked with small plastic tags for individual identification and were monitored during the breeding season to confirm hatching and collect eggshells before parental removal. Upon nest discovery, incubation length and embryonic development were determined using an egg flotation method described by Liebezeit *et al*., (2007) specifically for shorebirds, and each egg of a nest was numbered with a pencil. At the end of the incubation period, several eggshell fragments were collected and placed in polyethylene sachets properly identified. In the Tagus Estuary, eggshells from an egg of each clutch was collected at random from predated or flooded nests of these two species.

In the laboratory, eggshells were washed vigorously with deionized water, with the help of a soft brush to remove any internal and external contamination. Following wash, they were placed in the oven at 60 ºC for 24h to dry. The samples include the inner membrane due to the difficulty in remove it from the eggshell. Since the laying order of eggs from the Tagus Estuary was unknown, an attempt was made to replicate the sampling by selecting 1 egg from each nest in the Ria de Aveiro, using a random sample generator. Finally, eggshells of the eggs previously selected were crushed using an agate mortar, weighed to the nearest 0.01 mg directly in nickel boats with an analytical balance (AND GH – 252), and placed in the mercury analyser for mercury quantification.

2.2.3) Faeces

Faeces *of H. himantopus* were collect monthly at Salina da Troncalhada in Ria de Aveiro, from October 2019 to May 2020, except April due to strict lockdown measures imposed that month due to COVID-19 pandemic. Due to flooding of saltpans, some sampling days were cancelled and rescheduled for the following days, always attempting to keep regular intervals between monthly samples. During October, six field visits were performed to understand which dry sections of the saltpan were used most by the target species and suitable for sampling. Once determined, in the beginning of each sampling day these sections were cleaned with a broom to remove any waste and older faeces that might be present. Then, individuals of *H. himantopus* were observed for up to one hour exclusively using the previously cleaned areas and at the end this period, fresh excrements were collected into an Eppendorf. Number of collected samples per month averaged 3.7 ± 0.1 (range: 3-5).

After collection, the samples were frozen. Then the Eppendorfs were punctured and immediately placed in the lyophilizator (Telstar- LYOQUEST- 80% Plus Eco) for 48h, to dehydrate the samples at high pressure. Each sample was then grounded with an agate mortar and weight to the nearest 0.01 mg, in nickel boats, with an analytical balance (AND GH - 252) being ready for mercury analysis.

2.3) Mercury analysis

All samples were analysed for total mercury because 80-90% of the mercury present in birds' tissues is in the form of methylmercury. Mercury concentrations were determined by atomic absorption spectrophotometry with thermal decomposition using an advanced mercury analyser (LECO AMA 254) with a detection limit of 0.02 ng Hg. Inside the equipment, the samples pass through a combustion tube with an oxygen flux where they are dried first at 120 ºC and then thermally decomposed at 750 ºC. The resulting gases are transported over the catalyst, by oxygen carrier gas, being the impurities removed in the catalytic section and the mercury vapor sequestered in a gold amalgamator. Then, this amalgam is heated approximately to 900 ºC releasing Hg vapor to the detection complex and subsequent quantification system. Finally, mercury is quantified by atomic absorption spectrophotometry in a UV radiation detector at 253.6 nm and the results sent to the software (Costley *et al*., 2000).

At least three replicates were made for each sample until an RSD – residual standard deviation – of below 5% was achieved. A set of blanks (1 manual and 2 internal) were run before and after each sample to eliminate any internal contamination of residual mercury in the equipment that could influence the results of the following samples. Blanks were performed until the values obtained were always below the detection limit (0.01 ng Hg). Accuracy and quality of this method were checked daily by carrying out an analytical quality control that consisted of the analysis of certified reference material DOLT-5 (Dogfish liver: certified value = 0.44±0.18 ppm) according to the same conditions as the samples. Total mercury concentrations in this study are expressed in ppm and given on a dry weight (dw).

2.4) Data analysis

The data analysis was done using the software RStudio (version 1.3.1093). The normality of the distribution and the homogeneity of the variances were first examined with the Shapiro-Wilk and Levene tests, respectively. Normality was also visually checked using plot (QQ plot). Intra and inter specific differences were investigate for eggshells and feathers. Non-parametric procedures (Mann-Whitney tests) were applied to assess potential differences on eggshells mercury concentrations between *C. alexandrinus* and *H. himantopus*, and for this last species between wetlands during the breeding season. Since *C. alexandrinus* data followed a normal distribution, a T-test was used for intraspecific comparison. Relatively to feathers collected during the non-breeding season, data and/or residuals did not meet the assumptions of normality and homogeneity and therefore, were transformed by a logarithmic transformation (Log10). To assess whether there were significant differences in mercury concentrations in feathers among species, a parametric analysis of variance, One-Way ANOVA, was applied to species sampled on each wetland. A Two-Way ANOVA, was used for a subset of samples for species sampled in both wetlands, using species and wetland as fixed factors. For faeces samples, an One-Way ANOVA was performed to assess possible season variations. Each significant ANOVA test was followed by Tukey's Multiple Comparison of means tests (HSD), to further explore the significant differences among groups (i.e. distinguish significant differences among groups). The results were given with arithmetic mean ± SE for all analyses and the significant level was considered at 0.05%.

3) RESULTS

3.1) Mercury concentrations in shorebird's feathers

A total of 125 feathers of 10 shorebirds species, wintering at Tagus Estuary (n=57) and Bijagós (n=68), were analysed for total mercury concentration. Not all species are present in both wetlands and the number of feathers sampled for each species and wetland varied. The mean mercury concentration and their respective standard deviation and ranges are showed in Table 1. Overall, in this study, mercury concentrations on shorebird feathers ranged, individually, from 0.803 to 13.995 ppm dw on Tagus Estuary and from 0.540 to 4.229 ppm dw on Bijagós (Tab. 1).

Table 1: Mercury concentrations (mean ± standard error and range; ppm dw) in feathers of wintering shorebirds from Tagus Estuary and Bijagós Archipelago. *P-values* of Post Hoc Test (Tukey HSD) are also presented for intraspecific differences in mercury levels of shorebirds in both wetlands. Significant differences (*p-value* < 0.05) are in bold.

	Tagus Estuary		**Bijagós Archipelago**		
Species	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**p-value**
Calidris alba	9	5.947 ± 0.361 (4.756 - 8.337)	10	0.966 ± 0.074 (0.634 - 1.301)	**< 0.001**
Calidris canutus	10	3.542 ± 0.384 (1.548 - 5.198)	8	1.374 ± 0.122 (1.023 - 1.984)	**< 0.001**
Charadrius hiaticula	9	4.605 ± 0.560 (2.873 - 6.820)	6	1.223 ± 0.100 (0.936 - 1.643)	**< 0.001**
Tringa totanus	9	9.918± 1.166 (4.165 - 13.995)	10	2.338 ± 0.188 (1.145 - 3.226)	**< 0.001**
Calidris alpina	10	3.289 ± 0.306 (1.882 - 5.455)			
Limosa limosa	10	2.106 ± 0.215 (0.803 - 3.136)			
Actitis hypoleucos			9	2.773 ± 0.261 (1.756 - 4.229)	
Arenaria interpres			8	2.193 ± 0.145 (1.617 - 2.784)	
Calidris ferruginea			10	1.251 ± 0.114 (0.540 - 1.899)	
Pluvialis squatarola			7	0.932 ± 0.139 (0.591 - 1.559)	

3.1.1) Tagus Estuary

The feathers sampled in Tagus Estuary belong to 5 shorebird species (*Calidris alpina*, *Calidris alba*, *Charadrius hiaticula*, *Tringa totanus* and *Limosa limosa*). The highest mean mercury level was recorded for *T. totanus* (9.918±3.499 ppm dw) while *L. limosa* had the lowest (2.106±0.681 ppm dw) (Tab. 1; Fig. 2). There were significant differences in feathers mercury concentrations among species ($F_{(5, 51)}$ = 22.479, p-value < 0.001). Pair-wise comparisons (Tukey HSD) indicated several significant differences between species (Tab. 2), with mercury levels in *L. limosa* feathers being significantly different from all 4 other species from Tagus Estuary. *T. totanus* displayed mercury concentrations significantly different than all other species, except *C. alba* (Fig. 2). Furthermore, there was some species for which no differences were detected (Fig. 2; Tab. 2).

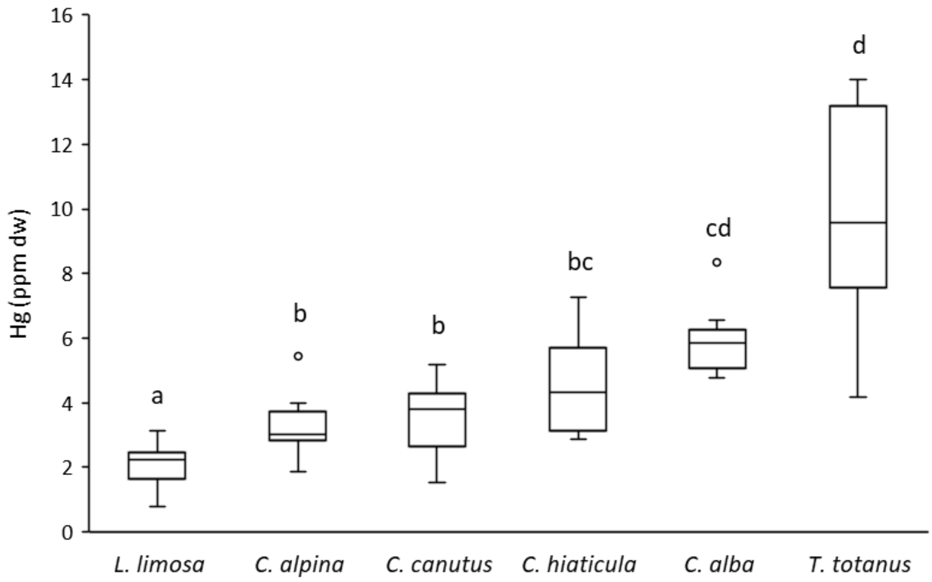

Figure 2:Variation of mercury concentrations (ppm dw) in feathers of wintering shorebirds from Tagus Estuary. The boxplots show median values (middle of the boxes), lower and upper quartiles (top and bottom of boxes) and minimum and maximum values (the whiskers), excluding outliers which are presented as the points above the boxes. Different letters represent significant differences (*p-value* < 0.05) from Post Hoc Test at α = 0.05. For statistical *p-values* between species please look to table 2.

Table 2: Tabuled *p-values* of Post Hoc Test (Tukey HSD) for interspecific differences in feathers mercury levels of shorebirds wintering at Tagus Estuary. ( ) = *p-value* ≥ 0.05, * = *p-value* < 0.05, ** = *p-value* < 0.01, *** = *p-value* < 0.001. Significant differences are in bold.

Species	*L. limosa*	*C. alpina*	*C. canutus*	*C. hiaticula*	*C. alba*	*T. totanus*
L. limosa	-	**0.042***	**0.019****	**< 0.001*****	**< 0.001*****	**< 0.001*****
C. alpina	-	-	0.999	0.372	**0.004****	**< 0.001*****
C. canutus	-	-	-	0.547	**0.010***	**< 0.001*****
C. hiaticula	-	-	-	-	0.455	**< 0.001*****
C. alba	-	-	-	-	-	0.074
T. totanus	-	-	-	-	-	-

3.1.2) Bijagós Archipelago

The feathers sampled in Bijagós belong to 8 wintering shorebird species (*Calidris ferruginea*, *Calidris alba*, *Charadrius hiaticula*, *Tringa totanus*, *Arenaria interpres*, *Actitis hypoleucos*, *Pluvialis squatarola* and *Calidris canutus*). *A. hypoleucos* had the highest mean mercury concentration (2.773±0.261 ppm dw), whereas the lowest was recorded for *P. squatarola* (0.932±0.139 ppm dw) (Tab.1; Fig. 3). The results from One-Way ANOVA indicated highly significant differences in the average concentrations of mercury amongst species ($F_{(7,\ 60)}$ = 19.694, p-value < 0.001). Through the Tukey post-hoc test (HSD), it was possible to observe the existence of two groups overall (Fig. 3; Tab. 3). The species *A. interpres*, *A. hypoleucos* and *T. totanus* did not show significant differences in their mercury concentrations but were significantly different from all other species. A similar pattern occurred for the other 5 species, which showed no differences among them (Tab. 3).

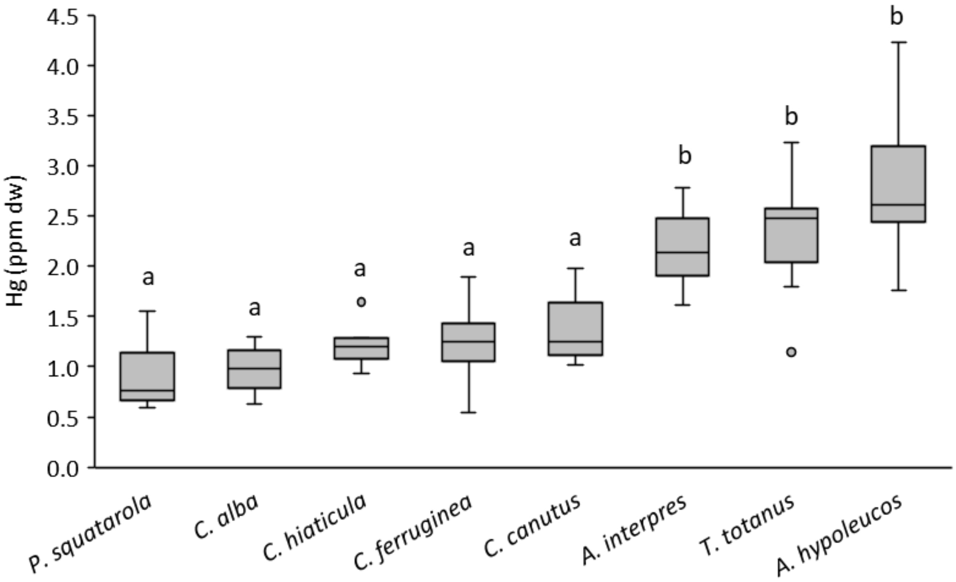

Figure 3: Variation of mercury concentrations (ppm dw) in feathers of wintering shorebirds from Bijagós Archipelago. The boxplots show median values (middle of the boxes), lower and upper quartiles (top and bottom of boxes) and minimum and maximum values (the whiskers), excluding outliers which are presented as the points above and bellow the boxes. Different letters represent significant differences (*p-value* < 0.05) from Post Hoc Test at $\alpha = 0.05$. For statistical *p-values* between species please look to table 3.

Table 3: Tabuled *p-values* of Post Hoc Test (Tukey HSD) for interspecific differences in feathers mercury levels of shorebirds wintering at Bijagós Archipelago. ( ) = *p-value* ≥ 0.05, * = *p-value* < 0.05, ** = *p-value* < 0.01, *** = *p-value* < 0.001. Significant differences are in bold.

Species	*P. squatarola*	*C. alba*	*C. hiaticula*	*C. ferruginea*	*C. canutus*	*A. interpres*	*T. totanus*	*A. hypoleucos*
P. squatarola	-	1.000	0.469	0.341	0.086	**< 0.001*** **	**< 0.001*** **	**< 0.001*** **
C. alba	-	-	0.680	0.543	0.154	**< 0.001*** **	**< 0.001*** **	**< 0.001*** **
C. hiaticula	-	-	-	1.000	0.997	**0.006** **	**0.001** **	**< 0.001*** **
C. ferruginea	-	-	-	-	0.989	**0.001** **	**< 0.001*** **	**< 0.001*** **
C. canutus	-	-	-	-	-	**0.025** *	**0.005** **	**< 0.001*** **
A. interpres	-	-	-	-	-	-	1.000	0.766
T. totanus	-	-	-	-	-	-	-	0.888
A. hypoleucos	-	-	-	-	-	-	-	-

3.1.3) Tagus Estuary vs Bijagós Archipelago

The species that winter in both wetlands were: *T. Totanus*, *C. hiaticula*, *C. canutus* and *C. alba* (Tab. 1). Overall, the ranges of mercury levels were higher for Tagus Estuary than Bijagós Archipelago and the Two-Way ANOVA indicated significant differences in mercury concentrations both at the level of species ($F_{(3, 63)}$ = 14.102; p-value < 0.001) and wetland ($F_{(1, 63)}$ = 167.364; p-value < 0.001). The interaction between these two factors also significant ($F_{(3, 63)}$ = 7,007; p-value < 0,001), indicated that differences are not identical between the two factors (species and wetland). Mercury levels on feathers were significantly higher in the Tagus Estuary than in the Bijagós Archipelago for all species. (Tab. 1; Fig. 4). *T. totanus* had the highest mean level of mercury in both wetlands whereas the species. *C. canutus* showed the most similar concentrations between wetlands (Fig. 4).

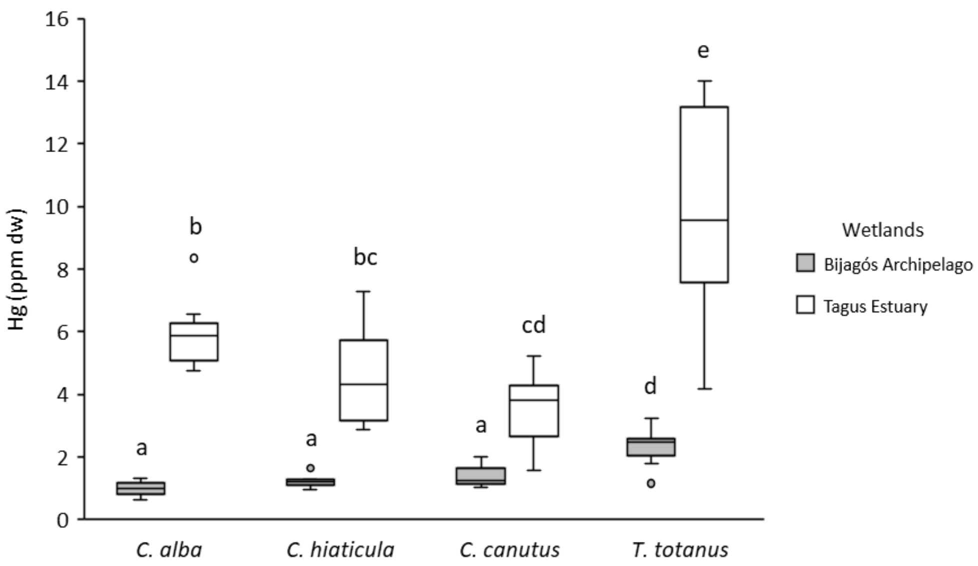

Figure 4:Variation of mercury concentration (ppm dw) in shorebird feathers between their wintering grounds. The boxplots show median values (middle of the boxes), lower and upper quartiles (top and bottom of boxes) and minimum and maximum values (the whiskers), excluding outliers which are presented as the points above and bellow the boxes. Different letters represent significant differences (*p-value* < 0.05) from Post Hoc Test at α = 0.05. For statistical *p-values* of intraspecific differences look at table 1 .

3.2) Mercury concentrations in eggshells

A total of 46 eggshells of *H. himantopus* (n = 26) and *C. alexandrinus* (n = 20), from the saltpans of Tagus Estuary and Ria de Aveiro, were collected and analysed. The mean mercury concentrations detected in the eggshells of the two species, and the respective standard error and ranges are presented in Table 4. The concentrations ranged between 0.004 to 0.063 ppm dw for *H. himantopus* and 0.004 to 0.069 ppm dw for *C. alexandrinus.* According to the Shapiro-Wilk test (W) and QQ plot, only *C. alexandrinus* data followed a normal distribution (p-value > 0,05).

Table 4:Mercury concentrations (mean ± standard error and range, ppm dw) in eggshells of *C. alexandrinus* and *H. himantopus* from Tagus Estuary and Ria de Aveiro. *P-values* of T-Test and Mann-Whitney test are presented for intraspecific differences of *C. alexandrinus* and *H. himantopus* respectively . Significant differences (*p-value* < 0.05) are in bold.

	Tagus Estuary		Ria de Aveiro		
Species	**n**	**Mean ± SE**	**n**	**Mean ± SE**	***p- value***
Charadrius alexandrinus	10	0.034 ± 0.006 (0.012 - 0.069)	10	0.037 ± 0.006 (0.013 - 0.069)	0.762
Himantopus himantopus	14	0.018 ± 0.004 (0.004 - 0.063)	12	0.009 ± 0.002 (0.003 - 0.030)	**0.046**

3.2.1) Intraspecific differences

The eggshells of the *C. alexandrinus* from Tagus Estuary showed slightly higher mercury levels than those from Ria de Aveiro. However, no significant differences were found between the eggshells sampled in the two wetlands ($t_{(18)}$ = 0.308; p-value = 0.762) (Tab. 4; Fig. 5). The same pattern occurred in the case of *H. himantopus*. The average mercury concentrations of *H. himantopus* eggshells from Tagus Estuary saltpans were two times higher than those from Ria de Aveiro and in this case, the Mann Whitney test indicated that these were significantly different (W = 45, p-value = 0.046) (Tab. 4).

3.2.2) Interspecific differences

In both wetlands, *C. alexandrinus* showed higher amounts of mercury on their eggshells than *H. himantopus* (Fig. 5). Mann-Whitney (W) non-parametric tests, indicated that the mercury levels present in eggshells of the two species were significantly different in both wetlands (p-value < 0.05 in both cases). The difference in average values between species found in both Ria de Aveiro (W = 112, p-value <0.001) than in Tagus Estuary (W = 108, p-value = 0.026). In Ria de Aveiro, *C. alexandrinus* had an average mercury concentration 4 times higher than the one observed for *H. himantopus*, whereas in the Tagus Estuary it was only 2 times higher (Fig. 5).

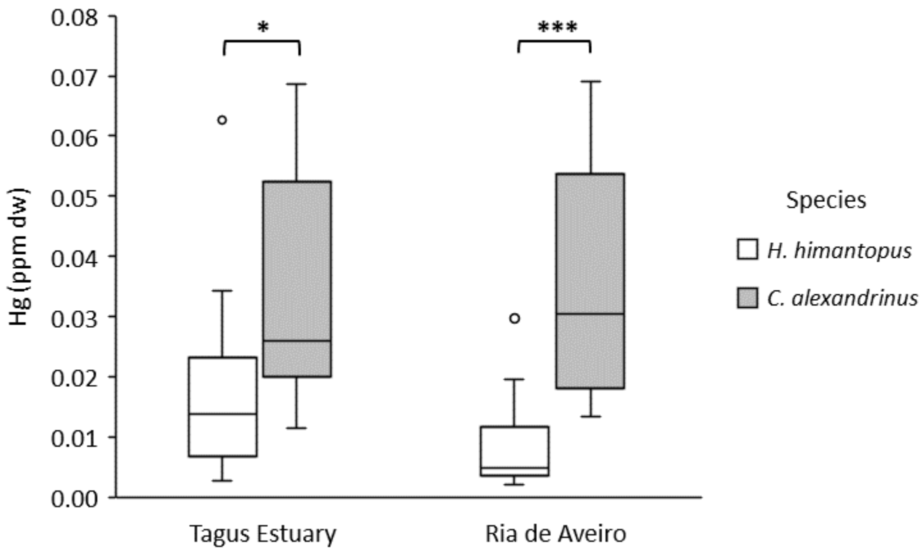

Figure 5: Variation of mercury concentrations (ppm, dw) in eggshells of *H. himantopus* and *C. alexandrinus* from Tagus Estuary and Ria de Aveiro. The boxplots show median values (middle of the boxes), lower and upper quartiles (top and bottom of boxes) and minimum and maximum values (the whiskers), excluding outliers which are presented as the points above the boxes. ( ) = *p-value* ≥ 0.05, * = *p-value* < 0.05, ** = *p-value* < 0.01, *** = *p-value* < 0.001.

3.3) Mercury concentrations in faeces

A total of 26 faecal samples of *H. himantopus* were collected at the saltpans of Ria de Aveiro across a 7 month period. Mean mercury concentrations ranged from 0.040 in October to 0.085 ppm dw in February. The samples with the highest (0.171 ppm) and lowest (0.014 ppm) mercury values were collected in November and December respectively. All samples followed a normal distribution and homogeneity of variance. According to the ANOVA test there were not any significant differences on mercury concentrations between months ($F_{(6, 28)} = 0.603$; p-value = 0.725) (Fig. 6).

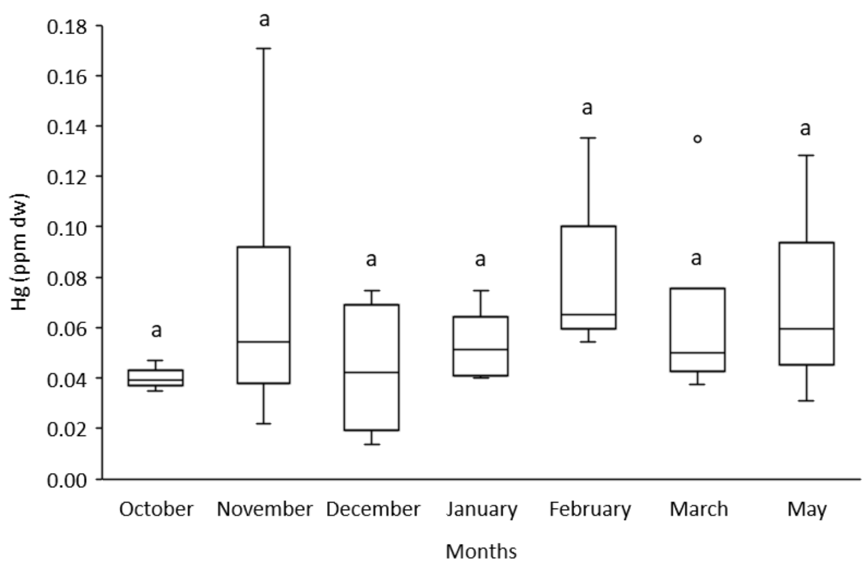

Figure 6: Variation of mercury concentrations (ppm, dw) in *H. himantopus* faeces between months. The boxplots show median values (middle of the boxes), lower and upper quartiles (top and bottom of boxes) and minimum and maximum values (the whiskers), excluding outliers which are presented as the points above the boxes. Different letters represent significant differences (*p-value* < 0.05) from Post Hoc Test at α = 0.05.

4) DISCUSSION

4.1) Mercury levels in feathers

Feathers proved to be a very useful tissue to assess mercury levels, presenting a large amount of this metal when compared to eggshells and faeces. Mercury levels in feathers reflect essentially the exposure during feathers growth but also the mobilization of mercury from internal tissues previously accumulated since the previous moult (Burger, 1993). Given that feathers examined in this study were sampled in second part of winter and shorebirds moult mainly on the winter grounds, mercury levels reported reflect mostly the exposure in Tagus Estuary and in Bijagós Archipelago. The results and comparisons with other studies must be interpreted carefully because mercury levels vary with feathers type and moulting sequence (Lewis and Furness, 1991). These factors were not a limitation since we used breast feathers which are not influenced by moulting sequence and are considered as the best feather type representative of mercury body burden.

Despite such limitations, the values reported in the literature for shorebirds from other geographic areas, were much lower than those found in the Tagus Estuary. In contrast, the ones from Bijagós showed similar values to those reported elsewhere (see Tab. 5 in Appendix). Lucia *et al*., 2014 recorded mercury concentrations of 1.83±0.280 and 2.18±0.349 ppm dw for *C. alpina* and *T. totanus* at Pertuis de Charentais, in France. In the same region, *L. limosa* and *C. canutus* displayed mean concentrations of 1.11±0.115 and 1.26±0.27 ppm dw respectively (Lucia *et al*., 2012). In Delaware Bay, Burger *et al*., 2015 reported concentrations of 0.428±0.058 for *Calidris pusilla*, 0.576±0.105 for *C. canutus* and 0.730±0.109 ppm dw for *C. alba*. In tropical areas, Fernández *et al*., 2018 reported levels of 1.98±0.06 ppm dw in *Calidris maurii* wintering at Sinaloa, Mexico. Despite the low values reported by these, high mercury levels were documented for several shorebird species in two tropical wetlands in India. Pandiyan *et al*., 2020 recorded mean concentrations of 5.50±0.44 for *C. ferruginea*, 6.01±0.50 for *T. totanus*, 8.30±0.33 ppm dw *for C. hiaticula*, among others. However, some species showed very low levels, namely C*alidris alpina* (0.50±0.07 ppm dw) and *Tringa stagnatillis* (0.05 ± 0.002 ppm dw).

4.1.1) Tagus Estuary vs Bijagós Archipelago

In this study, we found significant spatial differences in mercury levels, with all shorebirds wintering at Tagus Estuary showing higher feather mercury levels than their respective conspecifics wintering at Bijagós Archipelago. *T. totanus* from Tagus Estuary had almost 5 times more mercury in their feathers than those from Bijagós. This is in line with our prediction that, mercury exposure in these wetlands differs.

The differences found in this study might be related essentially to the discrepancies of mercury pollution and its bioavailability in these wetlands, resulting from different levels of human pressure, industrialization as well as abiotic conditions between wetlands. The Bijagós Archipelago is a little developed wetland, with relatively low human population, in which industrial activity is practically absent. This region is characterized by low levels of mercury in the environment. Coelho *et al*., 2016 recorded very low mercury concentrations on intertidal sediments (< 1-12ng/g) and in 2 bivalves species – *Tagelus andosoni* (0.09-0.12 µg/g dw) and *Senilia senilis* (0.12-0.14 µg/g dw) – which are consumed by shorebirds (Lourenço *et al*., 2017). The same authors concluded that mercury in the sediments is mainly of geogenic origin, reflecting thus the low anthropogenic and natural contamination status of the archipelago. The levels reported by Coelho *et al*., 2016 were very similar, or even below, to other west African coastal areas for both sediments and bivalves. Bodin *et al*., 2013 recorded for Petite Côte and the Sine Saloum Estuary (Senegal) mean mercury concentrations that ranged from 0.004 to 0.013 µg/g dw. Joiris *et al*., 1998 and Otchere, 2003 reported mercury levels of 0.02–1.3 µg/g and 0.06–0.34 µg/g dw, for *S. senilis* from Ghana and Nigeria, respectively. Conversely, Tagus Estuary is under strong human influence and one of the most contaminated with mercury in southern Europe. Two areas are identified as mercury hotspots, located at north channel (Vila Franca de Xira) and south margin (Barreiro), resulting from previous intense industrial activity (Figueres *et al*., 1985; Cesário *et al*., 2016). Fonseca *et al*., 2019 reported high mean mercury levels that ranged between 0.42-0,62 µg/g dw in sediments, 0.03-0.15 µg/g dw in plants, 0.20-0.47 µg/g dw in invertebrates and 0.15-1.03 µg/g dw in fish tissues. Boat's activity and other activities such as large scale bivalve collection can promote the resuspension of highly contaminated sediments which with the tide can be transported and deposited over the estuary increasing the mercury in several areas including intertidal mudflats where shorebirds forage. Shorebirds uptake mercury mostly from preys but also from the sediment that is attached to them. Therefore, sediments might be an additional source of pollutants for shorebirds in contaminated areas such as Tagus Estuary.

Shorebirds are very affected by food availability and some species show trophic plasticity being able to change their diet and habitat use across their range (Beninger *et al*., 2011; Quinn and Hamilton, 2012). Possible changes in trophic habits might have also contributed for geographical differences in feather mercury levels found in this study. Although there are no studies comparing the diets of wintering shorebirds between these wetlands, the analysis of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) allows to relate shorebird's diet, trophic position and mercury uptake. Catry *et al*., 2016 showed that shorebird's isotopic signatures and community structure varied geographically. Species from Tagus Estuary had higher $\delta^{15}N$ levels than their conspecifics from other African wetlands, including Bijagós. Their result might suggest that species explore different food resources and shorebirds wintering Tagus Estuary feed on higher trophic levels (enhanced $\delta^{15}N$). Since many studies demonstrates that $\delta^{15}N$ are positively correlated with mercury levels, it can be expected that higher mercury levels are recorded in shorebirds from Tagus Estuary. Therefore, both geographic differences in trophic resources use and local pollution levels, between wetlands explain the variations found in this study.

Spatial variation of mercury and other heavy metals among tropical and temperate wetlands have been found by other authors. Burger and Gochfeld, 1991 examined feathers heavy metals levels on terns breeding at Great Barrier Reef (Australia) and near Culebra (Puerto Rico), and compared them with values reported for terns nesting at temperate regions, where these birds forage in estuaries close to industrialized areas. They found that only terns breeding at Australia showed lower mercury concentrations than those reported at temperate areas. Thus, it is not always the case that waterbirds in tropical areas display lower mercury levels. Furthermore, Burger *et al*., 2018 documented higher mercury levels in blood of *C. pusilla* overwintering at Suriname than at Delaware Bay, suggesting an higher exposure in the tropical grounds. Blood levels provide information about a recent and local exposure (i.e. smaller time window). The high concentrations in shorebirds from Suriname might be related to the illegal gold mining that occurs in that region, which has consequently contributed to the increase of mercury pollution in the environment. As these results do not match those in the present study, it seems that mercury exposure is not always greater in temperate areas and that industrialized or polluted regions may occur anywhere even in tropical and less developed regions.

4.1.2) Interspecific differences

As expected, mercury concentrations varied significantly among species in both wetlands suggesting that they were exposed differently to this pollutant. The cause of such variation is likely related to diet composition (prey types and sizes) and foraging habitats at these wetlands. Shorebirds species examined appear to have broadly similar diet, based on varying proportions of benthic invertebrates, and use mostly intertidal mudflats for feeding, but they can explore several ecological niches. The great variety of bill shapes and lengths, as well as body size, allows them to forage on specific prey, resulting in a partition of food resources in response to interspecific competition that occurs when they forage in large and mixed flocks in a given area (Bocher *et al*., 2014). For example, *C. canutus* is very specialized, foraging mainly on marine molluscs on intertidal mudflats, whereas *T. totanus* is a generalist species, consuming a great variety of prey and exploring several habitats (Masero and Pérez-Hurtado, 2001; Sánchez *et al*., 2005; Lourenço *et al*., 2017). In the Bijagós Archipelago, *C. canutus* keeps its relatively limited prey selection foraging exclusively of bivalves, particularly *Dosinia isocardia* while other species have a broader diet such as: *T. tonanus*, *C. alba* and *C. ferruginea* that beside bivalves, also consume polychaetes and crustaceans (Lourenço *et al*., 2017). These authors concluded also that fiddler crab is an important prey for most shorebirds in Bijagós, while polychaetes are very important for species as *C. alba, C. ferruginea* and *C. hiaticula.* Mercury levels in prey themselves can vary, for example between filter suspension or deposition feeding benthos, potentially contributing with different loads of mercury for birds resulting in the differences found in feathers.

Shorebirds can also experience different exposures due to the wide range of foraging habitats that they use (intertidal mudflats, rocky/sandy beaches, salt marshes, mangroves, saltpans) where contamination might differ. Furthermore, shorebirds segregate spatially on foraging areas, occupying several micro-habitats within a habitat. Some species explore deeper waters while others spend most time on uncovered tidal flats. Others can even use more terrestrial habitats such rice-fields and wet grassland (although this is rare in south Europe and absent in West Africa). Upland areas typically have less methylmercury because methylation occurs primarily in aquatic environments (Wiener *et al*., 2003). Therefore, shorebirds exploring those areas might be less exposed to mercury contamination. Perkins *et al*., 2016 reported lower blood mercury values for shorebirds foraging at upland habitats than those at wet or aquatic ones. This might explain the low mean mercury concentration found, in this study, for *L. limosa* compared to the remaining species wintering at Tagus Estuary (Tab. 1; Fig. 2). It is known that this species, especially the subspecies *L. l. limosa*, uses rice-fields in the northern part of the Tagus Estuary particularly in late winter, where they forage essentially on rice seeds and other plant material (Lourenço and Piersma, 2008; Alves *et al*., 2010). Several studies have demonstrated that herbivorous species or organisms that have a diet rich in plant material tend to accumulate less mercury because they feed on lower trophic levels (Lucia *et al.,* 2010). Mercury uptake by foraging on primary producers (i.e., lower trophic levels), which are the base of food chain, is lower than the uptake of foraging of primary/secondary consumers (i.e., higher trophic levels). For example, in Gironde Estuary, Anser *anser,* an herbivorous bird, had much lower mercury levels than *P. squatarola* and *C. canutus* (Lucia *et al*., 2010). The lower mercury levels found in *L. limosa* feathers, which were similar to those in shorebirds in Bijagós, could also reflect mercury exposure in other wetlands, particularly along the African Coast, where this species also overwinter. It is possible that these individuals have spent the winter in african wetlands and when sampled in Tagus Estuary, were staging birds already migrating.

The relation between variations in mercury levels and trophic positions has been previously investigated for shorebirds. Lucia *et al*., 2014 found that mercury and trophic levels differed between shorebirds species and were positively correlated. That study indicates that *T. totanus* had the highest trophic level and mean mercury level among the 4 species examined (*L. limosa*, *C. alpina* and *C. canutus*). According to Lucia *et al.,* 2014, it appear that generalist species (e.g. *T. totanus*) tend to display higher mercury levels than specialized ones. Our results support this as *T. totanus* also showed the highest mercury levels in the Tagus Estuary, with those three same species also considered, and being significantly different from all of them. In the Bijagós it was also significantly higher than *C. anutus* (but not for *A. interpres* and *A. hypoleucos)*. This suggests that *T. totanus* has a more diversified diet than these species and/or is consistently feeding at a higher trophic level across the flyway.

4.2) Mercury in eggshells

Eggshells are an additional excretion pathway of contaminants in females reflecting dietary exposure days prior to eggs laying (Burger, 1994). In most cases, the assessment of mercury in eggs is done in the contents (i.e., yolk and albumen) instead of eggshells because they have a greater amount of mercury and its levels can be used to infer possible reproductive effects (Hargreaves *et al*., 2010; Peterson *et al*., 2017). Brasso *et al*., 2012 found that 92% of total mercury in eggs were in albumen, while the remaining 6.7%, 0.9% and 0.4% belonged to yolk, shells and membranes respectively. Tavares *et al*., 2004 recorded for *H. himantopus* eggs, from several Portuguese wetlands, mercury concentrations from 0.04 to 1.13 µg/g dw which were higher than the values of eggshells found in our study. Compared to other values reported in the literature for eggshells of birds species, the mercury levels documented in the present study are within the range being similar to certain values (see Tab. 6 in Appendix).

Acoording to our results mercury concentrations varied significantly between *C. alexandrinus* and *H. himantopus* and between wetlands for the last species. Similar results were observed in some studies but for adult and fledgling breast feathers. Pandiyan *et al*., 2020 found higher mercury levels in *C. alexandrinus* than *H. himantopus* feathers at two tropical wetlands in India. Tavares *et al.,* 2004, documented significant differences on mercury levels of *H. himantopus* fledglings feathers between and within wetlands including Tagus Estuary saltpans and ricefields. It should be noted that due to birds' mobility the mercury levels in eggshells might reflect exposure not only on the nesting location (e.g., saltpans) but also in other areas that female used for feeding. The relatively low values found in the current study were expected since, unlike feathers, eggshells are not the main excretion route for mercury and about 95% of the eggshell consistes calcite. Despite the low contribution of eggshells in mercury elimination it can be a significant excretory pathway for other trace elements, especially Sr, Ba, Co, and Tl (Rodriguez-Navarro *et al*., 2002; Agusa *et al*., 2005).

4.2.1)Intraspecific differences

Comparisons between wetlands showed only significant differences for *H. himantopus*. Eggshells collected in Tagus Estuary had twice as much mercury levels as those collected in Ria de Aveiro. This suggests that females of this species breeding on the Tagus Estuary are subject to a greater exposure to this pollutant.

As in feathers, the intraspecific variations of mercury levels might be related to possible dissimilarities in mercury pollution between study areas, in this case Tagus Estuary and Ria de Aveiro. Both wetlands are under a strong anthropogenic influence with a history of agricultural /industrial waste discharges in the past and high mercury levels recorded in these systems. However, Tagus Estuary is situated in a very anthropogenic/industrialized and highly populated area. Furthermore, the proximity to emission sources can be higher. Therefore, the exposure and consequent risk of contamination to mercury as well as to other heavy metals might be greater than in Ria de Aveiro. Although, Tavares *et al*., 2004 did not find any significant differences in mercury levels of *H. Himantopus* eggs between wetlands (Tagus Estuary, Mondego Estuary, Sado Estuary and Formosa Lagoon), they did for fledglings body feathers, with fledglings from Tagus Estuary having higher mercury levels (2.8±1.8 µg/g fresh weight) than in the other wetlands. Futhermore it also varied between sites within Tagus Estuary and Formosa Lagoon which indicates localized contamination and that exposure can also vary locally (Tavares *et al*., 2004). Since the *H. himantopus* chicks are nidifugous and precocial, as well as those of *C. alexandrinus,* they are limited to food resources of nest area until they are fully fledged, thus providing information about exposure in the nesting territory, whereas eggshells provide information about overall exposure in saltpans as well as from surrounding areas (e.g. saltmarsh, intertidal mudflats) due to adults' mobility.

Besides environmental contamination, mercury levels in macroinvertebrates and insects consumed by *H. himantopus* and *C. alexandrinus* might differ between Tagus Estuary and Ria de Aveiro. Tavares *et al*., 2008 found significant inter-site variation for mercury concentrations in potential *H. himantopus* preys in which macroinvertebrates from Vau saltpans (Tagus Estuary) had significantly higher levels than those from Vaia saltpans (Sado Estuary). Furthermore, mercury levels of macroinvertebrates were positively correlated with chicks' feathers, and chicks from Vaia saltpan had lower mercury levels. The possible spatial variation in *H. himantopus* preys' mercury levels might have contributed to the differences observed for *H. himantopus* eggshells since only this species showed significant differences between the two wetlands, with the highest levels recorded for samples from Tagus Estuary. Overall the results suggest that dietary contamination might differ between wetlands and *H. himantopus* females from Tagus might forage on highly contaminated preys than those from Ria de Aveiro. In the other hand, the lack of significant differences for *C. alexandrinus* eggshells reflects a similar dietary exposure in both wetlands.

4.2.2) Interspecific differences

For both saltpans of Ria de Aveiro and the Tagus Estuary the mercury concentration in shorebirds eggshells varied significantly between species, with *C. alexandrinus* showing higher concentrations than *H. himantopus.* This suggests that females of *C. alexandrinus* were more exposed to mercury. The same pattern between these two species has been documented in previous studies on chicks body feathers sampled in saltpans (Tavares *et al.,* 2009). Our results confirm that *C. alexandrinus* also has higher mercury concentrations on eggshells, which can be expected, as both the eggshell and its contents, that originate the embryo and chick, will receive similar levels of mercury from maternal input.

Since eggs are formed from nutrients acquired by female in the breeding grounds before laying eggs, the variation in mercury levels in the eggshells between species are likely due to differences in diet and foraging habitats. Both species overlap on dietary range, but *H. himantopus* feeds mainly on insects (*Coleoptera* and *Diptera*) and some molluscs (*Hydrobia sp*.), while the *C. alexandrinus* feeds mostly on benthos: molluscs, crustaceans (*Ostracoda*), and polychaetes (*Hediste sp*), while also including insects (*Coleoptera, Diptera, Lepidoptera*) (Cuervo, 1993; Perez-Hurtado *et al*., 1997; Pedro and Ramos, 2009). In fact, Perez-Hurtado *et al*., 1997 explicitly assign *H. himantopus* to the group of species that consume primarily insects and *C. alexandrinus* to the group of shorebirds that consume primarily benthos. While in the proximity of the nest, the spatial use in the saltpans by these two species also differs, as *H. himantopus* use practically the entire area of the saltpans, foraging mainly in the inundated pans or other deeper areas which it can exploit due to its long legs whilst C. *alexandrinus*, due to its much shorter legs, only uses certain microhabitats in the saltpans, namely the dry edges of pans, or pans with very low water levels or dry. However, *C. alexandrinus* might also explore other areas near the saltpans such as saltmarshes and intertidal mudflats, where the marine influence and mercury availability might be higher contributing therefore for a higher mercury uptake. Eagles-Smith *et al*., 2009 explained that the differences on feather mercury levels between *Himantopus mexicanus* and *Recurvirostra americana* might be attributable to distinct microhabitat use. Before breeding season *H. mexicanus* uses more managed and muted tidal pickleweed marshes whereas *R. americana* uses tidal mudflats and marshes.

As mentioned before for feathers, variation in mercury levels among species might be indicative of differences on trophic positions. Similar to our study but regarding body feathers of chicks sampled in the same habitat type (i.e. saltpans), Tavares *et al.,* 2009 found significantly higher mercury concentrations for *C. alexandrinus* than for *H. himantopus, Recuvirostra avosetta* and *Bubulcus ibis.* However they didn't find significant trophic differences between any of these species in the saltpans. But *C. alexandrinus* showed slightly higher N values than *H. himantopus* in C. Marim saltpans and Giganta ricefields. It is important to note that variations on heavy metal levels can occur within the same trophic level due to specific prey consumed. Tavares *et al.,* 2008 states that in the absence of trophic differences, the variation in mercury levels between these species might result from contamination status of their preys. Therefore this suggests that during pre-laying the foraging differences, with *H. himatopus* foraging mostly in insects and *C. alexandrinus* mostly on benthos, likely originated the higher levels of mercury on *C. alexandrinus* egg shells (and chicks). It also points towards the fact that insects in saltpans where *H. himantopus* feeds likely have lower mercury concentrations that benthos on intertidal sediments where *C. alexandrinus* feeds.

4.3) Mercury in faeces

Despite the low attention that faeces have received over the years, several studies have reveal its potential as a biological matrix in the assessment of heavy metals levels in birds and as an alternative suitable biomonitor tool (Yin *et al*., 2008, Signa *et al*., 2013). Faeces reflect essentially a recent dietary contamination, indicating metal levels in the remnants of multiple food items that were not fully digested, but also some mobilized from body tissues (Dauwe *et al*., 2004; Morrisey *et al*., 2005; Kenney *et al*., 2018). The results showed that *H. himantopus* faeces contain detectable mercury, proving that are an elimination pathway that can be used as an environmental contamination matrix just as eggshells and feathers. Although it was expected lower levels during the breeding season as females may also eliminate mercury via eggs, or via feathers during moult prior to the breeding season, there was no significant difference between months, suggesting that contamination by mercury via food did not vary seasonally. A similar result was observed by Signa *et al* 2013 concerning *Larus michahellis* faeces, in which there was a seasonal variation for all analyzed elements (As, Cd, Cr, Cu, Ni, Pb, V and Zn) except for mercury. On the other hand, Liu *et al*., 2019 found significant seasonal variation in droppings' mercury levels of captive *Nipponia nippon* in China. Temporal variations in mercury and other heavy metal levels in birds over seasons or months may be attributed to and reflect: 1) changes in the species' diet (e.g. shift from preys of lower trophic levels to preys of higher ones) in response to several factors such as food availability, life cycle or energy demands, 2) variation in prey heavy metal levels; and 3) variation in the metals bioavailability in the environment (Berglund *et al*., 2005; Signa *et al*., 2013; Finger *et al*., 2017). The lack of significant temporal variations might be due to the small sample size used here and given the sampling protocol, it should also be noted that it is impossible to sample faeces from females only and only 1 month of breeding season was sampled.

There is very little information about mercury levels in birds faeces. Overall mean mercury level in *H. himantopus* faeces recorded in this study (0.062 ± 0.008 ppm dw) were within the range reported in the literature (see Tab.7 in Appendix). Fu *et al*., 2014 and Wang *et al*., 2017 recorded, lower concentrations for several crane species (0.018 to 0.028 ppm dw) and *Cygnus cygnus* (0.010 ppm dw). These lower values are mainly due to their diet, which is composed of plant material, while *H. himantopus* forage on invertebrates which accumulate higher values of mercury. In contrast, mercury values in faeces of *Eudyptula minor* and *L. michahellis* recorded by Finger *et al*., 2017, Signa *et al*., 2013 respectively, were higher than those documented in the current study for *H. himantopus.* Penguins and gulls migh forage on higher trophic levels, specificaly fish, acumulating therefore high amounts of mercury. Futhermore *L. michahellis* can forage also on dumps and in other anthropogenic areas, close to mercury sources, that might be highly contaminated (Signa *et al*., 2013).

4.4) Potential deleterious effects of mercury

For migratory shorebirds and other birds, the bioaccumulation of mercury can be very problematic because it can result in loss of appetite, problems in coordination, little flight endurance, weight loss, and even influence decisions regarding the time to remain in the stopovers (Seewagen, 2018; Seewagen *et al*., 2019). All these effects can compromise migration success, their survival and ultimately contributing to the decline of shorebirds populations over the globe. Mercury concentrations above 5 ppm in feathers are associated to several sublethal effects (Burger and Gochfeld, 1997, Eisler, 2000). In this study, all shorebirds species from Bijagós Archipelago had mean mercury levels well below that threshold with a maximum of 2.773±0.784 ppm dw recorded for *A. hypoleucos*. However, in the Tagus Estuary, *T. totanus* and *C. alba* had mean mercury concentration that exceed 5 ppm. Therefore, these two species might be suffering or be at risk of sub-lethal effects. Although the remaining species wintering at Tagus Estuary had a mean level below the threshold, all had at least one individual with mercury levels above it, except for *L. limosa*, indicating that every species sampled is susceptible to accumulate higher mercury concentrations.

Breeding success can be considerably affected by mercury, so the threshold effect levels of this pollutant in birds' eggs have also been investigated. Mercury levels of 1.5 ppm in avian eggs, are associated to impaired reproduction (e.g., embryo malformations, lowered hatchability, eggshell thinning, among others) (Burger and Gochfeld, 1997). However, the threshold mentioned above, and others present in the literature, are related to whole egg or egg contents samples, and not only to eggshells. According to Brasso *et al*., 2012 findings, shells and membranes accumulate 0.9% and 0.4% respectively, of total mercury in the egg. Based on that and since we analysed shells with membrane, we can assume that levels recorded in our study account for those 1.3% of total mercury whereas the others 98.7% (contents) are approximately 1.367 and 0.683 ppm for *H. himantopus*, and 2.581 and 2.809 ppm for *C. alexandrinus* from Tagus Estuary and Ria de Aveiro, respectively. Contents of *C. alexandrinus* eggs had a speculative mean mercury concentration above the 1.5 ppm threshold. It should be noted that these data are speculative and need further confirmation. Moreover, given the levels of mercury found on feathers of other shorebirds species also foraging upon benthos on the intertidal habitats of the Tagus Estuary, (e.g., *C. hiaticula* and *C. canutus*), it is very likely that *C. alexandrinus* may present levels of mercury above the 1.5 ppm threshold being therefore at risk of impaired reproduction which might contribute for the declining of population.

5) CONCLUSION AND FUTURE CONSIDERATIONS

This work was very important because it allowed to assess mercury levels in three different non-invasive biological matrices (feathers, eggshells and faeces) and to compare mercury levels between species and exposure in tropical and temperate wetlands. All matrices used contained mercury, especially feathers, confirming their potential as depuration pathways and biomonitors tools. Mercury levels varied both among species and wetlands. We concluded that mercury exposure is higher in the Tagus Estuary than in the Bijagós Archipelago which was reflected by the low feather mercury levels found in the last wetland as expected. To our knowledge, this is the first assessment of mercury levels in shorebirds in the Bijagós Archipelago giving therefore an very important insight about shorebirds community exposure to mercury in this tropical region. Taking in account the results from other studies we can also conclude that mercury exposure it is not necessarily higher in temperate or industrialized regions. Concerning eggshells, we concluded that *C. alexandrinus* eggshells had more mercury than *H. himantopus* and that this last species are more exposed in Tagus Estuary than in Ria de Aveiro. Finally, there was no temporal variation in mercury levels in the faeces, thus suggesting a similar dietary contamination across months.

In terms of ecotoxicological risk, based on mercury effect threshold of 5 ppm for feathers, the results indicate that *T. totanus* and *C. alba* from the Tagus Estuary might be vulnerable to sublethal effects because exceeded the threshold. Considering the threshold of 1.5 ppm for eggs and the speculative mean concentration in eggs contents, *C. alexandrinus* might be also at risk. Furthermore the high values recorded in Tagus Estuary also warn about the possible high contamination of the foraging areas used by shorebirds. Therefore mercury pollution can be a serious problem in shorebirds conservation in that temperate estuary and futher studies concerning mercury biomonitoring and mercury impacts on shorebirds population are needed.

Differences in mercury bioavailability in each wetland as well as differences in trophic habits might be the cause of the variations of mercury concentrations between species and between wetlands observed in this study. Thus, we recommended in future works 1) an approach using stable isotopes to understand the influence of diet and habitat, as well as trophic levels on mercury concentrations; 2) comparisions of shorebirds's diet in different areas as well as the assessment of mercury levels in their prey. Furthermore, given the scarcity of information about mercury levels in shorebirds, especially in tropical latitudes, more studies are needed concerning not only mercury but also other equally dangerous heavy metals such as lead, cadmium among others.

6) REFERENCES

Agusa, T., Matsumoto, T., Ikemoto, T., Anan, Y., Kubota, R., Yasunaga, G., kunito, T., Tanabe, S., Ogi, H., and Shibata, Y. (2005). Body distribution of trace elements in black-tailed gulls from Rishiri Island, Japan: age-dependent accumulation and transfer to feathers and eggs. *Environmental Toxicology and Chemistry: An International Journal*, *24*(9), 2107-2120.

Aliakbari, A., Savabieasfahani, M., and Ghasempouri, S. M. (2011). Mercury in Egg and Eggshell of Whiskered Tern (*Chlidonias hybrida*) from Anzali Wetlands of the Caspian Sea, Iran. *Bulletin of Environmental Contamination and Toxicology*, *86*(2), 175–179. doi:10.1007/s00128-010-0155-1

Alves, J. A., Lourenço, P. M., Piersma, T., Sutherland, W. J., and Gill, J. A. (2010). Population overlap and habitat segregation in wintering Black-tailed Godwits *Limosa limosa*. *Bird Study*, *57*(3), 381–391. doi:10.1080/00063651003678475

Alves, J. A., Sutherland, W. J., and Gill, J. A. (2012). Will improving wastewater treatment impact shorebirds? Effects of sewage discharges on estuarine invertebrates and birds. *Animal Conservation*, *15*, 44–52. doi:10.1111/j.1469-1795.2011.00485.x

Arcia-Castañeda, G., Muñoz-Gil, J., Marín-Espinoza, G., Rondón-Sánchez, W., Hernández-Villarroel, M., Zabala-Marcano, R., and León-Ninin, J. (2019). CONTENIDO DE MERCURIO EN CÁSCARAS DE HUEVOS DEL ALCATRAZ (*PELECANUS OCCIDENTALIS*) EN UN ÁREA DE NIDIFICACIÓN EN EL GOLFO DE CARIACO, VENEZUELA. *The Biologist*, *17*(1), 31–39.

Beninger, P.G., Elner, R., Morançais, M., and Decottignies, P., (2011). Downward trophic shift during breeding migration in the shorebird *Calidris mauri* (western sandpiper). *Marine Ecology Progress Series*, 428, 259–269.

Beyer, W. N., and Day, D. (2004). Role of manganese oxides in the exposure of mute swans (*Cygnus olor)* to Pb and other elements in the Chesapeake Bay, USA. *Environmental Pollution*, *129*(2), 229–235. doi:10.1016/j.envpol.2003.10.026

Bocher, P., Robin, F., Kojadinovic, J., Delaporte, P., Rousseau, P., Dupuy, C., and Bustamante, P. (2014). Trophic resource partitioning within a shorebird community feeding on intertidal mudflat habitats. *Journal of Sea Research*, 92, 115-124. doi:10.1016/j.seares.2014.02.011

Bodin, N., N'Gom-Kâ, R., Kâ, S., Thiaw, O. T., Tito de Morais, L., Le Loc'h, F., Rozuel-Chartier, E., Auger, D., and Chiffoleau, J.-F. (2013). Assessment of trace metal contamination in mangrove ecosystems from Senegal, West Africa. *Chemosphere*, *90*(2), 150–157. doi:10.1016/j.chemosphere.2012.06.019

Brasso, R. L., Abel, S., and Polito, M. J. (2012). Pattern of Mercury Allocation into Egg Components is Independent of Dietary Exposure in Gentoo Penguins. *Archives of Environmental Contamination and Toxicology*, *62*(3), 494–501. doi:10.1007/s00244-011-9714-7

Braune, B. M., and Gaskin, D. E. (1987). A Mercury Budget for the Bonaparte's Gull during Autumn Moult. *Ornis Scandinavica*, *18*(4), 244.

Burger, J. (1993). Metals in avian feathers: bioindicators of environmental pollution. *Rev Environ ContamToxicol*, *5*, 203–311.

Burger, J. (1994). Heavy metals in avian eggshells: Another excretion method. *Journal of Toxicology and Environmental Health*, *41*(2), 207–220. doi:10.1080/15287399409531837

Burger, J., and Gochfeld, M. (1991). Lead, mercury, and cadmium in feathers of tropical terns in Puerto Rico and Australia. *Archives of Environmental Contamination and Toxicology*, *21*(2), 311–315. doi:10.1007/bf01055351

Burger, J., and Gochfeld, M. (1997). Risk, mercury levels, and birds: Relating adverse laboratory effects to field biomonitoring. *Environmental Research*, *75*(2), 160–172. doi:10.1006/enrs.1997.3778

Burger, J., and Gochfeld, M. (2001). Effects of chemicals and pollution on seabirds. In *Biology of marine bird* (pp. 503-544). CRC press. doi:10.1201/9781420036305.ch15

Burger, J., Gochfeld, M., Sullivan, K., Irons, D., and McKnight, A. (2008). Arsenic, cadmium, chromium, lead, manganese, mercury, and selenium in feathers of Black-legged Kittiwake (*Rissa tridactyla*) and Black Oystercatcher (*Haematopus bachmani*) from Prince William Sound, Alaska. *Science of The Total Environment*, *398*(1–3), 20–25. doi:10.1016/j.scitotenv.2008.02.051

Burger, J., Mizrahi, D., Tsipoura, N., Jeitner, C., and Gochfeld, M. (2018). Mercury, lead, cadmium, cobalt, arsenic and selenium in the blood of semipalmated sandpipers (*Calidris pusilla*) from Suriname, South America: Age-related differences in wintering site and comparisons with a stopover site in New Jersey, USA. *Toxics*, *6*(2), 1–11. doi:10.3390/toxics6020027

Burger, J., Seyboldt, S., Morganstein, N., and Clark, K. (1993). Heavy metals and selenium in feathers of three shorebird species from Delaware bay. *Environmental Monitoring and Assessment*, *28*(2), 189–198. doi:10.1007/bf00547037

Burger, J, Tsipoura, N., Niles, L., Gochfeld, M., Dey, A., and Mizrahi, D. (2015). Mercury, Lead, Cadmium, Arsenic, Chromium and Selenium in Feathers of Shorebirds during Migrating through Delaware Bay, New Jersey: Comparing the 1990s and 2011/2012. *Toxics*, *3*, 63–74. doi:10.3390/toxics3010063

Catry, T., Alves, J. . A., Andrade, J., Costa, H., Dias, M. P., Fernandes, P., Leal, A., Lourenço, P. M., Martins, R. C., Moniz, F., Pardal, S., Rocha, A., Santos, C. D., Encarnação, V., and Granadeiro, J. P. (2011). Long-term declines of wader populations at the Tagus estuary, Portugal: a response to global or local factors? *Bird Conservation International, 21,* 438-453. doi:10.1017/S0959270910000626

Catry, T., Figueira, P., Carvalho, L., Monteiro, R., Coelho, P., Lourenço, P. M., Catry, P., Tchantchalam, Q., Catry, I., Botelho, M. J., Pereira, E., Granadeiro, J. P., and Vale, C. (2017). Evidence for contrasting accumulation pattern of cadmium in relation to other elements in *Senilia senilis* and *Tagelus adansoni* from the Bijagós archipelago, Guinea-Bissau. *Environmental Science and Pollution Research*, *24*(32), 24896–24906. doi:10.1007/s11356-017-9902-8

Catry, T., Lourenço, P. M., Lopes, R. J., Bocher, P., Carneiro, C., Alves, J. A., Delaporte, P., Bearhop, S., Piersma, T., and Granadeiro, J. P. (2016). Use of stable isotope fingerprints to assign wintering origin and trace shorebird movements along the East Atlantic Flyway. Basic and Applied Ecology, 17(2), 177–187. doi:10.1111/1365-2435.12506

Cesário, R., Monteiro, C. E., Nogueira, M., O'Driscoll, N. J., Caetano, M., Hintelmann, H., Mota, A. M., and Canário, J. (2016). Mercury and Methylmercury Dynamics in Sediments on a Protected Area of Tagus Estuary (Portugal). *Water, Air, and Soil Pollution*, *227*(12), 475. doi:10.1007/s11270-016-3179-2

Coelho, J.P., et al., Estimation of mercury background values in sediment and biota of the Bijagós archipelago, GuineaBissau, Marine Pollution Bulletin (2016), doi:10.1016/j.marpolbul.2016.06.053

Costa, R. A., Eeva, T., Eira, C., Vaqueiro, J., and Vingada, J. V. (2012). Trace Elements in Faeces of Great Tit Nestlings in Relation to Breeding Performance in Coastal Areas in Central Portugal. *Archives of Environmental Contamination and Toxicology*, *63*(4), 594–600. doi:10.1007/s00244-012-9798-8

Costley, C. T., Mossop, K. F., Dean, J. R., Garden, L. M., Marshall, J., and Carroll, J. (2000). Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation.*Analytica Chimica Acta*, *405*(1-2), 179-183. doi:10.1016/S0003-2670(99)00742-4

Cuervo, J. J. (1993). *Biología reproductiva de la avoceta (Recurvirostra avosetta) y la cigúeñuela (Himantopus himantopus) en el sur de España.* Universidad Complutense de Madrid.

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R., and Eens, M. (2003). Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environmental Pollution*, *124*(3), 429–436. doi:10.1016/S0269-7491(03)00044-7

Dauwe, T, Janssens, E., Bervoets, L., Blust, R., and Eens, M. (2004). Relationships between metal concentrations in great tit nestlings and their environment and food. *Environmental Pollution*, *131*(3), 373–380. doi:10.1016/j.envpol.2004.03.009

Delany, S., Scott, D., Dodman, T., and Stroud, D. (2009). An atlas of wader populations in Africa and Western Eurasia. *British Birds*, *102*, 639–642.

Dias, J. M., Lopes, J. F., and Dekeyser, I. (1999). Hydrological characterisation of Ria de Aveiro, Portugal, in early summer. *Oceanologica Acta*, *22*(5), 473–485. doi:10.1016/S0399-1784(00)87681-1

Dodman, T., and Sá, J. (2015). *Monitorização de aves aquáticas no Arquipélago dos Bijagós , Monitorização de aves aquáticas no Arquipélago dos Bijagós, Guiné-Bissau*. Wetlands International, Dakar / Gabinete de Planificação Costeira / ODZH, Bissau.

Driscoll, C. T., Mason, R. P., Chan, H. M., Jacob, D. J., and Pirrone, N. (2013). Mercury as a global pollutant: sources, pathways, and effects. Environmental science and technology, 47(10), 4967-4983. doi:10.1021/es305071v

Eagles-Smith, C. A., Ackerman, J. T., De La Cruz, S. E. W., and Takekawa, J. Y. (2009). Mercury bioaccumulation and risk to three waterbird foraging guilds is influenced by foraging ecology and breeding stage. *Environmental Pollution*, *157*(7), 1993–2002. doi:10.1016/j.envpol.2009.03.030

Eisler, R. (2000). *Handbook of chemical risk assessment: health hazards to humans, plants, and animals*. Lewis Publishers.

Espín, S., Ruiz, S., Sánchez-Virosta, P., and Eeva, T. (2016). Effects of calcium supplementation on growth and biochemistry in two passerine species breeding in a Ca-poor and metal-polluted area. *Environmental Science and Pollution Research*, *23*(10), 9809–9821. doi:10.1007/s11356-016-6219-y

Fernández, G., García-Hernández, J., Cruz-Acevedo, E., and Castillo-Guerrero, J. A. (2018). Patterns of Mercury Accumulation in Tissues of Western Sandpipers (*Calidris maurii*) Wintering on the Coast of Sinaloa, Mexico. *Waterbirds*, *41*(4), 438-442. doi:10.1675/063.041.0408

Figueres, G., Martin, J. M., Meybeck, M., and Seyler, P. (1985). A comparative study of mercury contamination in the Tagus estuary (Portugal) and major French estuaries (Gironde, Loire, Rhône). *Estuarine, Coastal and Shelf Science*, *20*(2), 183–203 doi:10.1016/0272-7714(85)90037-X

Finger, A., Lavers, J. L., Dann, P., Kowalczyk, N. D., Scarpaci, C., Nugegoda, D., and Orbell, J. D. (2017). Metals and metalloids in Little Penguin (*Eudyptula minor*) prey, blood and faeces. *Environmental Pollution*, *223*, 567–574. doi:10.1016/j.envpol.2017.01.059

Fitzner, R. E., Rickard, W. H., and Hinds, W. T. (1982). Excrement from heron colonies for environmental assessment of toxic elements. *Environmental Monitoring and Assessment*, *1*, 383–386. doi:10.1007/bf0040383

Fonseca, V. F., França, S., Duarte, B., Caçador, I., Cabral, H. N., Mieiro, C. L., Coelho, J. P., Pereira, E., and Reis-Santos, P. (2019). Spatial Variation in Mercury Bioaccumulation and Magnification in a Temperate Estuarine Food Web. *Frontiers in Marine Science*, *6*,117.

doi:10.3389/fmars.2019.00117

Fu, J., Wang, Q., Wang, H., Yu, H., and Zhang, X. (2014). Monitoring of non-destructive sampling strategies to assess the exposure of avian species in Jiangsu Province, China to heavy metals. *Environmental Science and Pollution Research*, *21*(4), 2898–2906. doi:10.1007/s11356-013-2242-4

Furness, R. W. (1993). Birds as monitors of pollutants. In Furness, R. W., and Greenwood, J. J. (Eds.). (1993). *Birds as monitors of environmental change.*, (Issue 1987, pp. 86–143).

Furness, R. W., Muirhead, S. J., and Woodburn, M. (1986). Using bird feathers to measure mercury in the environment: Relationships between mercury content and moult. *Marine Pollution Bulletin*, *17*(1), 27–30.

Gaudêncio, M. J., and Cabral, H. N. (2007). Trophic structure of macrobenthos in the Tagus estuary and adjacent coastal shelf. *Hydrobiologia*, *587(1)*, 241–251. doi:10.1007/s10750-007-0686-6

Grajewska, A., Falkowska, L., and Reindl, A. (2019). Evaluation of claws as an alternative route of mercury elimination from the herring gull (*Larus argentatus*). *Oceanological and Hydrobiological Studies*, *48*(2), 165–173. doi:10.1515/ohs-2019-0015

Grajewska, A., Falkowska, L., Szumiło-Pilarska, E., Hajdrych, J., Szubska, M., Frączek, T., Meissner, W., Bzoma, S., Bełdowska, M., Przystalski, A., and Brauze, T. (2015). Mercury in the eggs of aquatic birds from the Gulf of Gdansk and Wloclawek Dam (Poland). *Environmental Science and Pollution Research*, *22*(13), 9889–9898. doi:10.1007/s11356-015-4154-y

Gutiérrez-Estrada, J. C., Vasconcelos, R., and Costa, M. J. (2008). Estimating fish community diversity from environmental features in the Tagus estuary (Portugal): Multiple Linear Regression and Artificial Neural Network approaches. *Journal of Applied Ichthyology*, *24*(2), 150–162. doi:10.1111/j.1439-0426.2007.01039.x

Hargreaves, A. L., Whiteside, D. P., and Gilchrist, G. (2010). Concentrations of 17 elements, including mercury, and their relationship to fitness measures in arctic shorebirds and their eggs. *Science of the Total Environment*, *408*(16), 3153–3161. doi:10.1016/j.scitotenv.2010.03.027

Hartman, C. A., Ackerman, J. T., Herzog, M. P., and Eagles-Smith, C. A. (2017). Season, molt, and body size influence mercury concentrations in grebes. *Environmental Pollution*, *229*, 29–39. doi:10.1016/j.envpol.2017.05.058

Honda, K., Nasu, T., and Tatsukawa, R. (1986). Seasonal changes in mercury accumulation in the black-eared kite, *Milvus migrans lineatus*. *Environmental Pollution Series A, Ecological and Biological*, *42*(4), 325–334. doi:10.1016/0143-1471(86)90016-4

INE. (2021). *Censos 2011. Instituto Nacional de Estatística*. http://censos.ine.pt.

Joiris, C. R., Azokwu, M. I., Otchere, F. A., and Ali, I. B. (1998). Mercury in the bivalve *Anadara (Senilia) senilis* from Ghana and Nigeria. *Science of The Total Environment*, *224*, 181–188. doi:10.1016/s0048-9697(98)00355-6

Kenney, L. A., Kaler, R. S. A., Kissling, M. L., Bond, A. L., and Eagles-Smith, C. A. (2018). Mercury concentrations in multiple tissues of Kittlitz's murrelets (*Brachyramphus brevirostris*). *Marine Pollution Bulletin*, *129*(2), 675–680. doi:10.1016/j.marpolbul.2017.10.055

Klaassen, M., Lindström, Å., Meltofte, H., and Piersma, T. (2001). Arctic waders are not capital breeders. *Nature*, *413*(6858), 794–794.

Lam, J. C. W., Tanabe, S., Lam, M. H. W., and Lam, P. K. S. (2005). Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence from trace elements in eggs. *Environmental Pollution*, *135*(3), 481–490. doi:10.1016/j.envpol.2004.11.021

Lavoie, R. A., Jardine, T. D., Chumchal, M. M., Kidd, K. A., and Campbell, L. M. (2013). Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis. *Environmental Science and Technology*, *47*(23), 13385–13394. doi:10.1021/es403103t

Lewis, S. A., and Furness, R. W. (1991). Mercury accumulation and excretion in laboratory reared black-headed gull *Larus ridibundus* chicks. *Archives of Environmental Contamination and Toxicology*, *21*(2), 316–320.

Lewis, S. A., Becker, P. H., and Furness, R. W. (1993). Mercury levels in eggs, tissues, and feathers of herring gulls *Larus argentatus* from the German Wadden Sea Coast. *Environmental Pollution*, *80*(3), 293–299. doi:10.1016/0269-7491(93)90051-o

Liebezeit, J. R., Smith, P. A., Lanctot, R. B., Schekkerman, H., Tulp, I., Kendall, S. J., Tracy, D. M., Rodrigues, R. J., Meltofte, H., Robinson, J. A., Gratto-Trevor, C., Mccaffery, B. J., Morse, J., and Zack, S. W. (2007). Assessing the development of shorebird eggs using the flotation method: Species-specific and generalized regression models. *Condor*, *109*(1), 32–47.

Liu, Q., Chen, Y., Maltby, L., Zheng, Y., and Ma, Q. (2019). Trace elements exposure of endangered crested ibis (*Nipponia nippon*) under in situ and ex situ conservations. *Environmental Pollution*, *253*, 800–810. doi:10.1016/j.envpol.2019.07.078

Liu, X., Zhao, S., Sun, L., Yin, X., Xie, Z., Honghao, L., and Wang, Y. (2006). P and trace metal contents in biomaterials, soils, sediments and plants in colony of red-footed booby (*Sula sula*) in the Dongdao Island of South China Sea. *Chemosphere*, *65*(4), 707–715. doi:10.1016/j.chemosphere.2006.01.043

Lourenço, P. M., Catry, T., and Granadeiro, J. P. (2017). Diet and feeding ecology of the wintering shorebird assemblage in the Bijagós archipelago, Guinea-Bissau. *Journal of Sea Research*, *128*, 52–60. doi:10.1016/j.seares.2017.08.004

Lourenço, P. M., and Piersma, T. (2008). Stopover ecology of Black-tailed Godwits *Limosa limosa*

limosa in Portuguese rice fields: a guide on where to feed in winter. *Bird Study*, *55*(2), 194–202. doi:10.1080/00063650809461522

Lucia, M., André, J. M., Gontier, K., Diot, N., Veiga, J., and Davail, S. (2010). Trace element concentrations (mercury, cadmium, copper, zinc, lead, aluminium, nickel, arsenic, and selenium) in some aquatic birds of the southwest atlantic coast of France. *Archives of Environmental Contamination and Toxicology*, *58*(3), 844–853. doi:10.1007/s00244-009-9393-9

Lucia, M., Bocher, P., Chambosse, M., Delaporte, P., and Bustamante, P. (2014). Trace element accumulation in relation to trophic niches of shorebirds using intertidal mudflats. *Journal of Sea Research*, *92*, 134–143. doi:10.1016/j.seares.2013.08.008

Lucia, M., Bocher, P., Cosson, R. P., Churlaud, C., and Bustamante, P. (2012). Evidence of species-specific detoxification processes for trace elements in shorebirds. *Ecotoxicology*, *21*(8), 2349-2362. doi:10.1007/s10646-012-0991-3

Masero, J. A., and Pérez-Hurtado, A. (2001). Importance of the supratidal habitats for maintaining overwintering shorebird populations: How redshanks use tidal mudflats and adjacent saltworks in Southern Europe. *Condor*, *103*(1), 21–30.

Morera, M., Sanpera, C., Crespo, S., Jover, L., and Ruiz, X. (1997). Inter- and Intraclutch Variability in Heavy Metals and Selenium Levels in Audouin's Gull Eggs from the Ebro Delta, Spain. *Archives of Environmental Contamination and Toxicology*, *33*(1), 71–75. doi:10.1007/s002449900225

Morgado, R., Nobre, M., Ribeiro, A., Puga, J., and Luís, A. (2009). A Importância do Salgado para a Gestão da Avifauna Limícola Invernante na Ria de Aveiro (Portugal). *Revista de Gestão Costeira Integrada*, *9*(3), 79–93.

Morrison, R. I. G., and Hobson, K. A. (2004). Use of Body Stores in Shorebirds After Arrival on High-Arctic Breeding Grounds. *The Auk*, *121*(2), 333–344. doi:10.2307/4090397

Morrissey, C. A., Bscendell-Young, L. I., and Elliott, J. E. (2005). Assessing trace-metal exposure to American dippers in mountain streams of southwestern British Columbia, Canada. *Environmental Toxicology and Chemistry*, *24*(4), 836–845. doi:10.1897/04-110r.1

Múrias, T., Gonçalves, D., and Lopes, R. J. (2017). Evaluating the impacts of a new railway on shorebirds: a case study in Central Portugal (Aveiro Lagoon). In L. Borda-de-Água, R. Barrientos, P. Beja, and H. Pereira (Eds.), *Railway Ecology* (pp. 197–214). Springer, Cham.

Newton, I. (2009). Moult and plumage. *Ringing and Migration*, *24*(3), 220-226.

Olivero-Verbel, J., Agudelo-Frias, D., and Caballero-Gallardo, K. (2013). Morphometric parameters and total mercury in eggs of snowy egret (*Egretta thula*) from Cartagena Bay and Totumo Marsh, north of Colombia. *Marine Pollution Bulletin*, *69*(1–2), 105–109.

doi:10.1016/j.marpolbul.2013.01.013

Otchere, F. A. (2003). Heavy metals concentrations and burden in the bivalves (*Anadara (Senilia) senilis*, *Crassostrea tulipa* and *Perna perna*) from lagoons in Ghana: Model to describe mechanism of accumulation/excretion. *African Journal of Biotechnology*, *2*(9), 280–287. doi:10.5897/ajb2003.000-1057

Pandiyan, J., Jagadheesan, R., Karthikeyan, G., Mahboob, S., Al-Ghanim, K. A., Al-Misned, F., Ahmed, Z., Krishnappa, K., Elumalai, K., and Govindarajan, M. (2020). Probing of heavy metals in the feathers of shorebirds of Central Asian Flyway wintering grounds. *Scientific Reports*, *10*, 1–13. doi:10.1038/s41598-020-79029-z

Pedro, P., and Ramos, J. A. (2009). Diet and prey selection of shorebirds on salt pans in the mondego estuary, western Portugal. *Ardeola*, *56*(1), 1–11.

Perez-Hurtado, A., Goss-Custard, J. D., and Garcia, F. (1997). The diet of wintering waders in Cádiz Bay, southwest Spain. *Bird Study*, *44*(1), 45–52. doi:10.1080/00063659709461037

Perkins, M., Ferguson, L., Lanctot, R. B., Stenhouse, I. J., Kendall, S., Brown, S., Gates, H. R., Hall, J. O., Regan, K., and Evers, D. C. (2016). Mercury exposure and risk in breeding and staging Alaskan shorebirds. *The Condor*, *118*(3), 571–582. doi:10.1650/CONDOR-16-36.1

Peterson, S. H., Ackerman, J. T., Eagles-Smith, C. A., Hartman, C. A., and Herzog, M. P. (2017). A critical evaluation of the utility of eggshells for estimating mercury concentrations in avian eggs. *Environmental Toxicology and Chemistry*, *36*(9), 2417–2427. doi:10.1002/etc.3777

Picado, A., Dias, J. M., and Fortunato, A. B. (2010). Tidal changes in estuarine systems induced by local geomorphologic modifications. *Continental Shelf Research*, *30*(17), 1854–1864. doi:10.1016/j.csr.2010.08.012

Picone, M., Corami, F., Gaetan, C., Basso, M., Battiston, A., Panzarin, L., and Volpi Ghirardini, A. (2019). Accumulation of trace elements in feathers of the Kentish plover *Charadrius alexandrinus*. *Ecotoxicology and Environmental Safety*, *179*(2018), 62–70. doi:10.1016/j.ecoenv.2019.04.051

Quinn, J. T., and Hamilton, D. J. (2012). Variation in diet of Semipalmated Sandpipers (*Calidris pusilla*) during stopover in the upper Bay of Fundy, Canada. *Canadian Journal of Zoology*, *90*(9), 1181-1190. doi:10.1139/z2012-086

Rodriguez-Navarro, A. B., Gaines, K. F., Romanek, C. S., and Masson, G. R. (2002). Mineralization of Clapper Rail Eggshell from a Contaminated Salt Marsh System. *Archives of Environmental Contamination and Toxicology*, *43*(4), 449–460. doi:10.1007/s00244-002-0266-8

Roodbergen, M., Klok, C., and van der Hout, A. (2008). Transfer of heavy metals in the food chain earthworm Black-tailed godwit (*Limosa limosa*): Comparison of a polluted and a reference site in The Netherlands. *Science of The Total Environment*, *406*(3), 407–412 doi:10.1016/j.scitotenv.2008.06.051

Rutkowska, M., Płotka-Wasylka, J., Lubinska-Szczygeł, M., Różańska, A., Możejko-Ciesielska, J., and Namieśnik, J. (2018). Birds' feathers–suitable samples for determination of environmental pollutants. *TrAC Trends in Analytical Chemistry*, *109*, 97-115. doi:10.1016/j.trac.2018.09.022

Salvig, J. C., Asbirk, S., Kjeldsen, J. P., and Rasmussen, P. A. F. (1994). Wintering waders in the Bijagos Archipelago, Guinea-Bissau 1992-1993. *Ardea*, *82*, 137–142.

Sánchez, M. I., Green, A. J., and Castellanos, E. M. (2005). Seasonal variation in the diet of Redshank *Tringa totanus* in the Odiel Marshes, southwest Spain: a comparison of faecal and pellet analysis. *Bird Study*, *52*(2), 210–216. doi:10.1080/00063650509461393

Seewagen, C. L. (2010). Threats of environmental mercury to birds: knowledge gaps and priorities for future research. *Bird Conservation International*, *20*(2), 112-123. doi:10.1017/S095927090999030X

Seewagen, C. L. (2018). The threat of global mercury pollution to bird migration: potential mechanisms and current evidence. *Ecotoxicology*, *29*(8), 1254–1267. doi:10.1007/s10646-018-1971-z

Seewagen, C. L., Ma, Y., Morbey, Y. E., and Guglielmo, C. G. (2019). Stopover departure behavior and flight orientation of spring-migrant Yellow-rumped Warblers (*Setophaga coronata*) experimentally exposed to methylmercury. *Journal of Ornithology*, *160*(3), 617–624 doi:10.1007/s10336-019-01641-2

Signa, G., Mazzola, A., Tramati, C. D., and Vizzini, S. (2013). Gull-derived trace elements trigger small-scale contamination in a remote Mediterranean nature reserve. *Marine Pollution Bulletin*, *74*(1), 237–243. doi:10.1016/j.marpolbul.2013.06.051

Silva, A. (2010). Estratégia para uma gestão sustentável do Salgado de Aveiro. Secção Autónoma de Ciências Sociais, Jurídicas e Políticas. Universidade de Aveiro.

St. Clair, C. T., Baird, P., Ydenberg, R., Elner, R., and Bendell, L. I. (2015). Trace elements in pacific Dunlin (*Calidris alpina pacifica*): patterns of accumulation and concentrations in kidneys and feathers. *Ecotoxicology*, *24*, 29–44. doi:10.1007/s10646-014-1352-1

Su, T., Lin, X., Huang, Q., Jiang, D., Zhang, C., Zhang, X., Dingle, C., Goodale, E., Que, P., Wang, R., and Liu, Y. (2020). Mercury exposure in sedentary and migratory *Charadrius* plovers distributed widely across China. *Environmental Science and Pollution Research*, *27*(4), 4236–4245. doi:10.1007/s11356-019-06873-6

Sutherland, W. J., Alves, J. A., Amano, T., Chang, C. H., Davidson, N. C., Max Finlayson, C., Gill, J. A., GillJR, R. E., González, P.M., Gunnarsson, T. G., Kleijn, D., Spray, C. J., Székely, T., and Thompson, D. B. (2012). A horizon scanning assessment of current and potential future threats to migratory shorebirds. *Ibis*, *154*(4), 663-679. doi:10.1111/j.1474-

919x.2012.01261.x

Tavares, P. C., Kelly, A., Maia, R., Lopes, R. J., Serrão Santos, R., Pereira, M. E., Duarte, A. C., and Furness, R. W. (2008). Variation in the mobilization of mercury into Black-winged Stilt *Himantopus* himantopus chicks in coastal saltpans, as revealed by stable isotopes. *Estuarine, Coastal and Shelf Science*, *77*(1), 65–76. doi:10.1016/j.ecss.2007.09.015

Tavares, P. C., McGill, R., Newton, J., Pereira, E., Duarte, A., and Furness, R. W. (2009). Relationships between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. *Waterbirds*, 311-321. doi:10.1675/063.032.0211

Tavares, P. C., Monteiro, L. R., Lopes, R. J., Correia Santos, M. M., and Furness, R. W. (2004). Intraspecific Variation of Mercury Contamination in Chicks of Black-Winged Stilt (*Himantopus* himantopus) in Coastal Wetlands from Southwestern Europe. *Bulletin of Environmental Contamination and Toxicology*, *72*(3), 437–444. doi:10.1007/s00128-004-0265-8

Thompson, D. R. (2001). Mercury in Bar-Tailed Godwit (*Limosa lapponica*) and Lesser Knot (*Calidris canutus*): Spatially Explicit Information from Non-Breeding Birds in New Zealand. *Bulletin of Environmental Contamination and Toxicology*, *66*(6), 707–713. doi:10.1007/s00128-001-0066-2

Thompson, D. R., Furness, R. W., and Lewis, S. A. (1993). Temporal and spatial variation in mercury concentrations in some albatrosses and petrels from the sub-Antarctic. Polar Biology, 13(4), 239-244.

Tiller, B. L., Marco, J. D., and Rickard, W. H. (2005). Metal Concentrations, Foraging Distances, and Fledging Success of Great Blue Herons Nesting along the Hanford Reach of the Columbia River. *Environmental Monitoring and Assessment*, *104*(1–3), 71–79. doi:10.1007/s10661-005-1603-y

Tjerngren, I., Meili, M., Björn, E., and Skyllberg, U. (2012). Eight Boreal Wetlands as Sources and Sinks for Methyl Mercury in Relation to Soil Acidity, C/N Ratio, and Small-Scale Flooding. *Environmental Science and Technology*, *46*(15), 8052–8060. doi:10.1021/es300845x

van Aswegen, J. D., Nel, L., Strydom, N. A., Minnaar, K., Kylin, H., and Bouwman, H. (2019). Comparing the metallic elemental compositions of Kelp Gull *Larus dominicanus* eggs and eggshells from the Swartkops Estuary, Port Elizabeth, South Africa. *Chemosphere*, *221*, 533–542. doi:10.1016/j.chemosphere.2019.01.013

Wang, F., Xu, S., Zhou, Y., Wang, P., and Zhang, X. (2017). Trace element exposure of whooper swans (*Cygnus cygnus*) wintering in a marine lagoon (Swan Lake), northern China. *Marine Pollution Bulletin*, *119*(2), 60–67. doi:10.1016/j.marpolbul.2017.03.063

Wang, Q., Zhang, Z., Zhou, X., and Lu, X. (2013). Mercury distribution and accumulation in typical wetland ecosystems of Sanjiang Plain, Northeast China. *Chinese Geographical Science*, *23*(1), 49–58. doi:10.1007/s11769-013-0553-x

Whitney, M. C., and Cristol, D. A. (2017). Impacts of sublethal mercury exposure on birds: a detailed review. *Reviews of Environmental Contamination and Toxicology Volume 244*, 113-163. doi:10.1007/398_2017_4

Wiener, J., Krabbenhoft, D., Heinz, G., and Scheuhammer, A. (2003). Ecotoxicology of Mercury. In J. J. C. Hoffman, David. J., Ratter, Barnett. A., Jr. Allen Burton. G. (Ed.), *Handbook of Ecotoxicology, Second Edition* (2nd ed., pp. 409–465). Lewis Publishers.

Wolfe, M. F., Schwarzbach, S., and Sulaiman, R. A. (1998). Effects of mercury on wildlife: a comprehensive review. *Environmental Toxicology and Chemistry: An International Journal*, *17*(2), 146-160. doi:10.1002/etc5620170203

Yin, X., Xia, L., Sun, L., Luo, H., and Wang, Y. (2008). Animal excrement: A potential biomonitor of heavy metal contamination in the marine environment. *Science of the Total Environment*, *399*(1–3), 179–185. doi:10.1016/j.scitotenv.2008.03.005

Zamani-Ahmadmahmoodi, R., Esmaili-Sari, A., Savabieasfahani, M., Ghasempouri, S. M., and Bahramifar, N. (2010). Mercury Pollution in Three Species of Waders from Shadegan Wetlands at the Head of the Persian Gulf. *Bulletin of Environmental Contamination and Toxicology*, *84*(3), 326–330. doi:10.1007/s00128-010-9933-z

7) APPENDIX

Table 5: Mercury concentrations in feathers of shorebirds reported in literature for Temperate and Tropical latitudes.

Latitude	Species	Hg	Site	Year	Reference
Temperate	*Arenaria interpres*	1900 (a)	Southampton Island, Nunavut	2008	Hargreaves et al., 2010
	Calidris alba	5.947	Tagus Estuary, Portugal	2012-2014	**This study**
		2.813	Delaware Bay, New Jersey, USA	1991	Burger et al., 1993
		2.007 (b)	Delaware Bay, New Jersey, USA	1991-1992	Burger et al., 2015
		0.730	Delaware Bay, New Jersey, USA	2011-2012	Burger et al., 2015
	Calidris alpina	3.289	Tagus Estuary, Portugal	2012-2014	**This study**
		1.83	Pertuis Charentais, France	2003-2011	Lucia et al., 2014
		2.6	Boundary Bay, Fraser River Delta	2011	St. Clair et al., 2015
	Calidris canutus	3.542	Tagus Estuary, Portugal	2012-2014	**This study**
		2.00	New Zealand	1999-2000	Thompson, 2001
		1.85	New Zealand	1999-2000	Thompson, 2001
		1.26	Pertuis Charentais, France	2007-2011	Lucia et al., 2012
		1.163	Delaware Bay, New Jersey, USA	1991	Burger et al., 1993
		0.791 (b)	Delaware Bay, New Jersey, USA	1991-1992	Burger et al., 2015
		0.576	Delaware Bay, New Jersey, USA	2011-2012	Burger et al., 2015
		2.09	Gironde Estuary, France	2005-2007	Lucia et al., 2010
	Calidris pusilla	0.021	Delaware Bay, New Jersey, USA	1992	Burger et al., 1993
		0.420 (b)	Delaware Bay, New Jersey, USA	1991-1992	Burger et al., 2015
		0.509 (b)	Delaware Bay, New Jersey, USA	1995	Burger et al., 2015
		0.428	Delaware Bay, New Jersey, USA	2011-2012	Burger et al., 2015
	Charadrius alexandrinus	8.710 (c)	Cavallino-Treporti Peninsula, Italy	2018	Picone et al., 2019
		1.129 (a)	Northern/Central China	2016	Su et al., 2020

(Continues in the next page)

	Charadrius dealbatus	2.08 (a)	Southern China	2016	Su et al., 2020
	Charadrius hiaticula	4.605	Tagus Estuary, Portugal	2012-2014	**This study**
	Himantopus himantopus	1.8-2.8 (d)	Tagus Estuary, Portugal	1997-1999	Tavares et al., 2004
		1.1-2.1 (d)	Sado Estuary, Portugal	1997-1999	Tavares et al., 2004
		0.7-0.9 (d)	Mondego Estuary, Portugal	1997-1999	Tavares et al., 2004
		1.2-2.5 (d)	Formosa Lagoon	1997 and 1999	Tavares et al., 2004
		6.6	Shadegan, Iran	2006-2007	Zamani-Ahmadmahmoodi et al., 2010
	Himantopus mexicanus	9.46	San Francisco Bay, California, USA	2004-2006	Eagles-Smith et al., 2009
	Limosa lapponica	2.13	New Zealand	1999-2000	Thompson, 2001
		2.53	New Zealand	1999-2000	Thompson, 2001
	Limosa limosa	2.106	Tagus Estuary, Portugal	2012-2014	**This study**
		1.11	Pertuis Charentais, France	2007-2010	Lucia et al., 2012
		0.52	Blokland, The Netherlands	2002-2004	Roodbergen et al., 2008
		0.52	Zeevang, The Netherlands	2002-2004	Roodbergen et al., 2008
	Pluvialis squatarola	1.18	Gironde Estuary, France	2005-2007	Lucia et al., 2010
	Recurvirostra americana	2.59	San Francisco Bay, California, USA	2004-2006	Eagles-Smith et al., 2009
	Tringa totanus	9.918	Tagus Estuary, Portugal	2012-2014	**This study**
		2.18	Pertuis Charentais, France	2003-2011	Lucia et al., 2014
	Vanellus indicus	0.8	Shadegan, Iran	2006-2007	Zamani-Ahmadmahmood et al., 2010
	Vanellus leucurus	1.9	Shadegan, Iran	2006-2007	Zamani-Ahmadmahmood et al., 2010
	Haemantopus bachmani	1.240	Prince William Sound, Alaska	2004	Burger et al., 2008
Tropical	*Actitis hypoleucos*	2.773	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
		0.2	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
		5.4	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020

(Continues in the next page)

Arenaria interpres	2.193	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
Calidris alba	0.966	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
Calidris alpina	0.5	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	0.9	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Calidris canutus	1.374	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
Calidris ferruginea	1.251	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
	5.4	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	5.5	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Calidris mauri	1.98 (a)	Sinaloa, Mexico	2010-2011	Fernández et al., 2018
Calidris minuta	6.07	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	0.1	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Calidris temmenckii	0.05	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	0.2	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Charadrius alexandrinus	8.3	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	7.2	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Charadrius hiaticula	1.223	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
	5.2	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	8.3	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Charadrius mongolus	7.7	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	5.8	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Himantopus himantopus	6.1	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	5.2	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Numenius arquata	5.3	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	7.7	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Pluvialis squatarola	0.932	Bijagós Archipelago, Guinea-Bissau	2019	**This study**

(Continues in the next page)

Tringa erythropus	7.8	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Tringa erythropus	7.5	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Tringa glareola	7.7	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	5.1	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Tringa stagnatillis	0.05	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	0.2	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
Tringa totanus	2.338	Bijagós Archipelago, Guinea-Bissau	2019	**This study**
	6.01	PCWS, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020
	5.6	PMF, Tamil Nadu, India	2015-2017	Pandiyan et al., 2020

a: flight feathers
b: samples analysed in 2013
c: tail feathers
d: fledglings feathers

Table 6: Mercury concentrations (ppm) in avian eggshell reported in the literature.

Species	Hg	Site	Year	Reference
Charadrius alexandrinus	0.037	Ria de Aveiro, Portugal	2020	**This study**
	0.034	Tagus Estuary, Portugal	2020	**This study**
Himantopus himantopus	0.009	Ria de Aveiro, Portugal	2020	**This study**
	0.018	Tagus Estuary, Portugal	2020	**This study**
Chlidonias hybrida	0.003	Anzali Wetlands, Caspian Sea, Iran	2008	Aliakbari et al., 2011
Ichthyaetus audouinii	0.220	Ebro Delta, Spain	1992	Morera et al., 1997
Larus argentatus	0.01	Long Island, New York, USA	1992	Burger, 1994
Larus dominicanus	0.015	Port Elizabeth, South Africa	2012	van Aswegen et al., 2019
Onychoprion anaethetus	0.004	Hong Kong	2000-2002	Lam et al., 2005
Saundersilarus saundersi	0.024	Dongtai, Jiangsu, China	2012	Fu et al., 2014
Sterna dougalii	0.02	Long Island, New York, USA	1992	Burger, 1994
Sterna hirundo	0.054	Mewia Lacha Bird Sanctuary, Poland	2010-2012	Grajewska et al., 2015
Thalasseus sandvicensis	0.050	Mewia Lacha Bird Sanctuary, Poland	2010-2012	Grajewska et al., 2015
Brachyramphus brevirostris	0.016	Agattu Island, Alaska, USA	2008-2011	Kenney et al., 2018
Ardea cinerea	0.022	Sanjiang Plain, Heilongjang, China	2010	Wang et al., 2013
Egretta alba	0.016	Wuxi, Jiangsu, China	2012	Fu et al., 2014
	0.016	Sheyang, Jiangsu, China	2012	Fu et al., 2014
	0.021	Sanjiang Plain, Heilongjang, China	2010	Wang et al., 2013
Egretta garzetta	0.023	Wuxi, Jiangsu, China	2012	Fu et al., 2014
	0.022	Xinghua, Jiangsu, China	2012	Fu et al., 2014
	0.071	Hong Kong	2000-2002	Lam et al., 2005
Egretta thula	0.007	Totumo Marsh, Colombia	2005	Olivero-Verbel et al., 2013
	0.020	Cartagena Bay, Colombia	2005	Olivero-Verbel et al., 2013
Nycticorax nycticorax	0.016	Wuxi, Jiangsu, China	2012	Fu et al., 2014
	0.019	Sheyang, Jiangsu, China	2012	Fu et al., 2014
	0.056	Hong Kong	2000-2012	Lam et al., 2005
Pelacanus occidentalis	0.023-0.027	Sucre, Venezuela	2016	Arcia-Castañeda et al., 2019
Rallus creptidans	0.368	Brunswick, Georgia, USA	2000	Rodriguez-Navarro et al., 2002
	0.105	Blythe Island, Georgia, USA	2000	Rodriguez-Navarro et al., 2002

Table 7: Mercury concentrations (ppm) in birds faeces reported in the literature.

Species	Hg	Site	Year	Reference
Himantopus himantopus	0.040-0.085	Ria de Aveiro, Portugal	2019-2020	**This study**
Larus michahellis	0.800-1.020	Sicily, Italy	2008-2009	Signa et al., 2013
Saundersilarus saundersi	0.055	Dongtai, China	2012	Fu et al., 2014
Ardea herodias	0.280	Lake Chatcole, Idaho, USA	1978	Fitzner et al., 1982
	0.100	Hanford Reach, Washington, USA	1996	Tiller et al., 2005
Nipponia nippon	0.160	Yangxian County, China	2013	Liu et al., 2019
	0.300	Yangxian County, China	2013	Liu et al., 2019
Sula sula	0.108	Dongdao, South China Sea	2003	Liu et al., 2006
Brachyramphus brevirostris	0.006	Adak Island, Alaska	2011	Kenney et al., 2018
Eudyptula minor	0.140-0.310	Port Philip Bay, Australia	2011-2013	Finger et al., 2017
Ciconia boyciana	0.080	Sheyang, China	2012	Fu et al., 2014
Grus grus	0.019	Sheyang, China	2012	Fu et al., 2014
Grus japonensis	0.022	Sheyang, China	2012	Fu et al., 2014
Grus vipio	0.022	Sheyang, China	2012	Fu et al., 2014
Grus virgo	0.018	Sheyang, China	2012	Fu et al., 2014
Cygnus cygnus	0.010	Swan Lake, Rongcheng, China	2014-2015	Wang et al., 2017
Cygnus olor	0.038	Chesapeak Bay, USA	1997	Beyer & Day, 2004
Cinclus mexicanus	0.036	British Colombia, Canada	2000-2001	Morrissey et al., 2005
Ficedula hypoleuca	0.100-0.150	Harjavalta, Finland	2014	Espín et al., 2016
Parus major	0.080-0.120	Harjavalta, Finland	2014	Espín et al., 2016
	0.024	Antwerp, Belgium	2000	Dauwe et al., 2004
	0.220-0.350	Figueira da Foz, Portugal	2009	Costa et al., 2012