Bulletin of Environmental Contamination and Toxicology (2023) 110:89
https://doi.org/10.1007/s00128-023-03729-1

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

Jorge Vizuete[1] · Marcos Pérez-López[1,3] · Ana López-Beceiro[2] · Luis Eusebio Fidalgo[2] · Francisco Soler[1,4] · María Prado Míguez-Santiyán[1,3] · David Hernández-Moreno[5]

Received: 6 October 2022 / Accepted: 20 April 2023 / Published online: 4 May 2023

Abstract

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

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

Introduction

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

✉ David Hernández-Moreno
david.hernandez@inia.csic.es

1 Toxicology Area, Faculty of Veterinary Medicine (UEX), Caceres 10003, Spain

2 Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine (USC), Lugo 27003, Spain

3 INBIO G+C Research Institutes, Madrid, Spain

4 IPROCAR Research Institutes, Caceres, Spain

5 Department of Environment and Agronomy, National Institute of Agriculture and Food Research and Technology (INIA), Spanish National Research Council (CSIC), Ctra A Coruña Km 7, Madrid, Spain

Published online: 04 May 2023

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

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

Materials and Methods

Study Areas and Species

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

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

Sampling Method

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

Liver and Kidney Metals Analysis

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

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

OCPs and PCBs Analysis

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

Biomarker Analyses in Liver and Kidney

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

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

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

Statistical Analyses

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

Results and Discussion

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

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

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

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

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

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

Malondialdehyde (MDA)

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

Reduced Glutathione (GSH)

In metal-induced oxidative stress, glutathione metabolism has an essential role because the functional thiol group of glutathione serves as a binding site for many metals (Pinto et al. 2003). The levels of GSH (Table 1) in the liver were lower than in kidneys (1.13 ± 0.07 and 1.34 ± 0.11 nmol/mg protein, respectively). There is a diversity of results found in literature regarding this specific biomarker. In this sense, the exposure to metals has been associated to tGSH increased levels in different species of birds, such as Shaoxing ducks, mallards and starlings, whereas other studies did not find any difference on tGSH levels in birds inhabiting a contaminated area respect to the same species of a selected reference area (Koivula and Eeva 2010). In this sense, the samples of the present study did not show any correlation between GSH and the studied contaminants. There were statistically significant differences in both livers and kidneys between samples from Gijón and A Coruña ($p < 0.05$) or

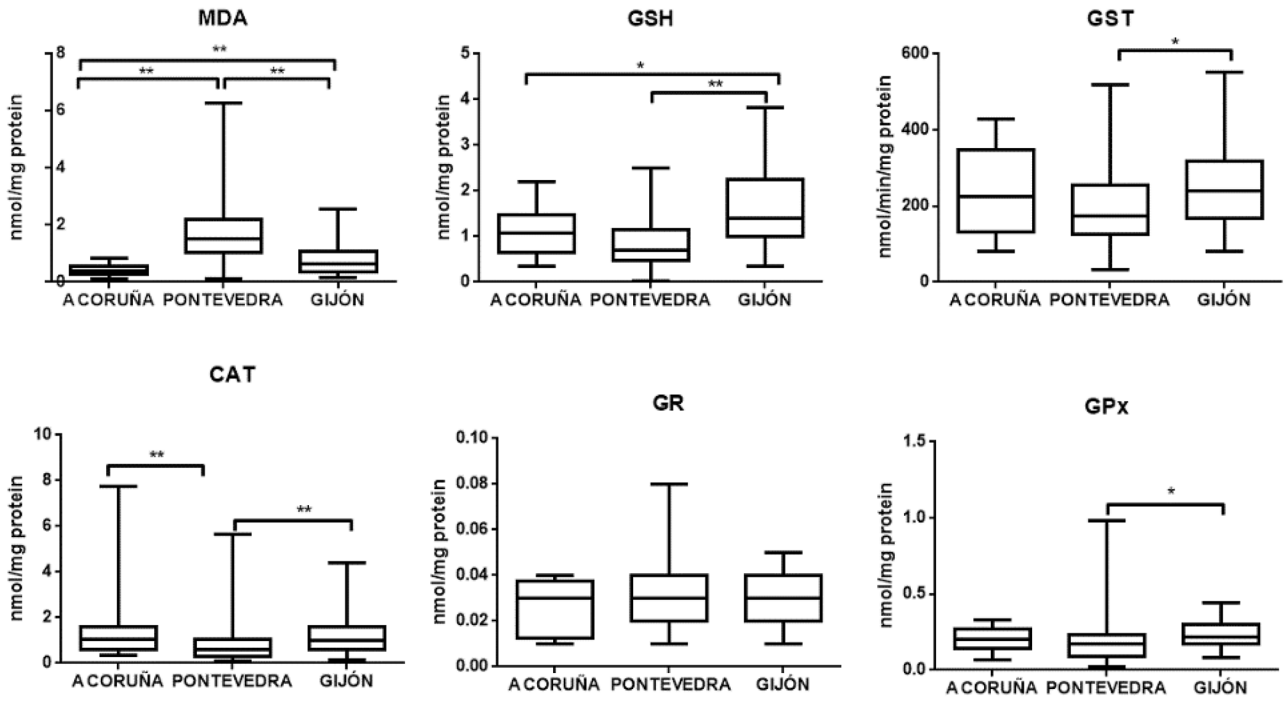

Fig. 1 MDA, GSH, GST, CAT, GR and GPx levels in liver of Yellow-legged gull from three different zones: Pontevedra (n = 21), A Coruña (n = 58) and Gijón (n = 30). Statistical significance *: $p < 0.05$; **: $p < 0.01$

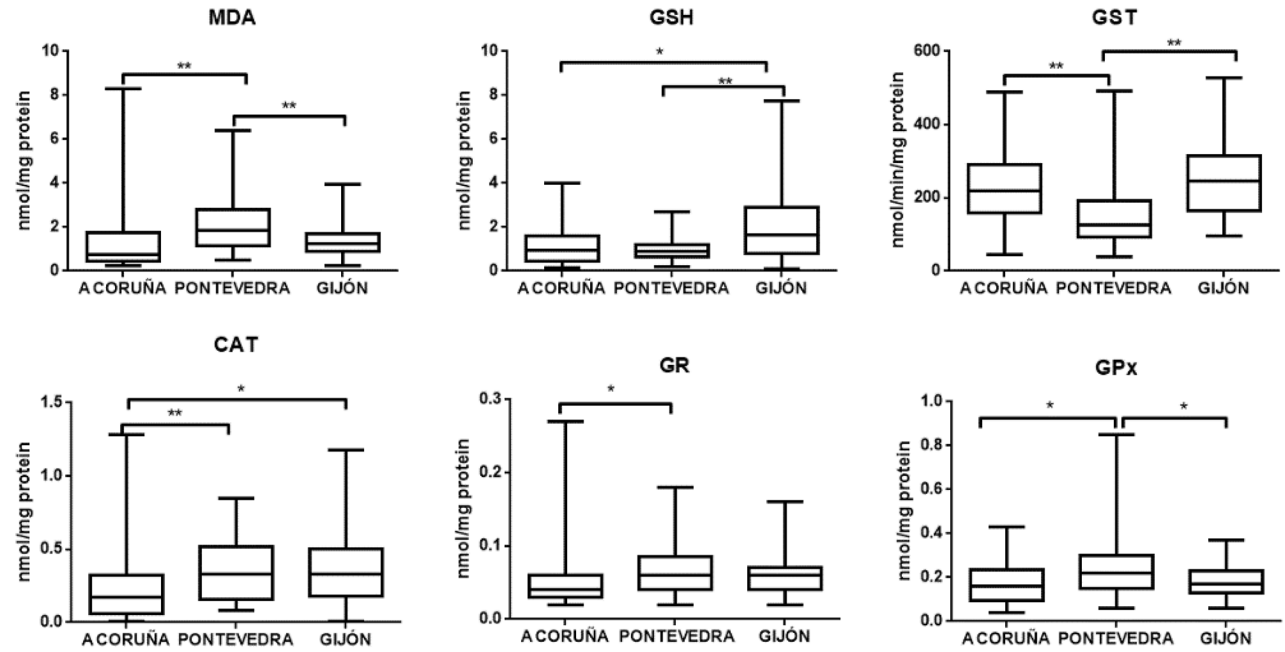

Fig. 2 MDA, GSH, GST, CAT, GR and GPx levels in kidney of Yellow-legged gulls from three different zones: Pontevedra (n = 21), A Coruña (n = 58) and Gijón (n = 30). Statistical significance *: $p < 0.05$; **: $p < 0.01$

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

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

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

Catalase (CAT)

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

Glutathione Reductase (GR)

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

Glutathione Peroxidase (GPx)

Both liver and kidney samples showed similar GPx activities, 0.22 ± 0.02 nmol/min/mg protein and 0.21 ± 0.01 nmol/min/mg protein, respectively. The reaction pathway followed by this enzyme involves the use of H_2O_2 as a substrate. The fact that Cd exposure increases H_20_2 levels (Pillai et al. 2002), extrapolated to birds, could explain a possible

correlation between GPx activity and Cd concentration in these animals. Indeed, Espín et al. (2014) found this significant correlation in vultures in Alcoy, Spain. However, it was not possible to observe a relationship in the present study. In liver, results showed a significant difference between Pontevedra and Gijón ($p < 0.05$). In kidneys, the significant differences were found in Pontevedra with respect to Gijón and A Coruña ($p < 0.05$). As for GR levels, a slight correlation was found between *As* and GPx in the kidneys ($p < 0.05$) (Table S1).

Correlation Study Applied to Biomarkers

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

Relationships Between Factors

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

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

Conclusion

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

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-023-03729-1.

Funding The work was funded by the Consejería de Economía e Infraestructuras of Junta de Extremadura (Convocatoria de Proyectos de investigación en los centros públicos de I+D+i de la Comunidad Autónoma de Extremadura, 2016; Ref. IB16112); project code: 2017/00206/001.

Declarations

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

References

Abdullah M, Fascola M, Muhammad A, Chemosphere et al (2015) https://doi.org/10.1016/j.chemosphere.2014.06.068

Ashraf MA (2017) Persistent organic pollutants: a global issue, a global challenge. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-015-5225-9

Beiras R, Fernández N, González JJ et al (2002) Mercury concentrations in seawaters and wild mussels from the coast of Galicia (NW Spain). Mar Pollut Bull 44:340–349

Berglund ÃMM, Sturve J, Förlin L et al (2007) Oxidative stress in pied flycatcher (*Ficedula hypoleuca*) nestlings from metal contaminated environments in northern Sweden. Environ Res. https://doi.org/10.1016/j.envres.2007.06.002

Besada MV, Fumega J, Cambeiro B (1997) Variación anual delas concentraciones de Hg, Pb, Cd, Cu y Zn en mejillon Silvestre de la Ria de Vigo. In: Prego, R., Fernández, J.M. (Eds.), Procesos biogeoquimicos en sistemas costeros Hispano-Lusos. Provincial de Pontevedra, Pontevedra, pp. 95–99, ISBN: 84-89690-27–8

Bianchi N, Ancora S, di Fazio N et al (2008) Cadmium, lead and mercury levels in feathers of small passerine birds: non-invasive sampling strategy. Environ Toxicol Chem. https://doi.org/10.1897/07-403.1

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem. https://doi.org/10.1006/abio.1976.9999

Clairborne A (1985) Catalase activity. In: Greenwald RA (ed) CRC Handbook of methods in oxygen radical research. CRC Press, Boca Raton, Floride (USA, pp 283–284

Congiu L, Chicca M, Pilastro A et al (2000) Effects of chronic dietary cadmium on hepatic glutathione levels and glutathione peroxidase activity in starlings (*Sturnus vulgaris*). Arch Environ Contam Toxicol. https://doi.org/10.1007/s002449910047

Cribb AE, Leeder JS, Spielberg SP (1989) Use of a microplate reader in an assay of glutathione reductase using 5, 5′-dithiobis (2-nitrobenzoic acid). Anal Biochem. https://doi.org/10.1016/0003-2697(89)90188-7

Duffus JH (2001) Heavy metals: a meaningless term? Chem Int 23:6. https://doi.org/10.1515/ci.2001.23.6.163

Elia AC, Galarini R, Dörr AJM et al (2005) Polychlorinated biphenyls and antioxidant enzymes in liver of *Cyprinus carpio* from Lake Trasimeno. Italian J Zool. https://doi.org/10.1080/11250000509356645

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal induced oxidative damage. Curr Top Med Chem 1:529–539. https://doi.org/10.2174/1568026013394831

Espín S, Martínez-López E, Jiménez P et al (2014) Effects of heavy metals on biomarkers for oxidative stress in Griffon vulture (Gyps fulvus). Environ Res. https://doi.org/10.1016/j.envres.2013.11.008

Fossi MC, Leonzio C, Focardi S et al (1991) Modulation of mixed-function oxidaseactivity in black-headed gulls living in anthropic environmentse biochemical acclimatization or adapation. Environ Toxicol Chem. https://doi.org/10.1002/etc.5620100910

Grant PJ (1986) Gulls: a guide to identification. Buteo Book, Vermillion, South Dakota. ISBN 13: 9781408138311

Guideline (2005) Validation of analytical procedures: text and methodology Q2 (R1). In International conference on harmonization, Geneva, Switzerland (pp 11–12)

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-Transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem https://doi. https://doi.org/10.1016/S0021-9258(19)42083-8

Halliwell B, Gutteridge J (2007) Free radicals in biology and medicine, Fourth edn. Oxford University Press, New York

Hernández-García A (2010) *In vitro* evaluation of effects induced by lead, cadmium and their binary mixtures on erythrocytes of three species of wild birds. Doctoral thesis, University of Murcia. http://hdl.handle.net/10201/34539.l

Hissin PJ, Hilf R R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem. https://doi.org/10.1016/0003-2697(76)90326-2

Hoffman DJ (2002) Role of selenium toxicity and oxidative stress in aquatic birds. Aquat Toxicol. https://doi.org/10.1016/s0166-445x(01)00263-6

Hoffman DJ, Heinz GH, Krynitsky AJ (1989) Hepatic glutathione metabolism and lipid peroxidation in response to excess dietary selenomethionine and selenite in mallard ducklings. J Toxicol Environ Healt–A. https://doi.org/10.1080/15287398909531296

Hoffman DJ, Heinz GH, Sileo L et al (2000) Developmental toxicity of lead-contaminated sediment in Canada geese (*Branta Canadensis*). J Toxicol Environ Health–A. https://doi.org/10.1080/009841000156916

Isaksson C, Ornborg J, Stephensen E et al (2005) Plasma glutathione and carotenoid coloration as potential biomarkers of environmental stress in great tits. Eco Health. https://doi.org/10.1007/s10393-005-3869-5

Koivula MJ, Eeva T (2010) Metal-related oxidative stress in birds. Environ Pollut. https://doi.org/10.1016/j.envpol.2010.03.013

Koivula MJ, Kanerva M, Salminen JP et al (2011) Metal pollution indirectly increases oxidative stress in great tit (*Parus major*) nestlings. Environ Res. https://doi.org/10.1016/j.envres.2011.01.005

La Merrill M, Emond C, Kim MJ et al (2013) Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ Health Perspect. https://doi.org/10.1289/ehp.1205485

Mahondas J, Marshall JJ, Duggin GG et al (1984) Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res PubMed: 6149017

Mateo R, Hoffman DJ (2001) Differences in oxidative stress between young Canada geese and mallards exposed to lead-contaminated sediment. J Toxicol Environ Health-A. https://doi.org/10.1080/15287390152627228

Mateo R, Beyer WN, Spann W, Hoffman DJ (2003) Relationship between oxidative stress, pathology, behavioral sign of lead poisoning in mallards. J Toxicol Environ Health-A. https://doi.org/10.1080/15287390306390

Ookhtens M, Karplowitz N (1998) Role of the liver in interorgan homeostasis of glutathione and cyst(e)ine. Seminars in liver disease. Thieme Medical Publishers, Inc, pp 313–329

Osičková J, Banďouchová H, Kováčová V et al (2014) Oxidative stress and liver damage in birds exposed to diclofenac and lead. Acta Vet Brno. https://doi.org/10.2754/avb201483040299

Pamplona R, Costantini D (2011) Molecular and structural antioxidant defences against oxidative stress in animals. Am J Physiol Reg Integr. https://doi.org/10.1152/ajpregu.00034.2011

Paskova V, Paskerova H, Pikula J et al (2011) Combined exposure of japanese quails to cyanotoxins, Newcastle virus and lead: oxidative stress responses. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2011.07.014

Pillai A, Laxmi PN, Gupta S (2002) Effects of combined exposure to lead and cadmium on pituitary membrane of female rats. Arch Toxicol. https://doi.org/10.1007/s00204-002-0399-6

Pinto E, Sigaud-Kutner TCS, Leitão MAS et al (2003) Heavy metal-induced oxidative stress in algae. J Phycol. https://doi.org/10.1111/j.0022-3646.2003.02-193.x

Recknagel RO, Gelende EA Jr, Waller RL, Lowrey K (1982) Lipid peroxidation: Biochemistry. Measurement and significance in liver cell injury. In: Toxicology of the liver (Eds.: Plaa GL and Hewitt WR). Raven Press, New York. pp 213–241

SEO (2018) https://www.seo.org/ave/gaviota-patiamarilla/

Vitula F, Peckova L, Bandouchova H et al (2011) *Mycoplasma gallisepticum* infection in the grey partridge *Perdix perdix*: outbreak description, histopathology, biochemistry and antioxidant parameters. BMC Vet Res. https://doi.org/10.1186/1746-6148-7-34

Vizuete J, Hernández-Moreno D, Fidalgo LE et al (2018) Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (*L. michahellis*) from Spain: role of gender and age. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2018.08.060

Vizuete J, Hernández-Moreno D, López-Beceiro A et al (2022) Heavy metals and metalloid levels in the tissues of yellow-legged gulls (*Larus michahellis*) from Spain: sex, age, geographical location differences. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-022-19627-8

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, New Jersey: Prentice Hall.