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Spatial variation and biovectoring of metals in gull faeces

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ABSTRACT

We assessed the spatial variation in concentrations of ten metals in faeces of the lesser black-backed gull (LBBG) *Larus fuscus* wintering at seven localities in South-West Spain. We found high concentrations of metals in gull faeces, with several elements (As, Cu, Mo, Pb, Zn) locally exceeding (by 2 to 11 times) derived Lowest Effect Level (LEL) values. We also found strong spatial variation, related to the main pollution sources associated with the different sites. Faeces from Chipiona Port (Gulf of Cádiz) showed the highest levels of As; Cetina saltpans (Bay of Cádiz) ranked first for Pb, Zn and Mo, which was consistent with historic mining and industrial pollution; Doñana ricefields showed the highest levels of Mn, a highly available element in flooded areas; while landfills ranked first for Cd, Co, Cr, Cu and Ni, potentially associated with electronic waste. Furthermore, we demonstrate how faecal analysis can be used to quantify biovectoring of metals into specific localities, using LBBG movement ecology and census data. At Fuente de Piedra, a shallow, closed-basin lake important for waterbirds, we show that metal inputs by LBBG have increased in recent years, and long-term deposition (e.g., of Pb) may impact aquatic communities and ecological processes in this Ramsar site.

1. Introduction

Anthropogenic activities (e.g., industrial processes, urban and agricultural practices) are increasingly contributing to environmental pollution worldwide (Baby et al., 2010; Vareda et al., 2019). Aquatic ecosystems are particularly vulnerable because pollutants not only cause direct impacts in biota, resulting in lethal or sub lethal effects, but also a variety of indirect perturbations through trophic cascades that can result in dramatic changes in food webs, ecosystem structure and nutrient fluxes (Fleeger et al., 2003; Baby et al., 2010). Among the most prevalent and harmful contaminants in aquatic ecosystems are heavy metals (Deb and Fukushima, 1999). Many are highly toxic and persistent and can bioaccumulate and biomagnify through food webs (Goodyear and McNeill, 1999). Understanding how toxic metals and metalloids enter and distribute within aquatic environments, and identifying potential sources of contamination in the environment, are critical points in evaluating the risks they pose to the environment, wildlife and human health.

While pathways and entry routes of metals into aquatic ecosystems via abiotic (physical) mechanisms are well characterized, the role of biological transport has been widely overlooked (Blais et al., 2007). Biovectors are often considered as negligible when looking at pollution transport pathways in a global context (see Kallenborn and Blais, 2015, for a recent review). However, there exists increasing evidence of pollutant transport within and among ecosystems via biota (Michelutti et al., 2010), which can, in some cases, even exceed that mediated by

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abiotic pathways (Xie and Sun, 2008; Chu et al., 2019). For example, several studies have shown a relationship between contaminant distribution and migration of vertebrates at regional and inter-continental scales (Evenset et al., 2007; Michelutti et al., 2009; Kallenborn and Blais, 2015).

Despite this recent interest, there are important gaps in knowledge that make it difficult to evaluate the magnitude and widespread character of this phenomenon. For example, the main focus of previous research on biovectors is in marine and terrestrial environments (Kallenborn and Blais, 2015; Shoji et al., 2019), whereas inland waters have received consideration in fewer studies (Shahbaz et al., 2013; Ullah et al., 2014). At the same time, such systems can be among the most diverse and threatened aquatic ecosystems and may be particularly susceptible to contaminants (whether by abiotic or biotic processes) (Dudgeon et al., 2006). In particular, contaminants are readily concentrated in closed-basin lakes without an outflow. On the other hand, most available studies have been performed in Arctic and Northern areas (Kallenborn and Blais, 2015; Mallory, 2018), and have focused on persistent organic pollutants (Kallenborn and Blais, 2015; Wang et al., 2019) and other emergent contaminants (Desjardins et al., 2019). Studies on metals are much more scarce, except for mercury (Leonzio et al., 2009; Kickbush et al., 2018; Yao et al., 2019). Moreover, many studies focus on tissues and other structures (Chen and Hale, 2010), but studies of biotransport through faeces remain limited (Evenset et al., 2007; Desjardins et al., 2019).

The role of waterbirds as biovectors of metals can be investigated using their excreta (Michelutti et al., 2009; Martinez-Haro et al., 2011). They are important components of aquatic ecosystems, and are able to move between water bodies and across boundaries between terrestrial and aquatic systems (Green and Elmberg, 2014; Soininen et al., 2015; González-Bergonzoni et al., 2017), transporting and subsequently releasing contaminants into receiving ecosystems (Blais et al., 2007).

Gulls in particular are of significant interest because they feed opportunistically and are widely adapted to utilise a range of anthropogenic habitats (such as ports and landfills) whilst commonly returning to roost in otherwise comparably unpolluted waterbodies (Winton and River, 2017; Martín-Vélez et al., 2020). As a result of their highly gregarious behaviour, roosting and feeding in large flocks, they then have the potential to generate "hotspots" of contamination by incorporating nutrients and other contaminants from human-influenced feeding habitats and releasing these into their wetland roost sites (via their faeces). In Spain, gull guano has been shown to contribute to eutrophication in the Fuente de Piedra lake, a major roosting site for gulls during the wintering period (Martín-Vélez et al., 2019). Given the ability of omnivorous gulls to ingest and potentially concentrate pollutants (as a result of their high trophic position and feeding habits; Ramos et al., 2013; Peterson et al., 2017), similar processes to those at play for nutrients may also operate with respect to other contaminants (Choy et al., 2010).

The lesser black-backed gull *Larus fuscus* (subsp. *intermedius* and *graellsi*, hereafter LBBG) is a long-distance migrant (Baert et al., 2018) whose population has experienced a progressive expansion throughout its range during the second half of the 20th century, and it is currently the second most abundant wintering species in Andalusian wetlands after the northern shoveler *Anas clypeata* (Martín-Vélez et al., 2020). The success of this generalist species is widely attributed to its high adaptability, plasticity and opportunistic feeding character (e.g. rubbish, fish discards, invertebrates etc; Gyimesi et al., 2016; Martín-Vélez et al., 2021), which allows it to efficiently use human-modified habitats (Gyimesi et al., 2016; Martín-Vélez et al., 2019, 2020). Thus, it represents a good model system to study local transport of contaminants during the winter. As far as we know, there are no studies quantifying the role of gull faeces in biovectoring of metals in wetland ecosystems. Furthermore, there are also no studies of metals in LBBG faeces and very few on other gull species. Given the abundance and local movements of gulls, such data would be important to monitor potential changes in the environment, particularly in wetlands or agricultural systems relevant for human health.

The aim of this study was to investigate the spatial variation in metals in LBBG faeces and the potential for LBBG to act as biovectors of metal contamination (including heavy metals and the metalloid Arsenic) using their excreta. We provided data on the concentrations of ten elements (including both essential and toxic metals) in faeces collected from sites in south-west Spain with different degrees of anthropogenic influence and used regularly as roosting and feeding habitats during wintering. The specific objectives were:

(1) To study spatial differences in the content of metals in gull samples from different foraging and roosting sites, including protected and unprotected wetlands, landfills and important areas for human food production such as fishing ports, ricefields and saltpans, during the wintering season. We anticipated that metal concentrations in gull faeces should reflect key pollution sources associated with these different environments.
(2) To quantify metal biotransport to one of Spain's most important natural lakes, Fuente de Piedra, based on faecal analysis, censuses and GPS movement data.

2. Materials and methods

2.1. Study area

This study was carried out across seven sites with different degrees of human influence, used for roosting and feeding by LBBG (see Fig. 1). Foraging sites largely determine dietary exposure to metals and act as 'sources' of metals that may ultimately be deposited in wetland roosting sites, which may then act as metal 'sinks' (Simpson et al., 1983).

These sites were:

(1) Doñana Ricefields in the Guadalquivir marshes (Seville, 37000 ha), the largest area devoted to rice production in Spain, accounting for up to 42% of total national harvest. This area is located in one of the most important wetlands for migratory waterbirds in the Western Palearctic (Rendón et al., 2008). Doñana ricefields provide important habitat for LBBG and other waterbirds (Toral and Figuerola, 2010) both for feeding (e.g., on the alien crayfish *Procambarus clarkii*) and roosting (Martín-Vélez et al., 2020, 2021). Up to 15000 gulls are regularly counted in the ricefields west of the Guadalquivir river (Rendón et al., 2008).
(2) Fuente de Piedra lake (Malaga, 1350 ha) is the largest natural lake in Andalusia, protected at regional (Natural Reserve), European (Special Protection Area) and international (Ramsar site) levels. It hosts one of the largest flamingo nesting colonies in the western Mediterranean (Bechet et al., 2012) and is a roosting area for over 20000 LBBG in winter (Martín-Vélez et al., 2020). These LBBG feed mostly at landfills beyond the lake catchment area, and their excreta are a major cause of lake eutrophication in winter (Martín-Vélez et al., 2019).
(3) Chipiona Port (2605 m^2), in the southern part of the Gulf of Cádiz, is an important feeding area for LBBG during the non-breeding season (Ramírez et al., 2015). Gulls benefit from high marine productivity nearby (LaFuente and Ruiz, 2007), which commonly peaks in late winter (around 400 individuals peak in January; F. Hortas pers. comm.). In this period, gulls concentrate close to the port to feed on fishery discards (Bartumeus et al., 2010; Ramírez et al., 2015).
(4) Cetina saltpan complex (also in the Gulf of Cadiz; 1100 ha), created in 2014, one of the biggest saltpan complexes in Spain, and one of the most important for salt production. It is listed as an Important Bird Area (IBA 251) (Infante et al., 2011), being an important unprotected feeding habitat for many species of waterbirds (Masero and Pérez-Hurtado, 2001), but LBBG use it

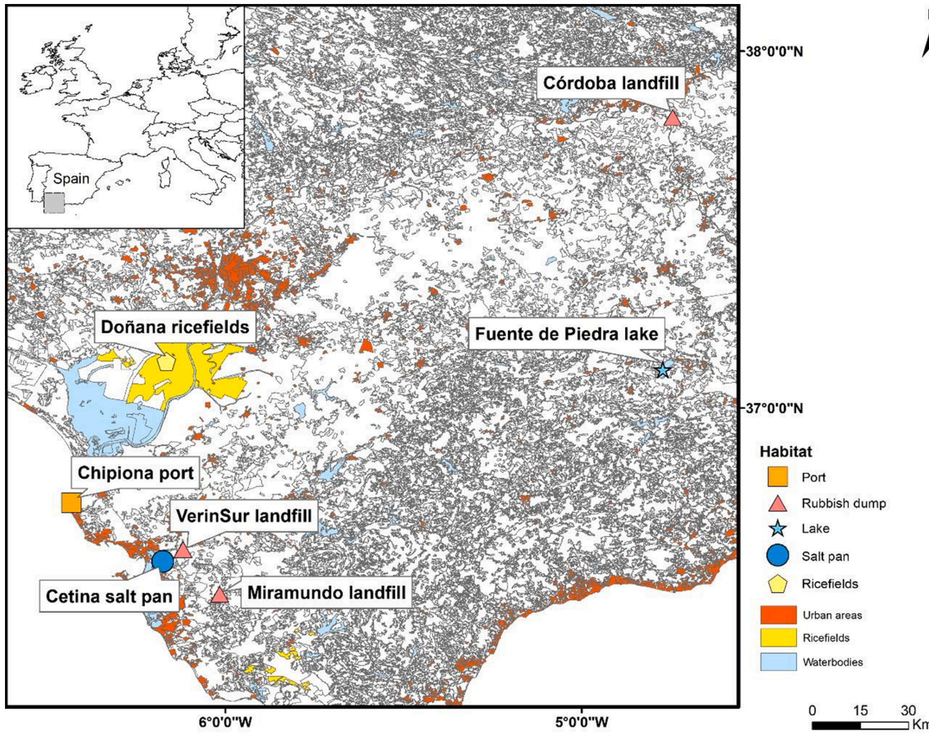

Fig. 1. Study area showing the seven locations where LBBG faecal samples were collected in 2017–2018. Fuente de Piedra lake and Cetina salt pan are important roosting sites whereas Córdoba, VerinSur and Miramundo are landfills and foraging areas. The Donana ricefields are used for both foraging and roosting. Polygons correspond to CORINE LAND COVER 2012.

mainly as a roosting site. In the Cadiz Bay in which Cetina Salt pan is included, an average of 5882 individuals were counted from 2010 to 2017 (Martín-Vélez et al., 2020).

(5) Landfill sites (two in Cadiz (VerinSur and Miramundo) and one in Córdoba). These landfills are used by large numbers of LBBG for feeding and are known to be connected with important roosting areas for LBBG (Martín-Vélez et al., 2019, 2020). These landfills receive waste from surrounding urban areas and contain elevated levels of heavy metals (de la Casa-Resino et al., 2014; Cabo et al., 2012). Cordoba landfill holds an average of 3536 individuals (data from 2010 to 2017, Junta de Andalucía).

2.2. Sample collection

All seven sites were visited by us between November 2017 and January 2018 and sample sites were chosen based on species counts from the region (Junta de Andalucía). Thirty faecal samples were collected at each site ($n = 210$). Only fresh faeces (i.e., visibly 'wet') were collected, after detecting monospecific LBBG flocks. Samples were taken from points separated by at least one meter, in order to increase the likelihood that they were from different individuals. Intra-individual variation in metal concentration was not considered in this study and was not expected to be significant. Faeces were collected with spatulas, avoiding collecting the part of the excreta in contact with the soil to avoid contamination. Samples were individually stored in labelled zip-lock bags and preserved in the freezer (at −20 °C). Prior to sample digestion for metal analysis, they were dried at 60 °C (for 24 h).

2.3. Sample digestion and ICP-OES analysis

For sample digestions, trace metal grade nitric acid (>67%) and hydrogen peroxide (>30%; Fisher Scientific) were used. Mixed standards for ICP-OES calibration and analysis were made using dilutions from certified 1000 ppm stock solutions (Sigma-Aldrich) of each element of interest: arsenic (As), cadmiun (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb) and zinc (Zn). Certified Reference Materials (CRMs) were used to assess recovery of elements during the digestion and analytical process. The CRM's used were 'Lobster Hepatopancreas' (TORT-2; NRC) and 'Bushes, Branches and Leaves' (DC73348; NCS). The concentrations of each element in these CRMs along with their associated uncertainties are displayed in Table S1.

All samples, CRMs and blanks were digested using the same procedure. Approximately 0.3–0.4 g of sample was accurately weighed into a disposable sample weigh-boat before being added to a 50 ml PTFE microwave digestion vessel. This was repeated to make up a 24-vessel digestion carousel which consisted of 21 samples, two CRMs and one blank (made using 0.35 ml of Milli-Q water). To each digestion tube, 3.5 ml of trace metal grade concentrated nitric acid was added and vessels were left overnight to pre-digest (cap placed loosely on top to allow fumes to escape). Each vessel then had 1 ml of trace metal grade hydrogen peroxide and 1 ml of Milli-Q water added the next day. The 24 vessel carousel was then placed into a microwave digestion reaction system (Anton Paar, Multiwave PRO), set to a program which ramped up to 110 °C over 20 min, held for 15 min, ramped to 180 °C over 15 min, held for 30 min, before finally cooling to 55 °C using the maximum cooling fan setting (giving a total digestion time of approximately 1 h and 40 min per 24 vessel carousel). Digestion vessels were then allowed to cool to room temperature before caps were carefully removed to allow the vessel pressure to release gradually. The digest liquid was then poured into a 15 ml sample tube. The reaction vessel was then further rinsed with Milli-Q (three times), adding each rinse to the sample tube, before finally topping up to the 15 ml mark with Milli-Q water. Digest vessels were then thoroughly washed with Type II deionised water (twice) and then placed in a wash bath made up with 3% DECON® for 24 h. They were then rinsed twice with Milli-Q and placed in an acid bath (5% nitric acid) for 24 h before washing again with Milli-Q (3 times) and drying overnight. The above was then repeated until all samples were digested.

ICP-OES analysis was performed on a Varian 720-OES (Agilent) instrument. All standards were prepared in diluted trace metal grade nitric acid to matrix-match the acidity of the primary sample digests. To account for instrumental drift during analytical runs, a re-slope was undertaken every 10 samples using an intermediate standard (alongside a blank). All measurements were performed by analysing three 'potential' wavelengths for each element. Wavelengths used for the final data were selected based on CRM recovery as well as sensitivity (i.e., signal intensity) and the final Limit of Detection (LOD).

2.4. Statistical analyses

For statistical analysis metal values below the LOD were replaced by 0.5*LOD. We used centered scaled data from the ten elements in the seven sites sampled from November 2017 to January 2018. To test differences in element concentrations between sites we carried out a PERMANOVA test with *vegan* package in R. We chose PERMANOVA over other tests (e.g. ANOSIM, Mantel) because PERMANOVA performs better when there are differences in variances between balanced groups (Anderson and Walsh, 2013). We carried out an NMDS ordination plot to visualize differences between groups. NMDS was carried out through *metaMDS* function which scales the results and works with random starts to make interpretation easier (Oksanen, 2015). Afterwards, we also performed a similarity of percentages analysis (SIMPER) to identify the element that contributed most to differences between pairs of sites based on Bray-Curtis similarities between samples. We performed all three analyses through the vegan package in R (Oksanen, 2015). Finally, we carried out a Hierarchical Cluster Analyses (HACA) after scaling all element concentrations to identify associations between elements that may occur together. We carried out HACA through *pvclust* package in R that conducts multiscale bootstrap resampling to calculate p-values in bootstrap probabilities ($>0.95\ \alpha$) for each cluster based on agglomerative average clustering (Suzuki and Shimodaira, 2006). We set up the analysis based on Euclidean distance and Ward clustering method.

Secondly, data were log-transformed to reduce the influence of outliers and normalize their distribution. Differences between locations were tested using one-way ANOVAs. If differences were significant, we carried out post-hoc Tukey tests. All analyses were performed in R (v.3.4.1) with package *multcomp* (Hothorn et al., 2017). To evaluate the environmental/toxicological significance of the metal levels measured in our samples, we compared our data with several widely used SQGs (sediment quality guidelines) (Persaud et al., 1993; de Deckere et al., 2011; Kabata-Pendias and Mukherjee, 2007; Buckman, 2008). These were based on consensus values taking into account ecotoxicological values (Threshold Effect Levels (TEL): the concentration below which adverse biological effects are expected to occur rarely) and ecological values (Lowest Effect Levels (LEL): level of sediment contamination that can be tolerated by most benthic organisms) for freshwater ecosystems (Table 1). We also compared our data with probable effect levels (PELs) from MacDonald et al. (2000), but measured values for all metals and metalloids were far below these threshold, so we did not include them in Table 1.

2.5. Estimations of metal biotransport

We estimated Metal Load (ML) to Fuente de Pieda Lake (the main midwinter roosting site for LBBG in Andalusia) via LBBG faeces for seven winters (from 2010 to 2017). We adapted a nutrient quantification methodology used in a previous study (Martín-Vélez et al., 2019) to determine metal transport to the lake. We provided estimations in g ha $^{-1}$ related to the area that the specific roosting sites cover within the Lake (calculated in ArcMap based on satellite maps), as well as relative to the whole lake surface (Fig. 2). Roosting sites were identified after Martín-Vélez et al. (2019), which is also the source of our data inputs for gull counts and gull movements to and from landfills used for feeding.

We estimated each element load as follows:

$$ML = TS*ER*ND*MN*EC_{faeces}$$

Where TS = Time Spent, i.e., the average roosting time per year, for every year (Fig. S1; Martín-Vélez et al., 2019). ER = Excretion Rate per individual per day (g day^{-1}), which was considered as a fixed parameter (21.06 g day^{-1}), calculated using equation 3 from the Hahn et al. (2007) model for nutrient transport by carnivorous or omnivorous birds:

$$ER = \alpha \times \frac{DER}{E \times AM}$$

Wherein α = the intake to excretion ratio of 0.395 (Dobrowolski et al., 1993; Nixon and Oviatt, 1973). *DER* = daily energy requirement:

Table 1

Summary table of geometric means (and 95% CI; in μg/g of faeces) for the ten elements analysed (As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb and Zn) in faecal samples (N = 30 per site) among seven locations in SW Spain, with samples collected in November 2017 (in ricefields), December 2017 (Cordoba, VerinSur and Miramundo landfill) and January 2018 (Fuente de Piedra, Chipiona and Cetina). 'Thresholds' based on consensus values using both ecotoxicological values (Threshold Effect Levels (TEL)) and ecological values (Lowest Effect Levels (LEL)) were reported where available in the literature. Levels above the thresholds were marked with [1] (for LEL) and [2] (for TEL). Other in Cobalt: apparent effect threshold, i.e. background value from screening table from inorganics in sediment (Buckman, 2008). Probable Effect Levels (PELs) are not included because values for all measured metals and metalloids were far below these thresholds.

Element	Threshold		Chipiona port	Cordoba landfill	Fuente de Piedra lake	Miramundo landfill	Cetina salt pan	Doñana ricefields	Verinsur landfill
As	LEL	7.9	10.3[1,2] (8.6–12.3)	3.8 (3.3–4.3)	1.9 (1.5–2.4)	4.2 (3.7–4.9)	7.6[2] (4.4–13.0)	5.2 (4.7–5.7)	3 (2.3–3.9)
	TEL	5.9							
Cd	LEL	0.71	0.1 (0.1–0.6)	0.6 (0.5–0.7)	0.3 (0.2–0.4)	0.4 (0.4–0.5)	0.5 (0.4–0.8)	0.3 (0.2–0.3)	0.7 (0.5–1.1)
	TEL	1.2							
Co	Other	10	0.4	2.1	1.2	3	1.8	2.4	1.1
			(0.3–0.6)	(1.8–2.5)	(0.9–1.6)	(2.5–3.7)	(1.1–3.0)	(2.0–2.8)	(0.8–1.4)
Cr	LEL	25	3.3	14.8	9.3	21.2	8.6	11.3	12.8
	TEL	26	(2.5–4.2)	(12.6–17.3)	(6.6–13.0)	(17.9–25.2)	(6.1–12.1)	(9.4–13.6)	(9.6–17.2)
Cu	LEL	13	19.2[1,2]	79.5[1,2]	32[1,2]	26[1,2]	67.7[1,2]	41.1[1,2]	26.2[1,,2]
	TEL	16	(13.7–27.0)	(60.4–104.7)	(22.7–45.1)	(20.2–33.5)	(35.8–127.7)	(32.7–51.7)	(18.5–37.1)
Mn	LEL	460	52.8	155.8	105.9	191.6	100	345.2	83.4
	TEL	460	(43.3–64.3)	(134.6–180.3)	(85.6–130.9)	(161.6–227.3)	(83.8–119.4)	(304.5–391.3)	(70.4–98.9)
Mo	LEL	1.1	0.2	0.5	0.8	1.1[1]	12.2[1]	0.2	1.6[1]
	TEL	–	(0.1–0.3)	(0.4–0.7)	(0.6–0.9)	(0.8–1.2)	(5.7–26.0)	(0.1–0.3)	(1.0–2.4)
Ni	LEL	15	0.8	10.1[2]	6.5	10.7[2]	5.1	7.3	5.9
	TEL	7.5	(0.5–1.4)	(8.8–11.7)	(4.8–8.8)	(8.8–13.0)	(3.8–6.9)	(6.2–8.6)	(4.6–7.8)
Pb	LEL	19	5.2	14.5	11.8	16.8	27.6[1]	7.6	16
	TEL	31	(3.7–7.2)	(12.1–17.3)	(8.6–16.2)	(12.6–22.3)	(14.8–51.5)	(6.3–9.4)	(10.0–25.5)
Zn	LEL	129	157.1[1]	129.9[1]	114.9	129.6[1]	203.7[1,2]	69.6	120.3
	TEL	163	(122.6–201.2)	(109.1–154.7)	(88.6–148.9)	(96.0–174.8)	(130.7–317.5)	(58.4–83.0)	(86.1–168.3)

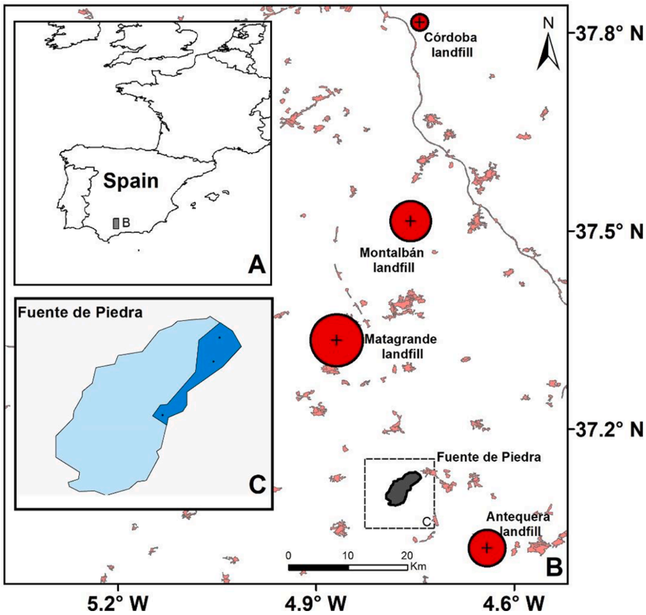

Fig. 2. a) Location of Fuente de Piedra lake in the Iberian Peninsula. (b) Four landfills used as foraging sites by gulls making daily movements from Fuente de Piedra. The size of the circle is proportional to the total cumulative time gulls spent in each landfill. Pink polygons show categorized urban areas based on Corine Land Cover 2012. (c) Dots show the location of the three main roosting sites of gulls within Fuente de Piedra. The basin of the lake is shown in light blue, except for the dark blue area used to quantify the impact of metal inputs from roosting sites. Figure . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) adapted from Martín-Vélez et al., 2019

968.66 kJ (Nagy et al., 1999), assuming LBBG have a mean body mass of 792 g (Hahn et al., 2007). E = food energy content: 23.9 kJ g^{-1}, based on a landfill diet (Winton and River, 2017). AM = the metabolizable energy coefficient: 0.76 (Karasov, 1990).

Further, ND = Number of Days per non-breeding period (181 days, from September to February based on the time that marked gulls were in the lake). We selected September from February based on the GPS data available and census for Fuente de Piedra lake (Martín-Vélez et al., 2019); while MN = Daily Mean Number of individuals (from 2010 to 2017) of LBBG present, calculated from census data (provided by Junta de Andalucía and corrected from a database of GPS of movements from 2010 to 2017; Martín-Vélez et al., 2019). EC = the average Element Content measured in micrograms per gram of dry faeces (collected in 2018).

3. Results

3.1. Analysis of metals in gull faeces

Permanova analyses showed significant differences in metal concentrations between sites ($F = 7.675$, $R^2 = 0.185$, $P < 0.001$, Fig. 3). Chipiona port, Cetina saltpan and Doñana ricefields showed the most dissimilarity in element concentrations in comparison to other sites (Fig. 3). On the other hand, landfills of Córdoba, Miramundo, Verinsur and Fuente de Piedra overlapped most (Fig. 3). Stress value associated to NMDS analyses was 0.12, which is validates the reduced dimensions (Clarke, 1993). SIMPER analyses also revealed high variability of contributing elements to differences between pairs of sites (Table S2). HACA clustering showed significant (based on bootstrap probability) associations of the elements As-Cu-Mo and Cr-Ni, whereas other elements did not show a significant association between them (Fig. S2).

Overall, all the elements significantly differed between locations,

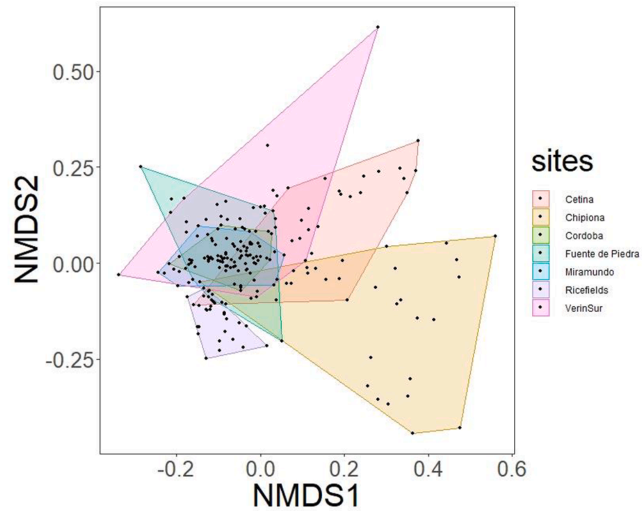

Fig. 3. Nonmetric multidimensional scaling (NMDS) representation of metal concentrations patterns of ten elements in faeces from different sites (Cetina saltpan, Chipiona port, Cordoba landfill, Fuente de Piedra lake, Miramundo landfill, ricefield and VerinSur landfill). Concentrations of each sample were standardized and distances were calculated based on Bray-Curtis dissimilarity.

both in general ($F = 12.71$;$p < 0.001$) and individually (Fig. 4; Table S3). Zn was least variable, showing high values in most of the sites (four of them exceeded the LEL values and the TEL value was exceeded for Cetina). More than 60% of the faecal samples from Chipiona port and Cetina saltpan were above the LEL threshold for Zn. Cu was also high, exceeding the LEL and TEL in all sites (Table 1). From 60% to 100% of samples exceeded the LEL threshold, depending on the site. Faecal samples collected at Chipiona port (Gulf of Cádiz) had the highest level of As, surpassing the LEL value in 66% of the samples (Table 1, Fig. 4); however, it showed the lowest concentrations of Cd, Co, Cr, Cu, Mn, Ni and Pb. Faeces from the Cetina saltpans (Bay of Cádiz) ranked first for Pb, Zn and Mo, with overall high values for all elements (four of them exceeded LEL values, with Mo exceeding the LEL value by more than 11 times). For example, 53% and 90% of the samples from Cetina were above the LEL threshold for Pb and Mo concentrations respectively. Samples collected at landfills ranked first for Cd, Co, Cr, Cu and Ni (Table 1), and Ni exceeded TEL values for Córdoba and Miramundo landfills (76% and 70% respectively). Faecal samples collected at the Doñana ricefields showed the highest levels of Mn and moderately high levels for most of the remaining elements (except for Zn, where the lowest concentrations occurred) (Table 1). Fuente de Piedra Lake showed similar values of metals to the landfills, although it had the lowest values for As, and significantly lower Cr in comparison to the landfills (Fig. 4).

3.2. Metal input into the Fuente de Piedra lake via gulls

Based on the average metal content in faeces, the percentage of time LBBG spent at Fuente de Piedra lake per winter, and the corrected census data from Martín-Vélez et al. (2019), we determined the metal loading caused by LBBG faeces into Fuente de Piedra lake over seven winters (Fig. 5). Metal load strongly varied between years, primarily because of increased gull numbers and an increase in the time spent roosting in recent years. Our estimations of metal load via gull faeces assigned highest values (in g ha^{-1}) to the essential elements Mn and Zn and lowest values to Cd and Mo (Fig. 5b). By way of example, in terms of the toxic heavy metal Pb, the total amount deposited in the roosting area over the first year year (2010–2011) was estimated to be 30.09 g ha^{-1}, compared to 76.88 g ha^{-1} (a 155% increase) by the last year (2016–2017). During the last year, this would translate to 10.18 g ha^{-1} to the whole lake basin (solely due to LBBG faecal inputs), and 13.76 Kg

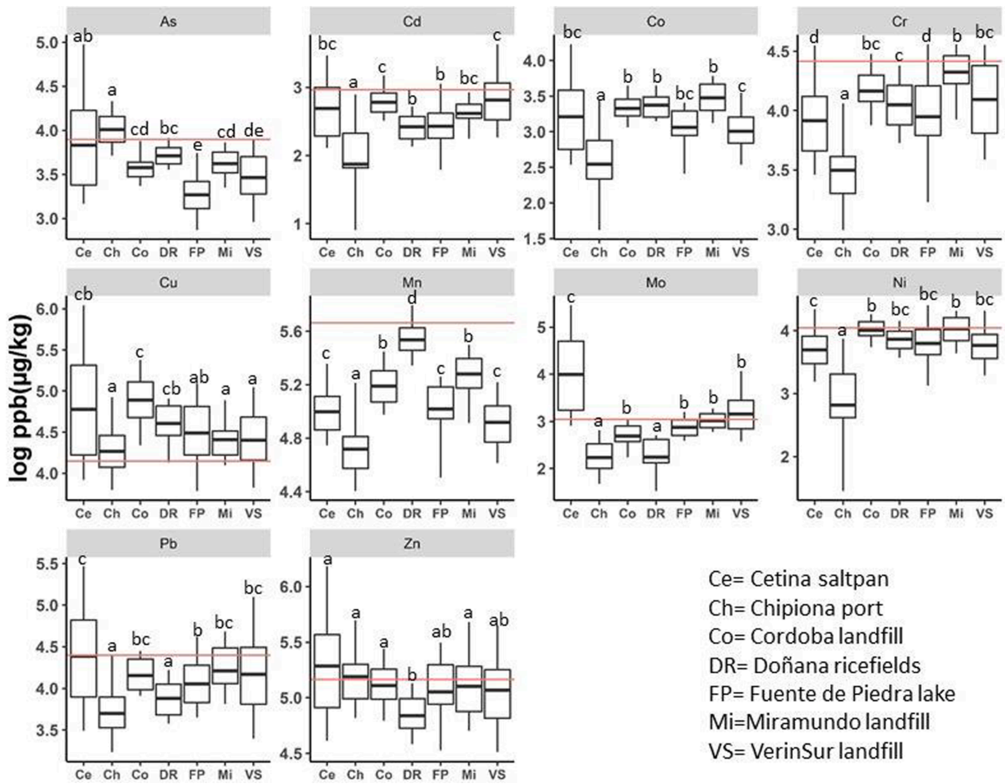

Fig. 4. Comparison between seven localities for each of the ten elements. Boxplots show the geometric mean of log-transformed data with the 25 and 75th quartiles, whereas whiskers show the 95% CI. The red line shows average consensus combined values for Lowest Effect Level (LEL) and Threshold Effect Levels (TEL). Significant differences ($p < 0.05$) between locations in the concentrations of the different elements are represented by different letters above the bars, based on a Tukey post-hoc test. Note: due to log-scale and similarity of the TEL and LEL levels, only one single average red line is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in total mass (see Fig. 2 for the differences between the roosting area and the whole basin).

4. Discussion

4.1. Spatial variation in metals in gull faeces

In this paper we show that LBBG can excrete high levels of metals in their faeces and that this reflects spatial differences in metal concentrations, most probably due to diet variation at different foraging sites. In some cases, gulls were foraging at the sites where we sampled faeces, but in other cases they were foraging elsewhere and only roosting at our sampling sites. As expected, and similar to other studies (e.g., Yin et al., 2008), essential elements (such as Zn) showed low variability among sites. Zinc is an essential trace metal fundamental for physiological functions in all living organisms (Kabata-Pendias, 1993), but it can be toxic above certain levels (Eisler, 1993). As part of the body, it can be actively regulated and eliminated, at least up to certain concentrations (Muyssen and Janssen, 2002). However, other essential elements (such as Cu and Mn), whose physiological functions in vertebrates are also fundamental (Kabata-Pendias, 1993), showed large variation among sites.

The highest concentrations of As were recorded in faeces from Chipiona Port (where it exceeded the LEL value) and Cetina saltpans, both in the Gulf of Cádiz. These sites sit hydrologically downstream of some of the most heavily mined areas in Europe – i.e., the Iberian pyrite belt (rich in As, Pb, Cu, Zn, etc.) – which has been mined for millennia, depositing large amounts of heavy metal-laden sediments and contaminated water via the Tinto and Odiel rivers, into the Gulf of Cadiz (Periáñez, 2009; Nieto et al., 2007). The Huelva estuary, formed by the union of the mouths of the rivers Tinto and Odiel, in the north west part of the Gulf of Cadiz, is also a heavily industrialized area discharging high concentrations of heavy metals into the Atlantic Ocean (Pérez-López et al., 2011). Arsenic load in particular, has been estimated at about 60 kg yr^{-1} and 2.7 t yr^{-1} for the Tinto and Odiel rivers, respectively (Sarmiento et al., 2009). This huge amount of highly toxic inorganic arsenic (and other metals) produces a plume of contaminants into the Gulf of Cadiz (Palanques et al., 1995), even reaching the Mediterranean Sea through the Strait of Gibraltar (Elbaz-Poulichet et al., 2001; Periáñez, 2009; Pérez-López et al., 2011).

LBBG diet at Chipiona Port is based around a natural diet of marine discards (e.g., of gadiforms, clupeiforms, benthonic fish, crustaceans, bivalve molluscs, etc; Sotillo et al., 2019; Oro, 1996), but these can contain high levels of As (Suñer et al., 1999) and other metals (Sarasquete et al., 1997). While the As accumulated in marine food chains is mainly present in organic forms, which are of low toxicity to gulls (Ahrar et al., 2014), these can be degraded back to inorganic As in the soil and some degree of demethylation from organic to inorganic forms of As could occur during the bird digestive process, thus increasing toxicity and retention (Huang et al., 2011; Yoshida et al., 2001).

The high concentrations of metals in samples from the Cetina saltpans are also likely related to wastewater discharges from surrounding urban settlements (especially for Pb and Cd), and effluents from naval and aeronautical industries (particularly for Zn and Cu, which both surpassed the LEL values) in adjacent areas (Ponce et al., 2000). This saltpan was also part of a large area of marshland which was historically used for hunting (Ruiz and Hortas, 2014), so high levels of Pb in samples from Cetina may be connected with the presence of Pb shot contamination in sediments in the area (Martínez-Haro et al. 2011).

Gull faeces collected at landfills near the Cetina saltpans, (i.e., at VerinSur and Miramundo), contained high levels of Cd and Cr which may be linked to the presence of electronic waste (Adelekan and Alawode, 2011). Electronic waste is an important emerging problem worldwide (Needhidasan et al., 2014). It contributes approximately 70% of the metals detected in landfill leachates (Li et al., 2009) and gulls may play an important role in transporting this to less contaminated areas (Martín-Vélez et al., 2020).

The expansion of open landfills in Andalusia since the 1980s has driven a major increase in the numbers of LBBG wintering inland in this region. Martín-Vélez et al. (2020) identified the 12 most important

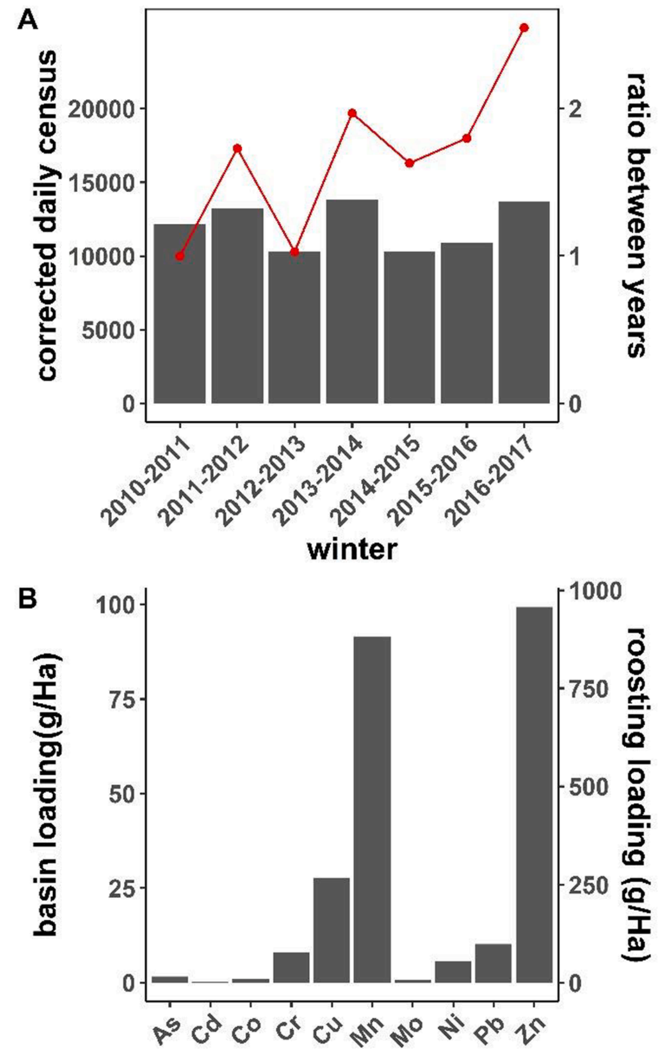

Fig. 5. A) Grey bars show the corrected daily mean counts in Fuente de Piedra between 2010 and 2017; the red line shows a ratio index of metal loading by LBBG in Fuente de Piedra lake (South Spain) between the seven study years, taking the first winter/year 2010–2011 as reference. B) Estimated element inputs (in grams per hectare) by LBBGs over the most recent winter (2016–2017) for both roosting sites and the whole lake basin. The two y axes show the same element loading in relation to two different measures of surface area: the whole lake basin to the left, and the area holding gull roosts to the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

landfills used by LBBG's, which then roosted in different wetlands around Andalucia (facilitating metal transport through their guano). These wetlands included many reservoirs and natural lakes, as well as ricefields (in late winter, after the harvest is complete). Fuente de Piedra lake, the most natural site in our study area, is one of the most important wintering roosting sites for LBBG in Spain. These birds feed in surrounding landfills (10.6% of the visits to landfills identified through GPS movements were to the Córdoba landfill 78.3 km away; Martín-Vélez et al., 2019) whose faeces present high levels of metals (Cd, Cu or Ni) that could subsequently be deposited in the lake (see section below).

The Doñana ricefields area is located in one of the most important wintering areas for waterbirds in the western palearctic (Rendón et al., 2008), and acts as both a foraging and roosting site for LBBG (Martín-Vélez et al., 2020, 2021), with more than 15,000 LBBG here each day during the harvest time (Rendón et al., 2008). Faeces collected in the Doñana ricefields showed the highest values for Mn and the lowest values for Zn. This pattern is consistent with the anaerobic processes occurring in the reduced flooded ricefield soils – which causes increased manganese availability (because of reductions of Mn^{4+} to Mn^{2+}) and decreased availability of Zn (Fageria et al., 2011). As expected (Carey et al., 2019), levels of As in gull faeces were also relatively high in this area. LBBG in Doñana ricefields feed mainly on alien crayfish *Procambarus clarkii* (Martín-Vélez et al., 2021), which is a good bioindicator of environmental contamination in the ricefields because it bioaccumulates huge quantities of metals (e.g. As, Pb, Cd, Zn, Cu), with potential to transfer these to higher trophic levels (Alcorlo et al., 2006; Suarez-Serrano et al., 2010). Contrary to As, levels of Cd were low in the ricefield samples, which is consistent with the reduction in solubility of Cd (i.e., decreased bioavailability) in reduced soil conditions (Moreno-Jiménez et al., 2014).

In general, levels recorded in our study are not that high in comparison with other habitats associated with anthropogenic impacts on waterbirds, but they are high in comparison with other gull studies in terms of Arsenic (Fu et al., 2013). This is important because of the high toxicity of this metalloid, ranked No.1 on the priority substances list (ATSDR, 2019). Regarding Pb%, (ranked as a 2nd priority toxicant; ATSDR, 2019), the levels from our study fall below other gull studies (Otero-Pérez, 1998; Fu et al., 2013).

4.2. Metal input at Fuente de Piedra lake

Biological transport of contaminants has typically been overlooked, but increasing evidence suggests that it can be substantial, and even the main pathway for contaminants in many contexts (Martín-Vélez et al., 2019). Gregarious animals that are exposed to contaminants and then move to congregate in specific areas are good candidates to be effective biovectors of contaminants (Blais et al., 2007). LBBG provides an example of a bird with such characteristics: i.e., it is commonly exposed to high levels of contaminants acquired in feeding areas, and then moves to concentrate in high numbers in roosting areas (depositing contaminated excreta at that site).

Here, we provide a detailed estimation of metal inputs by LBBG in the most important roosting sites, in SW Spain, Fuente de Piedra lake (1350 ha; see Fig. 2 for site details). Movement data has shown that LBBG feed in landfills (e.g., Córdoba landfill here, amongst others) where they are exposed to high levels of metals and then move to Fuente de Piedra lake for roosting (Martín-Vélez et al., 2019). In our approach, we based our calculations on monthly gull counts from seven years (2010 to 2017) corrected with movement data from Martín-Vélez et al., (2019) to estimate time spent at the lake. We modelled potential inputs of metals in Fuente de Piedra lake by LBBG, although levels in general fell below the thresholds for ecological contamination (except for Cu) (Table 1). Loads of Mn and Zn to roosting areas surpassed 700 g ha^{-1} during the last year studied (in 2017); while Cu reached 250 g ha^{-1} and for the most toxic element, i.e., Pb, values were 77 g ha^{-1}.

Interestingly, metal loads have increased in recent years, which can be explained by the increase in both the proportion of time spent at the lake and the number of gulls wintering at the lake (Martín-Vélez et al., 2019). LBBG have been gathering in increasing numbers to roost here during the non-breeding period since the 1980s, so long term deposition may be especially important for certain metals (such as As, Pb and Cd), as even low concentrations can be highly toxic (ATSDR, 2019). We also did not consider other potential sources of metal load via LBBG, such as pellets, which are also an important source of nutrients in Fuente de Piedra Lake (Martín-Vélez et al., 2019), or moulted feathers, which are known to bioacccumulate large amounts of certain metals (Abbasi et al., 2015; Szumiło-Pilarska et al., 2017; Einoder et al., 2018). The impacts of excess nutrients in the lake (as previously demonstrated) may be further exacerbated if synergic effects occur with metal loads within this ecosystem (Conde-Álvarez et al., 2012).

5. Conclusions

In this study we reported high concentrations of metals in gull faeces (among the highest recorded in any gull study), with several elements locally exceeding LEL values. We also found strong spatial variation, likely related to the main pollution sources associated with the different sites. We also provide the first data supporting the role of LBBG as biovectors of metals in a variety of aquatic ecosystems via their faeces. A similar approach could readily be adopted for other gull species with similar movement data (e.g., Ahlstrom et al., 2019; Navarro et al., 2016).

With the numerous anthropogenic pressures facing the environment, and the increasing availability of anthropogenic food resources for gulls, the contamination burden transported by birds to natural areas is expected to increase. Incorporating movement data of frequently tracked birds, such as LBBGs, will allow us to increase our ability to quantify biologically mediated contaminant flux and its implications for ecosystems and human health. From an ecosystem and health perspective, more attention should be paid to the role of gulls as vectors of pollutants in the long term, particularly in inland wetland ecosystems, which are used by millions of gulls across North America alone (Winton and River, 2017), and are less studied when compared with marine and other terrestrial ecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

VMV collected and prepared the samples, performed data analyses and figures and wrote the first draft; FH collected the samples and reviewed the draft; MAT and NOH analysed the samples in the lab and reviewed the draft, AJG contributed with sampling design and reviewed the draft, and MIS contributed to the sampling design and co-wrote the draft.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107534.

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