



Microlophus atacamensis as a biomonitor of coastal contamination in the Atacama Desert, Chile: An evaluation through a non-lethal technique[☆]



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ARTICLE INFO

Article history:

Received 23 March 2020

Received in revised form

12 September 2020

Accepted 25 September 2020

Available online 6 November 2020

Keywords:

Environmental pollution

Heavy metals

Non-lethal biomonitor

Lizard

ABSTRACT

In this report, we investigated the accumulation of heavy metals in the lizard *Microlophus atacamensis*, in three coastal areas of the Atacama Desert, northern Chile. We captured reptiles in a non-intervened area (Parque Nacional Pan de Azúcar, PAZ), an area of mining impact (Caleta Palitos, PAL) and an active industrial zone (Puerto de Caldera, CAL). Our methods included a non-lethal sampling of reptiles' tails obtained by autotomy and a few sacrificed animals to perform a stomach contents analysis. The concentrations of lead, copper, nickel, zinc and cadmium were measured by atomic absorption spectrophotometry in both soil and prey and compared to those recorded in the lizards' tails. Data obtained from lizard tails captured in PAL showed significantly high concentrations of Pb, Cu, Ni, and Zn compared to the other two sites PAZ and CAL. We did not find statistically significant differences among PAZ, PAL and CAL soils, probably due to the similar geological composition of the sites. However, the regional background values for Pb indicate contamination or at least metal enrichment in soils of the three sites, for Cu the global background values indicate contamination for the three sites, and for Cd both the regional and global background values show high values. The analysis of the stomach content showed differences in the food sources of the lizards among the sites studied. The concentration of heavy metal in lizard tissues versus prey delivered values of the Trophic Transfer Factor higher than one (1), suggesting that food may be a primary source of metals in the tissues of *M. atacamensis*. Calculations of the Bioaccumulation Factor (BAF) and the Ecological Risk (IR) resulted in values higher than one (1) indicating the relevance of this process in the sites studied. In this article, we report relationships between environmental contaminants, mainly putative preys, and concentrations found in lizard tails, which is more substantial in areas with historical heavy metal contamination such as PAL where the non-lethal technique developed in this research suggests a process of metal bioaccumulation in *M. atacamensis*.

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1. Introduction

The Atacama Desert, in Northern Chile, is one of the oldest deserts of the planet and has been arid to semi-arid for millions of

[☆] This paper has been recommended for acceptance by Maria Cristina Fossi.

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years (Ortlieb et al., 2000). Additionally, it is one of the wealthiest territories in the world in terms of porphyry copper deposits, whose heavy mining industry generates waste that significantly affects environmental sustainability (Fuenzalida, 2017; Oyarzún and Oyarzún, 2011; Valladares, 2012). The greater availability of heavy metals and metalloids in the environment can promote biomagnification processes in trophic webs (Ali et al., 2019; Tchernitchin et al., 2008; Valladares, 2012; Zhuang et al., 2009), and eventually increase the environmental hazards in addition to a higher risk for human health (Kim and Lee, 2012; Lind et al., 2012; Mendy et al., 2012).

Few studies have described the possible movements of heavy metals between the aquatic and terrestrial food webs through the coastal zone of the Atacama region (Clavijo-Calderón and Cázarez-Rodríguez, 2016; Nasri et al., 2015; Soucek et al., 2013). In this sense, new efforts can contribute to understanding the extent of the effects of heavy metals released into natural environments (Ali et al., 2019).

One of the most polluted ecosystems in the world is the Chañaral Bay located in northern Chile, (26° 22' 25.28" S 70° 20' 17.81" W) (Tapia, 2016). Through the El Salado river, this bay received a direct discharge of about ~150 million tons of mine tailings effluents, from Potrerillos refinery and El Salvador copper mine between 1938 and 1990, flowing into the Pacific Ocean, first directly into Chañaral Bay and, from 1975 diverted 8 Km north to the small coastal cove Caleta Palitos (PAL) (Tapia, 2016). These tailings caused, among many other problems, the siltation of the port, the filling of the bay with mining waste, and the disappearance of several marine species (Tapia, 2016; Vergara, 2011). Despite this situation, studies on the impact of this situation are still insufficient. Almost all studies on this type of metals on rocky intertidal communities in the Atacama region only consider localities that belong to these hotspots of mining waste (Fariña et al., 2008), leaving aside places that may be influenced by this condition despite being located at a greater distance or having little human population. Even so, researchers have importantly contributed with descriptions of the flora and vegetation (Diaz et al., 2018), micromammals of arid areas (Espinosa, 2009), birds in the Atacama desert (Torres-Mura et al., 2015) and reptiles in northern Chile (Fariña et al., 2008; Plaza and Lambertucci, 2017). Previous research also contributes with information on the state of health of places enriched or contaminated with heavy metals, particularly animals that feed on intertidal and terrestrial prey such as amphipods, algae, flowers, insects and molluscs (Fariña et al., 2003; Vidal and Ortiz, 2004a, 2014b).

Due to their presence in a variety of habitats, their wide geographical distribution, their longevity and, in many cases, small home ranges, reptile species are feasible to be considered as environmental biomonitors (Lambert, 2005). Previous reports have provided information on the use of squamates in environmental assessments of heavy metals (Fletcher et al., 2006; Mann et al., 2007; Nasri et al., 2015). Moreover, lizards inhabiting areas with a higher degree of anthropic disturbance or areas with significant environmental impairment have greater exposure to heavy metals, which could mean more content of metals in their bodies, surroundings, and prey (Fuenzalida, 2017; Salas, 2017). In contrast, lizards inhabiting in the coastal zone, away from productive companies, and that feed at a greater distance from polluting processes can be less compromised (Markert et al., 2003). Thus, reptiles have traditionally been used as biomonitors during the last twenty years, albeit through the sacrifice of animals which could affect populations and prevent repeated evaluation of the same animals for future reference (Campbell and Campbell, 2000; Hopkins et al., 2005; Nasri et al., 2015).

An abundant and common intermediate omnivorous predator

in the coastal zone of the Atacama Desert is the reptile *Microlophus atacamensis*, which has excellent plasticity to inhabit different environments including coastal rocks and slightly inland areas (Ortiz, 1980a; Vidal et al., 2002; Vidal and Ortiz, 2004a, 2014b). Through a process of autotomy, *M. atacamensis* can release its tail, which is a combination of fat, blood, muscle, bone, and skin tissues. Its tail could provide an overall assessment of the accumulation of metals throughout the organism, avoiding the sacrifice of the animal and positioning this species as a potential biomonitor (Fletcher et al., 2006).

This study aims to determine the content of Pb, Cu, Ni, Zn and Cd metals in soil, prey and tails of *M. atacamensis* collected from three sites with different degrees of heavy metal contamination caused by anthropic disturbance. As lizards are abundant and permanent inhabitants of this environment, capturing the heavy metals from their surroundings, in this research we propose a non-lethal technique to assess their metal accumulation that can become a novel tool for environmental and human health risk assessment in the Atacama Desert coast.

2. Materials and methods

2.1. Study site

The fieldwork was carried out in three sectors located near the city of Chañaral (26° 22' 25.28" S 70° 20' 17.81" W) in the coastal area of the Atacama Desert, northern Chile. This town and its surroundings have been considered one of the areas most affected by pollution worldwide due to the direct coastal discharge of mine tailings effluents (Tapia, 2016; Vergara, 2011). The selected sites have different levels of anthropic intervention: PAZ: National Park Pan de Azúcar (26° 08' 59" S 70° 39' 02" W) located 30 km north of Chañaral; PAL: Caleta Palitos (26° 16' 29" S 70° 39' 36" W), located 8 km north of Chañaral and which has received tailings from El Salvador Mine for over 45 years (Castilla and Nealler, 1978; Tapia, 2016; Toro, 2017), and CAL: Puerto de Caldera (27° 04' 00" S 70° 49' 00" W), a mining and fishing port located 100 km south of Chañaral. The total distance between PAZ and CAL is 130 km (Fig. 1). The three selected sites were chosen based on previous studies (Cáceres, 2015; Ramirez et al., 2005; Tapia et al., 2018). The results descriptions were made from north to south in ubication geographic terms PAZ, PAL and CAL. Considering PAZ as a low anthropic intervention site, PAL as a high anthropic intervention site, and CAL as an intermediate anthropic intervention site (Marschik and Fontboté, 2001; Vergara, 2011).

2.2. Study organism

Microlophus atacamensis (Donoso-Barros, 1960) is distributed in Chile from the south of the Loa River Antofagasta Region (range still under discussion) (Ibañez, 2014; Victoriano et al., 2003) until the north of the Coquimbo Region (Ortiz, 1980b) including an introduced population in La Serena (Sepúlveda et al., 2008). It is an oviparous species that can reach a maximum length ~300 mm (Vidal and Ortiz, 2004a; 2014b), and it is spatially segregated by sex and age (Heisig, 1993; Ortiz, 1980a; Vidal et al., 2002). This species is characterized by prowling within a radius of 100–150 m from their places of shelter (Ortiz, 1980a), and well known for being present in the intertidal zone (Nuñez and Veloso, 2001). It inhabits places free from anthropic disturbance, and it also coexists closely in areas with significant industrial, residential activity and productive sectors of the coastal border (Fariña et al., 2008; Fernández et al., 2000; Luoma, 1990; Sepúlveda et al., 2008). These animals prefer the soil over the rock surfaces since it is dry and can harbour

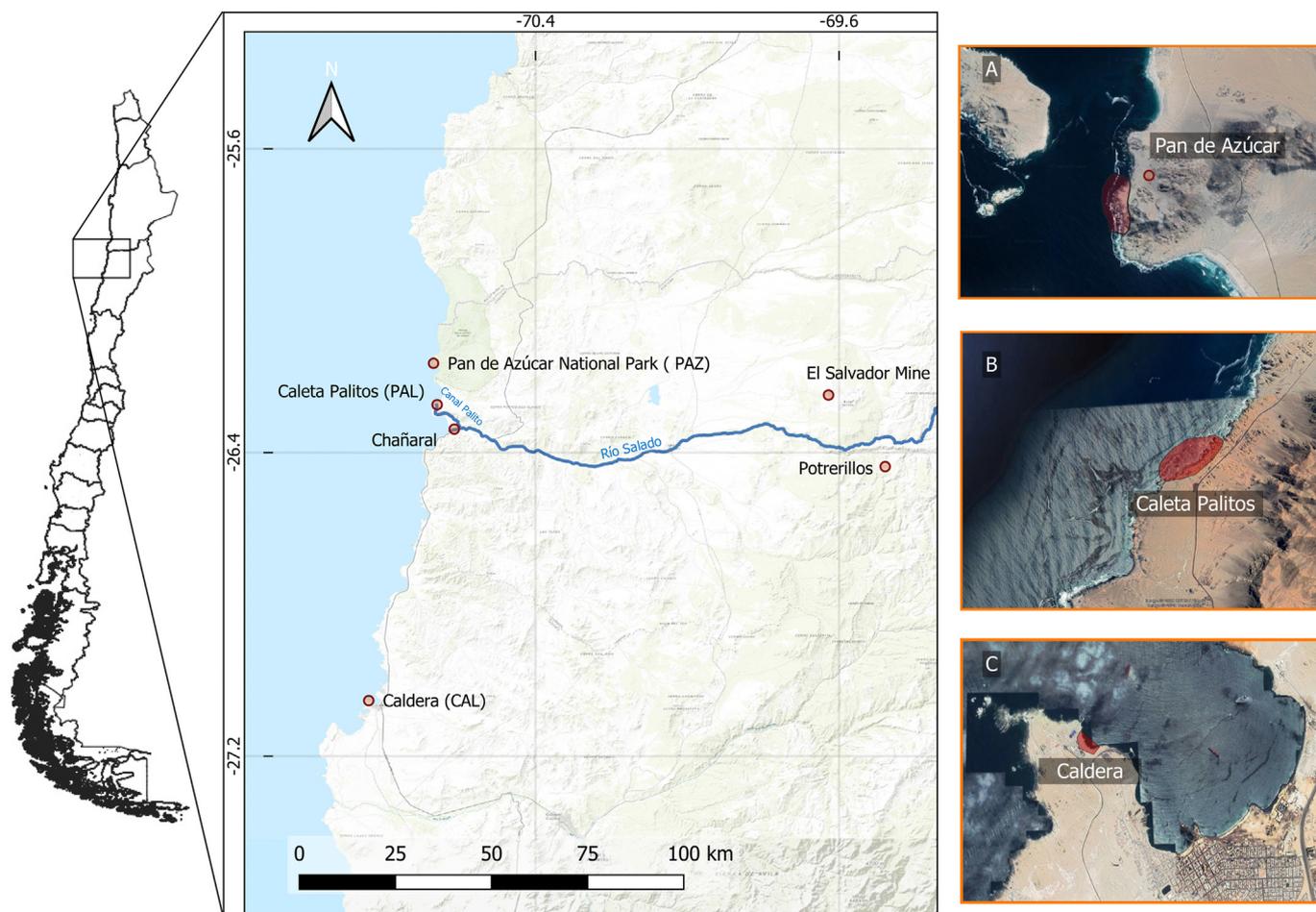


Fig. 1. Aerial view of the sites sampled is shown relative to a map of South America. The three sampling sites from North to South are National Park Pan de Azúcar (A, PAZ), Caleta Palitos (B, PAL) and Puerto de Caldera (C, CAL). The target taxon *M. atacamensis* is primarily present in the intertidal zone. These images correspond to a mosaic generated using Google Maps-Digital Globe Company. The images are native 30 cm resolution imagery. The average position of these images is 5 m CE90 in lat/long.

macroalgae brought by the waves with living moving food that can be caught. Besides, due to their omnivore habits, lizards can patrol 100 or 150 m parallel or perpendicular to the coast (Ortiz, 1980a) in search of food. Since they are very agile and fast, they receive the common name of *Corredor de Atacama* (Atacama runner). *M. atacamensis* has been considered a top predator in the intertidal ecosystems of northern Chile (Yañez, 1951), feeding mainly on intertidal invertebrates and macroalgae (Cerdeña and Castilla, 2001; Contreras-Porcía et al., 2011a; Fariña et al., 2008; Vidal et al., 2002). It has also been described as a species with cannibalistic behaviour (Donoso-Barros, 1948; Ibañez, 2014; Ortiz, 1980b). As of today, no studies have been found in the literature on the presence of pollutants in this lizard.

2.3. Sample collection

2.3.1. Soil

In order to determine the metal content in the soil of each sampling location, a total of 28 samples were obtained manually and stored in plastic bags previously rinsed with HCl and dried. Sampling transects were the same 300 m long where the lizards were captured, located approximately 10 m away from the breaker zone since animals do not naturally enter the water. The samples were obtained by digging holes of about 5–10 cm deep in places where there is soil and the animals usually roam. This study reports

analyses of all metals in comparison to their background levels according to local environmental values (CENMA, 2014) and background values of global soils (Alloway, 1995) (Table 1 and Supplementary Table 1).

2.3.2. Prey

Putative preys were obtained manually at the three study sites during January 2017 and November 2018 (Table 4, Supplementary Table 1) using hand searches and hand nets when necessary (e.g. for flying insects). Samples were returned to the laboratory, identified and, where necessary soft tissues removed from inorganic carapaces (decapods) or shells (molluscs). Samples were then dried (60 °C for 48 h) before processing for subsequent analysis for metal concentrations.

2.3.3. Diet analyses

A total of 27 animals were sacrificed for diet analysis (PAZ = 10, PAL = 7, CAL = 10, Table 4). In the laboratory, the stomachs were dissected from each animal, and the other organs (lungs, liver, heart) were removed and stored for further analysis not considered in this study. In addition to field observations at each site, the prey consumption by gastric content analysis was compared with previous reports (Fariña et al., 2008; Vidal et al., 2002). In this way, once the literature review and the field and laboratory analyses were completed, the most consumed prey by *M. atacamensis* were

Table 1Background soil concentrations expressed in mg kg⁻¹. RB (Regional Background) WB (World Background).

Background values	Pb	Cu	Ni	Zn	Cd
Background values (CENMA, 2014, RB)	12.7 mg kg ⁻¹	91.6 mg kg ⁻¹	41.7 mg kg ⁻¹	75.9 mg kg ⁻¹	1.2 mg kg ⁻¹
Background values (Alloway, 1995, WB)	32 mg kg ⁻¹	18.5 mg kg ⁻¹	20 mg kg ⁻¹	64 mg kg ⁻¹	1 mg kg ⁻¹

captured. (Table 4-Fig. 4).

2.3.4. Tails

A total of 72 adult *M. atacamensis* lizards (CAL n = 20, PAL n = 22, PAZ n = 30) were captured randomly up to 5 m each side of an established transect during the hottest hours of the day (11:00–15:00 h) (Tellería, 2006), of which, as indicated in the previous section, only 27 were sacrificed, with the remaining 45 animals released as explained later in the text. Each transect was 300 m long, parallel to the coastline, approximately 10 m from the breaker zone. The three study areas had substrates of soil and rocks. Across study areas the coast is quite uniform with a similar geological formation (Valdés and Tapia, 2019), where the animals are on the dark rocks or in the soil basking in the sun, hunting or moving from one rock to another with frequent soil contact. The coasts of Atacama are generally flat without elevations, although there may be a mixture of soil and sand. The water line shows a visible layer of metallic oxide residues, more significant in PAL due to their direct dumping of mining waste during the last forty five years, less abundant in CAL, an economically active and environmentally regulated port with minerals embarkments, fishing, aquaculture and tourism, and minimal in PAZ because it is a protected national park.

Each animal was carefully captured using a rod with a sliding lasso, ensuring that the process of autotomy had not taken place, preserving their original tails (Aguilera et al., 2012; Aguirre-León, 2011; Vidal et al., 2002). Subsequently, the individuals transferred to the laboratory were sexed, measured, and weighed according to methodology described by (Aguirre-León, 2011; Knudsen, 1972). The Supplementary Table 1 and Table 3 summarizes information on the lizards captured at each site. All individuals demonstrated autotomy of their tails; thus, there was no need for surgical removal. All the animals not sacrificed were returned 24 h later to their capture sites, without their tails. Tails were weighted and kept in vials frozen (–25 °C) to await subsequent processing and analysis for heavy metals.

2.4. Analytical procedure

2.4.1. Stomach content

In the lab, the animals were dissected, with gut removal and stomach separation. The prey items were spread in Petri dishes using tweezers, and then fixed and preserved in 70% ethanol and 10% formaldehyde. Finally, items were identified under a stereomicroscope to the lowest possible taxonomic level, usually to Order level. Some digested prey items were identified by structures or were reconstructed as pincers and carapaces (decapods), shells and radulae (molluscs), type of mouthpiece, antennae and wing shapes (insects), flowers, pollen and stamens (plants), thallus or part of the blade (algae). We used keys and identification guides (Gillott, 2005; Hiriart et al., 2019; Tapia-Mendez, 2002; Zuñiga-Romero, 2002). The frequency of occurrence (FO) that reports the nutritional habits of a population (Cailliet, 1977) would correspond to the percentage of full stomachs that contain a certain category of prey.

2.4.1.1. Sample preparation and metal analyses (lead, copper, nickel, zinc and cadmium). For the study of metals in soil, intertidal preys

and lizard tails by site, the methodology described by (Guiñez et al., 2015) and (Castillo and Valdes, 2011) was followed for the analytical pre-treatment, respectively (Supplementary Table 1).

Soil: the metal analysis was determined in the fraction <63 µm, after drying the material at 40 °C until constant weight. For this, between 0.2 and 0.6 g of dry soil was disaggregated in a MARS-X microwave digester (CEM model 350) with a mixture 12 ml of HNO₃:HCl (3: 1 ratio) at 150 °C for 20 min according to the US- EPA 3051 A procedure (Link et al., 1998). Subsequently, the resulting solution was filtered with a 0.45 µm filter and diluted to 25 ml with deionized water. Acid digestion with HNO₃:HCl was considered because only metals present in the organic fraction are recovered, where bioavailable metals are concentrated (Cox and Preda, 2003).

Putative preys and tails: in the tails the analysis of the metals was carried out considering the complete tail (tissue, bone and skin), while in putative preys only the soft parts were used, removing the hard structures (e.g. shells, exo-skeletons, etc.). All samples (tails and putative preys) were homogenized separately in an agate mortar until obtaining a wet paste. Between 0.5 and 1.0 g of the wet sample was added to a Teflon digestion vessel with 10 ml of concentrated HNO₃ (ultrapure) and digested in a microwave oven MARS-X (CEM model 350) at 180 °C for 10 min, according to the US-EPA method 3051 A. Subsequently, the resulting solution was diluted with 25 ml of deionized water.

For biological samples (putative prey and tails) and soils, the analysis of Pb, Cu, Ni, Zn and Cd was carried out using an atomic absorption spectrophotometer (Shimadzu AA-6300) by flame technique. The analytical procedure was checked using the reference material Dorm-3 (biological samples) and Mess-3 (soils) obtained from the National Research Council (NRC, Canada). In both cases the analytical error was less than 5% (Supplementary Table 2). The concentrations were expressed in mg Kg⁻¹ of dry weight (soils) and wet weight (biological samples), respectively (Supplementary Table 2).

2.4.2. Calculation of the bioaccumulation factor (BAF), potential ecological risk (RI), and Trophic Transfer Factor (TTF)

To calculate the bioaccumulation factor (BAF) the metal concentration detected in the lizard tails (*C*_{biota}, mg kg⁻¹) was divided by the concentration of the metal measured from the soil (*C*_{soil}, mg kg⁻¹), following the equation: BAF: $C_{biota}, \text{mg kg}^{-1} / C_{soil}, \text{mg kg}^{-1}$ (Mortuza and Al-Misned, 2015) (Table 2). On the other hand, the calculation of the potential ecological risks RI of total heavy metals toxicity applied the following equation (Hu et al., 2018; Pan et al., 2016), where *Tr* is the toxic response factor for a particular heavy metal, this factor was 30, 5, 5, 5, and 1 for Cd, Cu, Ni, Pb, and Zn, respectively (Baghaie and Aghilii, 2019; Soliman et al., 2019; Wu et al., 2010). *C_i* is the metal concentration in soil (Pb, Cu, Ni, Zn and Cd expressed in mg kg⁻¹, respectively). *C_r* is the background value of heavy metals in soil (Pb, Cu, Ni, Zn and Cd expressed in mg kg⁻¹ for PAZ, PAL and CAL) (Alloway, 2012). *Er* is the individual potential ecological risk factor, *RI* is a composite index that indicates the potential ecological risk of total heavy metals in soils (sum of each of the *Er*), and *n* is the total number of the estimated heavy metals (Pb, Cu, Ni, Zn and Cd) (Franco-Uría et al., 2009; Zhou et al., 2014) (Table 3).

Trophic transfer refers to the passage of a contaminant in food

Table 2
BAF (Bioaccumulation Factor) values for sites indicating, in grey, values > 1.

Sites	Pb	Cu	Ni	Zn	Cd
PAZ	3.71>1	1.72>1	0.90	2.26>1	0.18
PAL	5.56>1	2.82>1	1.33>1	5.03>1	0.25
CAL	4.23>1	1.23>1	0.49	2.36>1	0.24

Table 3
TTF (Trophic Transfer Factor) and RI (Potential Ecological Risk). TTF values between tissue and prey indicating values > 1 in grey, which would indicate transfer of metals. Ecological Risk Index Values RI for PAZ, PAL and CAL. In this case, all values present a moderate risk. There are no values in Considerable Risk or High Risk.

Sites	TTF					RI
	Pb	Cu	Ni	Zn	Cd	Moderate Risk 150<RI<300.
PAZ	1.66>1	1.63>1	1.62>1	0.99	0.19	297
PAL	2.22>1	2.59>1	2.93>1	3.49>1	0.38	286
CAL	0.91	1.24>1	1.76>1	1.08>1	1.79>1	291

Table 4
Preys for each evaluated site, mean and frequency determined in the stomachs of sacrificed animals. n = number of animals sacrificed per site in PAZ, PAL and CAL.

Prey	CAL (n = 10)		PAZ (n = 10)		PAL (n = 7)	
	Mean	frequency %	Mean	frequency %	Mean	frequency %
Amphipod	5	22	9	40	–	–
Decapod	1	11	5	20	1	14
Echinolittorina	1	11	–	–	3	29
Ulva	25	44	41	70	27	71
Luche Algae (Porphyra sp.)	–	–	11	20	–	–
UID insecta	12	33	–	–	3	14
UID Lepidoptera	3	22	–	–	–	–
UID diptera	17	44	2	10	27	100
UID Coleptera	10	11	0	0	–	–
Tenebrionidae	–	–	3	20	–	–
Microlophus	–	11	9	10	–	–
Flowers	25	44	–	–	40	86
Fish	–	–	2	10	–	–
Sand	–	–	19	90	–	–

chains from one trophic level to the next (Ali et al., 2019). The ratio of metal concentration in an organism to the concentration in its prey allows assessment of the potential of this metal to biomagnify at different steps in the food chain (Reinfelder et al., 1998; Wang and Fisher, 1999). The following equation allowed the calculation of the Trophic transfer factor (TTF) (Deforest et al., 2007):

3.4.2.1. TTF: metal concentration in the organism’s tissue/metal concentration in the organism’s food. A TTF value > 1 indicates a possibility of biomagnification, while values < 1 suggest that biomagnification is unlikely. For the TTF calculations, a range of assimilation efficiencies and ingestion rates standard for all organisms were considered (Mathews and Fisher, 2008) (Table 3).

2.5. Data analysis

Before statistical analysis, the data processing verified the normality by Shapiro-Wilk test and the Levenne homogeneity of

the variance. The software R Core Team was used to conduct all statistical tests and analyses (2019). Given the significant difference detected between the variances, it was determined to use ANOVA Welch tests for these cases. The data collected was first used to verify the existence of significant differences between the tails of all sites, process replicated for preys and soils samples using an ANOVA Welch (alpha = 0.05), followed by a post hoc Tukey HSD (Supplementary Table 4) to investigate whether metal concentrations varied significantly between sites. Finally, a principal component analysis (PCA) summarized the relationship of the metals studied to the tails, prey and soils between the sites.

3. Results

3.1. Metal accumulation in soil

Our results did not show significant differences for any of the metals at the study sites in soil (P > 0.05 for all metals; Fig. 2). Only

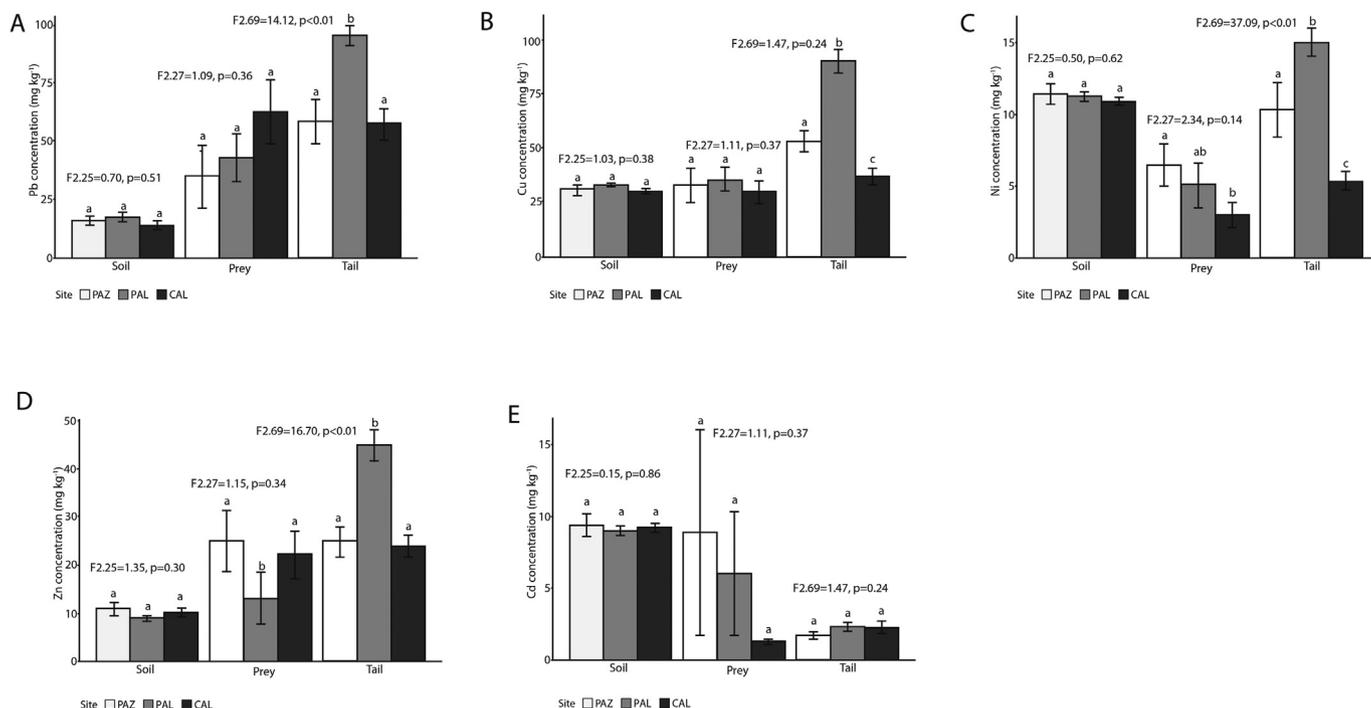


Fig. 2. Average concentrations (\pm SD) of A) Pb, B) Cu, C) Ni, D) Zn and E) Cd in the substrates, preys and tissues of the lizards sampled in PAZ, PAL and CAL. The F ratios and the associated *p* values are derived from the ANOVAs of the metal concentration depending on the site, prey and tail. The letters on the bars show the results of the Tukey post hoc test for the group mean.

the Pb values detected from soil of the three sites (PAZ, PAL and CAL) exceeded the regional background, while the values of Cu and Cd exceeded the world background (Alloway, 1995). Ni and Zn were below regional and international background values (Table 1 and Supplementary Table 1). The averages of the metals with their respective standard deviations are shown in the Supplementary Table 1.

3.2. Metal accumulation in *M. atacamensis* prey

The analyses of the stomachs revealed different proportions of prey consumed by the animals in the three sites (PAZ, PAL, CAL). The consumption of decapods (mostly *Petrolisthes* sp.) and *Ulva* sp. was common at all three sites. For each site, and as the food supply was different, samples were grouped and taken according to what was found in the stomachs of the *M. atacamensis*. In this way, depending on what were the most consumed prey at each site, different samples were taken. For instance, samples of algae such as *Porphyra* sp. were taken in (PAZ), flowers such as *Nolana* sp. and *Cristaria* sp. in (PAL and CAL) and other prey such as amphipods were sampled in (PAZ and CAL) (Table 4). The consumption of fish and sand recorded at (PAZ) is a finding, since although animals do not commonly enter the water, they can effectively swim and consume prey from small ponds, exposed during low tides, where juvenile fish are found, this study is the first verified record of this behaviour. On the other hand, cannibalism is something documented (PAZ and CAL) and occurs frequently (Farina et al., 2003; Vidal et al., 2002) (Table 4). In this way, we grouped the preys to be able to compare for each metal and site. (Table 4 and Fig. 4).

The average values of metals in preys on a gradient from high to low per site were: For Pb (CAL), (PAL) and (PAZ); for Cu: (PAL), (PAZ) and (CAL); for Ni (PAZ), (PAL), and (CAL); for Zn (PAZ), (CAL) and (PAL); for Cd (PAZ), (PAL), and (CAL) (Supplementary Table 1). Although the results did not show significant differences in the

metal content of the preys among the sites studied for Pb, Cu and Cd, differences were found *a posteriori* between Ni concentrations and Zn concentrations in PAZ, PAL and CAL (Supplementary Table 4).

Even though the analysis of heavy metals in food items of the lizard *M. atacamensis* did not show statistically significant differences, the levels of Pb in CAL samples were superior compared to all other metals from the PAZ and PAL sites. Overall copper levels were higher in PAL than in PAZ and CAL. Ni values were higher in PAZ than in PAL and CAL. Zn in general was higher in PAL than in CAL and PAL. Cd was also higher in PAZ than in PAL and CAL (Supplementary Table 1 and Supplementary Table 4).

TTF values of Pb at PAZ and PAL sites were >1, but CAL does not exceed the factor. However, for Cu, Ni at all three sites was PAZ, PAL and CAL >1, Zn in PAL and CAL was >1, for Cd only CAL was >1 (Table 3).

3.3. Metal accumulation in *M. atacamensis* tails

Lizards' tails studied at the three sites showed high concentrations of Pb, Cu, Ni and Zn except for Cd (Fig. 2), compared to putative preys and soil. The average concentrations in a gradient from high to low are shown below by metal: PAL had the highest concentrations of Pb in tails at almost twice that of the other two sites PAL (95.3 mg kg⁻¹), Pb in PAZ site (58 mg kg⁻¹) was similar to Pb in CAL (57.3 mg kg⁻¹). For Cu the tails also presented the highest value for PAL over PAZ and almost triple over CAL, (PAL (90.3 mg kg⁻¹), PAZ (52.8 mg kg⁻¹), CAL (36.6 mg kg⁻¹)). For Ni, the tails values are higher in PAL than the other two sites (PAL (20.4 mg kg⁻¹), Ni PAZ (14 mg kg⁻¹), Ni CAL (7.29 mg kg⁻¹)). For Cd, the values are similar in the three sites evaluated, but PAL is still the highest PAL 2.32 \pm 1.24 mg kg⁻¹, CAL 2.25 \pm 2.18 mg kg⁻¹, PAZ 1.69 \pm 1.47 mg kg⁻¹ (Fig. 2). PAL concentrations of Pb, Cu, Ni and Zn were significantly higher in tails compared to those of the other

two sites (Supplementary Table 1).

Tails of *M. atacamensis* between sites showed significant differences in Pb concentration (ANOVA, $F = 14.12$, p -value <0.01), where the Pb average content and standard deviations are $95.3 \pm 21.8 \text{ mg kg}^{-1}$ (PAL), $58.0 \pm 52.1 \text{ mg kg}^{-1}$ (PAZ), and $57.3 \pm 28.6 \text{ mg kg}^{-1}$ (CAL), respectively (Fig. 2). On the other hand, there is a significant difference in the concentrations of Cu in the tails between sites (ANOVA, $F = 30.48$, p -value <0.01), where the means and their corresponding standard deviations are $52.8 \pm 27.0 \text{ mg kg}^{-1}$, $90.3 \pm 26.6 \text{ mg kg}^{-1}$, and $36.6 \pm 17.1 \text{ mg kg}^{-1}$ for PAZ, PAL and CAL respectively. These scenarios are similar for Ni and Zn (ANOVA, $F = 37.089$, p -value <0.01) and (ANOVA, $F = 16.70$, p -value <0.01), for Ni and Zn respectively. The means of Ni and their corresponding standard deviations of the tails examined are $14.0 \pm 14.3 \text{ mg kg}^{-1}$, $20.4 \pm 5.97 \text{ mg kg}^{-1}$ and $7.29 \pm 3.69 \text{ mg kg}^{-1}$ for PAZ, PAL and CAL respectively. For Zn, the average concentrations and their corresponding standard deviations are $24.8 \pm 17.5 \text{ mg kg}^{-1}$, $44.9 \pm 14.4 \text{ mg kg}^{-1}$ and $23.9 \pm 9.84 \text{ mg kg}^{-1}$ for PAZ, PAL and CAL respectively. In the *a posteriori* statistical analysis, there was a difference of Ni in tails for all the places. For Zn, differences were detected in PAL, which showed high concentrations, whereas PAZ and CAL do not differ. Regarding the concentrations of Cd present in the tails of *M. atacamensis*, they showed no significant differences (ANOVA, $F = 1.50$ $p = 0.24$), while for the Cd concentrations, the means and standard deviations are $2.25 \pm 2.18 \text{ mg kg}^{-1}$, $2.32 \pm 1.24 \text{ mg kg}^{-1}$ and $1.69 \pm 1.47 \text{ mg kg}^{-1}$ for PAZ, PAL and CAL, respectively.

The BAF for Pb was higher in PAL than PAZ and CAL, recorded values of 3.71 (PAZ), 5.56 (PAL), 4.23 (CAL). For Cu, the BAF in PAL is higher than in PAZ and CAL, whereas for Ni the $BAF > 1$ was only observed in PAL. For Zn, the $BAF > 1$ were registered in all sites (PAL = 5.03, PAZ = 2.26, and CAL = 2.36). In the case of Cd none of the three places exceeded the value 1 (PAL = 0.25, CAL = 0.24 and PAZ = 0.18). The BAF analysis showed that *M. atacamensis* would be bioaccumulating Pb, Cu and Zn at all sites, Ni at PAL and Cd at none of the sites (Table 2).

The Ecological Risk delivered moderate values for the three sites, even though they are at the upper limit of the second level out of four. The values for each site were PAZ:296.83, PAL:285.61 and CAL:290.59 (Table 3).

PCA analysis (Fig. 3) showed that the two main components explain 72% of the variance, showing a strong relationship between Pb and Zn, and between Cu and Ni, for preys and tails of PAZ, PAL and CAL. For their part, soils are grouped independently. In general, Pb, Cu, Ni and Zn contributed the most to PC1, while Cd contributed mainly to PC2.

4. Discussion

In this article we reported the soil concentration of heavy metals (Pb, Cu, Ni, Zn and Cd) sampled from the northern and southern Chañaral coasts, and their accumulation in the prey and tails of the *M. atacamensis* lizards obtained from three sites with some degree of local mining influence in the Atacama region (Marschik et al., 2003; Tapia, 2016; Vergara, 2011).

4.1. Metal accumulation in soil

The samples were taken in soil, as explained, approximately 10 m from the breaker zone, this to allow comparison with other published studies (Valdés and Tapia, 2019; Valladares et al., 2013) and to establish how the minerals can be transported from the ocean to the interior (Dold, 2014). The PAZ site, being a National Park, is located in an industry-free zone and apparently it is less

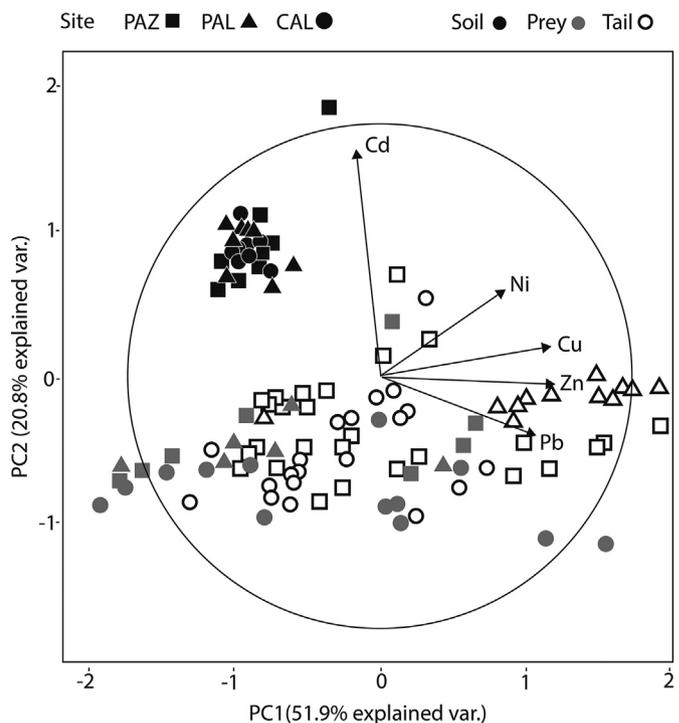


Fig. 3. Biplot of individual scores extracted by principal component analyses (PCA) and element loadings on the two principal axes (PC1 and PC2) with heavy metals per site, prey and tail in Atacama Desert. Abbreviations: PAZ (Pan de Azúcar National Park), PAL (Caleta Palitos), CAL (Puerto de Caldera).

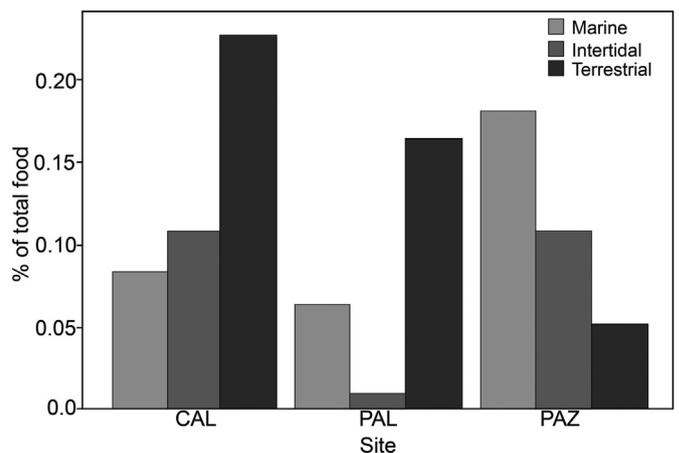


Fig. 4. Total food intake. It describes how the animals feed in the sites PAZ, PAL and CAL, considering intertidal, marine or terrestrial sectors.

affected by soil contamination (Tapia et al., 2018a). PAL, on the other hand, is a highly intervened area with mining pollution for over 45 years (Tapia et al., 2018b; Tapia, 2016; Vergara, 2011), but it does not show significant differences with the other two sites (PAZ and CAL). Even though CAL is a more recent industrialized area, it presents similar values to PAZ, probably due to the geological characteristics of the sites (Tapia et al., 2018b; Valdés and Tapia, 2019). Although the three sites did not show significant differences between them, this does not mean that they are not contaminated or enriched with metals, since when compared to the regional background values (CENMA, 2014), high Pb values were established for the three sites, as well as for Cd. While compared to the world background (Alloway, 1995) high values for Cu and Cd

were established in the three sites (Supplementary Table 1).

Thus, the average Pb values of the three sites do not vary considerably from what was recorded by other authors, but the Cu, Ni and Zn values in our study are lower on average in the three sites compared to the background regional values, being only Cd which presents a considerably higher value of the soil (CENMA, 2014; Sánchez, 2017; Valdés and Tapia, 2019). Only Cu and Cd surpassed the world background (Alloway, 1995), which is probably accounted for by the specificity of the chosen places, despite the fact that the levels are high in general, but similar to each other. When we compare the soil values measured in this study with the international Soil Quality Guidelines (Gallardo and Gonzalez, 2009), we can say that only Cu and Cd are at unacceptable risk levels. Pb, Ni and Zn are within acceptable risk ranges to the international community (Gallardo and Gonzalez, 2009), although this has been questioned, since Chañaral is in the 17th place of the global pollution hotspots, followed by PAL (Koski, 2012). In this way, and despite the fact that contamination in the aquatic habitat apparently does not physically reach the terrestrial soils, it is being incorporated into the terrestrial biota by means of transfer from metal-contaminated food. That is why new studies are required that establish the exchanges between the aquatic and terrestrial environment, and vice versa, that also determines the maximum levels and exchange of metals for the sectors anthropically intervened by the mining industry.

On the other hand, previous studies establish Cd values between 0.17 and 1.085 mg kg⁻¹ in uncontaminated places and places contaminated with mining pollution, respectively (Ramirez et al., 2005; Valladares et al., 2013), values well below those registered for PAZ, PAL and CAL (Supplementary Table 1), which are one order above. The same authors establish Pb values between 19 and 21.2 mg kg⁻¹, for uncontaminated sites and those contaminated with mining waste, respectively, values very close to those obtained at least in PAL, indicating that our measurements are correct since they are comparable. For Cu and Zn the ranges are so wide that our results are below what was previously evaluated, however Ni values are shown above the regional averages evaluated but below the background (Ramirez et al., 2005; Valdés and Tapia, 2019). Nevertheless, we must clarify that the traditionally used values, such as that of the upper continental crust (Rudnick and Gao, 2003; Turekian and Wedepohl, 1961), may correspond to geological references that do not normally represent the area evaluated, specifically.

The RI analysis suggested a moderate risk related to heavy metals concentration in soil, on the limit of the important risk score (Table 3) (Baghaie and Aghili, 2019). Moreover, solid waste deposited directly by mining companies in similar industrialized areas makes these places potential risk sites if the values rise to level three or four of the RI score, from moderate to important risk (Table 3) (Muñoz et al., 2005). Some authors have noted that subtidal environments of the Atacama Region present high heavy metals enrichment due to contaminants released into the sea by different long-standing mining industries (Tapia, 2016; Valdés and Tapia, 2019) and, therefore, the PAZ, PAL and CAL sites are no exception. These places would be enriched with heavy metals (Tapia et al., 2018b; Valdés et al., 2014), affecting the metal concentrations in wildlife (Gardner and Oberdörster, 2005; Hopkins et al., 2005), and human population (Cortés et al., 2015).

In concordance with previous studies of lizards in different locations worldwide (Ciliberti et al., 2013; Fletcher et al., 2006; Mann et al., 2007), one proportion of Pb, Cu, Ni and Zn content would enter the trophic chains (biological matrix), another part remains in suspension (water matrix), and the other fraction is incorporated into the seabed and in the coastal desert into the sediment and soil

matrix (Bea et al., 2010; Dold, 2006; Tapia et al., 2018a; Valdés and Tapia, 2019). For its part, Cd can accumulate in internal organs (Mann et al., 2007). Since reptiles are close to the ground, incidentally or intentionally, the consumption of soils is expected (Beyer et al., 1985; Ortiz-Santaliestro and Egea-Serrano, 2013; Salice et al., 2009) there being recent evidence of possible direct damage in lizards due to the regular ingestion of contaminated soil particles (Yang et al., 2020). Nevertheless, this has not been proven in *M. atacamensis* and we did not find a significant correlation for it, as previously published in other studies (Gardner and Oberdörster, 2005; Hopkins et al., 2005). The PAL site, long documented (Castilla and Nealler, 1978; Contreras-Porcía et al., 2011b; Vergara, 2011), seems to be exceptional (Cáceres, 2015; Fuenzalida, 2017; Toro, 2017), since the influence of mining and high metal concentrations in the bioavailability phase in beach soils, associated with mining tailings (Cáceres, 2015), make this place an example of biodiversity decline (Fariña et al., 2008; Ramirez et al., 2005). In the old Port of Chañaral Bay used as a landfill (1938–1974), about 150 million tons of fine sediments accumulated in the intertidal zone of the bay (the distance between the shore and the water is ~5 linear km) (Tapia, 2016). After that, through an approximately 100 km long canal, 25,000 tons of fine sediments per day were deposited to the north of the Port of Chañaral, about 8 km away in Caleta Palitos (PAL) between 1975 and 1990, 126–150 million tons of solids were deposited (Tapia, 2016), in addition to unknown amounts of chemical products (Cu, As, CN). From January 1975 to July 1976, this site received more than 13 million tons of sediment that have caused the deterioration of the coastal marine environment (Dold, 2014, Fig. 1), which can be seen with the naked eye on the entire coastline and up to ~100 m above the coastline, with the consequent loss of biodiversity (Castilla and Nealler, 1978; Fariña et al., 2008; Ramirez et al., 2005). Still, this genus has survived and is successful, but the reasons for this are still unknown.

These results are consistent with the general finding that the contamination of lizards by heavy metals is more significant the more anthropically disturbed the sites are (Dold, 2006, 2014), either by contemporary or historical intervention (Burger et al., 2005; Fletcher et al., 2006; Nasri et al., 2015).

4.2. Metal accumulation in *M. atacamensis* prey

Microlophus atacamensis is distributed from the north of Antofagasta to La Serena (Fariña et al., 2008; Ibañez, 2014). As previously reported, within this gradient the ambient temperature decreases from North to South and the intertidal zone is an unfavorable thermal environment because lizards lose heat rapidly by conduction, convection or evaporation when they feed on a wet rocky soil (Catenazzi et al. (2006); Sepúlveda et al., 2008). For this reason, lizards may face a compromise between feeding and thermoregulation (Fariña et al., 2008). So depending on the biological characteristics of *M. atacamensis*, the use of the intertidal zones, the marine and terrestrial feeding should change within the geographic area of this lizard (Fariña et al., 2008). Although in a subtle way, because the conditions between PAZ in the north and CAL in the south do not tend to vary abruptly in climatic terms. But in the northernmost regions of PAZ, where terrestrial productivity is almost nil but environmental temperatures are high, dependence on intertidal feeding is high and decreases to the South (where terrestrial productivity is higher and ambient temperature is lower), this would account for the use of intertidal zones as opposed to terrestrial. However, it is important to mention that another factor is the availability of prey, since the study areas, as mentioned, have a significant loss of biodiversity due to mining contamination, such as the case of PAL (Fig. 4). On the other hand,

the analysis of the feeding strategy showed that the population can be considered a generalist species (Huckembeck et al., 2014) and is consuming contaminated prey with heavy metals (Fariña et al., 2008).

In the northernmost PAZ area, and as this study demonstrates, lizards most frequently consumed *Ulva* sp., *Porphira* sp., Amphipods (e.g. *Emerita* sp., Gammaridae), decapods (*Petrolisthes* sp.), Diptera and tenebrionidae, since being a National Park, the area has a greater intertidal food supply (Fig. 4). Even though there is an important number of plants (eg. *Nolana* sp., *Cristaria* sp), these were not consumed, preferring elements closer to the intertidal zone. Although the animals do not enter into the open sea, there are isolated pools in the exposed intertidal zone that host juveniles of local fish, where it is possible for *M. atacamensis* to hunt these small fish. This is, however, unusual, and it had not been documented until this study, making this an important record. The further north animals are, the more frequently they feed from the marine environment (Fariña et al., 2008)(Fig. 4). In addition, it was also recorded that smaller individuals of the same species were cannibalized.

PAL for its part showed that the animals in fact consumed mainly marine materials such as drifting algae driven to the coasts by the waves in those areas where land productivity was low, and not enough to maintain these trophic networks, being coincident with other authors (Polis and Hurd, 1995, 1996). Their diet included *Ulva* sp. Algae, decapods (*Petrolisthes* sp.) and flies, but they also consumed molluscs present on rocks exposed to direct sunlight (*Echinolittorina peruviana*) and a significant volume of flowers (*Nolana* sp., *Cristaria* sp.), which are away from the breaker zone, generally in direct contact with the soil. This is relevant since our work is contrasted with other studies that highlight the importance of some physical components (the landscape and its degree of contamination) and biotic components (vector and receptor species) that regulate these links between ecosystems (Catenazzi et al., 2007; Sabo and Power, 2002a; 2002b).

Finally, in CAL, the southernmost section of this study, the animals consumed food from the intertidal zone, amphipods, decapods, and elements delivered by the breaker such as green macroalgae (e.g. *Ulva* sp.), where small flies gathered in great numbers. On the exposed rocks it consumed *Echinolittorina peruviana*, and on the ground flowers like *Nolana* sp. and *Cristaria* sp., where there are also coleopterans that were consumed, in addition to preying on smaller individuals (juveniles) of the same species.

Similarly, this study coincides with the findings of heavy metals in the tissues of marine animals, mainly molluscs (Calderón and Valdés, 2012; Nasri et al., 2015; Rabaoui et al., 2014) and fish (Messaoudi et al., 2008) that act as prey for lizards. *Microlophus atacamensis*, which consumes prey with high levels of heavy metal content, could be the cause of possible transfer towards land animals that inhabit coastal areas, although the information for northern Chile is currently deficient (Supplementary Table 1).

For PAL, no prey value exceeded tails, which would indicate a tendency for preys to accumulate metals, then delivering metals to *M. atacamensis*, demonstrating a trophic transfer from aquatic to terrestrial food chains affecting the terrestrial vertebrates health, including humans (Ali et al., 2019). Knowing that ingestion and, to a lesser extent, dermal contact are the two main routes of exposure of lizards to metals (Fox et al., 2007; Hopkins et al., 2001), we expected that lizards living in PAL would accumulate a higher concentration of heavy metals than those that lived in the other two sites. In general, our results supported this hypothesis.

Our study confirms that the movement of consumers depends on the energy balance of habitats (intertidal versus land productivity) and on some biological characteristics of the consuming species (for example, thermoregulation), (Farin et al., 2001). In

addition, within the geographic range of a species, it is possible to find a variation in the use of habitats and the trophic status (carnivorous to herbivorous or vice-versa, or from omnivorous to herbivorous), and in the relative importance of the available prey, for instance, marine relative to terrestrial prey.

4.3. Metal accumulation in *M. atacamensis* tails

The tail samples showed higher concentrations of Pb, Cu, Ni and Zn in PAL (Fig. 2), as opposed to PAZ and CAL. Cd is not significant in any of the three sites (Fig. 2). Concerning the distribution of metals in the tails, ANOVA-Welch was carried out showing the presence of statistically significant differences between sites, as it can be seen in the Supplementary Table 4. Tukey HSD shows the differences by groups, that is, for Pb and Zn there is no statistically significant difference between PAZ-CAL, while in Cu and Ni there is a statistically significant difference between all sites, coinciding with previous studies (Márquez-Ferrando et al., 2009), with different accumulation patterns (Fig. 2). These results are consistent with previous findings in other squamate reptiles, which may allow us to understand the mechanisms that influence trophic metal transfer, this being a critical step in the management of metal-contaminated ecosystems (Ciliberti et al., 2013; Mathews and Fisher, 2008). In general, to fully understand the cycle of metals through trophic levels, several factors that control the bioavailability of metals linked to consumer tissues must be considered and understood (for example, distributions and concentrations of metals, duration of exposure, nutritional status and the exposure history for the metal in question); (Monteiro and Soares, 2012).

The bioaccumulation factor “BAF” that compared the soil with the lizard tails showed accumulation values greater than 1 (Pb, Cu, Ni and Zn), which suggest a transfer from the environment (Cahuana and Aduvire, 2019; Guíñez et al., 2015). Although the soils do not show statistically significant differences between them, the comparisons with the regional and international backgrounds show that the soils present high levels of metals in general. In this way, and despite the fact that there are no significant differences between the soils of the sites, these metals play a role through the intake of terrestrial prey (Fariña et al., 2008; Fletcher et al., 2006; Nasri et al., 2015). Therefore they must have an effect, perhaps indirectly through the trophic chain of the terrestrial fauna, but also through intertidal marine fauna, as several studies show contamination in this type of fauna (Calderón and Valdés, 2012; Castro and Valdés, 2012; Valdés et al., 2014).

Our results allow us to infer that, as lizards inhabit restricted field areas, it is difficult for them to avoid contamination when it exists (Ortiz-Santaliestro and Egea-Serrano, 2013), this being the case of *M. atacamensis*, a low mobility animal (Ortiz, 1980a). As previously suggested, the lizards' tails provide a potent and non-lethal indicator of heavy metals (Fletcher et al., 2006; Kinney et al., 2008). This idea is supported by our results related to Pb, Cu, Ni and Zn, since they clearly show high levels of metals in the tails, coinciding with the most intervened sites. As for Cd, the tail samples showed relatively low levels, possibly due to basal concentrations of the Atacama desert coasts (J. Tapia et al., 2018b; Valdés et al., 2006). This is in line with conclusions of previous works that highlight the role of organs such as the kidney and liver that actively accumulate heavy metals such as Cd, (Liu et al., 2000; Nasri et al., 2015; Sabolic et al., 2001), explaining the low concentrations found.

The results of this study also demonstrate that the average concentrations of all metals in *M. atacamensis* tissues were generally higher than the concentrations found in preys, which is expected due to the fact that omnivorous/opportunistic lizards are

susceptible to the biomagnification of environmental pollutants within an ecosystem (Burger et al., 2000). This biomagnification may be due to the fact that reptiles are suitable transporters of contaminants in food chains because they have high conversion efficiencies owing to their physiological characteristics (Fletcher et al., 2006; Rezaie-Atagholipour et al., 2012).

In particular, the pattern of PAL tails differed from that of the other two sites in their concentrations of Pb, Cu, Ni and Zn, which is possibly related to the intake of amphipods and insects (Table 2), which have been independently used as indicators of metallic contamination in coastal environments (Guiñez et al., 2015) (Rainbow and Smith, 2010). The observations are in concordance with experimentally examined studies of trophic absorption of contaminants in squamate reptiles (Fletcher et al., 2006; Hopkins et al., 2001, 2005; Mann et al., 2007).

4.4. *Microlophus atacamensis* as a biomonitor

The lizards have been proposed as models to be used in ecotoxicological studies and environmental risk assessments because they present physiological, ethological and evolutionary characteristics that can be used to evaluate metals (Aguilera et al., 2012; Holem et al., 2006; Talent et al., 2002; Unrine et al., 2006). While this paper does not address the differences in metal content between the sexes, which could be addressed in other papers, there was no difference between sexes in terms of their degrees of contamination, as shown in the Supplementary Table 3, so we have to be cautious, but it could eventually be hypothesized that there is no relationship of contamination by gender differentiation.

With regard to sizes, although some PAZ tails are grouped with tails from PAL sites, the most contaminated site, the animals show a slight difference in average sizes (Table supplementary 3), in PAZ the animals were on average larger, in CAL slightly smaller and in PAL definitely on average smaller than in PAZ and CAL, always bearing in mind that all animals were adults. It should be noted that although lizards are smaller in PAL, these lizards of similar sizes accumulate more metals than lizards from PAZ and CAL. This may be due to the fact that their strategy is oriented towards reproduction, feeding, or survival over growth (Martin, 2002), given the extreme conditions in which they live (Martin, 2002). Future complementary studies would be required to ascertain the causes.

It is possible that the PAZ animals eat prey from the intertidal to a greater extent than terrestrial ones and as those are so enriched with metals it would be reflected in their tails (Fuenzalida, 2017). Another explanation has to do with the possible transport of the metals from Chañaral to PAL and PAZ, explaining its degree of contamination. Metals can be transported northward via local water bodies or winds (S-SW), whose prevailing northward direction can increase the levels of metals in PAL and subsequently in PAZ (Castillo et al., 2019; Castillo and Valdes, 2011; Stuu et al., 2007; Valdés and Castillo, 2014). It can be summarized that the deposit of waste rock on the coast of the Chañaral bay and the northern beaches (PAL) towards Pan de Azúcar National Park (PAZ), gave rise to the oxidation of sulphides and the formation of an acid oxidation zone on the waste rock surface (Dold, 2014). This, together with the climatic conditions of the Atacama desert, led to a wind transport of mainly Cu, Zn and Ni in mineral form (Bea et al., 2010; Dold, 2006) that can be accumulated by animals and humans (Dold, 2014), which seems feasible in our study.

The fact that the concentrations of heavy metals in the lizard tails corresponded strongly to prey items suggests that ingestion of macroalgae (*Ulva* sp, *Porphyra* sp), flora (*Nolana* sp., *Cristaria* sp), invertebrates (e.g. *Petrolisthes* sp.), and insects enriched with

metals contributes to the accumulation of metals in coastal lizards (Fig. 2) (Fariña et al., 2008). Pollutants are absorbed by macroalgae (Contreras et al., 2009) and also by terrestrial plants and then, through their roots, depending on the mobility of the chemicals, move to the vegetative parts (Madejón et al., 2004). Algae (marine environment) and plants (terrestrial environment) can be consumed by insects that are prey to reptiles or consumed directly by reptiles (Márquez-Ferrando et al., 2009), as would occur in PAL and CAL. The above is relevant since several studies have shown that the main route of acquisition of contaminants by reptiles is through ingestion of contaminated prey (Fletcher et al., 2006; Ghaleb, 2016; Márquez-Ferrando et al., 2009).

Microlophus atacamensis seems to provide a relevant link between marine and terrestrial food webs, based on the heavy metal transference ratio (Ali et al., 2019; Fariña and Castilla, 2001). There is a relationship between the lizards and the environment where they live, where they accumulate through trophic webs, and a transfer of metals could occur as it is shown by the TTF factor (Aguilera et al., 2012; Ali, 2019). Since the prey consumed were, in order of importance, flowers, algae, amphipods and insects, it could be inferred that foraging is effectively affecting the accumulation of metals (Mann et al., 2007; Markert et al., 2003; Mathews and Fisher, 2008). In summary, the intake of preys enriched with metals, as primary source along with possible dermal contact and accidental or intentional intake of sediments and seawater, promotes the expression of contamination through the tails of the animals.

5. Conclusions

When contrasted to the regional and world background levels of soil metal content, the limits were exceeded in three of the five metals evaluated. Although the comparison of the results of the sites' soils were not statistically significant, this does not mean that they are not contaminated or at least enriched with heavy metals. The three study areas presented a Bioaccumulation Factor (BAF) that exceeds the limit in four of the five metals at least in PAL and three of the five metals at all sites, so they should be evaluated permanently, given their possible impact on local fauna and flora. Although the Ecological Risk (IR) is moderate in terrestrial soil, and there are no significant differences in the soil, they are at the limit of a Considerable Ecological Risk. We also found evidence of a crucial trophic transfer to preys and tails of *M. atacamensis*, since the results of this study showed levels greater than one in eleven of the fifteen measurements of Pb, Cu, Ni, and Zn in prey and subsequently high in tails. The tails of the lizards of the three sites studied, given their levels of metal enrichment, suggest high contamination of the areas, maybe due to its opportunistic predatory behaviour that includes prey of the upper and middle intertidal, as well as terrestrial. This observation correlates the places studied and the tails of animals due to the intake of food contaminated or enriched with heavy metals. Our results also showed that lizard tails could be used in areas close to the industrial zones, particularly mining, of the Atacama Desert, where they can deliver values on pollution levels or at least enrichment, depending on the characteristics of the site, prey consumed and the metal evaluated. However, the lack of detailed knowledge about the effects of heavy metals on organisms makes it challenging to predict the biological importance of each metal or the existence of effects on populations living in these places. This reptile species could qualify as an initial evaluator of heavy metal contamination with a non-lethal methodology, avoiding its sacrifice; but future research is necessary to assess this possibility and propose it as a biomonitor.

Credit author statement

Yery Marambio-Alfaro: Developed the idea of using a bio-monitor, adapting existing techniques and methodology, and also wrote the manuscript, **Jorge Valdés Saavedra:** Developed the laboratory analysis of heavy metals and evaluated the characteristics of the methodology for sampling, **Luis Nacari Enciso:** Carried out the taxonomic review of the animals' diet with his own techniques, **Américo López Marras:** Contributed to the improvement of the protocol in the field and its application, **Antonio E. Serrano:** Reviewed and edited the manuscript and contributed to the improvement of the protocols, **Rodrigo Martínez Peláez:** Implemented and developed the statistical analysis in R, for a better visualization of the data, **Alexis Castillo Bruna** Applied his sedimentological knowledge to evaluate the sites where the samples were taken, **Gabriel Álvarez Ávalos:** Conducted the geomatic surveys for the location and analysis of the data, **Marcela Vidal Maldonado:** Contributed as the national expert on the species under study and applied her methodology for better ethological understanding.

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.

Acknowledgements

The authors thank the anonymous reviewers for their comments and suggestions as they contributed to improving the quality of the manuscript. Jorge Valdés was supported by MINEDUC -UA project, code ANT 1855. YM-A thanks Claudia Worner, Tomas Marambio-Alfaro, Javier Marambio A. and CONAF Atacama. Hugo Keith for the translation into English. Mafalda Paiva for the illustrations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2020.115739>.

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