doi: 10.13679/j.advps.2015.1.00001
March 2015 Vol. 26 No. 1: 1-7

Assessment of trace metals in droppings of Adélie penguins (*Pygoscelis adeliae*) from different locations of the Antarctic Peninsula area

José E. Celis[1]*, Winfred Espejo[2], Ricardo Barra[2], Daniel Gonzalez-Acuña[1], Francisca Gonzalez[1] & Solange Jara[2]

[1] Facultad de Ciencias Veterinarias, Universidad de Concepción, Casilla 537, Chillán, Chile;
[2] Facultad de Ciencias Ambientales, Universidad de Concepción, Casilla 160-C, Concepción, Chile

Received 6 August 2014; accepted 7 January 2015

Abstract In recent decades, polar regions of the planet have witnessed an increase in human presence. Antarctica is considered one of the most pristine regions of the world, but it could be affected by pollution owing to anthropogenic activities, particularly in the Antarctic Peninsula. Human presence can increase the levels of some trace metals in Antarctic environments, an issue that needs to be evaluated. To acquire data of trace metal contamination in the Antarctic Peninsula region, concentrations (dry weight) of Cd, Pb, As, Cu, Hg and Zn in fresh excrement of Adélie penguins were determined by atomic absorption spectrophotometry. During the 2012/2013 austral summer, samples were collected from four important nesting sites on the Antarctic Peninsula: Arctowski Base, Kopaitic Island (both sites in the northern Antarctic Peninsula), Yalour Island and Avian Island (both sites in the southern Antarctic Peninsula). Data showed that Adélie penguin excreta had significantly higher levels ($mg \cdot kg^{-1}$) of As, Cd, Hg, Pb and Cu at Arctowski Base and Kopaitic Island, both sites that have major anthropogenic activities that probably contributed to increased metal levels. The levels of trace metals in Adélie penguins were similar to those reported in excreta of Antarctic species in previous studies, and lower than those in excreta of other Antarctic animals. Data suggest that metals ingested by these penguin species that feed in the sea, end up in terrestrial ecosystems.

Keywords **heavy metals, seabirds, penguins, excreta, guano, marine pollution, Antarctica**

Citation: Celis J E, Espejo W, Barra R, et al. Assessment of trace metals in droppings of Adélie penguins (*Pygoscelis adeliae*) from different locations of the Antarctic Peninsula area. Adv Polar Sci, 2015, 26: 1-7, doi: 10.13679/j.advps.2015.1.00001

1 Introduction

Some studies have identified local chemical contamination in Antarctica, specifically trace metals derived from fuel spills, combustion processes, abandoned waste disposal sites, as well as chemicals disposed of through the sewage system near field stations[1]. These contaminants, especially the metals, could be affecting the Antarctic region[2]. In contrast, another study has stated that Antarctica is a remote polar region that still can be considered an unpolluted area[3]. Nevertheless, despite being remote, Antarctica is becoming a place where many human activities have increased noticeably in recent years[4]. Although concentrations of most chemical pollutants in Antarctic ecosystems appear to be very low, a continuous level of contamination could be affecting some marine ecosystems as well as Antarctica's endemic species[5].

Fauna living in Antarctica are less studied compared to animals from other continents because of its remoteness from other lands[3]. Penguins dominate the Antarctic avifauna and have a permanent ecological niche, and thus are one of the most suitable species for bio-monitoring[6]. Bio-monitoring is an essential tool for evaluating environmental changes as a consequence of contamination[5]. In particular, bio-monitoring

* Corresponding author (email: jcelis@udec.cl)

can provide useful information about the levels of metals in Antarctic animals like seabirds because they are easily monitored, abundant, wide-ranging, long-living, and feed at an elevated trophic level[6]. Adélie penguins are considered useful sentinels of the environmental changes occurring in Antarctic ecosystems[8], because they are seabirds occupying a high ecological position and their feeding habits are simple[9].

Excreta is an important route of metal elimination in penguins[10], thus their feces are a direct source of environmental contamination and suitable for bio-monitoring[3]. Some previous studies have showed excrements of Gentoo penguins (*Pygoscelis papua*) and Chinstrap penguins (*Pygoscelis antarctica*) are effective bio-monitors for metal contamination in Antarctica, showing that the levels of trace metals in excreta are an important criterion for environmental health, specifically for polar conditions[11-12]. However, trace metal data from Adélie penguin droppings in polar areas are still scarce. It is important to investigate baseline levels of chemical elements to predict possible future changes in the environment, particularly in the Antarctic Peninsula where most of the research stations are located and tourist visits occur[1,13]. The aim of this study is to add data on the concentration of trace metals such as Cd, As, Pb, Hg, Cu and Zn in excreta of Adélie penguins in the northern and southern Antarctic Peninsula.

2 Materials and methods

2.1 Sample collection

During the 2012/2013 austral summer, samples of fresh excreta from *Pygoscelis adeliae* were collected from four nesting areas on the West Antarctic Peninsula: Arctowski Base (Poland), Kopaitic Island, Yalour Island and Avian Island (Figure 1).

Geographical locations and sample sizes for each locality are shown in Table 1. Arctowski Base is a permanently staffed base where a large Adélie penguin colony inhabits the surrounding area. It is also frequently visited by tourists and therefore the immediate area is impacted by fuel tanks, personnel, researchers, tourists and their associated activities. Kopaitic Island is an island about 500 m from the Chilean O'Higgins Base, where a large number of Adélie penguins are found; it is frequently visited by researchers and tourists[12]. Yalour Island is a group of rocks where about 8 000 pairs of Adélie penguins breed around the low rocky islets. Avian Island is the home of the largest Adélie colony on the Antarctic Peninsula, containing a third of the total breeding population of the region with more than 35 000 pairs of this penguin species[14].

Excreta samples were handled with disposable plastic gloves and careful procedures were followed to avoid contamination. Penguin droppings were taken with plastic spatulas from the top of the mass of excrement deposited on the ground to avoid contact with any possible contaminant deposited in the soil. It was difficult to obtain an excrement sample for each individual. For that reason, each excrement sample of about 100 g was from many individuals of the penguin colony. Clean steel containers and sealed plastic bags were used to store samples. All samples were kept in sealed plastic bags at –20℃ for transport until trace metal analysis. All samples were collected with permits in accordance with Annex II, Article 3 of the Protocol on Environmental Protection of the Antarctic Treaty, and regulation from the Scientific Committee on Antarctic Research (SCAR) provided by the Chilean Antarctic Authorities (permits INACH 44/2012). The bioethics permit was provided by Universidad de Concepción which was required to obtain INACH permits.

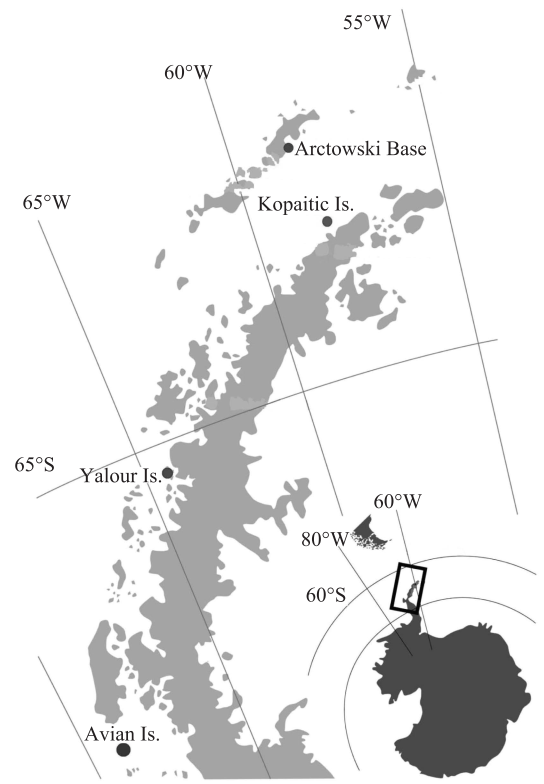


Figure 1 Location of sampling sites in the Antarctic Peninsula.

Table 1 Name of the locations, phical position and sample size

Name of the location	Geographical position	*n*
Arctowski Base	62°09′S, 58°28′W, King George Island, South Shetland Islands	27
Kopaitic Island	63°19′S, 57°55′W, Cape Legoupil, Graham Land	18
Yalour Island	65°15′S, 64°11′W, Wilhelm Archipelago	10
Avian Island	67°46′S, 68°54′W, Marguerite Bay	10

2.2 Metal analysis

The analytical method used in this study followed the one described by previous authors with some modifications[8,15].

The samples were washed with ultrapure water (18.2 MΩ·cm^{-1}) to eliminate adsorbed external contamination, dried at room temperature, and then ground with an IKA® A11 Basic Auto-miller to pass through a 24 mesh·dm^{-2} sieve. The elements Cd, Pb, As, Cu, Hg and Zn were determined by mass spectrometry with inductively coupled plasma (ICO-MS Optima 800 Perkin Elmer). All the reagents used were Suprapur (Merck).

According to availability, sub-samples between 0.02 and 0.45 g of the material were subjected to microwave digestion with high purity grade (GR) nitric acid, hydrochloric acid, and perchloric acid for Pb, Cd, As, Cu and Zn, but in nitric acid and 30% H_2O_2 (6:5, v/v) for Hg. The concentrations of elements in the samples were determined in the Laboratorio de Quimica Ambiental of the Universidad de Concepción (accredited ISO 17025). The detection limits (mg·L^{-1}) of the elements determined were as follows: 0.005 for Cd, Cu and Zn; 0.002 5 for Pb; 0.01 for Hg and As. All measurements were carried out in triplicate and resulting values were averaged. Non-detectable values were predicted from expected normal scores when more than 50% of all samples showed detectable levels within each data set[16]. To ensure quality control, a certified reference material (human hair, GBW07601) supplied by the National Research Center of China was used as an internal standard in a proportion of 10 % for each batch of samples. The determined results of the certified reference material showed a standard deviation <5% and were within the certified value ranges of the elements.

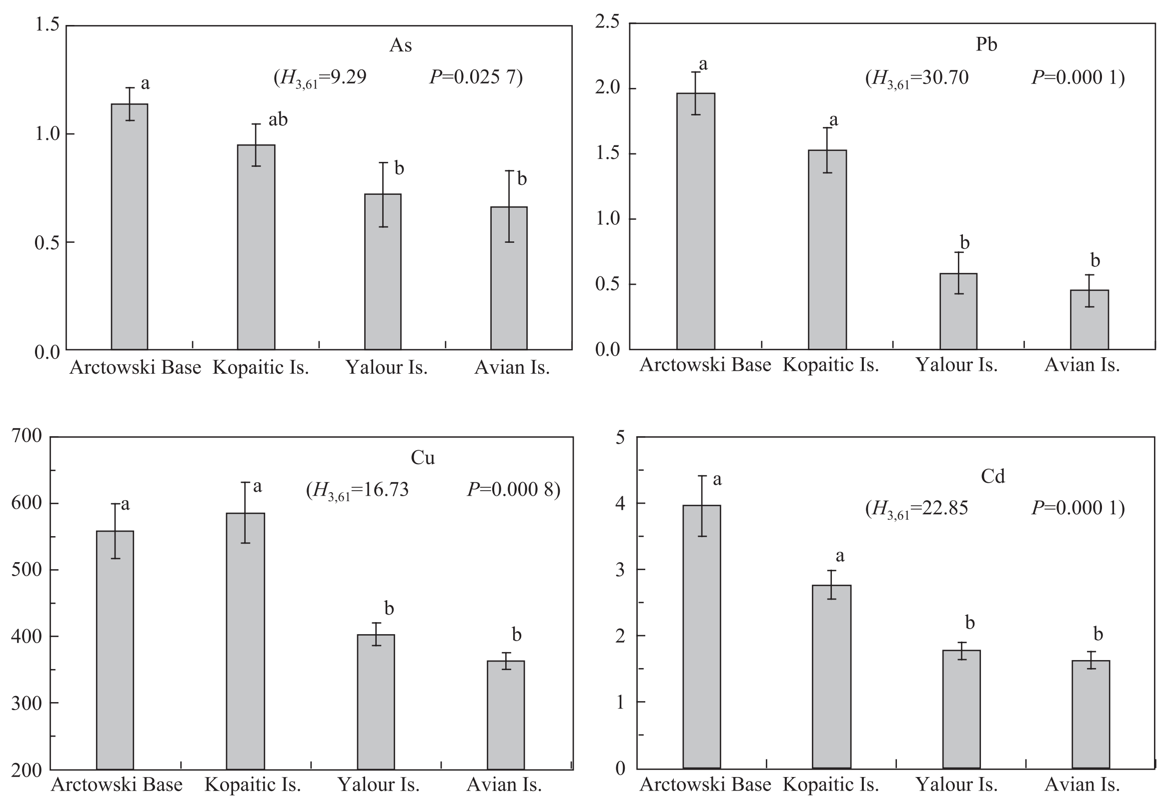
2.3 Statistical Analysis

The detected levels are presented as mean ± standard error in mg·kg^{-1} (dry weight). All data were first tested for normality and homogeneity of variance to meet statistical demands. Differences between trace metal concentrations among sites were assessed by means of non-parametric analysis of variance Kruskal-Wallis and Mann-Whitney U tests. Post hoc tests were carried out for Kruskal-Wallis analyses, using the critical differences of mean rank. Spearman rank correlation coefficients were calculated among trace metal levels. The differences were considered to indicate statistical significance when P values were less than 0.05. Statistical analyses were conducted using SPSS version 15.0 software (IBM Corp. Chicago, IL).

3 Results

3.1 Concentration of trace metals and geographical differences

The concentrations of Cd, Pb, As, Cu and Zn in excreta of Adélie penguins are shown in Figure 2. The excrement of *P. adeliae* showed significant geographical differences among trace elements from the studied locations.



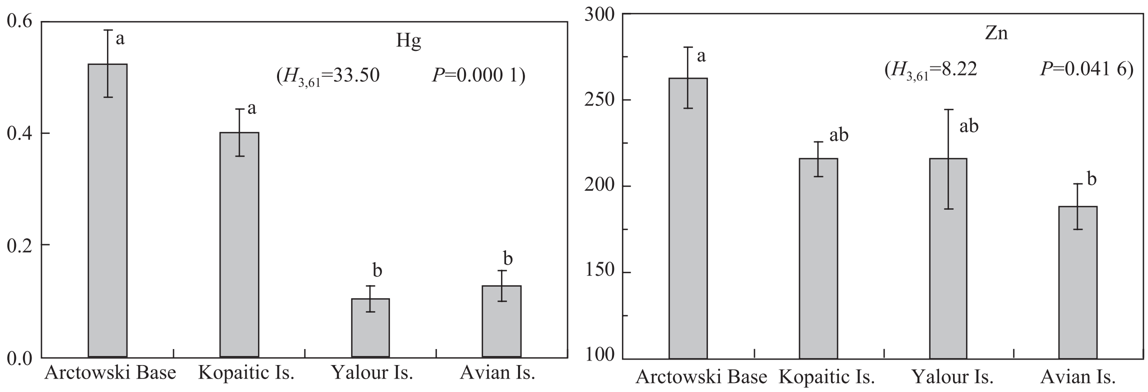


Figure 2 Trace metal levels (mg·kg-1, dry weight) in excreta of Adélie penguins (*Pygoscelis adeliae*) from different locations of the Antarctic Peninsula. Data shown are means ± standard error (Kruskal-Wallis *H* values and *P* level of statistical significances are shown in brackets).

Mean levels of As in feces of *P. adeliae* were significantly higher at Arctowski Base (1.14 mg·kg-1), compared to those levels found at Yalour Island (0.72 mg·kg-1) or Avian Island (0.66 mg·kg-1). The highest significant levels of Cd were found at Arctowski Base (3.96 mg·kg-1) and Kopaitic Island (2.77 mg·kg-1), and there were no significant differences between those locations. Both locations showed significantly higher Cd levels than on Yalour Island (1.78 mg·kg-1) and Avian Island (1.63 mg·kg-1). The highest levels of Cu were found on Kopaitic Island (585.98 mg·kg-1) and at Arctowski Base (558.29 mg·kg-1). The lowest levels of Cu were found on Yalour Island (402.99 mg·kg-1) and Avian Island (362.99 mg·kg-1). The levels of Hg were statistically higher at Arctowski Base and Kopaitic Island as compared to Yalour Island and Avian Island. With respect to Pb, *P. adeliae* excreta showed significantly higher levels in feces of Adélie penguin colonies at Arctowski Base (1.96 mg·kg-1) and on Kopaitic Island (1.53 mg·kg-1). The highest Zn levels were detected at Arctowski Base (262.66 mg·kg-1), which are statistically different from Zn levels found on Avian Island. There were no significant differences between Zn levels detected at Arctowski Base, Kopaitic Island or Yalour Island.

3.2 Correlations between metals

Considering all the sampling sites together, the following relationships among trace metal levels were noted in this study: Cu >Zn >Cd >Pb >As >Hg. Several significant positive correlations were observed between Cd-As, Cu-As, Zn-As, Cd-Cu, Cd-Hg, Pb-Cd, Zn-Cd, Hg-Cu, Zn-Cu, Pb-Hg and Zn-Hg (Table 2).

4 Discussion

4.1 Comparison of trace metal levels in excreta between species

Our maximum As levels were 1.5 times higher than those reported from King George Island (Maxwell Bay) in excreta of Adélie penguins a decade ago[17]. Also, As levels were 4.5 and 3.6 times lower than those found in excreta of Gentoo penguins 6 years ago at Livingston Island[3] and a decade ago at King George Island[17], respectively. Our As levels were 1.6 times higher than those reported in excreta of Chinstrap penguins a year ago at Barton Peninsula, King George Island[12]. When compared with Humboldt penguins (*Spheniscus humboldti*), a species of seabird that naturally inhabits the coast of Peru (5°S) and Chile (43°S), our As levels were 6.9 times lower[18]. The observed As levels were 2, 1.5 and 1.2 times lower than those from the southern giant petrel (*Macronectes giganteus*), elephant seal (*Mirounga leonine*) and fur seal (*Arctocephalus gazella*), respectively[17]. In our study, the higher As levels found at northern locations can be explained by local volcanic activity[15]. Additionally, As levels can be related to diet, owing to the fact that this metal is in the water and then accumulates in the food chain, passing to top predators such as penguins[3].

Table 2 Spearman correlations between trace metal levels in Adelie penguin droppings

	As	Cd	Cu	Hg	Pb	Zn
As	1					
Cd	0.43a	1				
Cu	0.37b	0.77b	1			
Hg	0.23ns	0.60a	0.49a	1		
Pb	0.22ns	0.25c	0.14ns	0.55a	1	
Zn	0.25c	0.60a	0.36b	0.27c	−0.03 ns	1

Notes: a—significant (p<0.001); b—significant (p<0.01); c—significant (p<0.05) ; ns = no significant.

Our maximum Cd levels were 1.4 times lower than the Cd levels found in excreta of Adélie penguins at Terra Nova Bay a decade ago[10]. By contrast, our Cd levels were 3.8 times higher than those levels found in Gentoo penguin excrement from Livingston Island six years ago[3]. Also, our Cd levels were 1.6 and 1.3 times higher than those levels found in excreta of Gentoo penguins at O'Higgins Base a year ago and levels detected in excreta of Chinstrap penguins at King George Island a year ago, respectively[12]. However, our results were 12 times lower than Cd levels found a year ago in Humboldt penguin feces at Pan de Azúcar Island, a site on the northern coast of Chile highly impacted by mining activities[18]. Cd levels in feces are linked to high dietary Cd intake[10], and is a toxic metal known to bioaccumulate in marine biota from both natural and anthropogenic sources[19].

Concentrations of Cu in Adélie penguin excrement were about 1.5 times higher than those reported from this species at several locations of Antarctica[8]. Also, our Cu levels were 5.6 times higher than those reported from excreta of Gentoo penguins at Livingston Island[3]. Our findings were 6 and 12 times higher than those levels found in excreta of giant petrel and fur seals in the Antarctic Peninsula[8]. There is evidence indicating that Cu levels in penguin droppings can be related to high Cu levels in Antarctic krill, their main prey[20]. The difference in Cu concentration among the penguin excrement from different geographical locations might have resulted from different food resources for the species.

Our maximum Hg levels detected at Arctowski Base were 2.5 and 1.7 times higher than those reported in excreta of Adélie penguins from East Antarctica six years ago[8] and from Terra Nova Bay a decade ago[10], respectively. When compared with data found in excreta of Chinstrap penguins at King George Island, Gentoo penguins from King George Island and King penguins from East Antarctica, our Hg levels were 5.2, 5.1 and 1.7 times higher, respectively[8]. Also, our Hg levels were 1.5 times lower than those reported in excreta of Humboldt penguins[18].

Our maximum Pb levels were 5 times higher than those levels found in Adélie penguin excrement from Terra Nova Bay twelve years ago[10], and similar to those levels reported six years ago from East Antarctica in excreta of the same species[8]. When compared with Gentoo penguins, our Pb levels were about 5 and 2 times higher than those levels reported from King George Island[3,8]. When compared with Chinstrap penguins, our Pb levels were 1.3 and 1.5 times higher than those levels six years ago[8] and a year ago from King George Island[12]. By contrast, when compared with other species of penguins that inhabit places far from Antarctica, our Pb concentrations were 6.5 times lower than those levels found in excreta of Humboldt penguins a year ago[18]. In addition, our highest Pb levels (2 $mg \cdot kg^{-1}$) were lower than those levels found in giant petrels (3.5 $mg \cdot kg^{-1}$), fur seals (3 $mg \cdot kg^{-1}$) and elephant seals (2.8 $mg \cdot kg^{-1}$) from King George Island six years ago[8]. Our maximum Pb concentrations were about five times lower than 10 $mg \cdot kg^{-1}$ (d.w.) in bird feces from other regions of the world with low human presence[21-23].

Concentrations of Zn in feces of Adélie penguins were 1.4 times lower in comparison with those levels reported in penguin droppings collected at different sampling locations from Antarctica six years ago[8]. Additionally, our levels of Zn were almost twice lower than those measured in excreta of Humboldt penguin colonies a year ago in the northern of Chile[18].

There is evidence indicating that bioaccumulation of trace metals in Adélie penguins is noticeably influenced by the accumulation of metals in Antarctic krill, their main prey[20]. Concentration of metals can differ among colonies of the same species that live far from each other owing to diet and the presence of pollutants in Antarctic waters[15]. The metal levels in feces vary depending on the diet and internal needs of the organism, especially diet, which represents a major factor of metal level variation[3].

4.2 Relationship among trace metals

Our finding on the relationship among trace metals (Cu>Zn >Cd>Pb>As>Hg) was similar to the descending order observed in Chinstrap penguin feces from the Antarctic Peninsula[12]. The average ratio of Cd/Pb, Cd/Hg and Pb/Hg in our study was 2.2, 8.7 and 3.9, respectively. These Cd/Pb and Cd/Hg ratios were 6 and 3 times lower respectively than those discovered in Adélie penguin excreta from Terra Nova Bay a decade ago, whereas the Pb/Hg ratio was 6.5 times higher[10]. Our average ratio Zn/Cd was 87.2, which is lower than the ratio reported in Gentoo penguin feces and the ratio in Chinstrap penguin droppings[12]. The Zn levels in seabirds can be related to Cd concentrations[24]. An increase in the Zn levels can reduce the toxic effects of Cd[25], which can explain the positive correlation ($P<0.001$) between these metals (Table 2).

4.3 Source of trace metals

Volcanic activity is an important natural input of As, Cd and Hg[15,26-27]. Local volcanism could explain the presence of these metals in the region because the South Shetland Islands and the northern Antarctic Peninsula concentrate volcanic activity[2]. In polar environments, Cd can also be produced by up-welling of Cd-rich waters and algal blooms[28-29].

Some anthropogenic sources can also explain the presence of metals in Antarctica. High Cd and Pb levels found in the Antarctic environment can be attributed to debris, runoff, fossil fuel combustion, shipping and sewage[5,30]. Zn is linked to anthropogenic sources such as mining, batteries, paints, electrical devices or metallurgical industries[1]. Sewage, oil spills, pesticides, and solid wastes can contribute to an increase in Cu and Hg levels in Antarctica owing to the increase in the presence of scientific stations, tourists and fishing[1]. There is evidence that Pb contamination is caused by scientific stations in Antarctica[15]. Some Antarctic locations adjacent to scientific bases are highly polluted[31]. Fuel combustion, waste incineration, sewage disposal, paint, accidental oil spills, impacts of the increase in tourism and research facilities with their associated activities have been particularly noted in the northern area of the Antarctic Peninsula[13, 32-33]. This can explain the higher trace metal levels detected in Adélie penguin feces at Arctowski Base and Kopaitic Island, both geographical locations with greater human presence than on Yalour Island and Avian Island. Higher Pb levels have been found in Adélie penguin feathers from the northern Antarctic Peninsula[15], and higher Pb, Cu and Zn levels have been found in Gentoo penguin excreta from the same area[12] and our results agree with these earlier studies.

In addition to local pollution, there is evidence that trace metals can be transported by air and could be reaching polar areas of the planet[2]. Cd levels in Antarctica could be linked to plastic industries, paints, batteries, smelters, corrosive coatings or P fertilizers, since Cd can be transported atmospherically for great distances[34-35]. Moreover, metals can be transported around the globe and move easily in water[3]. Considering the increase in population and industries in countries of the Southern Hemisphere, metals could be affecting in particular the Antarctic Peninsula, the nearest area of Antarctica to South America.

4.4 Deposition of penguin excreta on land

Similar to other penguin species, Adélie penguin colonies usually number in the tens of thousands. Although they feed almost exclusively in the sea, they nest on land. Levels of trace metals in penguin droppings vary depending on the diet and internal needs of the organism[3]. Most of the metals accumulate in the food chain, and because penguins are top predators they can biomagnify contaminants. Penguins then deposit metals by means of excretion on land, where the metals tend to accumulate[36]. Some studies have stated that deposition of seabird feces have had a significant effect on the geochemical composition of some polar terrestrial areas[17, 37-38].

The evidence indicates that Arctic soils next to nesting sites are strongly affected by Cd, Cr, Zn, Cu and Pb from gull feces[19,37,39]. In Antarctica, a study at Hope Bay (northern part of the Antarctic Peninsula) showed that soils had high levels of Cd, Cu, Pb and Zn as a consequence of contamination with oil, coal, alloys and feces from penguin colonies[40]. Another study found up to 92 $mg \cdot kg^{-1}$ Cu in soils near the Comandant Ferraz Station, King George Island[41]. Recently, a study reported Pb levels of about 281 $mg \cdot kg^{-1}$ and Cd levels up to 4.3 $mg \cdot kg^{-1}$ in soil next to O'Higgins Base[42].

Our findings suggest that the studied penguin colonies can transfer metals like Cd, As, Cu, Hg, Pb and Zn from the sea to the terrestrial ecosystem via droppings. These contaminants can reach coastal sediments by runoff. Research done on sediment quality indicates adverse effects on benthic organisms when exposed to Cd>1.2, Cu>34, Zn>150 and Hg>0.15 $mg \cdot kg^{-1}$[43]. Our detected metal levels are above the threshold values for Cd, Cu, Zn and Hg, indicating that Adélie penguins are possibly causing local contamination in some terrestrial areas of the Antarctic Peninsula, which could affect other living organisms, an issue that needs to be further investigated.

In conclusion, this study showed that Adélie penguin feces can be used as a non-invasive means of sampling trace elements, adding to the growing body of baseline data on metal contamination in Antarctica. In general, metal levels recorded were similar to those found previously in excreta of Adélie, Gentoo and Chinstrap penguins in the region, while they were lower than levels in excreta of other Antarctic sea animals. Even though it is not possible to support totally the idea that anthropogenic influence is responsible for the concentration of metals, our results showed the highest levels of Cd, As, Hg, Pb, Cu and Zn at sites with major human activities, which are located in the northern area of the Antarctic Peninsula. Additionally, Adélie penguins may be acting as bio-vectors of heavy metals from the marine ecosystem to the terrestrial ecosystems via excreta.

Acknowledgments This study was supported by project INACH T 18-09 granted to R. Barra, by project FONDECYT 1140466 and by project INACH RG 09-14 granted to J. Celis. Also, many thanks are due to Diane Haughney for her support on the manuscript translation from Spanish to English.

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