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Short Communication

Trace metal concentrations and body condition in adult Adélie penguins (*Pygoscelis adeliae*) from the western Antarctic Peninsula

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ABSTRACT. Adélie penguins (*Pygoscelis adeliae*) are seabirds that live exclusively in Antarctica, one of the planet's last pristine areas. However, this remote region is experiencing a continuing expansion of human activities that may affect Antarctic fauna. Trace metals constitute a menace to seabirds because they can adversely affect their health. There is a lack of studies relating to metals' levels in feathers with morphological parameters of seabirds. Trace metal levels were measured in Adélie penguin feathers and their body condition through the relative condition factor (ReCF) in adult individuals from two South Shetland Islands locations and two from Graham Land. Consequently, we determined the levels of some metals in feathers to see any relationship with morphological parameters linked to the bird's health. Our results showed significant differences in metals among locations studied and a significant relationship between ReCF with Cu and Zn in one of the South Shetland Islands. Also, penguins exhibited a significantly lower weight. We found a positive correlation between non-essential with essential metals, indicating that Cu acts as a detoxifying agent for Cr, Cd, and Pb. In contrast, Se could be for V and Pb. Although the relationship between most metals with ReCF was not significant, some site-specific factors may be influencing it, whereas metals may be affecting the organisms at low biological levels. Molecular, biochemical, and genetic studies are required to elucidate this issue.

Keywords: *Pygoscelis adeliae*; bioaccumulation; heavy metals; body condition; penguins; Antarctica

Antarctica is a cold continent that typically possesses marine ecosystems with still low human presence, although it can be exposed to global anthropogenic activities (Bargagli, 2008). Although metals in Antarctica are linked to a natural phenomenon caused by the different Antarctic areas geochemical characteristics (Sánchez-Hernández, 2000), several anthropogenic sources (oil spills, paints, open field garbage burning, or fuel combustion) contribute to increasing these natural levels (Tin *et al*., 2009).

Before the Protocol on Environmental Protection to the Antarctic Treaty (ratified in 1998), many human activities were carried out without considering this region's environmental health (Curtosi *et al*., 2010). Also, the increasing human activity in the Antarctic Peninsula area (which concentrates most of the research and tourism) could increase the accumulation of metals in the Antarctic biota (Lynch *et al*., 2010; Jerez *et al*., 2011, 2013a,b; Celis *et al*., 2015).

Aquatic birds can accumulate trace elements in different tissues; thus, they can be used indirectly to evaluate the marine ecosystem's toxicological status under study (Savinov *et al*., 2003). Particularly, penguins can be useful indicators of regional environmental health because they are superior predators, long-lived species, and present wide distribution ranges with abundant populations (Espejo *et al*., 2017). Additionally, penguins are extremely interesting as bioindicators because of their intense molting process (Carravieri *et al*., 2014), they can be finicky eaters with a restricted diet (Lescroël *et al*., 2004; García Borboroglu & Dee Boersma, 2013), and

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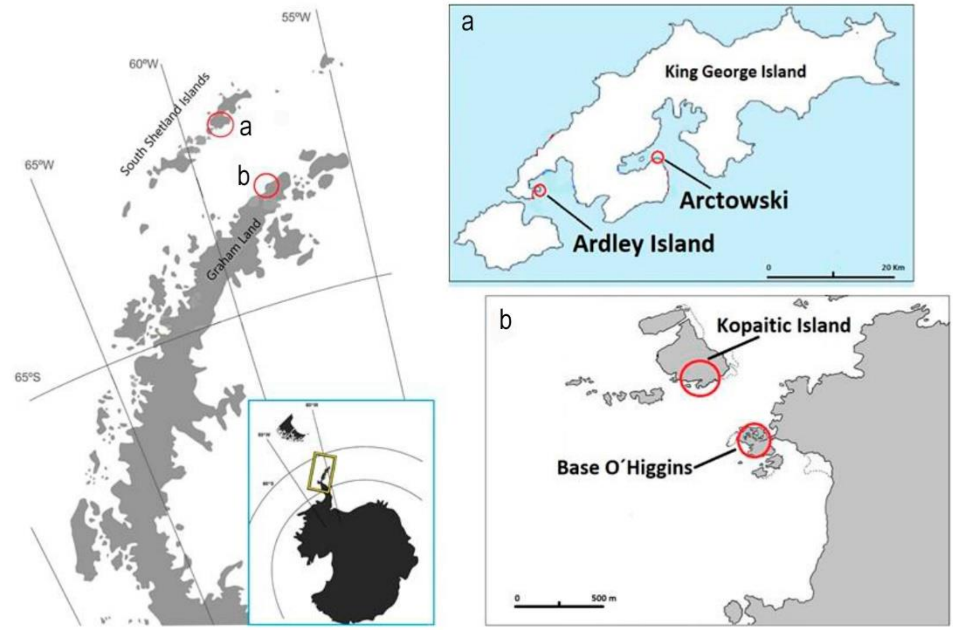

Figure 1. Sampling locations of Adélie penguin colonies from the western Antarctic Peninsula area: a) South Shetland Islands; b) Graham Land.

they represent an important part of the avian biomass in this region (Dee Boersma, 2008). Penguins inhabit exclusively in Southern Hemisphere, and about two-thirds of penguin species are seriously threatened by contamination, climate change, fishing, alterations of ecosystems, diseases, and even tourism (García-Borboroglu & Dee-Boersma, 2013; IUCN, 2016). The Adélie penguin (*Pygoscelis adeliae*) is a seabird species that only inhabit the entire coast of the Antarctic continent, feeding mainly of krill and fish (García-Borboroglu & Dee-Boersma, 2013).

It is well known that human activities can affect the health condition of birds (Burton *et al*., 2006; Johnson *et al*., 2006; Costantini *et al*., 2007; Madsen & Riget, 2007). Pollutants, such as heavy metals, oil, synthetic organic contaminants, and plastics, can cause oxidative stress and, consequently, affect seabirds' immune system (Briggs *et al*., 1996; Thompson & Hamer, 2000; Rainio & Eeva, 2010). Monitoring pollutants using feathers of seabirds has attracted increased attention among scientists (Honda *et al*., 1986; Ancora *et al*., 2002; Jerez *et al*., 2011, 2013a,b) because feathers are non-invasive for the bird and because they may be useful for evaluating metal levels in internal tissues of birds (Burger, 1993; Agusa *et al*., 2005). Metals can adversely affect captive birds' condition by reducing their body weight, damaging organs, altering their metabolism, or causing behavioral changes (Furness, 1996; Eisler, 2000). Some studies have been done on relating body condition to metal concentrations in different tissues (*e.g.*, muscles) of aquatic birds (Debacker *et al*., 2000; Takekawa *et al*., 2002; Wayland *et al*., 2002). There is evidence relating the body condition of birds to their survival (Bergan & Smith, 1993; Blums *et al*., 2005), reproduction (Blums *et al*., 2002; Bustnes *et al*., 2002), and behavior (Dufour & Weatherhead, 1991; Bachman & Widemo, 1999).

Some metals have a high affinity for the sulfhydryl groups of the feather's structural proteins (Metcheva *et al*., 2006). Although the accumulation of As, Pb, Hg, Cu, Zn, and Cd has received more attention in penguins, only a few studies have dealt with some other trace elements (Espejo *et al.*, 2017). In birds, it has previously observed that there is a relationship between muscle metal levels and morphological alterations (Eisler, 2000; Ohlendorf & Heinz, 2011), and also a positive correlation between muscle metal levels and feathers (Agusa *et al*., 2005; Espejo *et al*., 2017). To our knowledge, there are no studies in birds that show a relationship between metal concentrations in feathers and body condition. Although there is likely influence of other contaminants, the biological effects of metal contaminant mixtures are poorly understood and difficult to predict (Ohlendorf & Heinz, 2011). This study is the first approximation to understanding metals' effects on penguins' morphological conditions using feathers as a non-invasive matrix.

The study was carried out during the austral summer between January and February 2016. Samples consisted of feathers from adult Adélie penguins of four locations of the Antarctic Peninsula area, western Antarctica (Fig. 1): Ardley Island (62°13'S, 58°56'W) from the

King George Island, Base O'Higgins (63°19'S, 57°53'W) and Kopaitic Island (63°19'S, 57°55'W) from the west coast of Graham Land, and Arctowski (62°09'S, 58°28'W) from the King George Island. At each penguin rookery, the birds were captured using a long-handled net during molting and before foraging. All feather samples were collected using disposable plastic gloves, kept in sealed plastic bags, and stored at -4°C for transport until their laboratory analyses. Besides, birds captured were measured morphologically based on body length (cm) and body mass (kg).

In the laboratory, samples were washed with ultrapure water (18.2 MΩ cm^{-1}), dried at room temperature, and ground with IKA® A11 Basic Automiller for sieving (24 mesh dm^{-2}). Sub-samples (0.02 and 0.45 g) were digested in the microwave using high purity grade (GR) nitric acid, hydrochloric acid, and perchloric acid. All the reagents used were Suprapur (Merck®). The concentrations of metals were determined by using electrothermal atomic absorption spectrometry (ETAAS) ZEEnit 60 (Analytik Jena, equipped with Zeeman-effect BG correction system) at the Radioisotopes Lab. Biophysics Institute, Federal University of Rio de Janeiro (Brazil). Measurements were performed in triplicate, and then the values averaged. Quality control was carried out through blanks, which were proceeded through in the same way as the samples, using certified reference material Dolt-4 (dogfish liver), Dorm-3, and Dorm-4 (fish protein) NRCC. The relative condition factor (ReCF) was calculated using a regression of residuals of logarithmically transformed body mass (W) against body length (L), according to Labocha & Hayes (2012). The value of ReCF indicates whether a penguin has a good body condition (positive value) or a less than good body condition (negative value) (Brown, 1996).

An analysis of variance (ANOVA) was used to analyze the database, normality, and homogeneity of variance tests. Evaluated parameters included contaminant levels and analyzed morphological values (weight and length) in all four-study locations. As the data failed to pass the normality tests, a nonparametric statistical test (Kruskal-Wallis) was performed, and correlations for metals and condition factors calculated using Spearman's correlations. A value of $P \leq 0.05$ was considered significant. All analyses used InfoStat software (Di Rienzo *et al*., 2009).

The concentrations of metals in Adélie penguin feathers of the four locations studied here are shown in Table 1. Considering all the sampling sites together, metal burden in feathers varied as follows: Zn > Al > Fe > Cu > Se > Mn > Cr > Cd > Ni > V > Pb. The highest Al, V, Fe, and Se mean concentrations were obtained from Arctowski, while the highest Cr, Ni, Cu,

Table 1. Concentrations (mean value ± standard deviation) of trace elements (µg g^{-1}, dry weight) in feathers of adult *Pygoscelis adeliae* of the western Antarctic Peninsula area. Minimum and maximum values are shown in brackets. The same letter in the same column indicates the absence of a statistically significant difference ($P \leq 0.05$). ND: only one value was over the detection limit; n: number of detectable levels.

Location	Metal										
	Al	Cr	V	Fe	Mn	Ni	Cu	Zn	Se	Cd	Pb
Ardley Island	14.23 ± 18.37^b (1.35-73.58) n = 28	1.75 ± 0.18^a (1.36-2.12) n = 28	0.34 ± 0.10^b (0.59-0.20) n = 28	10.40 ± 16.08^a (2.56-89.55) n = 28	0.20 ± 0.20^b (0.00061-0.67) n = 15	0.65 ± 0.15^a (0.44-1.03) n = 28	9.92 ± 1.77^a (6.62-13.59) n = 28	60.00 ± 10.63^a (41.38-86.37) n = 28	5.99 ± 1.15^a (4.50-9.50) n = 28	1.12 ± 0.30^a (0.53-1.84) n = 28	0.032 ± 0.061^a (0.72-0.12) n = 4
Base O'Higgins	7.86 ± 7.40^b (1.07-36.75) n= 37	1.59 ± 0.16^{bc} (1.06-1.85) n = 37	0.33 ± 0.06^b (0.23-0.46) n = 37	5.50 ± 2.72^a (2.82-15.64) n = 37	6.38^a ND n = 1	0.38 ± 0.12^b (0.16-0.75) n = 34	8.42 ± 1.57^b (5.33-11.86) n = 37	60.79 ± 9.54^a (44.09-81.57) n = 37	5.65 ± 1.05^a (3.77-8.036) n = 37	0.60 ± 0.16^b (0.28-0.84) n = 37	0.005^a ND n = 1
Kopaitic Island	12.16 ± 10.39^b (2.39-59.31) n = 44	1.70 ± 0.22^{ab} (1.37-2.50) n = 44	0.36 ± 0.07^b (0.19-0.56) n = 44	8.25 ± 5.66^a (3.00-34.16) n = 44	0.38 ± 0.94^b (0.0036- 4.89) n = 29	0.39 ± 0.28^b (0.075-1.64) n = 44	7.71 ± 1.49^b (5.05-11.89) n = 44	60.98 ± 11.13^a (41.35-94.67) n = 44	5.85 ± 1.21^a (3.95-10.52) n = 44	0.44 ± 0.33^b (0.14-2.14) n = 40	0.002 ± 0.002^a (0.0006-0.009) n =11
Arctowski	27.11 ± 38.08^a (3.26-170.32) n = 20	1.54 ± 0.15^c (1.32-1.89) n = 20	0.44 ± 0.08^a (0.27-0.57) n = 20	11.31 ± 11.71^a (3.62-46.17) n = 20	0.20 ± 0.42^b (0.004-1.44) n = 11	0.15 ± 0.08^c (0.015- 0.37) n = 20	5.85 ± 1.50^c (4.03-9.03) n = 20	59.73 ± 16.38^a (40.00-112.10) n = 20	6.31 ± 1.33^a (3.86-8.45) n = 20	0.32 ± 0.12^b (0.25-0.49) n = 4	0.001^a ND n = 1

Cd, and Pb were found in Ardley Island. In contrast, the highest mean levels of Zn were determined at Kopaitic Island (ANOVA, $P \leq 0.05$). When comparing the metal concentrations in Adélie penguin feathers between the locations of the South Shetland Islands and Graham Land (Table 2), we noted that the levels of Al, V, Fe, and Cd levels were significantly higher in South Shetland Islands ($P \leq 0.05$). Higher concentrations of Al, V, Fe, and Cd in penguin feathers found in King George Island (South Shetland Islands) may be due to increased human presence. Here, the Antarctic scientific bases, heavy traffic vessels, airplanes, tourists, scientists, and support personnel are most concentrated (Tin *et al.*, 2009). Similarly, Jerez *et al.* (2011) reported higher levels of Al, Cr, Mn, Fe, Se, and Pb at King George Island than some Graham Land locations. When comparing with other studies on metals in Adélie penguin feathers, our Al, Cu, Cr, Fe, Ni, Pb, and Se levels were lower than those previously reported by previous studies in Antarctica (Table 5). Our Zn levels observed in the four localities were similar to those previously reported in Admiralty Bay, Avian Island, and King George Island, but below those reported in Zhongshan Station (140 µg g^{-1}, Table 5), a locality in the sub-Antarctic area of the Atlantic Ocean (Yin *et al.*, 2008). For Cd, this metal showed higher concentrations than previously reported (Table 5), and only the levels reported by Ancora *et al.* (2002) at Edmonson Point (0.30 µg g^{-1}) are similar to those we found at Artowski (0.32 ± 0.12 µg g^{-1}). Our Mn concentrations (0.20-6.38 µg g^{-1}) are higher than the levels previously reported in Adélie penguins (<0.01-2.01 µg g^{-1}) (Table 5), even though lower than the Mn levels detected in feathers of adult seabirds from industrialized and populated areas, such as the Brazilian coasts (11.4 µg g^{-1}, Barbieri *et al.*, 2010). Our increasing Mn levels in Adélie penguins seem to be related to its current use as an additive in combustibles in replacement of Pb (Burger & Gochfeld, 2000). At the same time, recent evidence shows that Mn levels in hepatic tissues of Antarctic penguins (Jerez *et al.*, 2013b) are slightly higher than those detected two decades ago (Honda *et al.*, 1986; Szefer *et al.*, 1993). Agusa *et al.* (2005) found V levels of 0.076 µg g^{-1} dry weight in feathers of black-tailed gulls (*Larus crassirostris*) from Japan, but in general, the levels of V in feathers of seabirds have been poorly investigated. At present, it is not possible to detect a geographical pattern in the concentrations of metals because data are fragmented, in which case any spatial analysis must be carried out with caution, following the recommendation indicated by Espejo *et al.* 2017.

Adult penguins at Arctowski exhibited a lower average weight (W) than the penguins in the colonies at

Table 2. Statistical differences of metal (mean values ± standard deviation (µg g^{-1} dry weight) between locations of South Shetland Islands (sector A) and Graham Land (sector B), western Antarctic Peninsula area (see Fig. 1 for sectors considered). The same letter in the same column indicates the absence of a statistically significant difference ($P \leq 0.05$); n is the number of detectable levels.

Sites	Metal										
	Al	Cr	V	Fe	Mn	Ni	Cu	Zn	Se	Cd	Pb
Sector a	19.6 ± 2.73^{a} (n = 48)	1.66 ± 0.03^{a} (n = 48)	0.38 ± 0.01^{a} (n = 48)	10.78 ± 1.37^{a} (n = 48)	0.21 ± 0.22^{a} (n = 25)	0.44 ± 0.40^{a} (n = 48)	8.22 ± 0.29^{a} (n = 48)	59.89 ± 1.66^{a} (n = 48)	6.13 ± 0.17^{a} (n = 48)	1.02 ± 0.05^{a} (n = 32)	0.03 ± 0.01^{a} (n = 5)
Sector b	10.17 ± 2.11^{b} (n = 81)	1.65 ± 0.02^{a} (n = 81)	0.34 ± 0.01^{b} (n = 81)	6.99 ± 1.05^{b} (n = 81)	0.58 ± 0.20^{a} (n = 30)	0.39 ± 0.03^{a} (n = 78)	8.04 ± 0.22^{a} (n = 81)	60.89 ± 1.28^{a} (n = 81)	5.76 ± 0.13^{a} (n = 81)	0.52 ± 0.04^{b} (n = 77)	0.002 ± 0.01^{a} (n = 12)
Significant level	0.007	0.81	0.006	0.030	0.20	0.21	0.61	0.63	0.08	<0.0001	0.14

Table 3. Values (mean ± standard deviation) of weight (W), length (L), and relative condition factor (ReCF) for Adélie penguins (*Pygoscelis adeliae*) from different locations of the Antarctic Peninsula area. The same letter indicates the absence of statistically significant differences ($P \leq 0.05$). N is the number of detectable levels.

Location	N	W (kg)	N	L (cm)	N	ReCF
Ardley Island	28	4.59 ± 0.61[ab]	28	66.50 ± 5.23[a]	28	-6.2 exp-6 ± 5.4 exp-2[a]
Base O'Higgins	36	4.80 ± 0.89[a]	37	64.87 ± 2.69[a]	36	-3.6 exp-2 ± 7.7 exp-2[b]
Kopaitic Island	44	4.71 ± 0.79[ab]	44	66.09 ± 3.71[a]	44	-6.1 exp-3 ± 5.8 exp-2[a]
Arctowski	18	4.24 ± 0.79[b]	20	64.64 ± 4.79[a]	20	-5.9 exp-2 ± 9.1 exp-2[b]

Table 4. Correlations coefficients between trace metal levels and relative condition factor (ReCF) in Adélie penguin (*Pygoscelis adeliae*) feathers of the Antarctic Peninsula area, western Antarctica (*significant values, $P \leq 0.05$).

	Metal											
	Al	Cr	V	Fe	Mn	Ni	Cu	Zn	Se	Cd	Pb	ReCF
Al	1											
Cr	-0.065	1										
V	0.215	0.146	1									
Fe	**0.600***	0.032	-0.017	1								
Mn	0.075	-0.025	-0.088	0.075	1							
Ni	0.003	0.210	-0.102	0.174	0.072	1						
Cu	0.009	**0.334***	-0.137	0.048	-0.062	**0.636***	1					
Zn	-0.060	0.230	-0.032	0.023	-0.064	-0.038	**0.253***	1				
Se	0.169	0.159	**0.240***	0.057	-0.171	-0.020	0.056	**0.245***	1			
Cd	-0.027	0.198	0.170	-0.003	0.054	**0.735***	**0.531***	0.006	0.023	1		
Pb	-0.171	**0.337***	**-0.235***	**0.645***	0.174	**0.614***	0.629*	-0.037	**0.443***	**0.349***	1	
ReCF	0.029	0.142	-0.117	0.004	-0.092	0.115	**0.237***	**0.183***	0.023	0.038	-0.105	1

Ardley Island and Base O'Higgins (ANOVA, $P \leq 0.05$, Table 3). Also, body condition values (ReCF) did not differ between Ardley Island and Kopaitic Island and between Base O'Higgins and Arctowski. However, ReCF values on both sites (Ardley and Kopaitic islands) did differ significantly from the ReCF values found at Base O'Higgins and Arctowski. There was no significant difference in mean length (L) between the sites studied ($P > 0.05$). Of all analyzed trace elements across the four study sites, only ReCF showed significant correlations to both Cu and Zn levels in feather samples (Table 4). Among locations, feather Cu and Zn in adult Adélie penguins were related positively ($P \leq 0.01$) to their body condition (ReCF) only at Arctowski (Fig. 2). On this site, penguins also exhibited a significantly lower weight (Table 1); possibly, both metals stores decreased by some physiological stress (Wayland *et al.*, 2002), caused by low food availability (Wiebkin, 2012). Considering that penguin rockeries at Arctowski are near the highest concentration of Antarctica's human activities (Tin *et al.*, 2009; Espejo *et al.*, 2014), food availability for these penguins colonies could negatively be affected (García-Borboroglu & Dee-Boersma, 2013). Studies in Antarctica have shown that impacted near-shores sites generally have lower species richness, biodiversity, and variability than control sites (Stark *et al.*, 2006). In any case, a low body condition of seabirds often implies little subcutaneous fat deposits and reduced pectoral muscle mass (Debacker *et al.*, 2000).

Some studies have found a relationship between feathers and muscle tissues for some trace elements in birds (Del Hoyo *et al.*, 1992), reaching up to 85% of similarity between the concentrations of Al, As, Cd, Pb, Hg, Cu, Zn and Mn in feathers and muscles of penguins (Espejo *et al.*, 2017). Additionally, we found significant positive correlations between Al-Fe, Cr-Cu, Cr-Pb, V-Se, Fe-Pb, Ni-Cu, Ni-Cd, Cu-Zn, Cu-Cd, Cu-Pb, Zn-Se, Se-Pb, and Cd-Pb, while a significant negative correlation between V-Pb. There is evidence showing that Se and Zn have a detoxifying effect on Cd and Hg in seabirds from the Arctic and Antarctica (Norheim, 1987; Smichowski *et al.*, 2006). Moreover, Se has shown to have some degree of detoxifying effect on Hg, Cd, and Pb in birds (Ohlendorf & Heinz, 2011) and penguins (Kehrig *et al.*, 2015). Our results indicate that probably Cu acts as a detoxifying agent for Cr, Cd, and Pb, whereas Se could be for V and Pb, which needs to be more investigated.

Table 5. Mean ± standard deviation of trace metal levels (μg g^{-1}, dry weight) in feathers of adult Adélie penguins (*Pygoscelis adeliae*) reported in previous studies. *Sample collection, †Juvenile, [1]South Shetland Islands (western Antarctica), [2]Subantarctic area of the Atlantic Ocean, [3]Victoria Land (east Antarctica), [4]Graham Land, Antarctic Peninsula (west Antarctica), [5]Adelaide, Antarctic Peninsula (west Antarctica), n/i: not informed.

Location	N	Al	Cd	Pb	Cu	Zn	Mn	Date*	References
Admiralty Bay[1]	>100	-	-	-	-	61.50	-	2004	Santos *et al*. (2006)
Zhongshan Station[2]	n/i	-	-	1.50	16.0	140.0	-	2001	Yin *et al*. (2008)
King George Island[1]	1	3.56	0.12	<0.01	16.21	70.41	0.21	2007-2010	Jerez *et al*. (2013b)
Avian Island[5]	2	0.71 ± 0.43	0.08 ± 0.01	0.06 ± 0.09	16.22 ± 0.51	60.59 ± 2.02	<0.01	2007-2010	Jerez *et al*. (2013b)
King George Island[1]	1†	52.44	0.01	<0.01	19.29	83.90	1.15	2007-2010	Jerez *et al*. (2013b)
King George Island[1]	5	64.3 ± 61.75	0.13 ± 0.08	0.24 ± 0.38	13.32 ± 8.22	61.11 ± 20.3	2.01 ± 0.52	2008-2009	Jerez *et al*. (2013a)
Edmonson Point[3]	4	-	0.30	0.50	-	-	-	1995	Ancora *et al*. (2002)
King George Island[1]	25	43.36 ± 69.03	-	0.64 ± 1.09	12.68 ± 7.09	50.84 ± 17.38	1.30 ± 1.16	2005-2007	Jerez *et al*. (2011)
Yalour Island[4]	21	8.62 ± 6.41	0.04 ± 0.05	0.32 ± 0.36	13.41 ± 2.6	82.45 ± 13.1	1.16 ± 1.26	2005-2007	Jerez *et al*. (2011)
Avian Island[5]	22	5.08 ± 3.03	0.04 ± 0.02	0.14 ± 0.21	13.16 ± 3.04	77.69 ± 15.17	0.34 ± 0.49	2005-2007	Jerez *et al*. (2011)

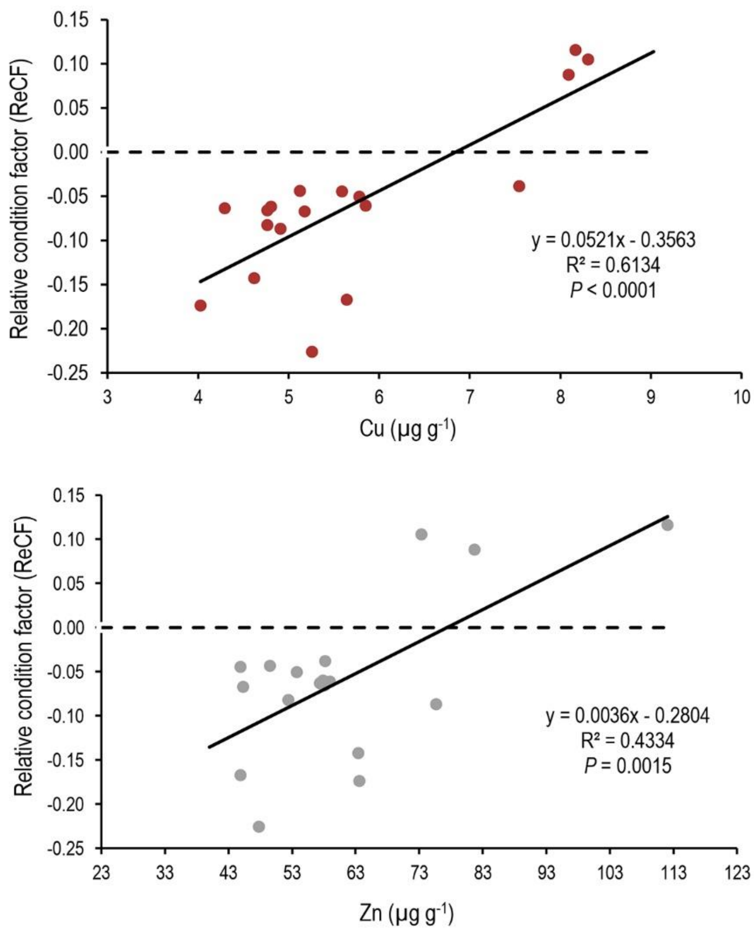

Figure 2. Correlation of relative condition factor (ReCF) to metal concentrations in Adélie penguins (*Pygoscelis adeliae*) at Arctowski, South Shetland Islands.

In conclusion, as indicated in Table 5, this study is the first report of Cr, V, Fe, Ni, and Se concentrations in Adelie penguin feathers. Further studies are needed to assess other metals in Adelie penguins. The levels of Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, and Zn found in this study indicate these metals are relevant in the Antarctic environment to bioaccumulate in Adélie penguin feathers. The results showed that, except for Cu and Zn, most of the metals in Adélie penguins' feathers showed no significant relationship with the body condition of the penguin rockeries studied here. However, this relationship may be site-specific, and metals might be affecting the organisms at low biological levels (*e.g.*, molecular, biochemical). Although feathers are a pathway for detoxification of the organism of penguins (Espejo *et al*., 2017), little is known about the interaction of metals with penguins' health; hence, genetic, molecular, and biochemical studies are required to assess those possible biological effects. Since feathers are non-invasive to birds, this study provides valuable information to validate them as a biological matrix for measuring birds' body health.

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