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SOIL SCIENCE

Influence of different seabird species on trace metals content in Antarctic soils

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Abstract: The behavior and feeding habits of different species of seabirds can influence the enrichment of trace metals in Antarctic soils. This study aimed to evaluate the influence of different species of seabirds on the concentrations of potentially toxic metals in Antarctic soils. For this, we collected soil samples in areas influenced by penguins, kelp gulls, and giant petrels. We analyzed the concentration of total organic carbon (TOC), total nitrogen (TN), available phosphorus (P) and metals by three different methods of extraction: USEPA 3051A, Mehlich-1, and distilled water. The concentrations of Cr and Hg presented positive correlations with P, TOC, and TN by the USEPA 3051A method, indicating the biotransport of these metals by seabirds. Soils influenced by penguins showed higher levels of P, TOC, TN, Cr, and Hg. Comparing the results from the different extractors, we found that Hg had the highest relative levels in the exchangeable fraction and the soil solution. Therefore, the soils with the influence of penguins present higher levels of biotransported trace metals, but this does not necessarily mean that these birds have a higher biotransport potential, since the concentration of trace metals in these soils may be related to their degree of ornithogenesis.

Key words: biotransportation, contamination, extractors, mercury, ornithogenic soils, heavy metals.

INTRODUCTION

The Antarctic flora and fauna are peculiar in the globe, and their establishment is hampered by extreme climatic conditions (Kim et al. 2018, Potapowicz et al. 2019, Ferrari et al. 2021). The vegetation is small and composed mainly of lichens, mosses, and terrestrial algae, and the animals are represented by seabirds and pinnipeds, highly dependent on the ocean (Ramírez-Fernández et al. 2019, Ferrari et al. 2021, Abakumov et al. 2021). The animals preferably occupy the coastal regions of Antarctic and islands close to this continent with mild climatic conditions and where are the few ice-free areas and soils (Park et al. 2012, Brooks et al. 2019).

Soils intensively influenced by seabird activity are called ornithogenic soils, these birds can deposit up to 10 kg m^{-2} per year of excrement, in addition to feathers, eggshells and corpses on these soils (Tatur 1989, Simas et al. 2007). Therefore, seabird activity can affect the soil physical, chemical and biological properties, resulting in high levels of phosphorus, organic matter, clay, and acid pH (Simas et al. 2007, Daher et al. 2019, Lopes et al. 2021, Rodrigues et al. 2021). The vegetation zonation patterns in Antarctica have been correlated with the nutrients from the intense activity of seabirds (Poelking et al. 2015, Zwolicki et al. 2015, Lachacz et al. 2018, Perfetti-Bolaño et al. 2018). However, the accumulation of guano in ornithogenic soils can also cause enrichment with pollutants, such as trace metals (Cipro et al. 2019a, b, Alekseev & Abakumov 2021).

Antarctic environments are sensitive to anthropogenic disturbances and there is a high concern to avoid contamination by trace metals (Brooks et al. 2019). Although several studies show that anthropic activity can contaminate soils with trace metals in this region (Abakumov et al. 2017, Fabri-Jr et al. 2018, Shi et al. 2018, Bueno et al. 2018), some authors also suggest that the activity seabirds can significantly enrich the soil with different trace metals (Huang et al. 2014, Santamans et al. 2017, Cipro et al. 2018, Perfetti-Bolaño et al. 2018, Castro et al. 2021). The enrichment by trace metals from the activity of seabirds is associated with several factors, such as diet, bird species and the composition and amount of excrement deposited in the soil (Celis et al. 2014, Ramírez-Fernández et al. 2019). However, information about the influence of specific seabird species on the enrichment of trace metals in Antarctic soil is still scarce.

It is extremely important to understand how different species of seabirds biotransport potentially toxic elements and its dynamics in the soil, whether they remain in exchangeable or non-exchangeable forms over time (Casalino et al. 2013, Castro et al. 2021). Penguins are the most studied seabirds, and there is little information about the biotransportation of trace metals by other seabirds, such as giant petrels and kelp gulls (Santamans et al. 2017, Cipro et al. 2018, Abakumov et al. 2021). Therefore, this study aimed to evaluate the contents and dynamics of potentially toxic elements in soils occupied by different species of seabirds in Antarctica. Specifically, we aimed to (i) analyze the contents of total organic carbon (TOC), total nitrogen (TN), available phosphorus (P), and potentially toxic metals in soil influenced by penguins, kelps gulls and petrels; and (ii) identify the solubility of potentially toxic metals in the soil using different extraction methods.

MATERIALS AND METHODS

Study area

For this study, we collected soil samples during the Brazilian Operation Antarctic XXXVI (February 2018) in areas under seabird influence in the Livingston Islands (Byers Peninsula) and Nelson (Stansbury Peninsula), both located in the South Shetland Islands archipelago, in Maritime Antarctica (Figure 1). Livingston Island is the second largest island (845 km^2) and with the biggest ice-free area in the archipelago (Almela et al. 2019). On this island, we collected the samples on the Byers Peninsula (62 ° 33’35”–62 ° 41’24” S, 61 ° 13’29”–60 ° 54’15” W), whose lithology is dominated by sedimentary (sandstones and conglomerates), volcanic, and volcanoclastic rocks (Moura et al. 2012). The climatic conditions are mild compared to the Antarctic continent, with average annual precipitation and temperature of 800 mm and -2°C, respectively, and in summer the average temperatures exceed 2°C and there are frequent liquid precipitations (Moura et al. 2012).

Nelson Island covers an area of 165 km^2, with 8 km^2 of ice-free area (Rodrigues et al. 2019). The lithology is mainly composed of Andesitic rocks with some intercalations of volcanoclastic sediments (Manfroi et al. 2015). Here, we collected samples on the Stansbury Peninsula (62 ° 14114 “ –62 ° 15’45 “ S, 58 ° 59’13 “–59 ° 02130 “W), an area that has not yet been studied and, therefore, without a weather station. Meanwhile, in the Fildes Peninsula that is located 4 km from the center of the Stansbury Peninsula, the average annual precipitation and temperature are 630 mm and -1.6 °C, respectively (Rodrigues et al. 2019).

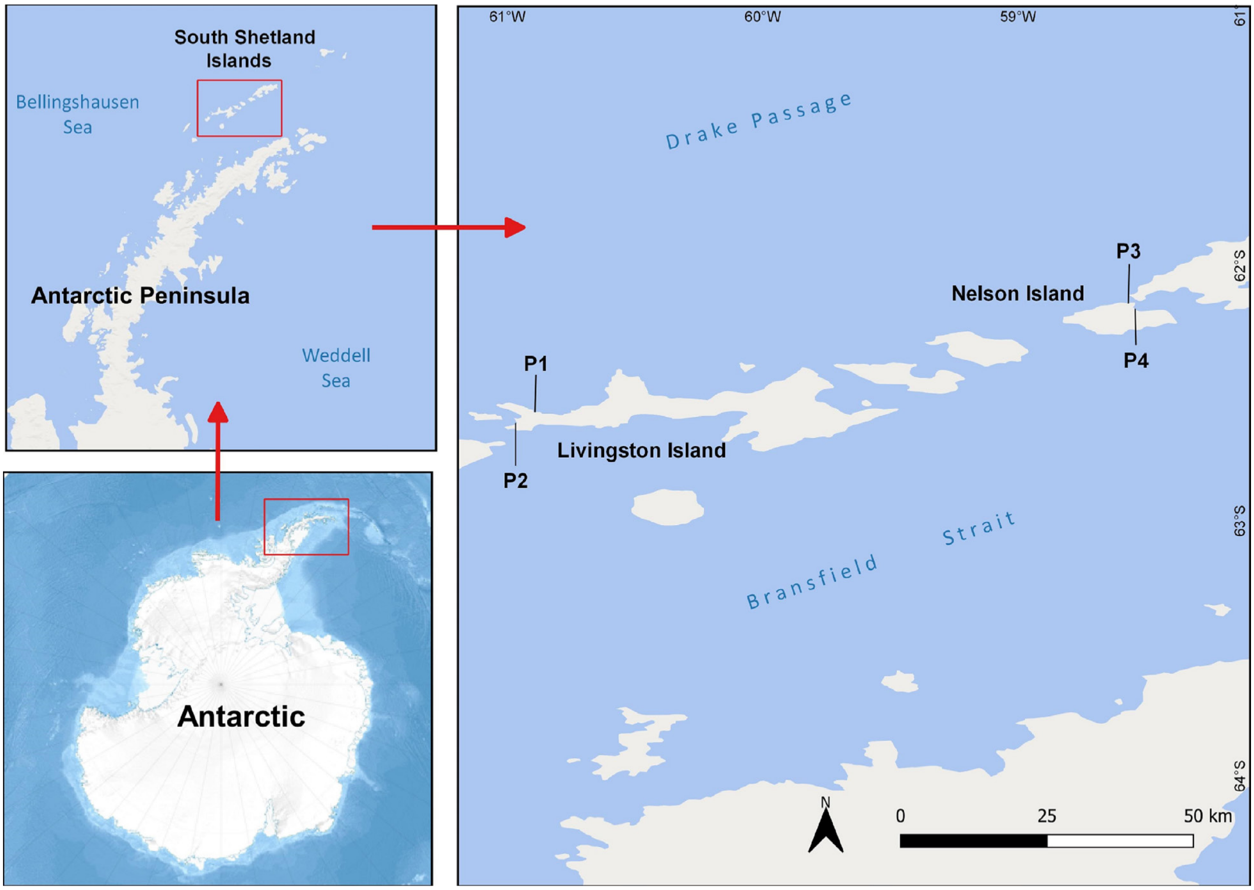

Figure 1. Location of soil sampling points (P1, P2, P3 and P4) with the influence of different species of seabirds in the Livingston and Nelson Islands, Antarctica.

The soil samples were collected in areas currently occupied (P1) and old nesting areas (P2) by gentoo penguins (*Pygoscelis papua* Forster) (Figure 2). In P1, there is a high lateral supply of phosphate and the vegetation cover is composed of algae, lichens and mosses. In the P2, there are bones and stones from nests up to 30 cm soil depth, and is currently occupied by nests of giant petrels (*Macronectes giganteus* Gmelim) and kelp gulls (*Larus dominicanus* Lichtenstein), but also used by gentoo penguins to access the sea. We also collected samples in areas with current activity of kelp gulls (P3) and abandoned brood of giant petrels (P4), both without the influence of penguin activity. In the Byers Peninsula (P1 and P2), we collected soil samples every 10 cm in a total of six layers to a depth of 60 cm, while in the Stansbury Peninsula (P3 and P4) we collected the soil samples based on the horizons of each profile (Figure 2). The samples were stored in plastic bags and kept frozen until they were analyzed. The detailed soil physical and chemical characterization of samples are presented in Table I.

Analysis of TOC, TN, P, and metals

To analyze the soil characteristics, we sieved (2 mm) the soil samples, macerated and passed through 0.149 mm (100 mesh) sieves. The total soil carbon (TOC) was determined according to Yeomans & Bremner (1988), and total nitrogen (TN) by the Kjeldahl method, modified by Tedesco et al. (1985). Available P was extracted by Mehlich-1 (Mehlich 1953) and determined

Figure 2. Representative pedons of soils influenced by gentoo penguins (P1 and P2) in the Livingston Island, and by kelp gulls (P3) and giant petrels (P4) in the Nelson Island, Antarctica. a) P1 - Haplic Leptsol (Ornithic, Skeletic); b) P2 - Haplic Leptosol (Ornithic, Humic); c) P3 - Lithic Skeletic Leptsol (Ornithic, Novic); d) P4 - Leptic Cambisol (Ornithic, Skeletic).

by spectrophotometer (UV-Vis). To analyze the contents (mg kg^{-1} dry weight) of Ba, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr, and Zn in the soil, we applied three different extraction methods: distilled water (Carvalho et al. 2021, Lima et al. 2019b), Mehlich-1 (Mehlich 1953) and the method 3051A (USEPA 1998, Carvalho et al. 2021, Castro et al. 2021). During the pseudototal extraction using the 3051A method, we added samples of the reference material with known trace metal levels (NIST SRM 2709a - San Joaquim Soil), and a control sample (blank) in each battery of 24 soil samples.

The levels of metals were quantified by inductively coupled plasma-optic emission spectrometry (ICP-OES), model Optima 8300 DV (PerkinElmer, Inc., Waltham, USA), also using the hydride generator, model VGA 77 (Agilent Technologies Inc., Santa Clara, USA) for the determination of Hg. The results of metal content of the control samples and reference material were used to calculate the detection limit (LD), according to the method proposed by the American Public Health Association (APHA 2012):

$$LD = (\bar{x} + t.s).d$$

where: $\bar{x}$ is the average content of the metal (mg kg^{-1}) of the blanks; t is the value of the Student distribution at 0.01 probability and n - 1 degree of freedom, n being the number of blanks used; s is the standard deviation of the blanks; and d is the dilution employed in the method. When below the LD, the metal levels were considered equal to half of the LD. We used the rate of recovery of the certified material used (NIST SRM 2709a - San Joaquim Soil) to analyze the reliability (precision and accuracy) of metals results. The leachate recovery rate was 70% for Cr and Fe; 75% for Ba and Co; 83% for Mn and Zn; 90% for Cu and Ni; and 103% for Pb. Although there was no Sr leachate content in the certified material, the total recovery rate was 62%. The results of the detection limit for the different extraction methods are in Table SI – Supplementary Material. The cadmium content was also analyzed, but its recovery rate in relation to the certified material was close to zero. Due to the lack of reliability of the Cd contents in the soil samples, it was decided not to include this element in the analyses.

Table I. **Soil physical and chemical characterization of samples influenced by gentoo penguins (P1 and P2) in the Livingston Island, and by kelp gulls (P3) and giant petrels (P4) in the Nelson Island, Antarctica.**

Horizon	Soil depth (cm)	pH water (1:2.5)	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	Gravel	Sand	Silt	Clay	Dp
			mg kg^{-1}	-------- cmolc kg^{-1} --------				%	-------- % --------			g cm^{-3}
P1 - Haplic Leptsol (Ornithic, Skeletic)												
-	0-10	4.49*	605	15.81	5.76	3.75	34.4	79	61	17	22	2.13
-	10-20	3.86	234	1.52	0.50	5.14	22.7	74	58	25	17	2.87
-	20-30	3.90	250	1.74	0.63	5.63	25.4	75	51	23	26	2.80
-	30-40	3.89	250	1.55	0.63	5.93	28.4	86	55	22	23	2.84
-	40-50	4.08	238	2.22	1.25	7.80	-	95	77	9	14	2.76
-	50-60	4.18	230	1.65	0.71	6.72	19.4	61	68	15	17	2.55
P2 - Haplic Leptosol (Ornithic, Humic)												
-	0-10	4.96	250	13.8	7.04	0.69	14.2	41	68	16	16	-
-	10-20	5.59	165	13.07	5.00	1.28	9.1	31	71	13	16	2.35
-	20-30	6.32	171	16.47	4.58	0.79	7.2	32	72	13	15	2.50
-	30-40	6.52	191	17.19	4.01	0.49	6.7	31	70	14	16	-
-	40-50	6.71	202	19.88	3.59	0.00	3.9	48	69	16	15	-
-	50-60	6.79	198	19.4	3.27	0.00	3.3	50	68	17	15	2.38
P3 - Lithic Skeletic Leptsol (Ornithic, Novic)												
A	0-5	4.77	150	2.23	1.89	24.33	38.2	0	44	34	22	2.53
B1	5-20	5.18	233	6.05	8.42	33.38	37.3	0	28	52	20	2.60
B2	20-35	5.81	281	19.36	26.36	6.72	8.7	0	32	51	17	2.61
B3	35-45	6.12	277	20.11	25.20	1.17	3.1	0	68	17	15	2.46
P4 - Leptic Cambisol (Ornithic, Skeletic)												
A	0-20	6.25	174	19.81	11.72	0.19	3.5	0	44	34	22	2.33
AC	20-35	6.95	138	20.42	11.88	0.00	1.6	0	61	17	22	2.30

*** pH water (1:10). Available K extracted by Mehlich^{-1} and determined by flame photometry; Ca^{2+}, Mg^{2+}, and Al^{3+} extracted with 1 mol L^{-1} KCl and determined by atomic absorption and volumetric determination; H+Al extracted by 0.5 mol L^{-1} calcium acetate at pH 7; Gravel determined by screening (2-20 mm); Clay, silt, and sand obtained by physical (50 rpm - 16h), and chemical dispersion (NaOH 0,1 mol L^{-1}); Dp: Particle density obtained by the volumetric flask method. Methods used according to Teixeira et al. (2017).**

Statistical analysis

We applied the correlation and principal component analysis (PCA) to the variables TOC, TN, P, and metals. 'The Pearson correlation analysis explore the relationships between the variables, considering p-values smaller than 0.10 significant by Student's t-test. We used descriptive statistics to compare the levels of potentially toxic metals obtained by the three different extraction methods: distilled water, Mehlich-1, and USEPA 3051A (Table SII, SIII and SIV). The software R version 3.4.1 was used to perform these statistical analyses (R Core Team 2017).

RESULTS

Contents of TOC, TN, P, and metals in the soil

The soils influenced by penguins (P1 and P2) presented the highest levels of TOC and TN, with the highest levels of TOC (> 110 g kg^{-1}) and TN (> 9 g kg^{-1}) in the superficial layer (0-10 cm). In P1, there was an increase in TOC and TN from 10 to 50 cm soil depth, indicating the illuviation of SOM in depth. In P3 and P4, which are influenced by giant petrels and kelp gulls, there were relatively low values of TOC (<20 g kg^{-1}) and TN (<1.5 g kg^{-1}) in the soil layers (Figure 3). The average levels of P were higher in P1 (559.83 ± 25.22 mg kg^{-1}) and P2 (312.73 ± 64.60 mg kg^{-1}) compared to P3 (61.90 ± 16.40 mg kg^{-1}) and P4 (198.35 ± 115.20 mg kg^{-1}), although P4 has a relatively high P content in the surface layer (Figure 3).

The soil P2 presented the higher values of Fe (17,683.5 ± 1,027 mg kg^{-1}); Ni (29.36 ± 2.72 mg kg^{-1}); Mn (542.82 ± 0.01 mg kg^{-1}); Pb (6.55 ± 0.09 mg kg^{-1}) and Zn (66.38 ± 3.86 mg kg^{-1}), while the mean values for these metals from the three others soils were lower: Fe (13,746.2 ± 579 mg kg^{-1}), Ni (6.72 ± 0.85 mg kg^{-1}), Mn (311.64 ± 102 mg kg^{-1}), Pb (2.55 ± 0.50 mg kg^{-1}) and Zn (33.22 ± 5.04 mg kg^{-1}). In general, the average levels of Fe, Ni, Pb and Zn had small variations with depth. However, the soil P1 showed the highest levels of Ni and Zn and the lowest Fe and Pb at 0-10 cm depth compared with the other layers. We also found that in P2 and P4, there were higher levels of Mn at the deeper layers. The soils P1 and P2 presented higher average values of Ba (15.78 ± 1.49; 16.43 ± 1.07 mg kg^{-1}) and Cr (9.51 ± 3.02; 9.80 ± 0.42 mg kg^{-1}) compared with P3 and P4. Similarly, the highest average values of Hg were found in P1 (0.07 ± 0.05 mg kg^{-1}) and P2 (0.06 ± 0.05 mg kg^{-1}), with P3 and P4 presenting values below the limit of detection. The soil P4 showed the highest mean values of Co (18, 32 ± 3.51 mg kg-1) and P1 the lowest value (6.42 ± 0.42 mg kg^{-1}), with a small variation in depth. The soil P4 also presented the higher values (99.35 ± 6.77 mg kg^{-1}), and P2 the lowest value (29.30 ± 1.21 mg kg^{-1}) for Cr. The Sr levels varied little between soils, with the highest average levels found in P3 (161.83 ± 0.85 mg kg^{-1}) (Figure 4).

Correlation and principal component analysis

Pearson's correlation analysis showed that only the trace metals Hg and Cr presented positive coefficients with TOC, TN, and P, indicating the association of these metals with seabird activity. We also found positive coefficients between Fe, Mn, Ni, and Pb and negative correlation between TOC, TN, and P with Fe, Co, and Mn (Figure 5).

Components 1 and 2 (PC1 and PC2) explained 67.1% of the variation in the data set (Figure 6). The axis PC1 showed a positive correlation

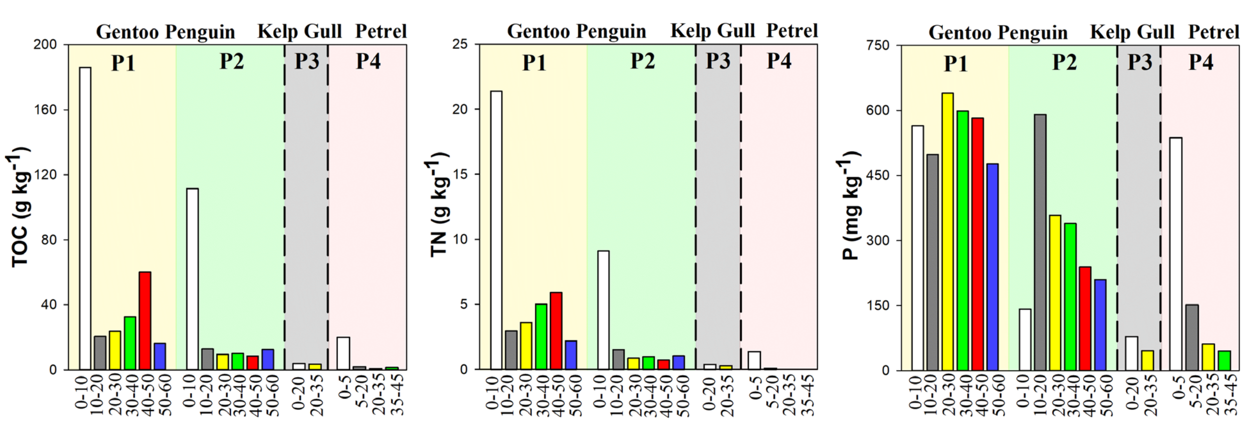

Figure 3. Contents of total organic carbon (TOC), total nitrogen (TN), available phosphorus (P) from different soil depths (cm) under influence of gentoo penguins (P1 and P2) in the Livingston Island, and by kelp gulls (P3) and giant petrels (P4) in the Nelson Island, Antarctica.

mainly with Fe, Ni, Pb, Sr, and Zn, while the PC2 axis correlated positively mainly with TOC, TN, P, Cr, and Hg. There was a clear separation of soil P2 from other soils, and P2 samples correlated positively with PC1 and being mainly influenced by Ba, Fe, Ni, Pb, Sr, and Zn. The P3 and P4 samples were negatively correlated with PC1 and PC2, but influenced by Cu, whereas the P1 samples were more influenced by TOC, TN, P, and Cu.

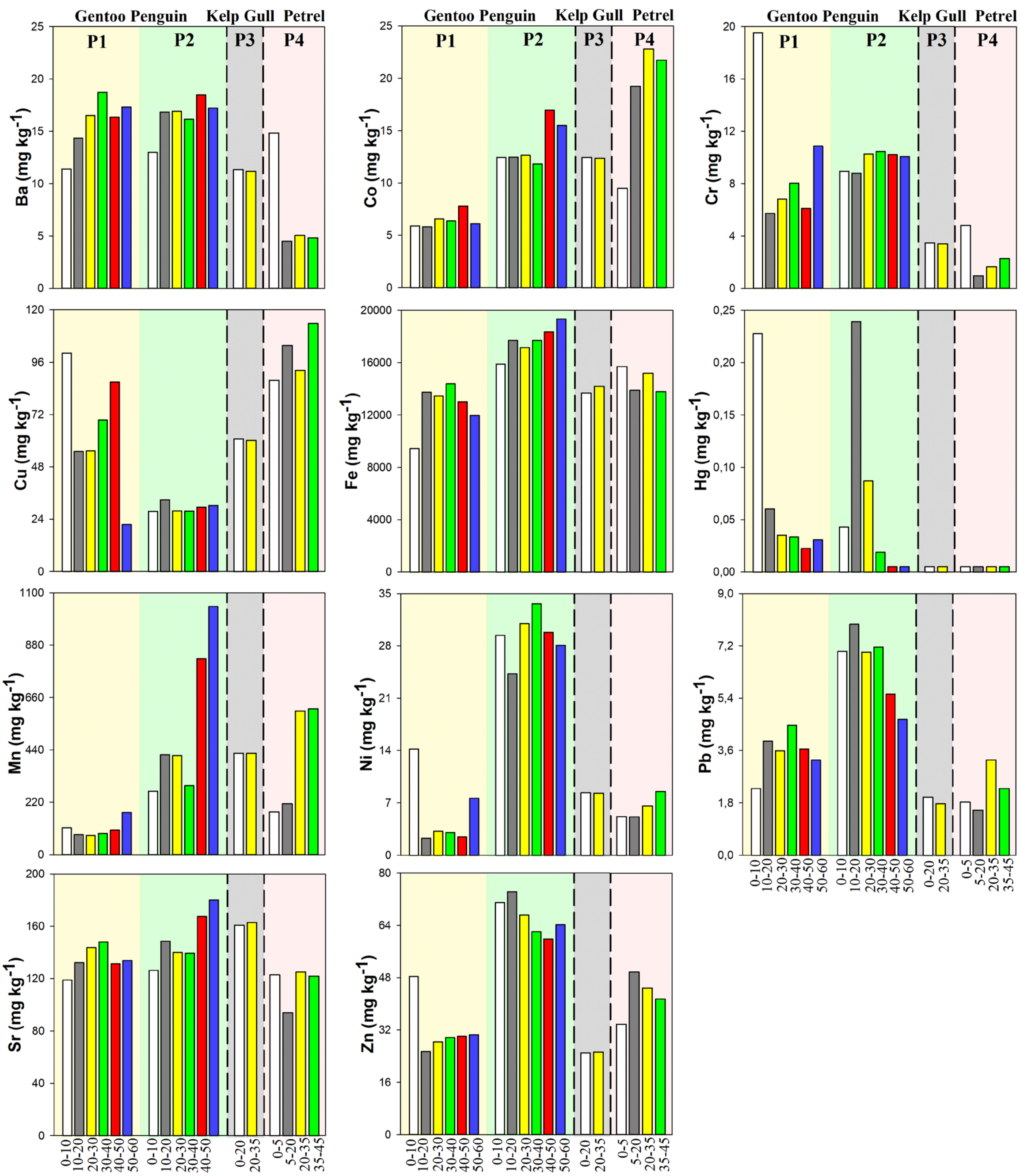

Figure 4. **Contents of metals (Ba, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr and Zn) from different soil depths (cm) under the influence of gentoo penguins (P1 and P2) in the Livingston Island, and by kelp gulls (P3) and giant petrels (P4) in the Nelson Island, Antarctica.**

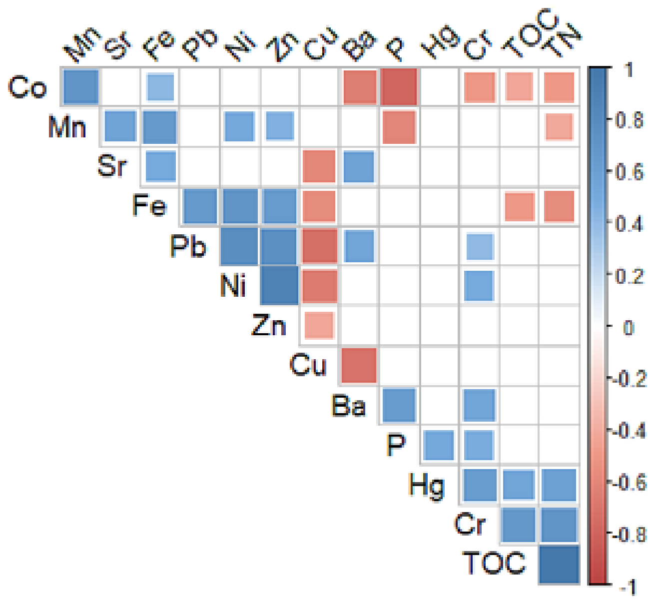

Figure 5. Pearson correlation coefficients (p <0.10) between the variables total organic carbon (TOC), total nitrogen (TN), available phosphorus (P) and metals (Ba, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr and Zn) under the influence of gentoo penguins (P1 and P2) in the Livingston Island, and by kelp gulls (P3) and giant petrels (P4) in the Nelson Island, Antarctica.

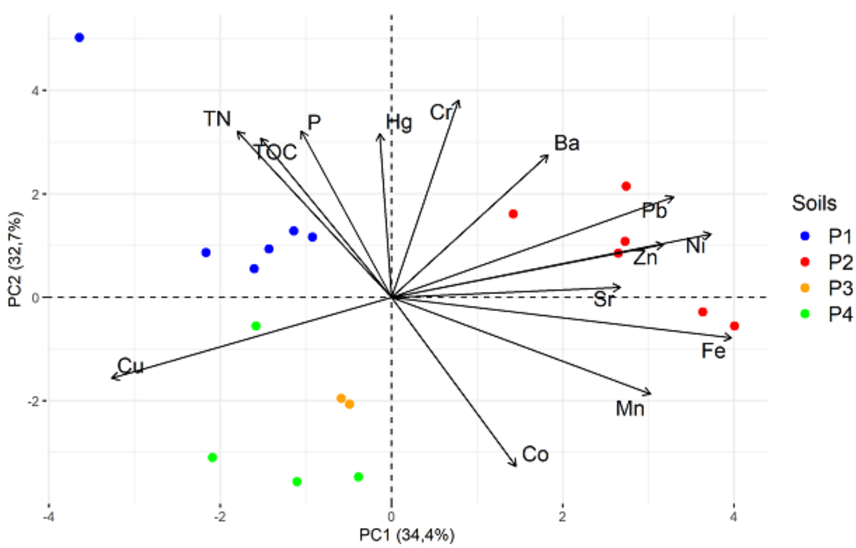

Figure 6. Principal component analysis with the variables total organic carbon (TOC), total nitrogen (TN), available phosphorus (P) and metals (Ba, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sr and Zn) under the influence of gentoo penguins (P1 and P2) in the Livingston Island, and by kelp gulls (P3) and giant petrels (P4) in the Nelson Island, Antarctica.

Solubility of the metals

In addition to the determination by the USEPA 3051A extraction method, our study explored the content of metals from soil samples influenced by seabird activity using distilled water and Mehlich-1 extraction methods (Figure 7). Here, we are emphasizing the results for P1 and P2, which had the highest levels of biotransported metals. We identified that Mehlich-1 extracted more than 20% of the levels extracted by USEPA 3051A only for Cu and Hg. The Hg extracted by Mehlich-1 in P1 represented 49% of the Hg extracted by USEPA 3051A, while in P2 this value was only 8%. The levels of metals extracted in distilled water did not exceed 4% of the levels extracted by USEPA 3051A, with Fe, Hg, and Pb the metals most dissolved in water (Figure 7).

DISCUSSION

Metals in soils with the influence of different species of seabirds

The high volume of guano produced by seabirds results in the accumulation of organic matter,

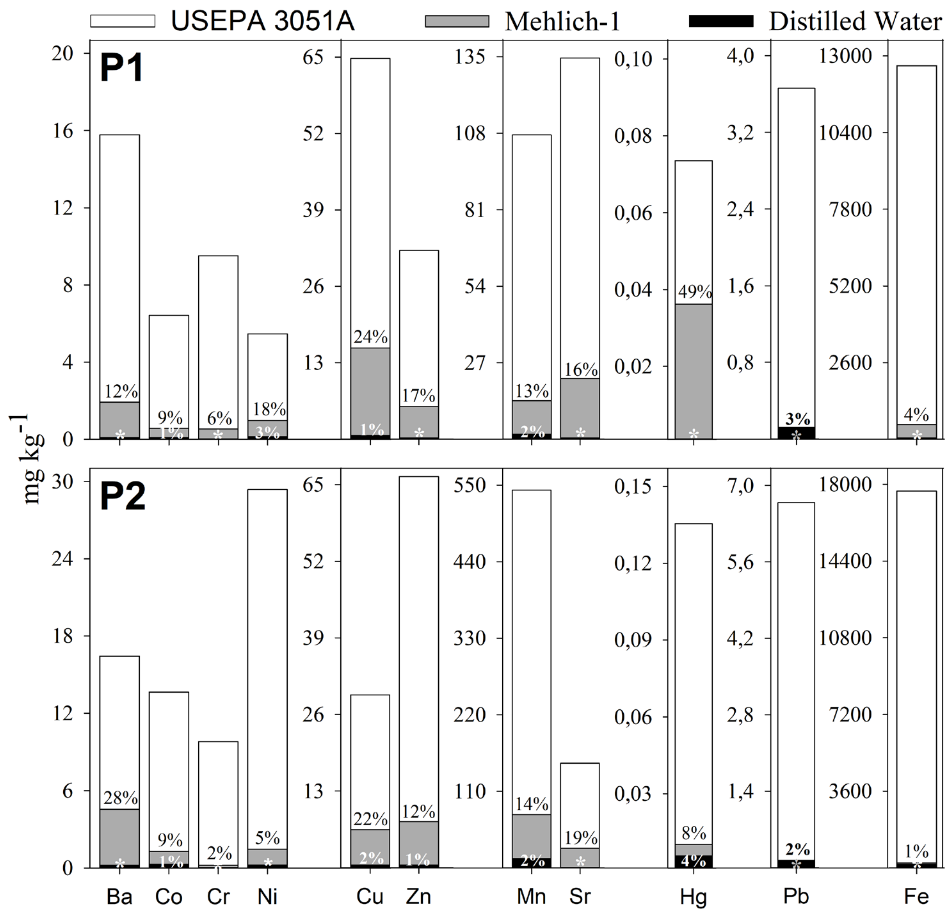

Figure 7. **Average values and percentage of the metals (Ba, Co, Cr, Ni, Cu, Zn, Mn, Sr, Hg, Pb and Fe) extracted by distilled water and Mehlich-1 compared with the USEPA 3051A method from soil samples influenced by gentoo penguins (P1 and P2) in the Livingston Island, Antarctica. * percentage lower than 1%.**

P, and trace metals (Santamans et al. 2017, Perfetti-Bolaño et al. 2018, Abakumov et al. 2021). In our study, we found that Cr and Hg had positive correlations with TOC, TN, and P, indicating the enrichment of these metals in the soil by the activity of seabirds. Soils influenced by gentoo penguins (P1 and P2), with higher P and organic matter content, showed the highest Cr and Hg levels, compared with P3 and P4 that had less P and organic matter accumulation. The alterations of soil characteristics and the contents of trace metals due to the activity of seabirds are associated with the stay period in a certain area and the amount of guano deposited and accumulated in the soil (Myrcha & Tatur 1991, Daher et al. 2019, Rodrigues et al. 2021). The higher levels of Cr and Hg in soils influenced by penguins may be related to the low locomotion capacity of these birds in the terrestrial environment, resulting in a large volume of guano deposited in small extensions of area (Ramírez-Fernández et al. 2019, Abakumov et al. 2021). Giant petrels and kelp gulls have different characteristics that permit to explore larger areas, depositing less guano in a single area and, consequently, causing fewer changes in the soil characteristics where they nest (Daher et al. 2019, Ramírez -Fernández et al. 2019).

Although soils influenced by penguin have the highest levels of biotransported metals, the comparison between species of seabirds in terms of their contribution to enriching the soil with trace metals must consider that the soils are in different stages of ornithogenesis (Myrcha & Tatur 1991, Simas et al. 2007). The P1 and P2 presented a high ornithogenic influence, with the highest levels of TOC, TN, and P. Although P2 is located in an area abandoned by penguins, the levels of TOC, TN, and P were high, which may be linked to the contribution of the old penguin nests, in addition to the vegetation and seabirds (giant petrels and kelp gulls) that currently occupy the site. The P3 did not present advanced ornithogenesis since presented low values of TOC, TN, and P (Simas et al. 2007). The P4 that has low TOC, TN, but high level of P influenced by the abandoned brood of giant petrel. According to Simas et al. (2007), ornithogenic soils of Antarctica should have at least three of these characteristics: clear morphological evidences of bird activity (e.g. fresh droppings, nests, or bones); presence of light grey horizons and/or whitish coatings on rock surfaces; Melich-1 extractable-P > 500 mg kg^{-1} for the < 2 mm fraction; and presence of crystalline or amorphous claysized phosphates. Furthermore, the lower levels of P in the guano of giant petrels compared with penguins` guano, may influence less on the soil ornithogenesis process (Poelking et al. 2015, Daher et al. 2019). The soil dynamics are also affected by the location giant petrel nests, which are located on rocky outcrop tops with a large part of the guano being lost from the nests and concentrated in fractures or the foothills of these outcrops (Rodrigues 2020, Abakumov et al. 2021). These factors mentioned influence the dynamics of the biotransported metals by different seabirds, mainly in the soil of these birds' nests.

The presence of Hg in Antarctic soils is mainly related to the burning of fossil fuels, seabird activity, atmospheric deposition, volcanic activity, and the source material (Cipro et al. 2018, Subhavana et al. 2019). The Hg has high ecotoxicity in terrestrial and marine environments (Blévin et al. 2013, Einoder et al. 2018, Carravieri et al. 2020) and special attention must be given to this element in the Antarctic environment. The positive correlation between Hg with TOC, TN, and P indicating the biotransport of this metal have been found also by Cipro et al. (2018, 2019a), with the level of Hg (0.028 ± 0.006 mg kg^{-1}) similar to the present study 0.24 ± 0.01 mg kg^{-1}.

The Hg biomagnifies in food chains and top animals in the chain, such as penguins, tend to accumulate it in their livers, feathers and excrement (Yin et al. 2008). The origin of this biomagnified Hg and deposited in the Antarctic soils is linked to the marine environment where the seabirds feed (Seco et al. 2019, Carravieri et al. 2020). The waters of the Southern Ocean have considerable levels of Hg mainly by atmospheric deposition, which can be directly associated with anthropic contamination (Fitzgerald et al. 2007, Blévin et al. 2013). Therefore, part of the Hg present in the waters of Antarctic Ocean is incorporated into the marine food chains, mainly by organisms at the base of the food chains (phytoplankton and krills), and is thus biomagnified between seabird species, reflecting in the seabirds guano and the ornithogenic soils of Antarctica (Fitzgerald et al. 2007, Cossa et al. 2011).

The contents of Cr in Antarctic soils are linked mainly with the source material and anthropic contamination (oil derivatives) (Celis et al. 2015, Santamans et al. 2017, Cipro et al. 2018, Xu et al. 2020). However, Espejo et al. (2017) demonstrated that in addition to the source material, the Cr levels in Antarctic soils may also be due to penguin activity, as we found in this study. Jerez et al. (2013) reported Cr levels higher than 7.0 mg kg^{-1} in krills (penguins' main food), while Celis et al. (2015) and Espejo et al. (2014) found Cr levels higher than 3.0 mg kg^{-1} in seabird droppings, showing that bird feeding as an important factor for the enrichment of Cr in the soil over time.

The levels of Cu did not present a positive correlation with TOC, TN, and P, indicating that the main source of this metal for the soil is the source material. The P3 and P4 showed relatively high average levels of Cu (60.52 ± 0.29 and 99.35 ± 6.77 mg kg^{-1}, respectively), but low levels of TOC and TN, excluding the possibility of the enrichment of this element by seabird activity (Huang et al. 2014). This argument is reinforced by Abakumov et al. (2017), who report soils without the influence of seabirds on the Stansbury Peninsula with higher levels of Cu (120.0 mg kg^{-1}) than in P3 and P4. However, the level of Cu in P1 presented a similar dynamic than TOC and TN in depth, indicating the presence of high levels of this element in the guano of seabirds and to the active colonies of penguins in this soil. The positive correlation of Cu with TOC and TN in ornithogenic soils has also been reported in the literature (Santamans et al. 2017, Cipro et al. 2018, Perfetti-Bolaño et al. 2018, Castro et al. 2021).

The elements Fe, Mn, Ni, Pb, and Zn were higher in P2 compared with the other soils, but there was no linkage to the activity of seabirds, since there was no positive correlations with TOC, TN, and P by Pearson's correlation or principal component analysis. These elements are probably more associated with the source material, even in the case of Antarctic ornithogenic soils (Abakumov et al. 2017, Santamans et al. 2017, Cipro et al. 2018). In areas without the influence of seabirds and same material of origin to soil P2, Santamans et al. (2017) reported levels of Fe (63,137.33 ± 23,013 mg kg^{-1}), Mn (527.60 ± 196.15 mg kg^{-1}), Pb (5.15 ± 0.48 mg kg^{-1}) and Zn (81.33 ± 15.76 mg kg^{-1}) similar to those found in this study in P2 (17,683.48 ± 668; 542.8 ± 182.6; 6.55 ± 0.70 and 66.38 ± 3.17 mg kg^{-1}, respectively). Similar to Fe, Ni, Pb and Zn, the variation in Ba, Co and Sr between soils are not due to the activity of seabirds, presenting no positive correlation between these metals and TOC, TN, and P. Navas et al. (2008) and Santamans et al. (2017) did not report enrichment of Ba, Co, and Sr in soils of the Byers Peninsula with seabird activity, suggesting the source material as the main source of these metals.

The biotransported trace metals had lower levels compared with the Canadian (CCME 2018) and Finnish standards (MEF 2007) for contaminated soils (Cr < 64 and Hg < 0.5 mg kg^{-1}), indicating that seabird activity did not result in a high contamination pressure (Table SV). Canadian and Finnish standards were used because they represent soils from cold climate regions, the latter being one of the most representative for European soils (Tóth et al. 2016, Shah et al. 2019, López et al. 2019). While these standards are not specific to the Antarctic environment, they provide guiding values for metals in soils that are considered contaminated by anthropic activity, that can assist in the interpretation of the results (Castro et al. 2021). However, special attention should be given to Hg, due to its high ecotoxicity (Yin et al. 2008, Cossa et al. 2011). Among the other metals, only Cu showed levels above the contamination limit (>100 mg kg^{-1}), which does not necessarily indicate that the soil is contaminated, as this element appears to come from natural sources and is not associated with anthropic activity (Huang et al. 2014). Finally, the variation in recovery rate for different metals also was found in other studies that used the same extraction method (Santos & Alleoni 2013, Abbruzzini et al. 2014, Roje et al. 2018, Castro et al. 2021). The fact that pseudototal extraction does not fully digest silicate minerals justifies the variations in the recovery rates of metals (Florian et al. 1998, Abbruzzini et al. 2014, Lima et al. 2018).

Extraction of soluble and exchangeable levels of metals

In general, Mehlich-1 extract cations adsorbed in the soil colloidal system, in this case, the exchangeable forms of metals in the soil (Nascimento et al. 2014, Lima et al. 2019a). Our results indicated that low percentages of Ba, Co, Cr, Ni, Zn, Mn, Sr, Pb, and Fe were in exchangeable form, with the majority in the soil mineral matrix (Casalino et al. 2013). Although the Cr presented a high positive correlation with TOC, TN, and P, most of this element must be in the mineral matrix or complexed by the soil organic matter, not available for Mehlich-1 extractor. The high levels of Cu in the exchangeable fractions of P1 and P2 may be related affinity of Cu with the soil organic matter, with some studies reporting the positive correlation in ornithogenic soils in Antarctica (Santamans et al. 2017, Gholami & Rahimi 2020, Castro et al. 2021).

The high content of Hg in the exchangeable fraction of the Antarctic soils is due to the affinity of this element with the soil organic matter and its dynamics in the environment (Andrade et al. 2012). A large amount of Hg in Antarctic soils is associated with atmospheric deposition and seabird activity, which can be absorbed by the soil's colloidal system and then the part of it can form complexes with the soil's organic matter or be incorporated into the structure of secondary minerals from phosphate (Nie et al. 2012, Lou et al. 2015). In P1, currently occupied by penguin, there was a constant deposition of guano in the soil, which explains the high relative contents of this metal in the exchangeable fraction of the soil. In P2, an abandoned penguin area, the Hg levels were higher compared to P1, but with lower Hg levels in the exchangeable fraction of the soil, indicating that the exchangeable Hg of this soil was lost and/or more strongly linked to soil organic matter and soil minerals over time.

The extraction by distilled water permits to quantify the levels of metals readily available in the soil solution (Reis et al. 2014, Lima et al. 2019a). In this study, we found low levels of metals extracted by distilled water, and the Hg was the trace metal presented relatively the highest levels in the soluble fraction of the soil in P2, which must be related to the decomposition of seabird guano (Cipro et al. 2018, Subhavana et al. 2019). Although present at low levels, Hg in the soil solution is very susceptible to leaching, and can become an important source of Hg for the ecosystem (Zvěřina et al. 2017).

CONCLUSIONS

Soils with the influence of penguins have higher levels of biotransported trace metals than those with the influence of giant petrels and kelp gulls. Nevertheless, our data does not necessarily indicate penguins as the largest biotransporters of trace metals. The soils with the influence of penguins present a more advanced stage of ornithogenesis, indicating a longer activity time for these birds in the same place compared to other seabirds.

Chromium (Cr) and Hg are the trace metals biotransported by seabirds, and Hg may have an indirect link with anthropic activities. The contents of Ba, Co, Cu, Fe, Mn, Ni, Pb, Sr, and Zn did not show any relation with the activity of seabirds and are associated with the source material of the soil. The metals studied are mainly in non-exchangeable forms in the soil and Hg has the highest levels in the exchangeable fraction and the soil solution, mainly in soil of active penguin colony, which must be related to the deposition and decomposition of the guano.

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SUPPLEMENTARY MATERIAL

Table SI. Recovery rate for metals from the certified soil material (NIST SRM 2709a - San Joaquin soil) extracted by USEPA 3051A and the detection limit (LD) obtained by distilled water extraction methods, Mehlich-1 and USEPA 3051A.

Table SII. Average levels and standard error of metals extracted by distilled water from soils influenced by gentoo penguins (P1 and P2), on Livingston Island, and kelp gulls (P3) and giant petrels (P4), on Island Nelson, Antarctica.

Table SIII. Average levels and standard error of metals extracted by Mehlich-1 from soils influenced by gentoo penguins (P1 and P2), on Livingston Island, and kelp gulls (P3) and giant petrels (P4), on Island Nelson, Antarctica.

Table SIV. Average levels and standard error of metals extracted by USEPA 3051A from soils influenced by gentoo penguins (P1 and P2), on Livingston Island, and kelp gulls (P3) and giant petrels (P4), on Island Nelson, Antarctica.

Table SV. Average levels (mg kg^{-1}) and standard error of metals in the topsoil layer of the present study, from different Antarctic soils studies, seabird guano and contamination reference.

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