

*Short Communication*

## Enrichment of trace elements by Humboldt penguin (*Spheniscus humboldti*) on nesting areas at Pajaros Niños Islet, central coast of Chile

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**ABSTRACT.** Seabirds are important biological pathways, moving pollutants from sea to land via their guano and feathers. This study investigated the influence of Humboldt penguin nesting activities on trace element concentrations in soils at Pájaros Niños Islet, central coast of Chile. Soil samples were collected from penguin nesting sites and control areas during July 2022 and analyzed for seven trace elements (Pb, Cu, Co, Cr, Zn, Ni, and As) using inductively coupled plasma optical emission spectroscopy (ICP-OES). Lead concentrations were significantly higher in nesting sites compared to control sites ( $3.57 \pm 0.64$  vs.  $2.66 \pm 0.70$  mg kg<sup>-1</sup>,  $P < 0.05$ ), while Cu, Zn, and Ni showed elevated but non-significant concentrations in nesting areas. The trace element hierarchy in nesting sites was Zn > Cu > Cr > Pb > As > Co > Ni, differing from typical crustal abundance patterns. Spearman correlation analysis revealed a strong positive correlation between Cu and Zn ( $r = 0.93$ ,  $P < 0.01$ ) and a moderate correlation between Co and As ( $r = 0.83$ ,  $P < 0.05$ ) in nesting sites. Principal component analysis revealed considerable overlap among site types, suggesting subtle geochemical differences. These findings suggest that while Humboldt penguins influence soil trace element composition through guano inputs, the magnitude of change is modest compared to natural variation. The results contribute to understanding seabird-mediated biotransport of trace elements and highlight the need for further research on the ecological implications of metal accumulation in coastal nesting habitats.

**Keywords:** heavy metals; seabird; sea-land; soil contamination; biotransport; nesting sites

### INTRODUCTION

Penguins are a family of aquatic birds found almost exclusively in the marine environments of the southern hemisphere (Boersma 2008). The Humboldt penguin (*Spheniscus humboldti*) establishes breeding colonies

along the coastal regions of southern Peru and northern Chile, with numerous populations distributed throughout this range. Pájaros Niños Islet, located off the central coast of Chile, is home to one of the most iconic breeding colonies for this species. As upper predators in marine food webs, Humboldt penguins serve critical

ecological functions in their habitats and serve as effective bioindicators for assessing environmental contamination across both marine and terrestrial ecosystems (Adesina et al. 2024).

Research has demonstrated that seabirds act as significant biological vectors, facilitating the biotransport of contaminants from marine to terrestrial ecosystems (Celis et al. 2022b, De La Peña-Lastra et al. 2022, Grant et al. 2024). A previous study found that Humboldt penguins substantially elevated trace element concentrations and nutrient availability in their nesting habitats through guano deposition (Espejo et al. 2017a). However, current literature presents a notable knowledge gap regarding the potential role of this species in mediating the biotransport of Pb, Ni, and As on ornithogenic soils.

In recent decades, trace elements including Pb, Cu, Co, Cr, Zn, Ni, and As have been widely used across numerous industrial sectors to meet escalating demands in manufacturing, electronics, automotive, construction, metallurgy, and agricultural applications (Nyika & Dinka 2023, Edo et al. 2024). These industrial developments have substantially increased the mobilization of trace elements such as Pb in batteries and pigments, copper in electrical wiring and plumbing, Co in rechargeable batteries, Cr in steel production and tanning, Zn in galvanization processes, Ni in stainless steel manufacturing, and as in pesticides and wood preservatives (Swaine 2000, Nordberg & Nordberg 2016). Consequently, these trace elements are being increasingly discharged into environmental matrices through mining operations, industrial processes, urban runoff, and agricultural practices (Swaine 2000). Comprehensive studies have reported elevated concentrations of trace elements in aquatic systems and marine environments (Mason 2013, Kolarova & Napiórkowski 2021), as well as bioaccumulation patterns in wildlife populations (Espejo et al. 2020, Celis et al. 2022a, 2025), demonstrating anthropogenic perturbation of natural biogeochemical cycling processes. Nevertheless, the comprehensive ecological implications of trace metal contamination remain inadequately characterized (Izah et al. 2023, Edo et al. 2024), despite mounting evidence that these pollutants can fundamentally alter ecosystem functioning and pose significant risks to environmental and human health (Kolarova & Napiórkowski 2021, Izah et al. 2023). At nesting sites, seabirds may serve as vectors of trace metal contamination into terrestrial habitats, as avian guano is an efficient mechanism for contaminant transfer to soils and a substantial source of biogeochemical inputs (De La Peña-Lastra et al. 2022,

Grant et al. 2024). This research aimed to assess the influence of Humboldt penguin guano deposition on the geochemical characteristics of trace elements in terrestrial environments.

A perennial rookery of the Humboldt penguin was selected from the central coast of Chile, situated within the Sanctuary of Nature. The Pájaros Niños Islet (33°21'37"S, 71°41'14"W), located approximately 150 m offshore from the coastal town of Algarrobo in the Valparaíso Region, is a small rocky formation measuring roughly 31 m<sup>2</sup>, characterized by sparse vegetation consisting primarily of endemic coastal shrubs and scattered succulent species. This islet supports a substantial Humboldt penguin rookery with 62 active nests and serves as a significant ecotourism destination for wildlife observation activities (Simeone et al. 2023).

Superficial soil was obtained from the uppermost 5 cm depth during July 2022, utilizing sterile plastic implements to collect samples directly from active nesting areas (guano-enriched substrates) and corresponding reference locations (non-guano-influenced soils situated adjacent to colonies but beyond the influence of avian activity). A total of 10 soil specimens were systematically collected from nesting zones, and an additional 10 samples were collected from reference sites for subsequent analytical procedures. All collected materials were preserved in hermetically sealed polyethylene containers and transported in sterile vessels until laboratory analysis.

In the laboratory, soil samples were initially dried to constant weight in an oven, after which 250 mg of dried soil was weighed and placed in 50 mL Teflon vessels. Subsequently, 2.5 mL of nitric acid (HNO<sub>3</sub>) at 1:1 dilution was added, carefully mixed, covered with a watch glass, and heated at 95°C for 15 min, avoiding boiling. The samples were then allowed to cool. Following this step, 1.25 mL of concentrated HNO<sub>3</sub> was added, the mixture was covered again with a watch glass, and refluxed for 30 min. After this time, the samples were heated to 95°C for 2 h, and then cooled. Then, 0.25 mL of grade 1 water and 0.75 mL of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added. Samples were covered with watch glasses until effervescence subsided and allowed to cool. An additional 30% H<sub>2</sub>O<sub>2</sub> was then added in 0.25 mL aliquots with heating until effervescence was minimal. Subsequently, samples were covered with watch glasses and heated at 95°C for an additional 2 h. Following this procedure, 2.5 mL of concentrated hydrochloric acid (HCl) was added, the mixture was covered again, and heated at 95°C for 15 min before cooling. The resulting solutions were

centrifuged at 2,000-3,000 rpm for approximately 10 min, and the supernatant was filtered using Whatman® No. 41 filter paper. The filtrate was collected in 25 mL volumetric flasks. Quantification of trace elements (Cu, Pb, Co, Cr, Zn, Ni, and As) was performed using inductively coupled plasma optical emission spectroscopy (ICP-OES). For quantification, 10-point calibration curves were prepared using multi-element standard solutions. Method validation was performed by analyzing certified reference materials (ERM-CC141, Joint Research Center), procedural blanks, and sample duplicates, thereby ensuring accuracy and precision. Recovery percentages for the certified standards ranged between 63 and 89.2%. The limits of detection (LOD) and quantification (LOQ) were established for each element. The obtained values were: Pb (LOD = 0.014  $\mu\text{g L}^{-1}$ ; LOQ = 0.048  $\mu\text{g L}^{-1}$ ), Cu (LOD = 0.102  $\mu\text{g L}^{-1}$ ; LOQ = 0.339  $\mu\text{g L}^{-1}$ ), Co (LOD = 0.831  $\mu\text{g L}^{-1}$ ; LOQ = 2.771  $\mu\text{g L}^{-1}$ ), Cr (LOD = 0.197  $\mu\text{g L}^{-1}$ ; LOQ = 0.656  $\mu\text{g L}^{-1}$ ), Zn (LOD = 1.220  $\mu\text{g L}^{-1}$ ; LOQ = 4.066  $\mu\text{g L}^{-1}$ ), Ni (LOD = 0.085  $\mu\text{g L}^{-1}$ ; LOQ = 0.282  $\mu\text{g L}^{-1}$ ), and As (LOD = 0.041  $\mu\text{g L}^{-1}$ ; LOQ = 0.138  $\mu\text{g L}^{-1}$ ). The analyses were conducted according to Chilean standard NCh 3634 at the Environmental Biogeochemistry Laboratory, Department of Soils and Natural Resources, Faculty of Agronomy, Universidad de Concepción.

Data normality and homoscedasticity were assessed using the Shapiro-Wilk and Levene's tests to determine whether parametric or nonparametric approaches were appropriate for subsequent analyses. The data were analyzed using nonparametric statistical tests due to violations of normality and homoscedasticity assumptions, even after data transformation. The Mann-Whitney U test was applied to compare element concentrations between nesting and control sites. Spearman's rank correlation coefficients were calculated to examine monotonic relationships between trace element concentrations. Principal component analysis (PCA) was performed to identify underlying patterns in the multivariate dataset and to reduce dimensionality while preserving variance structure. All statistical procedures were performed using Python 3.11.4, with a significance level of  $P < 0.05$ . The concentrations of seven trace elements were analyzed in soil samples from penguin nesting sites and control sites at Pájaros Niños Islet. The results are presented as mean  $\pm$  standard deviation ( $\text{mg kg}^{-1}$ ) and reveal distinct patterns between the two soil types (Table 1). Lead concentrations showed a statistically significant difference between nesting and control sites ( $3.57 \pm 0.64$  vs.  $2.66 \pm 0.70$   $\text{mg kg}^{-1}$ , respectively;  $P < 0.05$ ), the only element to show significant variation between

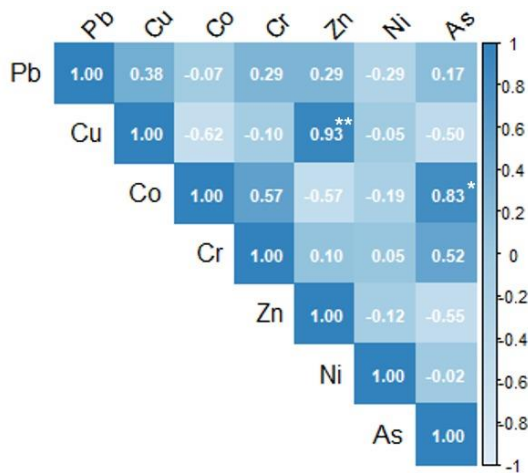
the two soil types. Copper, Zn, and Ni exhibited higher mean concentrations in nesting sites compared to control sites, with values of  $16.43 \pm 4.10$ ,  $200.41 \pm 58.09$ , and  $0.42 \pm 0.14$   $\text{mg kg}^{-1}$ , respectively, vs.  $15.54 \pm 6.76$ ,  $188.89 \pm 78.13$ , and  $0.38 \pm 0.41$   $\text{mg kg}^{-1}$  in control sites. Conversely, Co, Cr, and As showed higher concentrations in control sites ( $1.41 \pm 0.64$ ,  $6.77 \pm 5.12$ , and  $2.34 \pm 0.67$   $\text{mg kg}^{-1}$ , respectively) compared to nesting sites ( $0.94 \pm 0.56$ ,  $4.30 \pm 2.00$ , and  $1.90 \pm 0.71$   $\text{mg kg}^{-1}$ , respectively). However, these differences were not statistically significant ( $P > 0.05$ ). The highest trace element concentrations in penguin nesting sites corresponded to Zn ( $200.41 \pm 58.09$   $\text{mg kg}^{-1}$ ) and the lowest to Ni ( $0.42 \pm 0.14$   $\text{mg kg}^{-1}$ ). The following concentration hierarchy among trace elements was observed in nesting sites:  $\text{Zn} > \text{Cu} > \text{Cr} > \text{Pb} > \text{As} > \text{Co} > \text{Ni}$ . The hierarchy observed at the control site followed the same descending order. This pattern differs from typical crustal abundance values, in which elements such as Cr and Zn are generally more abundant than Cu and Pb in uncontaminated soils, suggesting that biological inputs from penguin colonies may alter the natural geochemical distribution of these elements in nesting areas (Zhu et al. 2014, Celis et al. 2015). A study demonstrated that guano-related geochemical alterations can affect Cu and other metals, leading to their enrichment in the surroundings; this enrichment occurs through bioaccumulation of metals in the organism by food consumption and depositing them in guano, followed by the microbial degradation of guano, which releases these metals into the environment (Miko et al. 2001).

Spearman correlation analysis (Fig. 1) revealed significant relationships between specific trace elements in nesting sites. A strong positive correlation was observed between Cu and Zn concentrations ( $r = 0.93$ ,  $P < 0.01$ ), indicating that these elements tend to co-occur in penguin nesting areas. Additionally, a moderate positive correlation was found between Co and As ( $r = 0.83$ ,  $P < 0.05$ ), suggesting potential common sources or similar geochemical behavior for these elements. Other notable correlations included moderate positive relationships between Cr and As ( $r = 0.52$ ,  $P < 0.05$ ), and between several element pairs, though these did not reach statistical significance. Negative correlations were observed between Co and Cu ( $r = -0.62$ ,  $P < 0.05$ ) and between Zn and As ( $r = -0.55$ ,  $P < 0.05$ ), indicating potential antagonistic relationships or different sources of origin.

PCA revealed considerable overlaps between nesting sites and control sites, indicating that the overall geochemical signatures of these two soil types are rela-

**Table 1.** Mean  $\pm$  standard deviation of trace elements ( $\text{mg kg}^{-1}$  dw) in surface soils ( $n = 15$ ) from Pájaros Niños Islet, central coast of Chile. Pájaros Niños Islet (nesting site: ornithogenic soils; control site: soils not having birds). Different letters between collecting sites indicate significance at  $P < 0.05$ . Maximum and minimum values in parentheses.

TEs	Nesting site	Control site
Pb	$3.57 \pm 0.64^a$ (4.66-2.44)	$2.66 \pm 0.70^b$ (3.52-1.32)
Cu	$16.43 \pm 4.10^a$ (23.93-11.37)	$15.54 \pm 6.76^a$ (26.04-6.24)
Co	$0.94 \pm 0.56^a$ (1.93-0.17)	$1.41 \pm 0.64^a$ (2.83-0.56)
Cr	$4.30 \pm 2.00^a$ (7.35-1.69)	$6.77 \pm 5.12^a$ (20.51-2.89)
Zn	$200.41 \pm 58.09^a$ (268.72-106.76)	$188.89 \pm 78.13^a$ (298.75-84.11)
Ni	$0.42 \pm 0.14^a$ (0.57-0.18)	$0.38 \pm 0.41^a$ (1.38-0.1)
As	$1.90 \pm 0.71^a$ (3.18-1.17)	$2.34 \pm 0.67^a$ (3.48-1.54)



**Figure 1.** Spearman correlation coefficient matrix of the trace elements for the soils with the presence of Humboldt penguins at Pájaros Niños Islet, central coast of Chile. \*Significant at  $P < 0.05$ ; \*\*significant at  $P < 0.01$ .

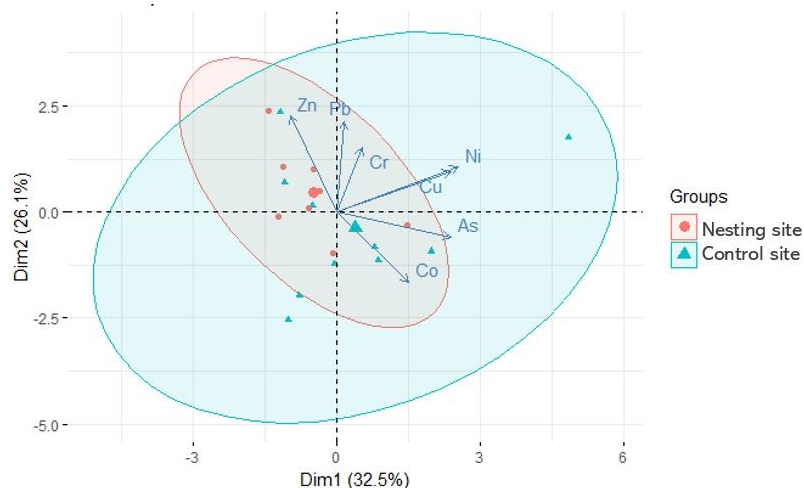
tively similar (Fig. 2). The PCA results suggest that while some differentiation exists between the sites, the separation is not pronounced, with Pb concentration representing the primary distinguishing factor between penguin nesting areas and control sites.

The lack of similar studies makes it very difficult to analyze our results. The present study represents the first evaluation of Pb levels in soils from colonies of this penguin species, revealing statistically significant differences between nesting sites ( $3.57 \pm 0.64 \text{ mg kg}^{-1}$ ) and control sites ( $2.66 \pm 0.70 \text{ mg kg}^{-1}$ ), thereby confirming the role of penguins as bio-vectors for this previously unassessed element. The high levels of Pb in ornithogenic sites can be influenced by human activities such as burning fossil fuels, incineration of solid waste, use of paints, and accidental oil spills

(Bargagli 2008). However, given that Humboldt penguins on Pajaros Niños Islet spend most of their time there and do not migrate to other places, and considering that penguins are known to be a biovector that transports nutrients and contaminants from the ocean to land (Huang et al. 2014, Celis et al. 2015), the enrichment of Cu, Zn, Pb, Cr, As, Co, and Ni in the islet is mainly influenced by penguin guano and feathers as a natural contaminant, exceeding possible anthropogenic contributions.

When compared with Espejo et al. (2017a) on Humboldt penguins in northern Chile and Adelie penguins (*Pygoscelis adeliae*) in the Antarctic Peninsula, the current findings present contrasting patterns for specific trace elements. While Espejo et al. (2017a) reported significant enrichment of Cd, Co, Cr, Cu, Mo, V, and Zn in penguin colony soils, the present study detected no statistically significant differences for most of these elements between nesting and control sites. Notably, Cu levels in this study ( $16.43 \pm 4.10 \text{ mg kg}^{-1}$  in nesting sites vs.  $15.54 \pm 6.76 \text{ mg kg}^{-1}$  in control sites) were considerably lower than those reported by Espejo et al. (2017a), who found Cu levels up to  $99.4 \text{ mg kg}^{-1}$  in non-guano affected soils at Ardley Island, Antarctica. Similarly, Zn concentrations showed no significant differences between sites ( $200.41 \pm 58.09$  vs.  $188.89 \pm 78.13 \text{ mg kg}^{-1}$ ), despite being notably elevated compared to Antarctic geochemical background values. The observed discrepancies may be attributed to differences in colony population density, local marine trophic chains, and geographical/environmental conditions.

Spearman correlation analyses revealed substantial differences in trace element relationships between penguin colony soils from different geographical loca-



**Figure 2.** Principal component analysis (PCA) based on the trace element concentrations in soil from nesting site (ornithogenic soil) and control site (soils not having birds).

tions. Espejo et al. (2017a) reported strong positive correlations between Cu-Co ( $r = 0.65$ ,  $P < 0.01$ ), Cu-V ( $r = 0.68$ ,  $P < 0.01$ ), and Zn-Cr ( $r = 0.63$ ,  $P < 0.01$ ) in northwestern Chilean and Antarctic Peninsula colonies. In contrast, the present study at Pájaros Niños Islet showed markedly different patterns, with an exceptionally strong Cu-Zn correlation ( $r = 0.93$ ,  $P < 0.01$ ) and negative correlations between Cu-Co ( $r = -0.62$ ,  $P < 0.05$ ) and Zn-Co ( $r = -0.57$ ,  $P < 0.05$ ). Additionally, while Espejo et al. (2017a) found positive Co-V correlations ( $r = 0.88$ ,  $P < 0.01$ ), the present study revealed strong Co-As associations ( $r = 0.83$ ,  $P < 0.05$ ). These contrasting correlation patterns suggest that local environmental conditions, including geological substrate, oceanographic influences, and colony-specific characteristics, significantly modulate trace element biogeochemical processes in penguin-influenced soils. The site-specific nature of these relationships underscores the importance of accounting for regional variability when assessing the role of seabirds as biovectors of trace elements in coastal ecosystems.

The overall similarity between nesting and control sites, as evidenced by the substantial overlap in PCA analysis, suggests that penguin nesting activities produce relatively subtle changes in soil geochemistry. This finding indicates that while penguins really influence their nesting environment, the magnitude of these changes is generally modest compared to the natural variation in soil trace element concentrations.

These results provide valuable insights into penguins' potential to act as biovectors of trace elements from sea to land, particularly regarding Pb

enrichment in nesting soils, warranting further investigation for a more comprehensive risk assessment. Most trace elements tend to bioaccumulate in birds' internal organs, such as the liver and kidneys. The main route of elimination of these contaminants is guano, followed by feathers (Espejo et al. 2017b).

Trace elements enter animal systems mainly through consumption, and their cycling is closely linked to biogeochemical processes in the environment. Their biogeochemical cycles imply interactions among the atmosphere, hydrosphere, geosphere, and biosphere, with organisms playing a fundamental role in the uptake, transformation, and release of elements (Tagliabue & Weber 2024). Some metals are crucial for biological functions, like Zn, which is important for immune function and enzyme activity and is regulated by the gastrointestinal system, whereas Cu is essential for enzyme function and cellular processes; however, both can be toxic at high concentrations (Espejo et al. 2017b). There is evidence that elevated concentrations of trace elements such as Pb, Cu, and Zn can alter soil microbial communities and promote metal tolerance in both marine-influenced and terrestrial ecosystems (Chen et al. 2019, Liu et al. 2020). Similarly, the significant Pb enrichment and co-occurrence of Cu and Zn observed in penguin nesting sites might enhance microbial adaptation to metal stress, potentially affecting soil biogeochemical processes that warrant deeper investigation.

Additionally, the strong positive correlation between Cu and Zn ( $r = 0.93$ ,  $P < 0.01$ ) suggests potential synergistic effects that could amplify their

combined environmental impact, particularly when considering interactions with other emerging contaminants in coastal ecosystems. More research is needed to assess the long-term ecological implications and potential risks associated with trace element accumulation in penguin nesting habitats. Future studies should also examine these potential sources, ideally using approaches such as metal isotope analysis.

### Credit author contribution

J. Celis: writing, conceptualization, methodology and original draft; W. Espejo: conceptualization, investigation, writing, review, editing, project administration and funding acquisition; P. Arce: investigation, resources and funding acquisition; C. Espinoza-Mendoza: investigation, data curation and visualization; M. Sandoval: resources and funding acquisition.

### Conflict of interest

The authors declare no conflict of interest.

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