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Short Communication

Assessing the influence of Humboldt penguin (*Spheniscus humboldti*) by excrements on the levels of trace and rare earth elements in the soil

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ABSTRACT. Rare earth elements (REE) and some transition metals (e.g. Nb) are a group of chemicals that have recently been widely used in industrial processes due to the increasing demand for new technologies. As a result, these chemicals are increasingly being released into the environment, which could mean that these pollutants could modify marine and terrestrial ecosystems. Seabirds, such as penguins, can biotransport pollutants and nutrients from the sea to land through excreta. However, there is no information about the role of the Humboldt penguin (*Spheniscus humboldti*) in bio-transporting emerging contaminants such as REE. This study aimed to assess any possible contribution of Humboldt penguins to the geochemical composition of some terrestrial areas. Excreta samples were collected from Chañaral Island, one of the most important sites in Chile for the conservation of Humboldt penguins. The results showed that this species tends to contribute to soil enrichment with REE (Ce, La, Nd, and Pr) and Nb through excreta, as well as with carbon. More studies are needed to see the potential impacts on the soil.

Keywords: *Spheniscus humboldti*; seabirds; sea-land transport; soil contamination; rare earth elements; emerging pollutants

Penguins are seabird species that exclusively inhabit the southern hemisphere (Boersma 2008). Humboldt penguins (*Spheniscus humboldti*) inhabit the coasts of southern Peru and northern Chile, where there are several colonies of this species. One of the most important colonies of Humboldt penguins is located at Chañaral Island, northwestern coast of Chile. The Humboldt penguin is a bird at the top of the food web in marine ecosystems and plays an important role in the ecology of the areas where it lives; and can be used to monitor environmental pollution in the marine and terrestrial environments as it feeds on the sea and nests on land (Celis et al. 2014).

Evidence shows that seabirds can biotransport contaminants and nutrients from the sea to land (Liu et al. 2006, Brimble et al. 2009, Mallory et al. 2015). Some evidence has shown that Humboldt penguins can contribute to increased heavy metal and nutrient loads at nesting sites through excreta (Espejo et al. 2017a). However, to our knowledge, no study shows that this species can biotransport rare earth elements (REE) to ornithogenic soils.

In recent years, REE and other less-known metals have been widely used to satisfy the growing demand for new technologies, such as renewable energy, cellular telephony, computers, biomedicine, space crafts,

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military technology, and even agriculture and animal husbandry (Eggert 2011, Filella & Rodushkin 2018). These advances in emerging technologies are using REE such as cerium (Ce), lanthanum (La), neodymium (Nd), praseodymium (Pr), and yttrium (Y), as well as some transition metals like niobium (Nb) that were previously stable in the Earth's crust (Hurd et al. 2012, Espejo et al. 2018a,b). Consequently, they are becoming more released into the environment by several anthropogenic activities (Ali 2014). Some studies have reported the presence of REE in rivers and seas (Kulaksiz & Bau 2013, Hatje et al. 2014) and the presence in the fauna of other lesser-known high-technology metals (Ricciardi et al. 2020), indicating that humans are affecting the geochemical cycles of emerging elements. However, the potential impacts of REE on ecosystems are still unknown (Cobelo-García et al. 2015, Pagano 2017), even though these contaminants could modify ecosystems and could have serious effects on wildlife and the environment (Heller et al. 2019, Bu-Olayan et al. 2020, Celis et al. 2020, Malhotra et al. 2020, Yin et al. 2021). Seabirds can negatively affect local habitats at breeding sites, as seabird guano is an effective vector of contaminants to soils and a major source of biogenic substances, such as organic matter (Liu et al. 2006, Brimble et al. 2009, Espejo et al. 2017a). The objective of the present study was to evaluate how the deposition of Humboldt penguin excreta affects the geochemical composition of some terrestrial areas with emerging contaminants.

A perennial colony of the Humboldt penguin was chosen from the northwestern coast of Chile, located within the Humboldt Penguin National Reserve. The Chañaral Island (29°02′S, 71°35′W), located about 7 km from the coastline and 100 km north of Coquimbo Bay, is a small circular island of about 6.55 km^2, with no trees and only cacti and bushes scattered every-where, sustaining a rockery for Humboldt penguins of approximately 22,000 individuals, frequently visited by tourists (Celis et al. 2014).

Surface soil samples were collected from the top (5 cm) during January 2016, using disposable plastic spatulas directly from nesting sites (manured soils) and control sites (non-manured soils, adjacent to the colonies but unaffected by birds, distant about 30 m). Usually, the colonies were spread out along the cliffs and shores, making it difficult to accurately determine the distance of the control sites from the colony. For analytical procedures, 15 soil samples from nesting sites and 15 from reference sites (control) were taken from each site. All samples were kept in sealed plastic bags and stored in very clean steel containers for transport until their analyses.

In the laboratory, all samples were freeze-dried until dry masses were constant and then homogenized, following the procedure described by Espejo et al. (2017a). Afterward, the samples were heated (oven at 100°C × 12 h) to remove adsorbed water prior to analysis (Fang et al. 2018). Organic C was determined by Walkley-Black wet digestion. Organic matter (OM) was determined as the percentage of organic C multiplied by 1.724. Then, the samples were analyzed for chemical elements according to the EPA method 6200 at the Chemical Laboratory of the Faculty of Agronomy, Universidad de Concepción (Chile), using a portable battery-operated energy dispersive X-ray fluorescence spectrometer (Thermo Scientific Niton XL3t 950 He GOLDD+). The instrument was set up with the instrument tip on a shielded laboratory test stand, which was remotely operated. The blank was a certified 99.99% SiO_2 dioxide analyzed every 20 samples. The precision and accuracy were verified using international reference standards Rare Earth Ore "CGL 124" (USZ-42 Mongolia Central Geological Laboratory), being the precision <2% and accuracy within 1-5% (Table 1).

Statistical analyses were done using SPSS version 15.0 software. Before analyzing the data, the Shapiro-Wilk test was done to determine the normality of the data. Since it did not pass normality, the Mann-Whitney test was used to determine the central tendency of the two groups (nesting *vs*. control). An α of 0.05 was used to assess significance. The differences were considered to indicate statistical significance when $P < 0.05$.

The lack of similar studies makes it very difficult to analyze our results. The data obtained here showed that the state of La, Ce, Pr, Nd, and Nb enrichment was higher in the ornithogenic soils sampled than in control sites, with Y being an exception (Table 2, $P > 0.05$). The highest REE levels here corresponded to Pr (320.8 ± 98.7 μg g^{-1}) and the lowest to Nb (6.1 ± 2 μg g^{-1}). The

Table 1. Average, standard deviation, and acceptable range of the certified reference material for Rare Earth Ore "CGL 124" (USZ-42 Mongolia Central Geological Laboratory). Ce: cerium, La: lanthanum, Nd: neodymium, Pr: praseodymium, Y: yttrium.

Elements	Unit	Average	Standard deviation	Acceptable range
Ce	% m m^{-1}	2.70	0.04	2.71 - 2.81
La	% m m^{-1}	2.01	0.01	2.00 - 2.22
Nd	% m m^{-1}	0.66	0.009	0.62 - 0.68
Pr	% m m^{-1}	0.23	0.005	0.2 - 0.26
Y	mg kg^{-1}	150	1.41	147 - 187

Table 2. Concentrations of trace and rare earth elements (µg g^{-1} dw) in surface soils (n = 15) from Chañaral Island, northwestern Chile (nesting site: ornithogenic soils; control site: soils not having birds). Different letters between collecting sites indicate significance at $P < 0.05$. Ce: cerium, La: lanthanum, Nb: niobium, Nd: neodymium, Pr: praseodymium, Y: yttrium. SD: standard deviation.

Elements	Nesting site		Control site	
	Mean ± SD	Max-Min	Mean ± SD	Max-Min
Ce	164.3 ± 62.1[a]	280.8 - 126.9	143.2 ± 82[a]	238.6 - 121.9
La	165.6 ± 81.3[a]	252.7 - 235.5	122.1 ± 94.9[a]	238.7 - 110.3
Nb	6.1 ± 2.0[a]	8.8 - 3.0	5.6 ± 1.5[a]	8.3 - 3.5
Nd	284.2 ± 99[a]	444.5 - 217.1	195.1 ± 201.3[a]	541.6 - 235.7
Pr	320.8 ± 98.7[a]	518.8 - 206.7	273.1 ± 123[a]	492 - 158.9
Y	23 ± 2.8[a]	28.7 - 17	26.3 ± 4.4[a]	32.7 - 19.1

following relations among REE levels were found in nesting sites: Pr > Nd > Ce > La > Y > Nb. In the control sites, the relations were: Nd > Pr > La > Ce > Y > Nd. Our findings differ a little from Nie et al. (2014), who reported a relation Ce > La > Nd > Pr > Y in ornithological soils from Antarctica.

Our concentrations of La and Nd found in the control soil (Table 2) are higher than La (134-77 µg g^{-1}) and Nd (98-51 µg g^{-1}) levels, whereas our Ce values are lower than the contents of Ce (291-216 µg g^{-1}) reported by Smith et al. (2000) for soils in Uganda. The Pr and Nb values in the control soil (Table 2) are higher than those levels found in Spain (32-1.3 µg g^{-1}, Ramos et al. 2016) and Finland (5-0.15 µg g^{-1}, Ladenberger et al. 2013), respectively. The range of Y is within those reported globally (169-3.8 µg g^{-1}, Laul et al. 1979). In soils, the natural levels of REE depend highly on the parent material, decreasing as follows: granite > basalt > sandstone (Zhu & Liu 1988). The texture of coastal soils of northern Chile is typically sandy loam or sand with gravel and stones (UChile 2013). However, environmental factors (rain, snow, wind, bio-transport by birds) and anthropogenic activities can contribute to these contents (Ramos et al. 2016, Espejo et al. 2017a).

When birds excrete, they can enrich the soil where they nest with REE and nutrients, which tend to remain adsorbed to soil particles. However, La, Y, and Pr elements can be released as soil pH decreases (Jones 1997, Ramos et al. 2016). In addition, a soil enrichment ($P < 0.05$) in macronutrients (such as NO_3, K, and P), OM, and acidity at sites with increased Humboldt penguin influence was found in Chañaral Island previously by Espejo et al. (2017a). Ornothogenic soils of Chañaral Island showed several significant correlations among the levels of REE (Fig. 1, $P < 0.05$). The concentrations of REE had significant positive correlations between La-Ce, La-Pr, La-Nd, Ce-Pr, Ce-Nd, Ce-Y, Pr-Nd, and Pr-Y. A positive relationship may indicate the same source or is closely related for each pair of elements.

Some studies have evidenced correlations between trace element levels and organic matter content, stating that organic matter plays a role in either remobilization (negative) or retention (positive) of certain elements in soils (Castro et al. 2021). According to Nie et al. (2014), negative correlations may be due to a dilution effect for REE in ornithogenic sediments. Here, we observed no correlations between OM and trace/REE (Fig. 2). More studies are required to elucidate this subject.

REE have become important metals in modern technologies, but discarding waste containing REE may increase the contamination in water bodies (Malhotra et al. 2020). Heavy metals in penguins occur mainly through food intake (Espejo et al. 2017b). Therefore, the entry of REE into the organism may also occur similarly (Malhotra et al. 2020). Additionally, physiological parameters may influence the elements to be available as freely dissolved ions or particle-bound metals (Drexler et al. 2003), an issue that needs deeper investigation.

Some evidence has shown that the mobility and bioavailability of REE in excreta deposited on land could be promoted by containing high organic carbon and low pH (Yin et al. 2020). The increased organic matter and acidity of the soil in the nesting sites found by Espejo et al. (2017a) in the same area can enhance the mobility and bioavailability of REE, which could affect some terrestrial invertebrate organisms, such as reproduction alterations and reduced locomotion (Egler et al. 2022). These results could contribute valuable knowledge about the potential for penguins to be vectors of lesser-known elements from the sea to the land, which can help to perform more accurate risk assessments through further studies.

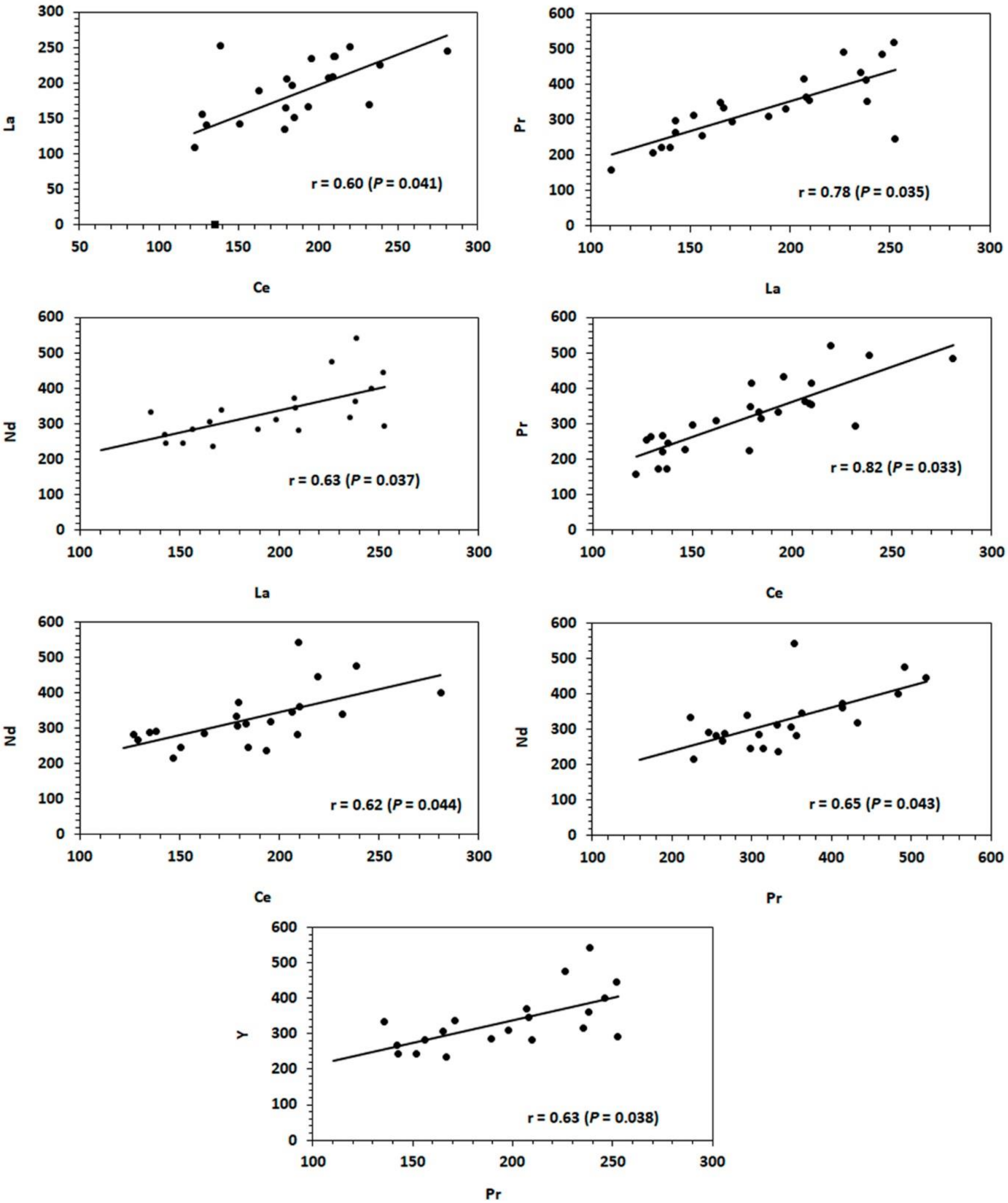

Figure 1. Significant relationships between the contents (μg g^{-1}) of rare earth elements. Lines correspond to the best-fit regression ones. Ce: cerium, La: lanthanum, Nd: neodymium, Pr: praseodymium, Y: yttrium.

Although there were no significant differences between the REE content for the sites studied (nesting *vs*. control soils), this was probably due to the small sample numbers. In any case, these findings may contribute valuable knowledge about the potential for penguins to be vectors of lesser-known elements from the sea to the land to perform more accurate risk assessments through further studies (Espejo et al. 2017b).

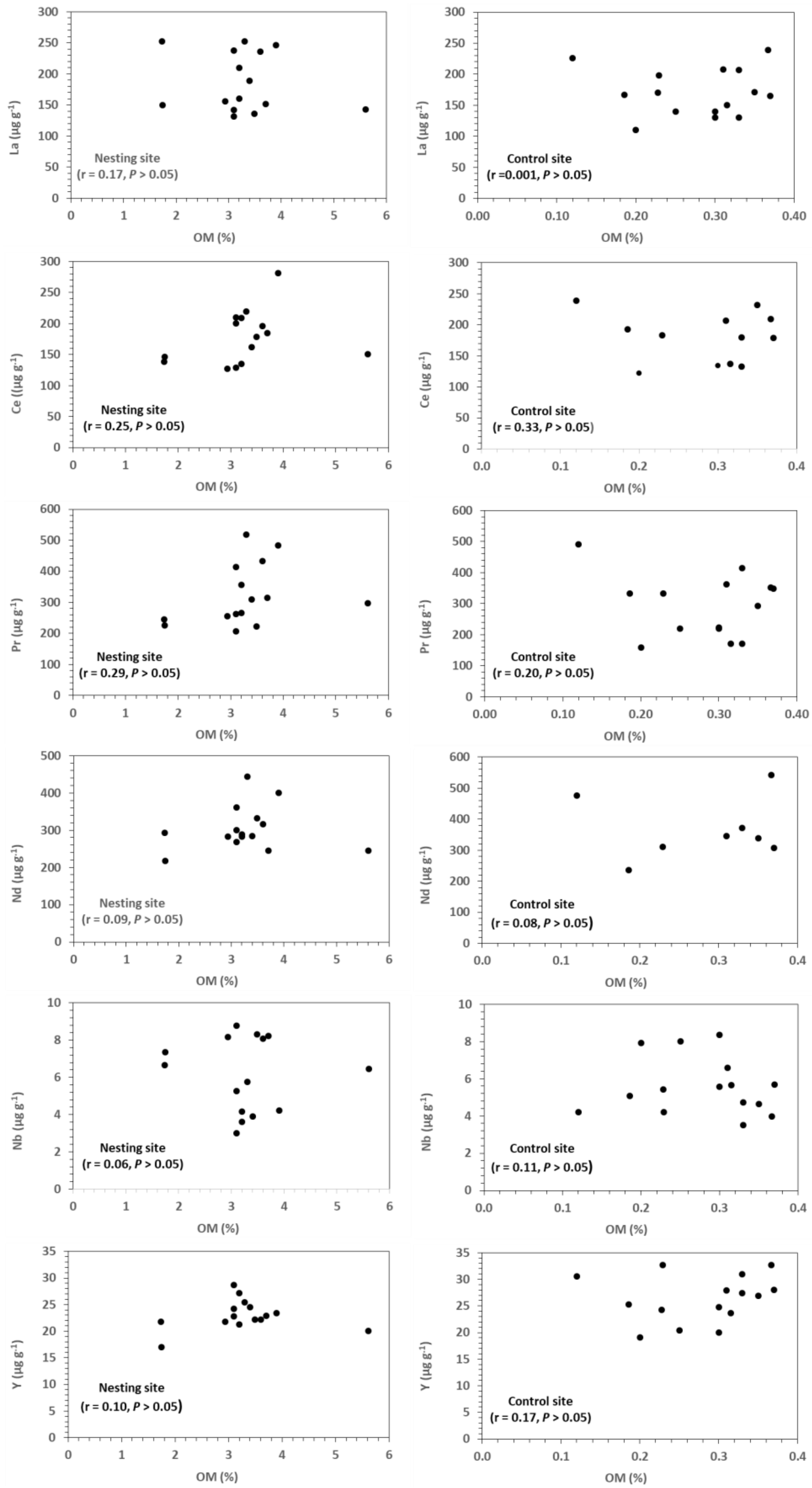

Figure 2. Relationships between trace and rare earth elements *vs.* soil organic matter (OM) content in nesting and control sites from Chañaral Island. Ce: cerium, La: lanthanum, Nd: neodymium, Pr: praseodymium, Nb: niobium, Y: yttrium.

Even though it is necessary to keep in mind that these are wild species that live in protected areas, more studies with increased sample size are needed to understand better seabirds' influence on rare earth element levels in soils.

Evidence shows that heavy metal contamination can promote bacterial resistance in marine and terrestrial environments (Baker-Austin et al. 2006, Espejo et al. 2017c). Similarly, the enrichment of soils with REE might enhance bacterial resistance to certain elements, a topic that needs to be investigated deeper. Additionally, certain interactions between REE and other emerging contaminants (e.g. microplastics) could cause potentially more risky combined impacts on ecosystem health (Cao et al. 2021). Thus, more research should be conducted to assess the ecological and human health risk related to the presence of REE in soils is required.

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