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# Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater

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# **Highlights**

- Guano contains leachable elements that are locally important for primary production.
- Released concentrations can be useful to feed Southern Ocean biogeochemical models.
- Kinetic release is modelled considering two pools in penguin guano compartment.
- Similar release % to sea- and snow-water are observed in both penguin species.

Total estimated kg/yr released in the order Cu>>Zn>Fe ≈Mn >As>>Ni>Mo>>Cd>Co.

# Abstract

The present work extends the scope of prior studies through analysis, modelling and simulation of the As, Cd, Co, Cu, Fe Mn, Mo, Ni and Zn release from Gentoo (*[Pygoscelis](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pygoscelis) papua*) and Chinstrap (*Pygoscelis antarcticus*) penguin guano to the [Southern Ocean](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/southern-ocean) seawater and to Antarctic snow [meltwater](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/meltwater). Laboratory experimental results have been modelled considering kinetic processes between water and guano using two element pools in the guano compartment; its application allows us to interpret behaviours and predict release concentrations of dissolved trace elements from guano which are potentially useful for incorporation as elements source into biogeochemical models applied in the Southern Ocean. Variations in quantities and release patterns depending on the type of guano and aqueous medium in contact have been identified. The release percentages from the guano to the aqueous medium, once the steady state has been reached, vary depending on the water medium and guano type in the ranges of 100–2.9% for Mo; 91.5–68.6% for Ni; 81.8–22.8% As; 52.0–43.9% Cu; 26.9–7.4% Mn; 24.9–5.4 for Co; 4.4–3.2% for Zn and 0.94–0.51% for Fe. Considering a penguin population of 774,000 Gentoo and 8,000,000 Chinstrap, the estimated annual mass released to the both seawater and freshwater would be ≈18,500kg for Cu, ≈1710kg for Zn, ≈1944kg for Fe, ≈1640kg for Mn, ≈499kg for As, ≈289kg for Ni, ≈155kg for Mo, ≈36.7kg for Cd and ≈8.1kg for Co. These contributions can be locally significant both in promoting *phytoplankton* growth and in their role as inhibitors of primary productivity.

# Graphical abstract



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### Keywords

Southern Ocean; Penguin excreta; Metalloids; Modelling; Annual flux; Dissolved fraction

# 1. Introduction

<span id="page-2-7"></span><span id="page-2-5"></span><span id="page-2-1"></span>Penguins play a key role in the [biogeochemical cycles](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/biogeochemical-cycle) of Antarctica. They effectively represent the **Antarctic ecosystem** by dominating the aquatic **[avifauna](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/avifauna)**, both in terms of their abundance (about 16 million individuals) and their widespread distribution ( Sparaventi et al., 2021). Their specialization in swimming and diving allows them to cover larger oceanic regions than other [bird species](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/bird-specie). Additionally, they feed within the top 100m of the water column (Wilson, 1989; Mahadevan, 2016), effectively representing the ocean surface, and furthermore, traverse long distances, facilitating the distribution of chemical compounds and elements that they excrete. Nevertheless, they nest on land and during the breeding period, potentially acting as biotransporters of trace elements and nutrients in the form of guano from ocean to terrestrial and lacustrine ecosystems ( Tovar-Sanchez et al., 2009; Emslie et al., 2014; Wing et al., 2014, Wing et al., 2017; Ratnarajah et al., 2018).

<span id="page-2-9"></span><span id="page-2-8"></span><span id="page-2-6"></span><span id="page-2-4"></span><span id="page-2-3"></span><span id="page-2-2"></span><span id="page-2-0"></span>The main food source of *[Pygoscelis](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pygoscelis)* penguins is [Antarctic krill](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/euphausia-superba) (*Euphausia superba*) which plays a crucial role in the [Southern Ocean](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/southern-ocean) ecosystem as a [keystone species.](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/keystone-species) Its significance stems from its dual role as a primary producer herbivore and a primary prey item for various [marine vertebrates](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/marine-vertebrate) in the Antarctic. This species serves as a vital link in the transfer of energy and nutrients through the food web. [Krill's](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/krill) importance in the ecosystem is highlighted by its position as a primary consumer of [phytoplankton](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phytoplankton) and other microscopic organisms. By feeding on these primary producers, krill helps regulate their populations and influences the overall productivity of the Southern Ocean. Additionally, krill serves as a major food source for several key predators in the region, including penguins, seals, whales, and fish. The abundance of krill directly impacts the populations and behaviours of these higher-order predators, making it essential for the stability and functioning of the Antarctic ecosystem. The central dependence of higher [trophic levels](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/trophic-level) on krill is unique to the Antarctic, highlighting the crucial role of Antarctic krill in [biogeochemical cycles](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/biogeochemical-cycle) ( Murphy et al., 2012; McCormack et al., 2021). Furthermore, certain elements concentrated

<span id="page-3-17"></span><span id="page-3-13"></span><span id="page-3-3"></span><span id="page-3-2"></span><span id="page-3-1"></span>in krill have been found to be transferred to higher-order marine animals upon consumption. Metals and other trace elements accumulated in krill (Chu et al., 2019a; Tovar-Sánchez et al., 2019; Wing et al., 2021; Sparaventi et al., 2021; Celis et al., 2015, Celis et al., 2023) can be recycled through the excretion of excess elements into the [surface](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/surface-water) [waters](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/surface-water) of the Southern Ocean by predators. This recycling process contributes to nutrient cycling and the redistribution of essential elements within the ecosystem of the Southern Ocean (Deheyn et al., 2005; Tovar-Sanchez et al., 2007; Ratnarajah et al., 2014, Ratnarajah et al., 2018). Despite being rich in nutrients, this region exhibit slow [phytoplankton](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/phytoplankton) biomass due to the limited presence of trace metals, particularly iron, which governs phytoplankton productivity and community structure (Wadley et al., 2014; Shatova et al., 2017; Viljoen et al., 2018).

<span id="page-3-16"></span><span id="page-3-15"></span><span id="page-3-14"></span><span id="page-3-12"></span><span id="page-3-11"></span><span id="page-3-4"></span>Some of the leached elements from penguin guano occur naturally in the ocean, are critical for marine life and therefore influence the functioning of ocean ecosystems and the global [carbon cycle](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/carbon-cycle). For instance, Cd, Co, Cu, Fe, Mn and Zn are critical for marine life and hence influence the functioning of ocean ecosystems and the global carbon cycle. Fe in particular plays an important role in supporting [primary production](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/primary-production) and nutrient consumption, with Fe limitation thought to be a major control on biogeochemical processes in the region ( Frew et al., 2001; Gall et al., 2001a, Gall et al., 2001b). In excess, these elements show a dual role and can, like other elements such as As, Hg and Pb among others negatively affect the health of ecosystems (Fraústo da Silva and Williams, 1991; Morel et al., 2003; Morel and Price, 2003).

<span id="page-3-10"></span><span id="page-3-9"></span><span id="page-3-8"></span><span id="page-3-7"></span><span id="page-3-6"></span><span id="page-3-5"></span>Most of the droppings of penguins are deposited either in the ocean during swimming or in [breeding sites](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/breeding-site) during the reproductive season. Nesting sites can be located in coastal or in inland areas at different elevation and distance to the coast, that could show anyways flat or steep terrain. Both during the breeding season and the non-breading season, guano can be in contact with rainwater, melted snow and runoff from [glaciers](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/glaciers) which wash elements, compounds and particles to the surrounding areas. The release compounds from guano can percolate into the terrain, be runoff to lacustrine areas or reach the sea depending on multiple environmental, terrain and biological combined factors (Ratnarajah et al., 2018; Sparaventi et al., 2021).

<span id="page-3-0"></span>The large volume of guano deposited by seabirds in Antarctic ornithogenic soils brings considerable amounts of exogenous elements from marine sources, and thus these soils incorporate trace metals and nutrients. This elements transference is growing due to the increased levels of surface [water runoff](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/water-runoff) (from snow/glacial melt) and/or melting of layers of [permafrost](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/permafrost) during summer that percolates through the soil (Celis et al., 2014;

<span id="page-4-13"></span><span id="page-4-12"></span><span id="page-4-8"></span><span id="page-4-1"></span>Romaniuk et al., 2018; Castro et al., 2021; Rodrigues et al., 2021; García-Veira et al., 2024). It is known that the large colonies on land and the huge amount of discharged guano significantly influences the structure of terrestrial flora communities, by enhancing biodiversity or creating zonation pattern of cyanobacteria, mosses and lichens (Ellis, 2005; Smykla et al., 2007; Cipro et al., 2018; Otero et al., 2018).

<span id="page-4-18"></span><span id="page-4-17"></span><span id="page-4-16"></span><span id="page-4-11"></span><span id="page-4-10"></span><span id="page-4-9"></span><span id="page-4-7"></span><span id="page-4-6"></span><span id="page-4-4"></span><span id="page-4-3"></span><span id="page-4-2"></span>In addition to the soil, penguin droppings could be directly discharged into the lakes, ponds and [meltwater](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/meltwater) streams, deposited into the lake by ice or snowmelt water or by the strong winds that can spread guano particles; elements in guano likely become transfer to the water and to the [lacustrine sediments](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/lacustrine-deposit) (Sun and Xie, 2001; Chu et al., 2019a; de La Peña-Lastra, 2021). Several authors (e.g., Zale, 1994; Chu et al., 2019b; Emslie et al., 2014; Martínez Cortizas et al., 2014; Chen et al., 2020; Joju et al., 2024) analyse nutrient and chemical contaminant contents in Antarctic lacustrine surface (0–2cm), short (≈20–60cm) and long (up to 300cm) [sediment cores](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/sediment-core). They observe significantly higher concentrations in samples than background levels from lakes not associated with penguin colonies, due to the transport of guano into the water system through runoff or dissolution. Lakes, where sediment accumulates near penguin colonies, also preserve biological and geochemical evidence of former breeding colonies and have been referred to as ornithogenic sediment. Mineral bio-element analyses on these kinds of sediments have been and will continue to be used to add new information on the long-term record of penguin occupations in Antarctica (Emslie et al., 2014).

<span id="page-4-15"></span><span id="page-4-5"></span>Trace elements and nutrient runoff from penguin colonies reach the surrounding seawater in the vicinity of island-based seabird colonies and may remain bioavailable for phytoplankton growth (Shatova et al., 2016). Leached material from guano can also influence the sea spray aerosol production in Antarctic coastal areas (Dall'Osto et al., 2022). The leaching of elements from guano, whether in soluble or colloidal forms, depends on the size of the fecal particles and the speciation of the elements. Additionally, processes such as the dissolution of elements, particle fragmentation over time, complexation by organic ligands, oxidation/reduction, and removal from the aqueous phase (through precipitation or sorption) also play an important role.

<span id="page-4-14"></span><span id="page-4-0"></span>Using measurements of trace metals in surface water influenced by penguin colonies, Sparaventi et al. (2021) estimated an annual release during the breeding season of 28, 56, 4, and 29t of Cu, Fe, Mn, and Zn respectively, for Chinstrap, Adélie (*[Pygoscelis adeliae](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pygoscelis-adeliae)*), and Gentoo penguins. More recently, Belyaev et al. (2023) estimated that the global Antarctic Chinstrap penguin population recycles 521t of iron per year into Antarctic waters. A previous work of the authors (Ruiz Gutiérrez et al., 2023) presents a two metal(loid)s pools

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kinetic model to interpret the release pattern and to estimate the quantitative contribution of dissolved trace metal(loid)s from Gentoo penguins guano to Antarctic seawater.

<span id="page-5-12"></span><span id="page-5-9"></span><span id="page-5-8"></span><span id="page-5-7"></span><span id="page-5-6"></span><span id="page-5-4"></span><span id="page-5-3"></span><span id="page-5-1"></span><span id="page-5-0"></span>Biogeochemical models and ecosystem global models have been widely used to evaluate effect on surface nutrient consumption and net primary production on coastal waters in Southern Ocean (Lancelot et al., 2000; Fujii et al., 2005; Muglia et al., 2017; Verdy and Mazloff, 2017; Nicholson et al., 2019; Person et al., 2021; Robinson et al., 2024). These models can incorporate more than ten distinct Fe supply mechanism ( Boyd and Ellwood, 2010; Boyd et al., 2012) among which are glacial iron supply from ice melt (Lancelot et al., 2009; Death et al., 2014; Wadley et al., 2014; Uchida et al., 2019; Twelves et al., 2021). However, to the best of the authors' knowledge, metal(loid)s enrichment from guano is not incorporated into global-scale ocean biogeochemical models. Alongside this aspect, the large quantities of guano deposited, along with its content of elements both beneficial and potentially harmful to the Antarctic environment, make the study of the elements' mobility both inland and offshore relevant.

<span id="page-5-11"></span><span id="page-5-10"></span><span id="page-5-5"></span><span id="page-5-2"></span>The overall objective of this study is to extend the scope of previous modelling results through the analysis, modelling, and simulation of the As, Cd, Co, Cu, Fe, Mn, Mo, Ni, and Zn release from Gentoo and Chinstrap penguin guano into seawater and snowmelt water. The specific objectives are as follows: i) provide a kinetic model of guano as a source of dissolved elements that can be incorporated into biogeochemical models applicable to the Southern Ocean; ii) determine the maximum amounts mobilized over long times in scenarios of guano in contact with seawater and meltwater; iii) identify variations in quantities and release behaviours depending on the type of guano and aqueous medium in contact; iv) obtain [partition coefficients](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/partition-coefficient) establishing the relative affinity of each element in the aqueous medium; an v) make a preliminary estimate of the annual quantities released into the ocean from the guano of both penguin species.

# 2. Experimental methodology

# 2.1. Penguin guano, seawater and snow meltwater sample collection

Fresh guano samples were collected from a Gentoo penguin colony and a Chinstrap penguin colony located on Livingston Island, South Shetland Islands in February 2022 during the Spanish Antarctic campaign 2021–2022 of the PiMetAn Project. The Gentoo penguin colony is located in Argentina Cove (62°40′11.1″S 60°24′20.8″W) and the Chinstrap penguin colony is located in Miers Bluff (62°43′12.3″S 60°26′11.3″W) [\(Fig. 1\)](#page-6-0). Sampling was carried out manually using a plastic scoop. Composite samples were obtained from several daily

samplings throughout the campaign. The Argentina Cove penguin colony was sampled three times, yielding a total of 2170g; the Miers Bluff penguin colony was sampled twice, yielding a total of 1765g. Sampling was conditioned by the duration of the Antarctic campaign and the availability of access to the penguin colonies. The samples were transported in polyethylene bags to the laboratory of the Antarctic Spanish Base (BAE) Juan Carlos I where they were homogenized, weighed, [moisture content](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/moisture) measured and stored frozen at −20°C and sent to Spain for analysis and use in kinetic experiments.

<span id="page-6-0"></span>

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Fig. 1. Location of the penguin guano and seawater sampling sites (Juan Carlos I Spanish Antarctic Base). Livingston Island, South Shetland Islands, [Antarctica.](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/antarctic-region) Penguin guano sampling; <sup>o</sup> Seawater sampling; <sup>o</sup> Snow water sampling.

Seawater was collected in the vicinity of the BAE Juan Carlos I (62°39'46"S 60°23'20"W) and snow from a snowfield on the slope of Mount Sofia (62°40′11″S 60°22′45″W) [\(Fig. 1\)](#page-6-0). Seawater and snow meltwater (named snow water hereinafter) were filtered through a 22μm pore size nitrocellulose filtration membrane (PTFE cartridge filter, 0.22μm; Sartobran® P-300, Sartorius 5231307H5—OO-B) and frozen at −20°C in 1L acid clean plastic containers for transfer and subsequent chemical analysis in Spain and its use in kinetic experiments.

# 2.2. Trace elements release kinetics experiment and modelling

Duplicate kinetic experiments were performed at the laboratory-controlled conditions. The experimental system was described previously in Ruiz Gutiérrez et al., 2023. In short, the system consisting a stirred glass-made 2-L jacketed vessel and a temperature controller (Polyscience) ([Fig. 2](#page-8-0)a) were used. All the experiments were carried out at a constant temperature of  $1.01\pm0.346^{\circ}$ C in experiments with sea water and at  $2.02\pm0.381^{\circ}$ C in experiments with snow water. Temperature of 1°C was selected as the average temperature measured in the experiments outside the BAE Juan Carlos I and similar to the measurements of the sea water in the vicinity of the Antarctic Base. The temperature of 2°C in the melted snow water experiments is the minimum temperature to prevent the freezing of water at the bottom of the reactor during the kinetic experiments. 6g of wet weight guano were added to the previously temperature conditioned 2L of filtered water.

<span id="page-8-0"></span>

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Fig. 2. (a) Experimental equipment used in the kinetic test (b) kinetic scheme proposed based on Ruiz Gutiérrez et al., 2023.

Samples at 0, 5min, 0.25h, 0.5h, 1h, 2h, 6h, 24h, 36h, 48h, 72h, 96h were taken using a plastic syringe and without stopping the mixing. Each sample was filtered through a 0.22 $\mu$ m pore size nitrocellulose filtration membrane, acidified to pH1.5–2 with HNO<sub>3</sub> and kept at 4°C until analysis. Blanks (seawater and snow water sample without guano at *t*=0) were obtained for each kinetic experiment.

The kinetic scheme presented by the authors in a previous work (Ruiz Gutiérrez et al., 2023) to describe the behaviour of metal(loid)s released from penguin guano (environmental compartment 1) to seawater (environmental compartment 2) has been used in the present work. The kinetic model considers that the elements are associated partially to a "labile pool" and partially to an "equilibrium pool" in the guano (environmental compartment 1). Elements release from both compartments to the seawater occurs according to order 1 parallel kinetic processes at different rates, either under an irreversible process or following an equilibrium process respectively. In this work it is proposed to use this kinetic model to model the release of metal(loid)s from penguin guano of two different species (environmental compartment 1) to seawater or snow water (environmental compartment 2) [\(Fig. 2](#page-8-0)b). The mathematical equations of the model proposed by Ruiz Gutiérrez et al. (2023) are shown in S1, page 1 of the supplementary material.

The resolution of the model and the estimation of the corresponding parameters are completed using [Aspen](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/populus) Custom Modeler software version 14 (AspenTech, Bedford, Massachusetts, USA) which solves rigorous models and simultaneously estimates parameters. The input variables required for solving the proposed model are the volume of water in the reactor, the mass of dry guano and the element rate coefficients. The model determines the element concentration in each guano pool and in water compartment over time. To solve it, it is necessary to input the element concentrations in the guano and water used as initial data, and the element rate coefficients must be estimated from the experimental results. The software used allows solving rigorous models and simultaneously estimating parameters. The adjustment of the model parameters was performed using an NL2SOL algorithm for the least-square minimization of the deviation between the experimental and theoretical data. The [correlation coefficient](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/correlation-coefficient) ( $\mathsf{R}^2$ ), standard deviation ( $\sigma$ ), coefficient of variation (CV), and relative and absolute error were used to check the validity of the model. This tool has been used previously by the authors to model the release of pollutants from sediments to seawater (Martín-Torre et al., 2015, Martín-Torre et al., 2016, Martín-Torre et al., 2017), to model the release pattern and the contribution of trace metals and inorganic nutrients from [sunscreens](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/sunscreen) to coastal marine waters ( Rodríguez-Romero et al., 2019, Rodríguez-Romero et al., 2022) as well as in Ruiz Gutiérrez et al. (2023) for the description of the metals release from guano to seawater.

# <span id="page-9-4"></span><span id="page-9-3"></span><span id="page-9-2"></span><span id="page-9-1"></span><span id="page-9-0"></span>2.3. Analysis

<span id="page-9-5"></span>In the laboratory, the guano samples were lyophilized and the elements (As, Cd, Co, Cu, Fe, Mn, Mo, Ni, and Zn) were extracted with a microwave [acid digestion](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/acid-digestion) system (MARS-V, CEM) in accordance with the SW-846 EPA Method 3051A (USA EPA, 2007). Trace elements extraction involved the digestion of approx. 0.2g of guano sample with 10mL of nitric acid (65%, Suprapur quality) in Teflon vessels. After digestion, samples were diluted to 45mL using Milli-Q water and then analysed using an inductively coupled plasma-mass spectrometry (ICP-MS, iCAP Thermo). Blanks and certified material for digestion and analysis were treated like the samples. The accuracy of the analytical procedure was checked using a certified reference material (Lobster [hepatopancreas](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/hepatopancreas) TORT-2, NRC-CNRC), with concentration measured (μg/g dry weight) and recoveries (%) for the certificated values of As: 22.96 (106.3%); Cd: 30.4 (114.05%); Co: 0.53 (104.6%); Cu: 100.7 (95.0%); Fe: 95.8 (91.3%); Mn: 13.81 (101.5%); Mo: 0.84 (88.9%); Ni: 2.4 (95.1%); and Zn: 192.0 (106.7%).

Concentration of trace elements in seawater were determined directly by ICP-MS (PerkinElmer ELAN DRC-e) previous dilution 1:10 (*v*/v) with 0.5M ultrapure nitric acid to avoid salt matrix interferences in the ICP- MS. During the analysis samples were spiked with Rhodium as internal standard to correct for variability between the calibration standards and the samples and improve the accuracy of the analysis.

The concentration of elements in kinetic experiments samples were determined by the same procedure as for the seawater and snow water samples.

# 3. Results and discussion

# 3.1. Penguin guano, seawater and snow water composition

The [moisture content](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/moisture) of the guano of each penguin species was determined prior to carrying out the kinetic experiments, resulting in 70.9%±0.725% for Gentoo penguin guano and 76.1%±0.0757% for Chinstrap penguin guano. Moisture content values are necessary to determine the mass content of dry guano added to the reactor in kinetic experiments and performed calculations in a dry basis.

Considering that 6g of wet guano are added in each kinetic experiment, the mass of dry guano will be 1.75g in the experiments with Gentoo penguin guano and 1.43g in the experiments with Chinstrap penguin guano. This means a slightly different Guano Concentration (Dry Guano Mass/Volume) in kinetic experiments: 0.875g/L dry Gentoo guano and 0.715g/L dry Chinstrap guano.

Three subsamples of each penguin guano used in the kinetic experiments were analysed. The average [concentration value](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/concentration-value) ( $\mu$ g/g) of the considered elements is shown in [Fig. 3](#page-11-0)a. Tables S1 and S2 in the supplementary material shows the concentration values measured in each subsample, the mean value, the standard deviation and the coefficient of variation.

<span id="page-11-0"></span>

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Fig. 3. Elements content of a) Gentoo and Chinstrap penguin guano, b) filtered (<0.22 um) seawater and snow water used in kinetic experiments. The errors bars represent the standard deviations.

Among the guano from different studied species, greater variations are observed in the results obtained with Gentoo penguin guano than with Chinstrap penguin guano (except for Cd). The coefficients of variation (CV), except for Cd, are higher in Gentoo penguin guano which shows greater heterogeneity between the analysed samples. The greatest variations

<span id="page-12-2"></span><span id="page-12-1"></span><span id="page-12-0"></span>are observed for Fe in both types of guano (CV: 29.4% in Gentoo penguin guano and CV: 12.4% in Chinstrap penguin's guano). Penguins, mainly of *[Pygoscelis](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pygoscelis)* species, can be classified as krill-dependent (Hill et al., 2007). Several authors have highlighted the high concentrations of iron in [krill](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/krill) as well as its significant role in iron cycling in the Southern Ocean (e.g., Tovar-Sanchez et al., 2007; Nicol et al., 2010; Ratnarajah et al., 2014; Sparaventi et al., 2021; Mirzoeva et al., 2022). Although the dependence of both species on krill is similar, their different [foraging behaviour](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/foraging-behavior) (Wilson and Peters, 1999) may lead to high variations in accumulated Fe in guano depending on the species and feeding site. For the other elements in both guanos the variations are <20%. The same order of magnitude of the average elements contents is obtained for both penguin species. One-way analysis of variance (ANOVA) shows a statistically significant difference (*p*<0.05) in averages of Cd, Cu, Mo and Zn concentrations in terms of guano type.

<span id="page-12-3"></span>Three and four seawater and snow water samples respectively corresponding to *t*=0 of the duplicate kinetic experiments were analysed. [Fig. 3](#page-11-0)b and Tables S3 and S4 in supplementary material show the average concentration values of the studied elements in both samples, the standard deviation and the coefficient of variation. The concentrations of the elements in snow water are lower than those measured in seawater. Except for Cd, Co and Mn, higher CV values are observed in the analysed seawater samples.

# 3.2. Released concentrations of the elements

The experimental results obtained for the elements release from Gentoo and Chinstrap penguin guano to seawater and snow water are shown in [Fig. 4](#page-13-0). The detail of the evolution in short times (up to 2h) is also shown. Mo concentration evolution is shown on two scales given the great difference in the obtained concentration with seawater and snow water.

<span id="page-13-0"></span>

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Fig. 4. Evolution of the concentration of released elements (μg/L) over time. Experimental data:  $(\bullet)$  using Gentoo penguin guano and sea water,  $(\bullet)$  using gentoo penguin guano and snow water,  $(\cdot)$  using Chinstrap penguin guano and sea water,  $(\cdot)$  using Chinstrap penguin 9/8/24, 5:40 PM Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater - ScienceDirect

guano and snow water. Simulated curves with the proposed model: ( ) using sea water, (**1990)** using snow water.

The results obtained show that the release of elements follow the same trend for the guano of the both species of penguins. Except for Fe and Zn, the concentration of the element increases, to a greater or lesser extent, until reaching an equilibrium value. Differences in behaviour are observed in the release rate and final equilibrium values depending on the species. In Fe and Zn, a rapid release of the element is observed and a subsequent decrease to an equilibrium value. Similar behaviour has been showed by Martín-Torre et al., 2015 in the trace metals release from contaminated [estuarine sediments](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/estuarine-sediment) using acidified water at pH between 4 and 8. In the same way, Martín-Torre et al., 2016 when studying the mobility of Fe from a contaminated sediment to acidified sea water concludes that the Fe, once released, was rapidly oxidized and precipitated as Fe (III), decreasing the available amounts in solution.

Comparing the results obtained with different waters, it is observed that with both Gentoo penguin guano and Chinstrap penguin guano, the released concentrations of Cd, Co, Mn, Mo, Ni and Zn are higher with seawater than with snow water. This may be due to the combined effect of a higher initial concentration in seawater along with an increased release of these elements. However, for As the concentrations released in the experiments with snow water are higher than in the experiments with seawater for both types of guano. For Cu, it is observed that similar concentrations are reached with both waters in the experiments with Chinstrap penguin guano and a slightly higher release with seawater in the experiments with Gentoo penguin guano. For Fe, similar concentration values are reached at long times with both waters and guanos. However, it is observed that the maximum concentrations reached are higher with seawater for both types of guano. To discuss properly the different release behaviours, it is necessary to analyse the kinetics of mobility by eliminating the effect of initial concentrations in both of them the water and the guano. This will be addressed in [Section 3.4,](#page-19-0) where the fractions of released elements will be studied.

Comparing the results from different guanos, it is observed that elements with statistically significant differences in concentration (see Tables S1 and S2 of the supplementary material) exhibit greater release from the guano with the higher concentration. This indicates a clear relationship between initial concentration and the extent of element release. For Cd and Cu, higher concentration values are observed in experiments conducted with Chinstrap penguin guano, whereas for Mo, the opposite behaviour is observed. For Zn, however, no clear influence is observed, likely due to its rapid decrease to equilibrium values. For those elements whose concentrations in both guanos are not statistically

9/8/24, 5:40 PM Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater - ScienceDirect

different (As, Co, Fe, Mn, and Ni), a similar or slightly higher behaviour is observed in experiments conducted with Gentoo penguin guano, with the effect being more pronounced for As. This could be due to the higher concentration of dry guano used in the Gentoo penguin guano experiments (0.870g dry guano/L) compared to the amount used in the Chinstrap penguin guano experiments (0.715g dry guano/L).

The mobility of elements between environmental compartments obeys multiple behaviours, depending on the main processes involved in their mobilization. Guano, like other environmental compartments, is a heterogeneous material. The trace elements contained in the guano can be linked to different compounds, with greater or lesser strength, depending on their affinity. Therefore, its release behaviour differs depending on the metal(loid)s-remaining guano components interaction and on the physicochemical characteristics of the element emitting and receiving environmental compartments ( Ruiz Gutiérrez et al., 2023). A rapid release and a subsequent decrease in the release of elements, with the possibility of adsorption and/or precipitation at high times, agrees with the results obtained by other authors who have studied the mobility of metals and trace elements between environmental compartments (Cappuyns et al., 2004a, Cappuyns et al., 2004b; Cappuyns and Swennen, 2008a, Cappuyns and Swennen, 2008b; Santos et al., 2010; Martín-Torre et al., 2016, Martín-Torre et al., 2017; Pasquet et al., 2018; Rodríguez-Romero et al., 2019; Klein et al., 2023 among others).

<span id="page-15-11"></span><span id="page-15-10"></span><span id="page-15-9"></span><span id="page-15-8"></span><span id="page-15-7"></span><span id="page-15-6"></span><span id="page-15-4"></span><span id="page-15-3"></span><span id="page-15-2"></span><span id="page-15-1"></span>Crucial biological functions have been identified for studied elements as Fe, Mn, Zn, Co and Cu among others in the growth of the oceanic plankton (Shatova et al., 2016; Roshan et al., 2018, Roshan et al., 2020). However, these elements can be both harmful and beneficial to marine plankton. Authors as Gall et al., 2001a, Gall et al., 2001b and Scharek et al. (1997) conclude that Fe used at concentrations starting from 0.1μg/L, is the most important trace metal for oceanic Antarctic [phytoplankton,](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phytoplankton) and other trace metals such as Mn (0.137  $\mu$ g/L), Co (0.0236 $\mu$ g/L), and Zn (0.131  $\mu$ g/L) appear to be of only minor importance. Brand et al., 1986 shows as relatively low concentrations of free Cu (i.e., [Cu <sup>2+</sup>]<6.36<sup>.</sup>10<sup>-3</sup>µg /l) can hinder plankton growth an even been toxic to eukaryotic [phytoplankton](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/phytoplankton) (Whitfield, 2001); de Baar et al. (1990) concludes that experiments with Cu  $(0.063\,\mu$ g/L) and Cd  $(0.112\,\mu$ g /L) may suppress rather than limit Antarctic phytoplankton growth. In the present work, the concentrations obtained in the kinetic experiments are higher than those reported to have negative effects on oceanic phytoplankton; this highlights the potential local impact of the mobilization of trace elements from guano into the sea.

# <span id="page-15-12"></span><span id="page-15-5"></span><span id="page-15-0"></span>3.3. Kinetic modelling of released concentrations of the elements

The simulated curves using the model proposed by Ruiz Gutiérrez et al., 2023 are shown in [Fig. 4](#page-13-0) together with the experimental data. It can be seen that the curves correctly describe the results obtained at short and long times.

The kinetic coefficients of the equilibrium process of each element i ( $\mathbf{k}_{i,1}$  y  $\mathbf{k}_{i,2}$ ), and the kinetic coefficient of the fast process of element i  $(k_{i,3})$  are shown in [Table 1.](#page-16-0) The standard deviation ( $\sigma$ ), [correlation coefficients](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/correlation-coefficient) ( $\mathbb{R}^2$ ) and the coefficient of variation (CV) between the results estimated by the model and the experimental data are also shown.

<span id="page-16-0"></span>Table 1. Estimated kinetic coefficients  $\mathbf{k}_{i,i}$  for each studied element and  $\sigma$ , R<sup>2</sup>, CV parameters between the experimental released concentrations and simulated using the proposed model.





The  $k_{i,3}$  coefficients, which show the rate of release of the element i from the labile pool, are all non-zero when using snow water ([Table 1b](#page-16-0) and d), except for Mo. However, in the experiments with seawater the  $k_{i,3}$  coefficient is zero for Cd, Co, Mo and As (in the experiments with Chinstrap penguin guano), which indicates that there is no release of these elements from the labile pool. Furthermore, for all elements,  $k_{i,3}$  in the snow water experiments is higher than the obtained coefficients for the seawater experiments. This

9/8/24, 5:40 PM Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater - ScienceDirect

means that the element is released more quickly from the labile pool when using snow water. It may be due to the different physical-chemical characteristics of the waters such as [salinity](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/salinity) and pH.

Comparing the  $k_{i,3}$  values between the experiments carried out with different guanos, it is observed that it is higher in the experiments with Gentoo penguin guano for As, Cd (snow water), Cu, Mn, Ni and Zn, while it is lower for Co (snow water) and Fe. For Cd and Co with sea water  $k_{i,3} = 0$ . On the other hand, it is observed that  $k_{i,3}$  values are higher in the experiments carried out with Gentoo penguin guano than with Chinstrap penguin guano, with the exception of Fe.

The rate of release of element i from the equilibrium pool is related to the coefficients  $k_{i,1}$ and  $k_{i,2}$ . The kinetic coefficient  $k_{i,1}$  represents the rate of the release process, while  $k_{i,2}$ represents the rate of the opposite process. For Ni, Cu in experiments with Gentoo penguin guano and As in experiments with snow water,  $k_{i,1} > k_{i,2}$ , which means that the process is favoured towards the release of the element. In all the other experiments  $k_{i,1} < k_{i,2}$ therefore the reverse process is favoured, that is, towards uptake in the solid.

In the experiments carried out with seawater, it is observed that for Mo  $k_{M_0,2} = k_{M_0,3} = 0$ , which means that all the Mo present in the guano is released into the seawater at a higher speed when the guano comes from Chinstrap penguins. However, for the experiments carried out with snow water  $\mathbf{k}_{\text{Mo},2} \neq 0$ , the release of Mo is described as an equilibrium reaction, strongly shifted towards non-release ( $k_{Mo,2} \gg k_{Mo,1}$ ).

The parity diagram obtained for the validation of the proposed model in terms of the experimental and simulated element concentrations is shown in [Fig. 5.](#page-19-1) The correlation coefficient  $(R^2)$  obtained from the experimental and simulated values by the model and considering the 396 experimental data is 99.9%, which indicates that the proposed model correctly describes the results obtained experimentally. A good fit of the proposed model is also confirmed by the fact that all experimental data are within a relative error of ±20% with respect to those predicted with the model. Only 16 experimental data (4.04%) are >10% different from those obtained with the proposed model.

<span id="page-19-1"></span>

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Fig. 5. Parity plot of the experimental vs. simulated released elements concentrations.

# 3.4. Released fraction of the elements

<span id="page-19-0"></span>The graphical representations shown in [Fig. 4](#page-13-0) show the released concentration evolution over time. However, the initial concentration of the elements in seawater, snow water and guanos are different [\(Fig. 3\)](#page-11-0). The moisture content of the guano used also changes (70.9% Gentoo guano and 76.1% Chinstrap guano), so the mass of dry guano used in each experiment differs.

To avoid the effect of these variables on the time behaviour of concentrations, we consider the fraction released from element i to the aqueous medium  $(x_{r,i})$  defined as:

<span id="page-19-2"></span>
$$
\boldsymbol{x}_{\mathrm{r},i} = 1 - \left[ \tfrac{M_{\mathrm{i},\mathrm{in}} - M_{\mathrm{i}}}{M_{\mathrm{i},\mathrm{in}}} \right]_{\mathrm{guano}} = 1 - \left[ \tfrac{C_{\mathrm{i},\mathrm{in}}^{\mathrm{G}} \bullet M_{\mathrm{G}} - \left(C_{\mathrm{i}} - C \boldsymbol{w}_{\mathrm{i}}\right) \, \boldsymbol{V}}{C_{\mathrm{i},\mathrm{in}}^{\mathrm{G}} \bullet M_{\mathrm{G}}} \right]_{\mathrm{guano}} \tag{1}
$$

where  $M_{i,in}$  is the initial content of element i in guano and  $M_i$  the content of element i in guano.  $M_{i, in}$  is determined multiplying the measured initial concentration of element i in the guano  $(C_{i, in}^G)$  by the mass of guano in each experiment  $(M_G)$   $(M_{i, in} = C_{i, in}^G \bullet M_G)$ .

 $M_i$  is calculated multiplying the difference in the aqueous concentration of element i at each moment  $(C_i)$  and its initial concentration in the aqueous phase  $(Cw_i)$  by the volume  $(V)$   $(M_i = (C_i - Cw_i) V)$ .

[Fig. 6](#page-21-0) shows the evolution of the release fraction  $\mathbf{x}_{r,i}$ . The simulated curves are obtained with the proposed model by adding Eq. [\(1\)](#page-19-2). A greater release of As, Cd, Fe Mo, Ni and Zn (seawater) is observed in the experiments carried out with Chinstrap penguins' guano. Comparing the experiments carried out with sea water and snow water, greater release of As, Co, Fe, Ni with snow water is observed in the experiments carried out with both types of guano, of Cd and Zn with Gentoo penguin guano and of Cu with Chinstrap penguin guano.

<span id="page-21-0"></span>

Time (h)

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Fig. 6. Evolution of the released fraction of the elements studied. Experimental data:  $(°)$ using Gentoo penguin guano and sea water,  $\left( \mathbb{I} \right)$  using Gentoo penguin guano and snow water,  $($ <sup>o</sup>) using Chinstrap penguin guano and sea water,  $($ <sup>o</sup>) using Chinstrap penguin guano 9/8/24, 5:40 PM Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater - ScienceDirect

and snow water. Simulated curves with the proposed model: ( ) using sea water,  $\left($   $\frac{1}{2}$  ) using snow water.

The release fractions of Cu, Fe, Ni and Zn follow similar trends in the experiments carried out with both guanos and both types of water. If the release percentages are determined at time =96h for these elements, ranges of 45.0–52.0% for Cu, 0.509–0.938% for Fe, 68.6–91.5% for Ni and 3.22–4.39% for Zn, are obtained, estimating the coefficient of variation <24.5% (corresponding to Fe). Variations in release percentages are mainly due to differences in initial concentrations between seawater and snow water and different initial concentrations in the guanos used ([Fig. 3](#page-11-0)).

For As and Co, a greater release throughout all the time is observed in the experiments carried out with snow water. The concentration of As and Co in snow water are much lower than in seawater (Concentrations in seawater:  $Cw_{As} = 2.78 \mu g/L$  and  $Cw_{Co} = 0.0928 \mu g/L$ ; and snow water:  $Cw_{As} = 0.0356 \mu g/L$  and  $Cw_{Co} = 0.00301 \mu g/L$  ([Fig. 3](#page-11-0)). However, the concentrations of As and Co observed at time=96h for the experiments in seawater and snow water [\(Fig. 4](#page-13-0)) differ much less than the initial ones in seawater or snow respectively. Therefore, there is a significantly higher release in the snow water experiments, probably due to a higher solubility of these elements.

However, an opposite behaviour is observed in Cd and Mn. The release of these metals is greater in experiments carried out with seawater. The initial concentrations of these metals in seawater are considerably higher: [Cd] $_{\rm{seawater}}$ =0.113µg/L vs. [Cd] $_{\rm{snow\ water}}$ =0.00228µg/L;  ${\rm [Mn]_{seawater}}$ =1.58 $\mu$ g/L vs.  ${\rm [Mn]_{snow\ water}}$ =0.467 $\mu$ g/L. No significant variations are observed in the initial concentration of Cd and Mn in the guanos with coefficients of variation of 21.8% and 21.9% respectively [\(Fig. 3\)](#page-11-0). It seems that the salinity of seawater does not significantly influence Cd and Mn release and that the changes are due to the different physical-chemical characteristics of the guanos.

<span id="page-22-1"></span><span id="page-22-0"></span>The obtained results may be relevant to the ecosystem under study, given that several authors have highlighted the impact of guano on Antarctic penguin colony ecosystems, particularly on freshwater bodies. Román et al. (2023) have observed, through spectral images, small water lagoon covered by [green algae](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chlorophyta) inside a Chinstrap penguin guano stain in Livingston Island. Otero et al. (2018) establish that nutrients can reach small lakes by dissolution in [runoff waters,](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/water-runoff) thus increasing the rate of [primary production](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/primary-production) of plant life in [coastal ecosystems](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/coastal-ecosystem) eutrophizing them with a regional impact; this process can become especially relevant in the Antarctic and Southern Ocean regions, where the majority of the main guano producers, represented by the penguin's population, is concentrated. In the Ross Sea, Liu et al. (2013) conducted a geochemical analysis of 22 bio-elements and carbon isotope distributions in five [sediment cores](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/sediment-core) taken in ponds and lakes near active [Adélie](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/pygoscelis-adeliae) penguin colonies on Ross and Beaufort Islands. The combined analyses provided a strong proxy for the influence of penguin guano versus algal biomass on the [geochemistry](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/geochemistry) of the sediment. Borghini et al. (2007) identifies the leaching of nutrients from the guano of nesting seabirds, among other factors, as responsible for the higher autotrophic biodiversity at Edmonson Point lake. Xiao et al. (2005) found a great impact of input penguin guano into a [lake sediment](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/lacustrine-deposit) on Ardley Island, Antarctica on the [bacterial community](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/bacterial-communities).

<span id="page-23-5"></span><span id="page-23-2"></span>Allende and Izaguirre (2003) study the Lake Boeckella in Hope Bay which exhibits a mesoeutrophic condition due to the input of nutrients of a nearby penguin rookery.

<span id="page-23-4"></span><span id="page-23-1"></span>Sun et al. (2000) demonstrate that deposition of penguin droppings had a significant effect on the geochemical composition of elements as sulphur, phosphorus, calcium, copper, zinc, selenium, strontium, barium and fluorine of the lake sediment core.

# 3.5. Equilibrium analysis

# 3.5.1. Determination of partition coefficients

[Fig. 4,](#page-13-0) [Fig. 6](#page-21-0) show that the steady state has practically been reached, except for Mo at time =96h in the experiments with seawater. That is, the state of equilibrium has been reached. Equilibrium concentrations can be determined by considering the variations of concentration with time equal to zero in the model equations (see S1, page 1, supplementary material). [Table 2](#page-24-0) shows the values of the equilibrium concentrations along with the partition or distribution coefficient for each element  $(Kd_i)$ , as a representation of the equilibrium relationship for the experimental conditions in which the experiments have been carried out:

$$
\mathrm{Kd}_{i} = \frac{\left[C_{i,\mathrm{guano}}\right]_{\mathrm{eq}}}{\left[C_{i,\mathrm{water}}\right]_{\mathrm{eq}}} = \frac{k_{i,2}}{k_{i,1} \bullet C_{\mathrm{guano}}}
$$
(2)

<span id="page-23-3"></span><span id="page-23-0"></span>where  $[C_{i,guano}]_{eq}$  and  $[C_{i,water}]_{eq}$  are the equilibrium concentrations of metal(loid) i in the guano and water respectively. The  $k_{i,1}$ ,  $k_{i,2}$ , are the rate coefficients of element i release by the equilibrium [\(1\),](#page-19-2) [\(2\)](#page-23-0) from equilibrium pool;  $C_{\text{guano}} = M/V$  with M the mass of penguin guano and V the seawater volume at each experiment. The partition coefficient ( $\mathbf{Kd_i}$ ) between sediments and water has been widely applied to determine the migration ability and transformation of elements in the field or lab tests.  $\mathbf{Kd_i}$  is one of the key parameters for assessing the potential migration of an element in the liquid phase that is in contact with a solid matrix (Sedeño-Díaz et al., 2020) being useful for modelling applications.

<span id="page-24-0"></span>Table 2. Equilibrium concentration in water, equilibrium in guano concentration of the studied elements and estimated partition coefficients  $Kd_i$ . Lowest  $Kd_i$  values in red colour and highest values in bold.



The obtained [partition coefficients](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/partition-coefficient)  $Kd_i$ , ([Table 2](#page-24-0)) depend on the nature of the solid and of the water specific characteristics of each element and solid-liquid contact conditions. For a single element,  $Kd_i$  distributions can cover several orders of magnitude. This variability is not surprising due to the complexity of solid-liquid exchanges and also to the empirical nature Boyer et al. (2018).

<span id="page-24-3"></span><span id="page-24-1"></span>According to Yavar Ashayeri and Keshavarzi (2019), and Nabelkova and Kominkova (2012),  $\mathbf{Kd_i}$ , provides crucial information for risk assessment:

- <span id="page-24-2"></span> $-$  Kd<sub>i</sub> > 100L/g indicate elements or compounds with affinity to bind or remain in the solid phase. Fe is the element with the highest  $Kd_i$  value ( $Kd_{Fe} > 100$  $L/g$ ). The Fe content in the guano is initially released and subsequently retained again in the guano, which implies a low Fe content in the final equilibrium state and its high  $\mathrm{Kd}_{\mathrm{Fe}}$  value. No significant influence is observed by the type of water.
- values in the range  $1 \leq K d_i \leq 10 L/g$  correspond to elements released from the solid phase. For Cd, Co, Cu, Mn, Zn, and As with seawater and Mo with snow meltwater,  $1 \leq K d_i \leq 10$ . Cd, Mn, and Zn show a greater influence from the type

9/8/24, 5:40 PM Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater - ScienceDirect

of water, while Co and Cu are more influenced by their initial concentration in the guano.

 $-$  Kd<sub>i</sub> < 1L/g indicate chemicals prevailing in the liquid phase. This is the case of Ni and As in the experiments with snow meltwater. Note that Mo in experiments with sea water is completely released from guano obtaining a value of  $\mathrm{Kd}_{\mathrm{Mo}}=0$ .

# 3.5.2. Equilibrium dependence on the initial water composition

Ruiz Gutiérrez et al. (2023) perform kinetic experiments at laboratory and at external natural conditions of temperature and light with the same Gentoo penguin guano of this work and seawater also coming from the vicinity of the BAE Juan Carlos I. The composition of the guano used match with the used in the present work ([Fig. 3a](#page-11-0), Table S1 of the supplementary material). However, the composition of seawater differs significantly in the concentration of some elements (Table S3 and S5 in the supplementary material). This variation could probably be due to the release of ice from the nearby [glaciers](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/glaciers) that flow into the bay and, depending on the currents, reach the coast near the BAE with the possible specific modification of the seawater composition.

[Fig. 7](#page-26-0) shows the time evolution of the studied elements concentration obtained in this work along with those obtained previously in Ruiz Gutiérrez et al. (2023). Furthermore, the black line represents the simulated curve with the proposed model when the Antarctic data are used, and the red line represents the curve simulated in the present work. It is observed that, for Cu, Fe and Ni, only one line (black one) is represented; this is because the initial concentrations of these elements in both sea waters, the used in Antarctica and the used at Spanish laboratory, are very similar and the behaviour of the three experiments is the same. However, this is not the case for the rest of the elements. The concentrations in the initial seawater are different and reach different concentration values in the final steady state.

<span id="page-26-0"></span>



Fig. 7. Evolution of the concentration of released elements ( $\mu$ g/L) over time in the experiments carried out with Gentoo penguin guano and seawater in this work and in those published in Ruiz Gutiérrez et al., 2023. (•) Results of the [Antarctica](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/antarctic-region) laboratory experiments; ( $\circ$ ) Results of the Antarctica external natural experiments; (<sup> $\bullet$ </sup>) Results of the Laboratory experiments in this study;  $($   $)$  curve simulated with the Antarctic experiments; (**In the experiments**) curve simulated with the experiments of this study.

[Table 3](#page-27-0) shows the Kd<sub>i</sub> values obtained in previous works (Ruiz Gutiérrez et al., 2023) and in this work. For Fe and Ni, the  $Kd_i$  values are similar because the concentrations of these

elements in the seawater used for both experiments (Antarctic and in this study) are similar (see Table S5 of the supplementary material). Cu has a relatively high release; reaches equilibrium values of 63.8μg/L in this study and 65.8μg/L in the Antarctic experiments. The difference in concentration between the initial value and the time of 96h is high enough that changes in the initial concentration of seawater are not significant. However, for the rest of the elements, important differences are observed, which are greater the difference in concentrations in water (Cd and Co) (Table S5 of the supplementary material).



<span id="page-27-0"></span>Table 3. Comparison of the  $\mathbf{Kd_i}$  values obtained in the Antarctic experiments ( Ruiz Gutiérrez et al., 2023) and in this work.

Heterogeneous equilibria commonly obey isotherms described by the Langmuir or [Freundlich equation](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/freundlich-equation) (Kleineidam et al., 2004; Diban et al., 2007;

<span id="page-27-6"></span><span id="page-27-5"></span><span id="page-27-4"></span><span id="page-27-1"></span>Bagal and Raut-Jadhav, 2021; Murphy et al., 2023). That is, the equilibrium situation will depend on the initial conditions such as the composition of the seawater. This explains the different values of  $\mathbf{Kd_i}$  obtained in both studies for Cd, Co, Mn and Zn. Therefore, it is advisable to consider the initial concentrations of the liquid medium in contact with the guano to more accurately estimate the amount of each element released into that medium.

# 3.6. Estimation of the annual released elements

<span id="page-27-7"></span><span id="page-27-3"></span><span id="page-27-2"></span>The Southern Ocean has received particular scientific attention, with several [ocean](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/ocean-fertilisation) [fertilization](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/ocean-fertilisation) experiments due to the high nutrient, low chlorophyll (HNLC) characteristic having the greatest potential to remove CO<sub>2</sub> from the atmosphere (Williamson et al., 2012). de Baar et al. (2005) and Boyd et al. (2007) summarized several in situ artificial Feenrichment experiments in the Southern Ocean highlighting the importance of Fe availability for phytoplankton communities in this region. These iron additions increased

the <u>[surface water](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/surface-water)</u> dissolved iron concentration from 3.35·10<sup>-3</sup>-5.58·10<sup>-3</sup>  $\mu$ g/L to 0.056-0.28μg/L. In situ experiment clearly showed that iron plays an important role in supporting phytoplankton biomass and nutrient consumption with a considerable impact on the microbial components of the food web in HNLC regions (Hall and Safi, 2001). Similar to anthropogenic fertilization, natural sources of iron affected nutrient utilisation and promote phytoplankton growth (Planquette et al., 2007; Mongin et al., 2008; Hernandez-Sanchez et al., 2010).

<span id="page-28-4"></span><span id="page-28-3"></span><span id="page-28-1"></span><span id="page-28-0"></span>Due to the accumulation of high densities of Fe-containing guano in the coastal ecosystem Belyaev et al. (2023) report Fe concentrations of up to  $3 \cdot 10^3$  times higher than the background in the waters surrounding the penguin rookeries. In the same way, our laboratory experiments [\(Fig. 4](#page-13-0)) show Fe concentrations released from guano at equilibrium, two orders of magnitude higher (10μg/L) than those reached in fertilization experiments. However, scarce information exists about the effects that penguin guano may have on Antarctic coastal marine ecosystem communities. Some of the current studies aimed to assess the fertilizing potential of [marine vertebrate](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/marine-vertebrate) excretion products in the waters of the Southern Ocean. For example, the results of guano-enrichment incubation experiments of Shatova et al. (2016), based on desirable final concentration of guano derived Fe in the seawater sample ( $> 0.084 \mu$ g/L), can have a positive effect on primary production by increasing phytoplankton biomass. Similarly, Smith et al. (2013) showed that blue whale faeces with a Fe concentration treatment that ranged from 4375 to 220,267μg/L enhanced the [photosynthetic efficiency](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/photosynthetic-efficiency) and community growth of three common marine Antarctic phytoplankton species. Only one study is aimed to study the toxicity of guano exposure, using the yellow-legged gull guano and the [sea urchin](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/sea-urchin) embryo test (Rial et al., 2016). They concluded that the binding of trace metals through organic ligand reduces the toxicity to marine organisms.

<span id="page-28-6"></span><span id="page-28-5"></span>Therefore, it is to be expected that the amounts of chemical elements released from guano into the water have environmental significance, making a preliminary estimation of these quantities relevant.

According to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species 2020 (IUCN, 2020) the number of individual Gentoo penguins is 774,000 and Chinstrap penguins is 8,000,000.

<span id="page-28-2"></span>According to Sun and Xie, 2001, 84.5g/day guano is excreted by one penguin per day during the breeding period. Furthermore, in this work we consider that the guano deposited directly into the sea is a conservative 10% and the remaining 90% stays on land during summer period of 120days; in addition, we consider that only 10% of the guano in the

breeding sites comes into contact with meltwater, which mobilizes the guano elements towards the sea. The remaining 245days of the non-breeding season, the totality of the produced guano, is considered to be released into the Southern Ocean for calculations (See Fig. S1 in Supplementary material).

Considering the total content of each metal(loid) in the guano,  $C_{i, guano}$ , showed in [Fig. 3a](#page-11-0), the amount of the element released at equilibrium  $[C_{i, guano}]_{ea}$  showed in [Table 2](#page-24-0) and the amount of guano excreted by a penguin per day,  $E$ , the amount of element excreted by a penguin in one day,  $\mathbf{F}_i$ , can be determined:

$$
\mathbf{F}_{i} = \left( \mathbf{C}_{i,\text{guano}} - \left[ \mathbf{C}_{i,\text{guano}} \right]_{\text{eq}} \right) \bullet \mathbf{E} \tag{3}
$$

<span id="page-29-0"></span>Amount released directly to sea water:

$$
M_i^{R,sea} = F_i^{sea} \bullet t^{summary} \bullet PE_i^{sumer} + F_i \bullet t^{winter} \bullet PE_i^{winter}
$$
\n
$$
\tag{4}
$$

Amount released into meltwater from breeding sites to the sea:

$$
M_i^{R,soil} = F_i^{soil} \bullet t^{summary} \bullet (1 - PE_i^{summary}) \bullet x_R
$$
\n
$$
(5)
$$

where  $M_i^{R,sea}$  and  $M_i^{R,soil}$  are the annual amount of element i released from the guano excreted to the sea and to the reproduction sites respectively,  $\mathbf{F}_i^{\text{sea}}$  and  $\mathbf{F}_i^{\text{soil}}$  are the fluxes excreted by the penguins to the sea and to the soil respectively (determined by the Eq. [\(3\)](#page-29-0)),  $t^{\text{summary}}$  and  $t^{\text{winter}}$  the days summer (120days) and winter (245 days) respectively,  $PE_i^{\text{summer}}$  and  $PE_i^{\text{winter}}$  the fraction excreted directly to the sea in summer (0.1) and winter (1) and  $x_R$  the fraction guano excreted to the soil that is contact with meltwater.

[Fig. 8](#page-30-0) shows our estimation of the annual amount of elements released to seawater and snow water by both penguin species. The release percentages of each element are also shown. It is observed that a greater content of Chinstrap penguin guano is released since their number is much greater. The percentages of release to seawater increase as penguins spend more time in the sea, making the direct contribution of guano to seawater for the studied elements significant. This is particularly relevant because penguins feed within the upper 100m of the water column, where primary production and photosynthetic [carbon](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/carbon-fixation) [fixation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/carbon-fixation) by phytoplankton occur (Wilson, 1989; Mahadevan, 2016). As and Co present the highest percentages of release to meltwater. These elements have a much higher release rate to meltwater than to sea water (see [Fig. 6\)](#page-21-0).

<span id="page-30-0"></span>

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Fig. 8. Estimation of the annual amount released by penguin species and water type. Percentage released of each element to each type of water.

<span id="page-31-6"></span><span id="page-31-3"></span>Although the amounts mobilized by snowmelt water range between 0.1% and 14.2% of the total contribution of elements from guano to the ocean [\(Fig. 8](#page-30-0)), this contribution can be locally significant. Additionally, an increase in snow melt-rates and meltwater runoff into the ocean is expected in a scenario of rising atmospheric temperatures (Vaughan, 2006). Several authors have studies snow meltwater surficial runoff and glacial ice melt as a local source of Fe into the ocean; however, authors such as McGillicuddy et al. (2015), highlight the heterogeneity and regionally dependent nature of these iron supply processes along the Antarctic [continental margin.](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/continental-shelf) Authors such as Death et al. (2014), van Wessem et al. (2017), Laufkötter et al. (2018) and Höfer et al. (2019) among others, suggest a significant local contribution of glacial iron and meltwater runoff iron fluxes to the Southern Ocean. Person et al. (2020) and Twelves et al. (2021) demonstrated the importance of the Fe release to surface seawater from melting Antarctic ice as a supply for phytoplankton activity in the Southern Ocean conditioning spatial patterns of productivity.

<span id="page-31-5"></span><span id="page-31-4"></span><span id="page-31-2"></span><span id="page-31-1"></span>If we add the annual content released by both penguin species (both to seawater and snow water) in summer and winter, considering the above-mentioned assumptions, the estimates shown in [Fig. 9](#page-31-0) are obtained. From greater to lesser, the total content released annually follows the following order: Cu, Zn, Fe, Mn, As, Ni, Cd, Co. Highlighting the high release of Cu, which is a degree of magnitude greater than that of Zn.

<span id="page-31-0"></span>

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Fig. 9. Estimation of the total annual amount released content of guano from Gentoo and Chinstrap penguins considering that only 10% of the guano in the [breeding sites](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/breeding-site) comes into contact with [meltwater.](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/meltwater)

Belyaev et al. (2023) considering the same population of Chinstrap penguins, estimates that with an average guano concentration of 3000μg/g Fe, it is recycling 521 tons of iro/year to the Southern Ocean taking into account that all the Fe content is released to seawater. In the present work, unlike the previous one, the release of metal(loid)s is evaluated considering that there are two possible "solvents" reaching the sea (meltwater through runoff and seawater or direct input to the sea) and that the differences between these two aqueous media are significant for certain elements. Therefore, in the global calculations of elements input from penguin guano, both element entry pathways must be bearing in mind. In Belyaev et al. (2023), no study of the kinetics and equilibrium of element release was conducted; instead, the estimation was made by considering the concentration of Fe measured in the water in front of the penguin colony and assuming that this concentration results from a specific discharge of guano into the water. This approach provided a valid initial approximation of the Fe flux from a penguin-inhabited area to the sea, as it considered the total Fe inputs, which, in addition to guano, could come from other solid matrices such as soil, sediments and glacial ice melt (e.g., Uchida et al., 2019; Twelves et al., 2021).

In the present work, the estimation is not based on [in situ measurements](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/in-situ-measurement) but on laboratory experiments, which demonstrate mobilizations of <1% of the Fe in the guano. Our estimations are made based on the following assumptions (see Fig. S1 in Supplementary Information): A maximum iron concentration in guano of 1126μg/g (compared to 3000μg/g reported by Belyaev et al. (2023)); that the guano deposited directly into the sea is a conservative 10% and the remaining 90% stays on land during summer period of 120days; in addition, we consider two possible scenarios of guano contact with meltwater which mobilizes the guano elements towards the sea at the breeding sites: 100% and a conservative 10%. The remaining 245days of the non-breeding season, the totality of the produced guano, is considered to be released into the Southern Ocean for calculations (See Fig. S1 in supplementary material). These assumptions, along with the observed Fe mobility value of <1% in equilibrium, result in different amounts of Fe recycled into the sea.

On the other hand, in the authors' previous work by Ruiz Gutiérrez et al. (2023), only direct input to the sea was considered, unlike in the present study. The input of Fe from guano deposited in the soil, which can be released and transported to the sea, was not considered. In the present work, considering only the input to the sea, a percentage of 0.68 is obtained, compared to 0.88 obtained in the previous work of Ruiz Gutiérrez et al. (2023). This small difference may be due to variations in the concentrations of elements in seawater and the guanos used in the experiments which leads to different equilibrium values, reflected in different values of  $\mathbf{Kd}_{\mathbf{Fe}}$ :  $\mathbf{Kd}_{\mathbf{Fe}}$  = 115L/g in Ruiz Gutiérrez et al., 2023 (Gentoo) in contrast to the values obtained in the present work of  $\mathbf{Kd}_{\mathbf{Fe}}$  = 132L/g (Gentoo) and  $\mathbf{Kd}_{\mathbf{Fe}}$  = 113L/g (Chinstrap).

Depending on the scenario considered for guano-meltwater contact, it is estimated that the total dissolved Fe reaching the seawater would be between 0.75% and 0.51% of the Fe content in the guano. While these amounts are small, the information obtained is valuable for estimating the iron fluxes to the coastal areas from the nesting areas during the summer season. The quantities of Fe released can be a significant source of dissolved Fe locally. In addition, these values are potentially useful for incorporation as elements source into biogeochemical models applied in the Southern Ocean.

These obtained quantities represent an estimation under the previously mentioned assumptions of penguin population, penguin periods on land and at sea, quantities excreted per individual, and the proportion of guano in contact with the liquid medium. The obtained estimations are locally conditioned by penguin diet, meteorological variables, the [orography](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/orography) of penguin colonies and coastal areas, distance from the colony to the sea and [edaphic factors](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/edaphic-factors) among others. Additionally, in a [climate change](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/climate-change) scenario, the availability of liquid water from melting ice and thawing of [permafrost](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/permafrost) soils may increase, promoting the [remobilization](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/remobilization) of trace elements from penguin guano.

### 4. Conclusions

This paper presents the contribution to Antarctic aqueous media, seawater and [inland water](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/inland-water) bodies, of As, Cd, Co, Cu, Fe, Mn, Mo, Ni and Zn released from guano of two different species (Gentoo and Chinstrap) penguins, after contact with seawater and ice or snow meltwater. The release pattern of elements has been interpreted and estimated under laboratorysimulated Antarctic conditions using a kinetic model involving two metal(loid) pools. The release of elements follows the same trend for the guano of the both species of penguins. The concentration of the element increases, to a greater or lesser extent, until reaching an equilibrium value except Fe and Zn which show a rapid release of the element and a subsequent decrease to an equilibrium value. The release percentages from the guano to the aqueous medium once the steady state has been reached are in the range of 100–2.9% for Mo; 91.5–68.6% for Ni; 81.8–22.8% As; 52.0–43.9% Cu; 26.9–7.4% Mn; 24.9–5.43 for Co; 4.4– 3.2% for Zn and 0.94–0.51% for Fe depending on the water medium and guano type.

Under equilibrium conditions the partition coefficient for each element has been obtained. For both types of guano and both aqueous media, Fe shows values higher by an order of magnitude compared to the rest of the studied elements, indicating its limited release to water at the equilibrium conditions. Ni in snow water and Mo in seawater have the lowest

9/8/24, 5:40 PM Kinetic and equilibrium analysis of penguin guano trace elements release to Antarctic seawater and snow meltwater - ScienceDirect

values of the partition coefficient indicating their prevalence in the liquid phase. The comparative analysis with previous values obtained by the authors shows the non-linearity of the equilibrium values and their dependence on the initial conditions of water composition.

Except for Cd, similar release percentages to seawater and snow water are observed in both penguin species. The annual content released by an estimated population of 774,000 Gentoo penguins and 8000,000 Chinstrap penguins is estimated to be ≈18,500kg for Cu, ≈1710kg for Zn, ≈1944kg for Fe, ≈1640kg for Mn, ≈499kg for As, ≈289kg for Ni, ≈155kg for Mo, ≈36.7kg for Cd and ≈8.1kg for Co.

These results represent an estimate of the trace elements release to the sea water, assuming that a penguin excretes 84.5g/day of guano per day along the 120-day breeding period, during which 10% of penguin dropping deposition takes place directly into the sea and the remaining 90% to the ground; consider two possible scenarios of guano contact with meltwater which mobilizes the guano elements towards the sea at the breeding sites: 100% and a conservative 10%. During the remaining 245days of the non-breeding season, all droppings are considered to be released into the sea. Regarding Fe, which is crucial in the process of primary production in the Southern Ocean, its estimated that only ≈0.75% - ≈0.51% of the Fe content in the guano would reach the seawater from both iron sources (direct to seawater and meltwater) depending on the scenario considered for guanomeltwater contact. While these amounts are small, they can be an important source of dissolved Fe locally, which should be considered when feeding biogeochemical models applied in the Southern Ocean. Additionally, the quantities of Fe released to coastal areas from nesting areas during the summer season may have a significant local impact that would be the subject of future study. Another remaining challenge is to evaluate, through in-situ work, the extent of the plume originating from the penguin colonies and to discern the different contributions of elements, especially Fe, in these plumes.

# CRediT authorship contribution statement

**Gema Ruiz-Gutiérrez:** Writing – original draft, Software, Methodology, Formal analysis. **Erica Sparaventi:** Methodology, Investigation, Formal analysis, Data curation. **Berta Galan Corta:** Writing – review & editing, Validation, Investigation. **Antonio Tovar-Sánchez:** Writing – review & editing, Validation, Resources, Funding acquisition. **Javier R. Viguri Fuente:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization.

# Declaration of competing interest

The authors declare that they have no know competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Appendix A. Supplementary data

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Supplementary material

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# Data availability

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