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Penguin guano trace metals release to Antarctic waters: A kinetic modelling

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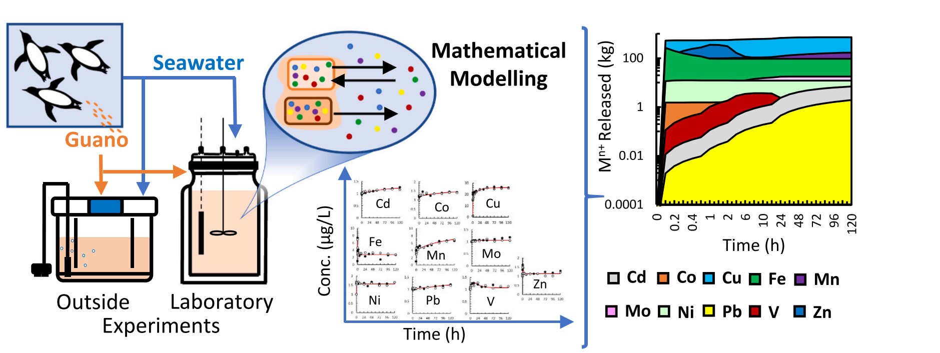
HIGHLIGHTS

- Kinetic modelling of trace metals release from Gentoo penguin guano to seawater
- Released mass of trace metals from Antarctic Gentoo penguin guano is estimated
- Iron and Ni reach 90 % of the maximum release after 10 min in seawater
- Fast Ni, Cu, Mo, Mn, slower Co, Cd, Pb release and Fe, Zn, V release-adsorption
- Releases >90 % of Mo and Cd, 65 %–46 % Co, Ni, Pb and Mn, but only 0.88 % of Fe

GRAPHICAL ABSTRACT



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ABSTRACT

Penguin guano has been considered as a suitable bioindicator of the exposure to environmental contaminants in Antarctic environment. Although trace metal content values in penguin guano have been widely reported, the kinetics of their mobility in seawater have not been determined. In the present study, we have estimated the release rate of dissolved Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, V, and Zn from Gentoo (*Pygoscelis papua*) penguins guano to Antarctic seawater by 120 h laboratory and at external natural conditions of temperature and light experiments. A mathematical model using two metal pools guano (labile and equilibrium) and seawater compartments considering pseudo-first-order kinetics, is proposed in order to interpret and predict the release of trace metals. A good statistical agreement between experimental and modelled concentration values allows us obtention of kinetic parameters and partition coefficients (Kd_i). These values allow to estimate releases into seawater from 5400 to 6.3 µg/day·penguin of Cu and V, respectively. More than 50 % of the initial content of all the studied elements are released during the first two hours, reaching 90 % release in the decreasing order of speed Ni ≫ Cu ≈ Mo > Mn > Co > Cd ≈ Pb; periods of up to one hour, Fe, V and Zn reach a maximum release and are then readsorbed. Equilibrium releases >90 % for Mo and Cd, and 55 % - 46 % for Co, Ni, Pb and Mn are obtained; Zn with 5.4 %, V with 1.7 % and Fe with 0.88 % show the lowest values. With an overwhelming growth of estimated population south of 60°S of 259.750 breeding pairs we estimate that the Gentoo penguin population is releasing annually in

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the Southern Ocean, 716 kg Cu, 188 kg Mn, 113 kg Fe, 102 kg Zn, 17.7 kg Mo, 12.0 kg Ni, 8.70 kg Cd, 4.59 kg Co, 6.27 kg Pb and 0.790 kg V of soluble metals.

1. Introduction

Biogeochemical cycling of nutrients and trace elements are essential for marine ecosystems. Some elements such as cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), among others, are essential for marine life and, therefore, influence the dynamics of ocean ecosystems. On the other hand, these elements can be present in excess, and together with other metals such as lead (Pb), can negatively affect the health of ecosystems.

The Southern Ocean (SO) surrounding the Antarctic continent is known as one of the world's largest "high-nutrient, low chlorophyll" oceans, characterized by an abundance of nutrients such as nitrates, phosphates and silicate, but with low phytoplankton biomass due to the scarcity of trace metals, especially Fe, which controls the productivity of phytoplankton and the structure of the community (Lancelot et al., 2009; Boyd et al., 2012; Wadley et al., 2014; Shatova et al., 2017; Viljoen et al., 2018).

Antarctic krill (*Euphausia superba*) is a keystone species in the Southern Ocean ecosystem with an important role in the dynamics of the Antarctic food web (Tovar-Sanchez et al., 2009). It is both an important primary productivity herbivore and a major prey item for many marine vertebrate predators in the Antarctic (penguins, seals, whales, fish). Some elements concentrated in krill have been shown to be transferred to higher-order marine animals when eaten and ultimately recycled through defecation of excess metals into the surface waters of the Southern Ocean (Deheyn et al., 2005; Tovar-Sanchez et al., 2007; Ratnarajah et al., 2014, 2018).

Penguins are sentinel seabirds of the Antarctic marine environment, as they are high in the Antarctic food chain and are long-lived. They are representative of the ecosystem due to their abundance and distribution, feeding in the sea, primarily on krill, and they nest on land, being able to act as biotransporters of trace elements and nutrients between the marine and terrestrial ecosystems. In fact, they are specialized in swimming and diving, and therefore impacting more directly the aquatic compartment than other birds. Thanks to its diet largely based on Antarctic krill (mainly for *Pygoscelis* species), which is a large reservoir of nutrients, penguin guano has a large amount of both macronutrients such as nitrates or phosphates, as well as micronutrients such as Fe, Co, Mn or Zn (Wing et al., 2014). Most of the droppings of these birds are deposited either in the ocean during swimming or in coastal areas during the breading season where rainwater, melted snow and runoff from glaciers wash large quantities to the sea. Once in the marine environment, these recycled trace metals may play a key role as bioavailable fraction.

Metal concentrations in penguin guano in Antarctica vary widely depending mainly on their diet (Metcheva et al., 2011). Concentration (μg/g) ranges of As (0.25–5.13), Cd (0.75–5.5), Cu (0.10–260), Co (0.23–9.23), Hg (0.035–62.97), Pb (0.08–45.9), Zn (108.7–573), Ni (0.63–20), V (1.76–9.62) and major elements as Fe (185–947.2), Mn (12.3–44.75) has been reported (Szefer et al., 1993; Bargagli et al., 1998; Ancora et al., 2002; Xie and Sun, 2008; Yin et al., 2008; Metcheva et al., 2011; Celis et al., 2012, 2015a, 2015b; Nie et al., 2012; Espejo et al., 2014; Shatova et al., 2016; Chu et al., 2019; Tovar-Sánchez et al., 2019; Sparaventi et al., 2021; Wing et al., 2021; Celis et al., 2023).

Penguins' guano can be an important source of recycled elements in the surface water of the Southern Ocean with potential influence in enhancing primary production (e.g., Wing et al., 2014, 2017; Shatova et al., 2016, 2017). Based in measurements of trace metals in surface water under the influence of penguins' colonies Sparaventi et al. (2021) estimated an annual release during the breeding season of 28, 56, 4 and 29 t of Cu, Fe, Mn and Zn respectively, for Chinstrap, Adélie and Gentoo penguins. More recently Belyaev et al. (2023) estimate that the Antarctic global Chinstrap penguin population is recycling 521 t of Fe yr^{-1} to the Antarctic waters.

Mathematical modelling has been used as an useful tool to explain, assess and predict contaminants release from solid matrices in contact with water, contributing to the exposure assessment stage for risk management studies. Model-based approach offers a valuable potential tool to better understand the risks associated with nutrients and metals released from solid matrices in freshwater and marine ecosystems.

Release of trace metals from soil, sediment and waste to water has been modelled widely (Cappuyns et al., 2004a; Ganne et al., 2006; Cappuyns and Swennen, 2008a, 2008b; Ho et al., 2012; Van Herreweghe et al., 2012) according to the two compartments model of Schwarz et al. (1999). More recently, the research group modelled the release of metals from polluted estuarine sediment (Martín-Torre et al., 2015, 2016, 2017) and from sunscreens (Rodríguez-Romero et al., 2019, 2022) to seawater considering that trace metals are associated to different pools (oxidized, reduced, labile or less labile pool); these models explain metals' trends, including initial release delay of elements, as well as adsorption and precipitation after an initial element release.

The aim of the present work is to obtain, analyse and simulate the experimental release of dissolved Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, V, and Zn from Gentoo (*Pygoscelis papua*) penguins guano to seawater from Livingston Island, Antarctica. Two set of long-term experiments lasting 120 h, at laboratory-controlled conditions and at external Antarctic natural conditions of temperature and light, have been conducted to evaluate dissolved (<0.22 μm) elements release behaviour. The comparison of the results of these two sets will allow to determine the validity and general application of the kinetic model obtained in laboratory conditions for its use at external Antarctic natural conditions of interest for tests with organisms in the Antarctic. A kinetic expression and its kinetic parameters are obtained to fit the experimental and modelled release of the elements. Proposed modelling aims to obtain the flow and mass of the released elements from the Gentoo penguin guano to the Southern Ocean; this may be of crucial relevance given the increasing presence of this species south of the 60°S parallel due to its diet and the effects of climate change in Antarctica.

2. Experimental methodology

2.1. Penguin guano and seawater sample collection and analysis

Fresh guano samples were collected manually with a plastic scoop from the gentoo penguin colony in Argentina Cove, on the Hurd Peninsula of the Livingston Island, South Shetland Islands in February 2022 during the PiMetAn Project 2021–2022 Spanish Antarctic campaign (Fig. 1a). The samples were transported in polyethylene bags to the laboratory of the Juan Carlos I Spanish Antarctic Base where they were homogenized, weighed, measured for moisture content and stored frozen at −20 °C until their use in kinetic experiments in Antarctica and their chemical analysis in Spain.

Seawater was collected in the vicinity of the Juan Carlos I Spanish Antarctic Base (Fig. 1a). Seawater was collected using an acid-washed Teflon tubing connected to a peristaltic pump and directly filtered on polypropylene cartridge filter (0.22 μm; MSI, Calyx®) and stored in an acid clean plastic container in a dark and refrigerated place (4 °C) before the experiments. Seawater was filtered in order to avoid the influences that the presence of organisms could introduce in the elements' mobility and in order to work with a homogeneous leaching fluid that allows comparability among long term experiments and experiments to be made outside Antarctica. Part of the water is used for kinetic tests and another part is acidified to pH < 2 with ultrapure HCL in acid clean plastic containers for transfer and subsequent chemical analysis in Spain.

In the laboratory, the guano samples were lyophilized and the metals (Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, V and Zn) were extracted with a microwave acid digestion system (MARS-V, CEM) in accordance with the SW-846 EPA Method 3051A (USA EPA, 2007). TMs extraction involved the digestion of approx. 0.1 g of guano sample with 10 mL of nitric acid (65 %, Suprapur quality) in Teflon vessels. After digestion, samples were diluted to 45 mL 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. Blanks presented metal signal always lower than 10 % of the samples. The accuracy of the analytical procedure was checked using

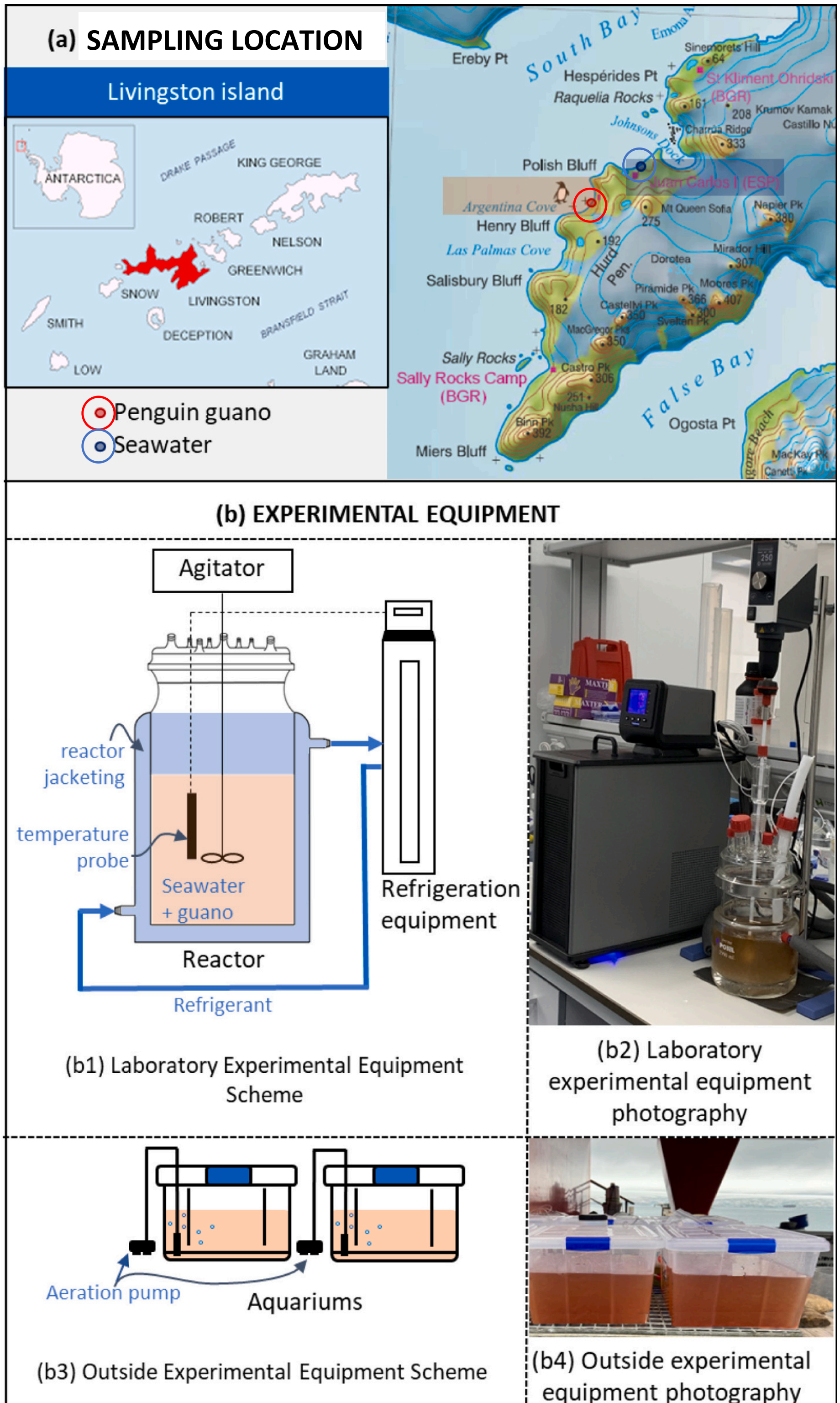

Fig. 1. a) Location of penguin guano and seawater sampling; b) Experimental equipment used in the kinetic tests.

a certified reference material (Lobster hepatopancreas TORT-2, NRC-CNRC), with concentration measured (μg/ g dry weight) and recoveries (%) for the certificated values of 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 %); Pb: 0.23 (64.8 %); V: 1.83 (111.4 %) and Zn: 192.0 (106.7 %).

Concentration of trace metals in seawater were determined directly by ICP-MS (PerkinElmer ELAN DRC-e) previous dilution 1:10 (v/v) with 0.1 M ultrapure nitric acid to avoid salt matrix interferences in the ICP-MS. During the analysis samples were spiked with Indium as internal standard to correct for variability between the calibration standards and the samples and improve the accuracy of the analysis.

2.2. Metals release kinetics experiment

Duplicate kinetic experiments were performed at the laboratory-controlled conditions. A system consisting of a stirred glass-made 2-L jacketed vessel and a temperature controller (Polyscience) (Fig. 1b) were used. All the experiments were carried out at a constant temperature of 1 °C. 3 g wet weight/L of guano is added to the previously temperature conditioned 2 l of filtered seawater. Relatively high amount of guano is used in order to avoid problems with detection limits for minor elements.

Samples at 0, 5 min, 0.25 h, 0.5 h, 1 h, 2 h, 6 h, 24 h, 36 h, 48 h, 72 h, 96 h and 120 h were taken using a plastic syringe and without stopping the mixing. Liquid/solid ratio changes were determined to be <15 % by measuring the volume of liquid and weighing the solid extracted after filtration of each sample. The pH of the sample was measured using a Basic 20 pH metre (Crison) with a special electrode for samples containing suspended solids; each sample was filtered through a 0.22 μm pore size nitrocellulose filtration membrane. From each sample, two subsamples were obtained, one for inorganic nutrient analysis (kept at −20 °C until analysis) and another subsampled for trace metal analysis (acidified to pH 1.5–2 with 1 M HCl) and kept at 4 °C until preconcentration and analysis. Blanks (seawater sample without guano at $t = 0$) were obtained for each kinetic experiment. Inorganic nutrients and metals in kinetic samples were determined as previously described to seawater samples.

Additionally, kinetic experiments at external natural conditions of temperature and light were carried out in duplicate, using 15 L polypropylene aquaria (acid-washed PP tanks), adding 3 g/L of wet guano, in order to validate the model for its use under outside conditions of interest for tests with organisms in Antarctica (Fig. 1b). The closed aquaria were kept under ambient conditions in an area close to the Juan Carlos I Spanish Antarctic Base for a period of 120 h. The agitation of the mixture is carried out with continuous aeration pumps for fish tanks. Samples for analysis were taken at the same times and following the same protocol as in laboratory experiments.

2.3. Kinetic model

A two pools analysis, with a rapidly extractable pool or "labile pool", and a less rapidly extractable pool or "less labile pool", has been considered previously to represent the potential mobile trace metals under EDTA-based kinetic extractions; this approach has been applied to assess the total mobile pools of trace metals from sediments, soils, soils mixed with organic wastes, organic amendments and compost, (Bermond et al., 2005; Brunori et al., 2005; Fangueiro et al., 2005; Labanowski et al., 2008; Santos et al., 2010; Pasquet et al., 2018; Merrot et al., 2022; Klein et al., 2023). Under the same approach, trace metals and inorganic nutrients release rate from sunscreens to seawater has been determined by a kinetic scheme that considers transfer between elements in organic material, colloidal suspension and seawater compartments (Rodríguez-Romero et al., 2019).

In the present work a kinetic scheme to describe the behaviour of metals release from the penguin guano (environmental compartment 1) to seawater (environmental compartment 2) has been considered (Fig. 2). 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 parallel kinetic processes at different rates, either under an irreversible process or following an equilibrium process respectively. While fraction of the element contained in the guano "labile pool" is weakly bound and it can be released into seawater by rapid irreversible processes such as dissolution, element fraction in the guano "equilibrium pool" will be released reversibly due to equilibrium processes, such as adsorption and desorption.

The reaction scheme and mass balances of the proposed model, considering first order kinetics are shown in Eqs. (1)–(3).

$$\frac{dC^{eq}_{i,guano}}{dt} = -k_{i,1} \bullet C^{eq\ pool}_{i,guano} + k_{i,2}\frac{V}{M} \bullet C_{i,water} \tag{1}$$

$$\frac{dC^{labile}_{i,guano}}{dt} = -k_{i,3} \bullet C^{lab\ pool}_{i,guano} \tag{2}$$

$$\frac{d\ C_{i,water}}{dt} = -k_{i,2} \bullet C_{i,water} + k_{i,1}\frac{M}{V} \bullet C^{eq\ pool}_{i,guano} + k_{i,3}\frac{M}{V} \bullet C^{lab\ pool}_{i,guano} \tag{3}$$

where $C^{eq\ pool}_{i,guano}$, $C^{lab\ pool}_{i,guano}$ and $C_{i,water}$ are the concentrations of metal i in the equilibrium pool, labile pool and seawater respectively. The $k_{i,1}$, $k_{i,2}$, are the rate coefficients of element i release by the equilibrium reactions (1) and (2) from equilibrium pool; the $k_{i,3}$ is the rate coefficient of element i release by reaction (3) from labile pool. M is the mass of penguin guano, V the seawater volume and t the time.

The modelling of this study and the estimation of the corresponding parameters are completed using Aspen Custom Modeler software (Bedford, Massachusetts, USA) which solves rigorous models and simultaneously estimates 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 (R^2), standard deviation (σ), 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, 2016, 2017) as well as to model the release pattern and the contribution of trace metals and inorganic nutrients from sunscreens to coastal marine waters (Rodríguez-Romero et al., 2019, 2022).

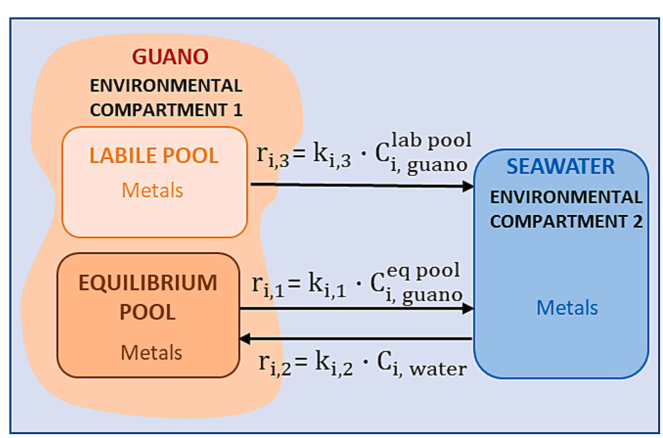

Fig. 2. Representation of the proposed kinetic scheme of studied chemical elements release from penguin guano to seawater.

3. Results and discussion

3.1. Penguin guano and seawater composition

Three subsamples of Gentoo penguin's guano used to the kinetic experiments were analysed. The mean value of the concentration (μg/g) of the considered metals is shown in Fig. S1a. Table S1 of the supplementary material shows the concentration values measured in each subsample, the mean value, the standard deviation, and the coefficient of variation. The greatest variations in the results are found in Fe with a CV of 29.4 % and V with a CV of 26.1 %; the remaining studied elements show CV below 20 %.

Two samples of seawater corresponding to the $t = 0$ of the duplicate kinetic experiment were analysed. Fig. S1b and Table S2 in supplementary material show the average concentration values of the elements studied in both samples, the standard deviation and the coefficient of variation. The greatest variations in the results obtained are observed in Fe with a CV of 26.1 %. For the other elements the CV is always <20 %.

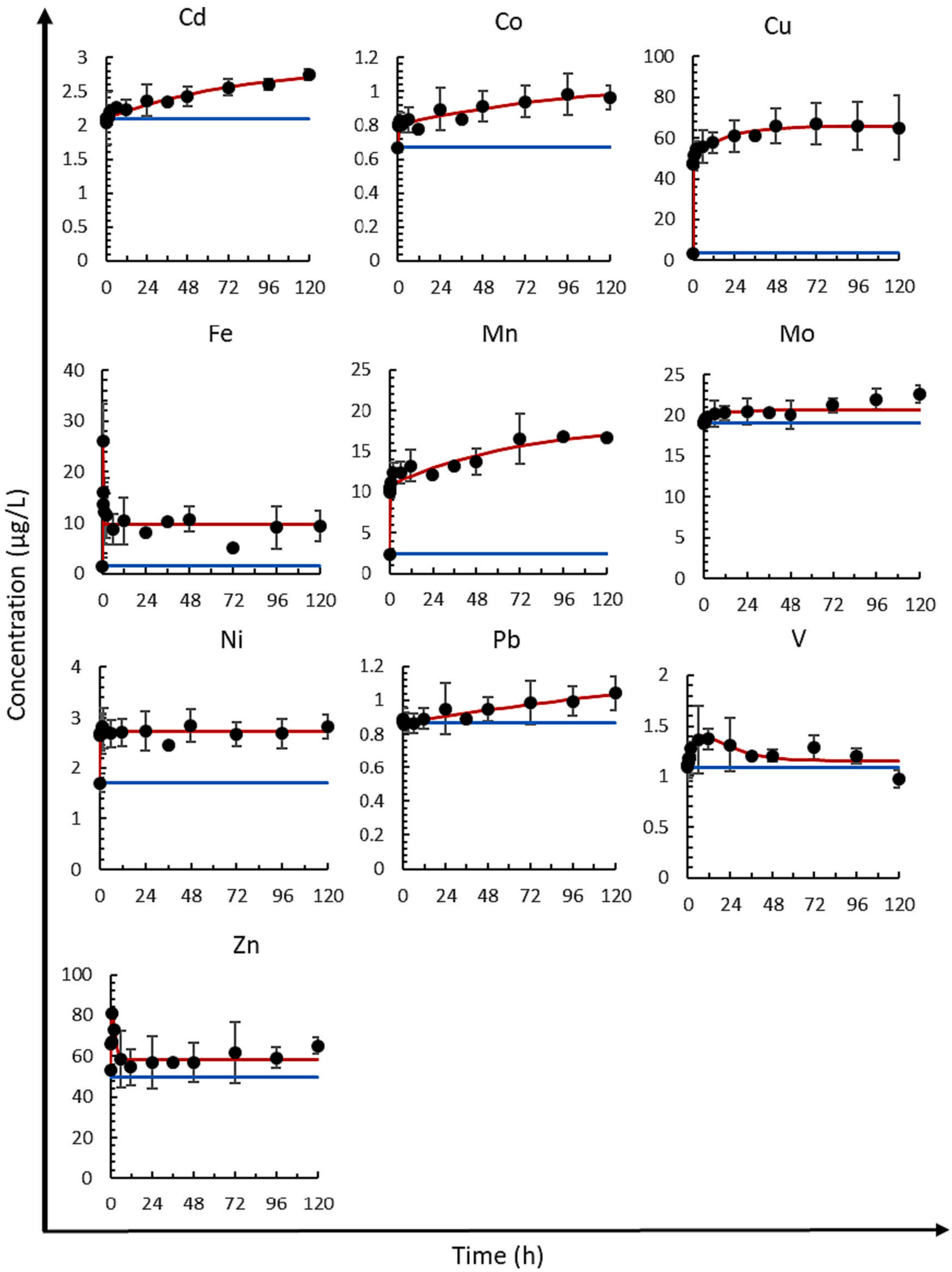

Fig. 3. Evolution of elements release concentration (mg/l) over time. The experimental concentration element release (•), error bars, element concentration in seawater (—) and simulated curves using the proposed model (—) are represented.

Although the area is not affected by any penguin colony, coastal surficial waters tend to have a higher concentration of metals than open areas due to a multitude of potential undetected sources, such as resuspension of sediments and melting ice, among others. In addition, as Gledhill and Buck (2012) point out, excess iron ligand concentrations, particularly in the truly soluble size fraction, seem to be consistently higher in the upper water column, and especially in Fe-limited waters.

3.2. Element's release

There is a growing interest on the scientific research in Antarctica in response to the emergent of trace metal pollution in the region. Numerous efforts have been exercised to investigate metal pollution in the Antarctic region in the past decades (e.g., Sparaventi et al., 2021; Celis et al., 2023; Darham et al., 2023). However, to the best of the authors' knowledge, no data have been published on modelling the mobility of trace metals from guano to the marine environment.

The experimental results in Fig. 3, showing the total content of each element, are simulated using the proposed model. Detail of the evolution at short times (up to 2 h) of the concentration of studied elements in seawater is shown in Fig. S2 (supplementary material). Obtained results show different time-dependent release behaviours. A first pattern (Cd, Mo, and Pb) in which the release of the element from guano to seawater occurs through a single equilibrium process. The initial value of the kinetic experiments coincides with the concentration of the element in seawater and its concentration in seawater increases over time until reaching a constant equilibrium value. This behaviour can be explained considering that all the element is in the equilibrium compartment proposed in the model.

In a second pattern, the elements Co, Cu, Mn and Ni show a very rapid release that decreases until equilibrium is reached (Fig. 3). This behaviour suggests that a fraction of the element is poorly bound to guano and is easily released quickly in short times (present in a so-called labile compartment), while another fraction is more strongly retained and its release is slower leading significantly more time (present in a so-called equilibrium compartment).

The mobility results of Fe, Zn and V show a third differentiated behaviour; firstly, a release of the element from the guano into seawater and latter a decrease in its concentration in solution. This behaviour, more pronounced in Fe and Zn than in V, suggests that the elements released rapidly in the seawater are then adsorbed back into the guano due to equilibrium processes. Similar behaviour has been showed by Martín-Torre et al. (2015) in the trace metals release from contaminated estuarine sediments 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.

The high concentration of guano used in kinetic experiments can promote the formation of aggregates characterized by a wide range of sizes and increase the dissolved organic matter, that can interact with trace metals through several complex processes; these will depend mainly on the surface properties and the structure of the organic matter, the speciation of the trace metals, the leaching agent and the contact conditions, among others. Processes such as complexation, adsorption, and precipitation of metallic elements have been previously mentioned as mechanisms that can explain their leaching behaviour.

According to Martín-Torre et al. (2015), the organic complexation of humic and fulvic acids and the adsorption to Fe- and Al-(hydr)oxides are the main mechanisms that can explain the leaching behaviour of As, Cd, Cr, Cu, Ni, Pb and Zn from contaminated estuarine sediments to seawater. Rodríguez-Romero et al. (2019) show that Al, Cd, Cu, Mn, Mo, Ni and Pb are initially released into seawater from sunscreen and can subsequently be adsorbed on organic material, forming a stable colloidal suspension. Klein et al. (2023) observe the tendency of Cu and Zn to form very stable complexes with organic ligands, which could explain the high concentrations of organically bound Cu and Zn in various compost matrices.

Although more experiments would be necessary to determine the reason for the decrease in concentration of Fe, Zn and V at high times, it is reasonable to conclude that the mechanism for this is adsorption to the organic compounds included in the guano composition.

The mobility of elements between environmental compartments obeys multiple behaviours, depending on the main processes involved in their mobilization. Guano, like the other environmental compartments, is a heterogeneous material. The metals 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-remaining guano components interaction and on the physicochemical characteristics of the metal emitting and receiving environmental compartments. A rapid release and a subsequent decrease in the release of metals, with the possibility of adsorption at high times, agrees with the results obtained by other authors who have studied the mobility of metals between environmental compartments (Cappuyns et al., 2004b; Cappuyns and Swennen, 2008a, 2008b; Santos et al., 2010; Martín-Torre et al., 2016, 2017; Pasquet et al., 2018; Rodríguez-Romero et al., 2019; Klein et al., 2023 among others).

The presence of dissolved Fe at concentrations beyond the inorganic solubility of Fe in seawater, estimated as 0.2 to 0.3 nM in seawater at S = 36 and 25C near a pH of 8 (Liu and Millero, 2002), is thought to be facilitated by organic complexation of Fe with a wide spectrum of stabilizing ligands. Organic Fe-binding ligands complex >99 % of dissolved Fe (Gledhill and Buck, 2012). In the kinetic experiments carried out, Fe moves from guano to seawater, observing a significant increase in the Fe-total concentration analysed in the first 5 min; in this period probably due to the complex speciation of Fe in seawater where various types of iron small ligands are involved. After this period, the adsorption processes of Fe with larger macromolecular complexes from guano, cause the measured Fe-total concentration dissolved (<0.22 μm) to decrease drastically.

3.3. Kinetic model of elements release

The experimental results in Fig. 3, showing the total content of each element, are simulated using the proposed model, allowing to estimate the kinetic coefficients of the release process of dissolved element i from the equilibrium pool ($k_{i,1}$, $k_{i,2}$) and kinetic coefficient ($k_{i,3}$) of the release process of element i from the labile pool. The rate coefficients of the kinetic reactions estimated, together with the standard deviation (σ), correlation coefficients (R^2) and coefficient of variation (CV) of the elements released over time, are shown in Table 1.

The parity plot obtained for the validation of the model proposed in terms of the experimental and simulated concentrations of elements is shown in Fig. S3 of the Supplementary material. The correlation coefficient (R^2) obtained from the experimental and simulated values by the

Table 1
Estimated kinetic coefficients $k_{i,j}$ for each studied element and σ, R^2, CV parameters of the relation between the experimental and simulated released concentrations using the proposed model.

Elements	$k_{i,1}$ (h^{-1})	$k_{i,2}$ (h^{-1})	$k_{i,3}$ (h^{-1})	σ (μg/L)	R^2	CV (%)
Cd	0.0129	0.000348	0	0.0533	94.4	2.33
Co	0.00806	0.00162	107	0.0273	91.2	3.23
Cu	0.0278	0.0303	36.1	1.73	99.0	3.21
Fe	0.0434	4.864	86.9	2.31	84.3	21.1
Mn	0.00646	0.00661	38.61	0.872	95.1	7.19
Mo	0.198	0	0	0.757	56.5	3.79
Ni	0.272	0.0623	26.2	0.0678	95.6	2.54
Pb	0.00233	0.000683	0	0.0199	90.1	2.18
V	0.0408	0.142	0.0699	0.0746	61.9	6.21
Zn	0.265	0.692	0.991	3.51	85.2	5.64

model and considering the 140 experimental data is 99.6 %, which indicates that the model proposed describes correctly the variation of the studied elements concentration from Gentoo penguin guano to seawater. A good fit of the proposed model is also confirmed by the fact that all of the experimental data lie within a model relative error of ±20 %, with the exception of three values corresponding to Fe at short times of 0.5, 1 and 72 h which is the element with the highest CV. Fe shows high concentration in guano and a high variability between samples (Table S1). The high variability found may be due to the great heterogeneity of the guano itself. Guano samples, although mechanically homogenized, have been added without homogenization treatment, such as lyophilization and subsequent grinding to achieve the same particle size.

The simulated curves from the estimated kinetic parameters are shown in Fig. 3. The worst fittings with lowest R^2 values are obtained for Mo (with mean value of 20.3 ± 1.10 μg/L) that shows low CV values and V (mean value of 1.22 ± 0.110 μg/L and moderate value of CV), both elements displaying less defined behaviours in a narrow range of concentrations.

Table 1 shows a value of $k_{i,3}$ of zero for Cd, Mo and Pb. According to the proposed model, this situation predicts their entire release from the equilibrium compartment, not having an easily available quantity of the element in the guano matrix. For Mo, the value of $k_{i,2}$ is also zero, indicating that all the Mo present in the guano is released into seawater through an irreversible reaction with a kinetic constant value of 0.198 h^{-1}.

3.4. Equilibrium analysis

At equilibrium, $dC_{i,guano}^{eq\ pool}/dt = dC_{i,guano}^{lab\ pool}/dt = dC_{i,water}/dt = 0$, therefore the system of differential equations of Eqs. (1)–(3), becomes a system of algebraic equations that allows to determine the equilibrium concentrations and the partition or distribution coefficient for each element (Eqs. (4)):

$$Kd_i = \frac{[C_{i,guano}]_{eq}}{[C_{i,water}]_{eq}} = \frac{[C_{i,guano}^{eq\ pool} + C_{i,guano}^{lab\ pool}]_{eq}}{[C_{i,water}]_{eq}} = \frac{k_{i,2}}{k_{i,1} \bullet C_{guano}} \quad (4)$$

where $C_{guano} = M/V$ with M the mass of penguin guano and V the seawater volume at each experiment.

Table 2 shows the equilibrium concentrations in guano and seawater and the partition coefficient for each studied element. Kd_i values show differences of up to five orders of magnitude with values in a decreasing order Fe ≫ V, Zn, Cu, Mn ≫ Pb, Ni, Co ≫ Cd. Iron had the highest value, suggesting that most of the metal is finally retained in the solid matrix. On the contrary, Pb, Ni, Co and Cd with $Kd_i < 1$, shows that the released fraction of these elements is greater than the fraction that remains retained in guano. Despite its low concentrations in guano, the relatively low values of Kd_i show a high release of the elements into seawater.

Table 2 also shows the release percentages of each metal to seawater

Table 2
Equilibrium concentrations of the studied elements in water, in Gentoo penguin guano, estimated partition coefficients Kd_i and released amounts of elements.

Elements	$[C_{i,water}]_{eq}$ (μg/L)	$[C_{i,guano}]_{eq}$ (μg/g)	Kd_i (L/g)	Released amount (%)
Cd	2.85	0.0787	0.0276	90.7
Co	1.07	0.2197	0.206	65.0
Cu	65.8	73.5	1.12	46.4
Fe	9.72	1120	115	0.885
Mn	18.7	19.6	1.05	46.0
Mo	20.6	0	0	100
Ni	2.75	0.644	0.235	62.3
Pb	1.42	0.436	0.307	56.0
V	1.16	4.13	3.56	1.67
Zn	58.8	157	2.67	5.42

once equilibrium conditions has been reached. Final releases >90 % are obtained for Mo and Cd, and between 65 % - 46 % for Co, Ni, Pb and Mn; Zn with 5.42 %, V with 1.67 % and Fe with 0.885 % show the lowest values.

These releases are relevant given, on the one hand, the high leachability of potentially toxic elements to the phytoplankton growth such as Cd and Pb and, on the other hand, the limited mobility of Fe, estimated <1 %, which plays a positive key role in the phytoplanktonic growth and consequently in the sequestration of atmospheric carbon (Echeveste et al., 2012; Shatova et al., 2017; Permana and Akbarsyah, 2021; Belyaev et al., 2023). Both of them, the kinetic behaviour over periods of days and the equilibrium concentrations reached, are crucial to estimate the solubilized metal fraction and therefore to assess the amounts of bioavailable metals. This is relevant in regions of special ecological interest with limited available Fe, such as some regions of the Southern Ocean.

Gentoo penguins are known for greater flexibility in diet composition which comprise a mix of Antarctic krill and fish, and foraging behaviour; in addition, occupy regions characterized by reduced ice-cover. Gentoo breeding colonies below 60°S have experienced a rapid growth over the last 40 years in contrast to the decline of Chinstrap and the little change on average of Adelie Penguin colonies (Talis et al., 2023; Wethington et al., 2023). Gentoo penguin population grown can be attributed largely to increases on the Antarctic Peninsula, which has experienced warming air temperatures, increasing precipitation and declines in the extent, seasonal duration, and thickness of sea ice. Estimated population of Gentoo Penguins (*Pygoscelis papua*) south of 60°S is 259.750 breeding pairs (Herman et al., 2020).

According to Sun and Xie, 2001, 84.5 g/day guano is excreted by one penguin per day during the breeding period. In addition, according to Belyaev et al. (2023), elements release from the breeding sites during a period of 120 days is calculated to be a conservative 10 % of all excreted element. The remaining 245 days of the non-breeding season, the totality of the produced guano, is considered to be released into the Southern Ocean for calculations. Considering the total content of each metal in the guano $C_{i,guano}$, (Table S1) and the not released metal amount after reaching equilibrium $[C_{i,guano}]_{eq}$ (Table 1), the maximum dissolved metal released to sea per penguin or flux to sea in one day can be estimated (Fig. 4a) (see calculations in Supplementary Table S3).

Elements such as Cd and Pb need >48 h to release at least 50 % of their content into water; other elements are released in >90 % in 10 min (Ni), in 12 h (Cu and Mo), or need >72 h (Mn and Co). The annual amounts released to the seawater when equilibrium is reached are shown in Fig. 4b, where the errors bars represent the standard deviations considering the variation of the metal concentration in guano. However, elements such as Fe, Zn and V are available in greater amounts in short times, so quantities of 297 kg Fe during 5 min, 937 kg Zn during 1.2 h and 16.3 kg of V during 10 h remain available in the aquatic environment before adsorbing (see calculations in Supplementary Table S3).

Considering a number of 519,500 Gentoo penguins individuals, and the average concentration of metals showed in Table S1, the annual amount of each dissolved metal released (kg) into the sea from the south of 60°S throughout the time of the kinetic experiment can be estimated as shown in Fig. 5.

4. Validation of the model with external natural conditions experiments

Numerous authors (e.g., Lehette et al., 2012; Kern et al., 2014; Camacho et al., 2015; Black et al., 2019; Angulo-Preckler et al., 2020; Alcamán-Arias et al., 2021; Saba et al., 2021; Camacho et al., 2022; Dall'Osto et al., 2022)) have carried out experiments maintaining Antarctic natural temperature and illumination conditions, in which adequate containers are placed outdoors in order to mimic natural conditions or manipulate the desired variables.

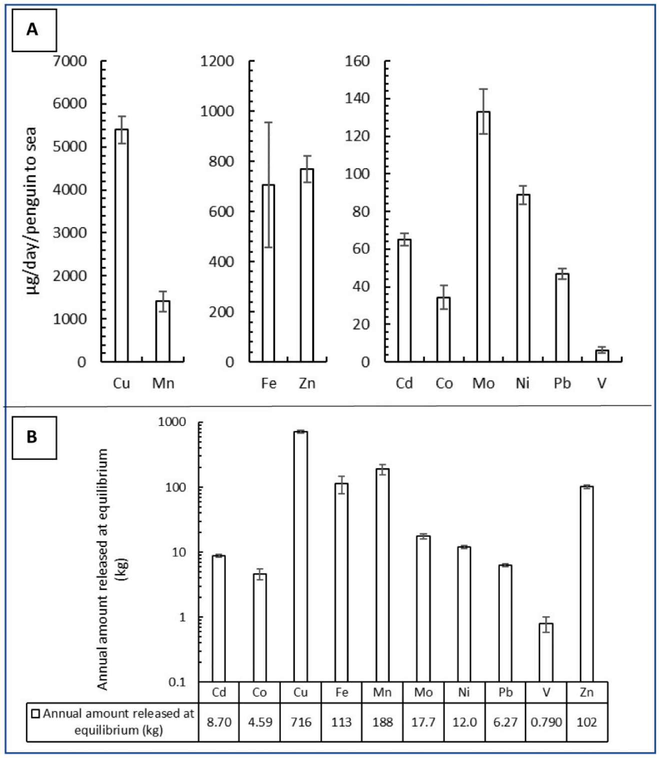

Fig. 4. a) Estimated amount of dissolved metals per day and Gentoo penguin, released into seawater; b) Maximum annual estimated amount of trace metals released from Gentoo penguin to seawater when guano-seawater equilibrium conditions are reached. The errors bars represent the standard deviations.

The evolution of the dimensionless concentration of element release (mg/l) over time in the external natural conditions' experiments (white dots) together obtained values at the laboratory experiments (black dots) and simulated curves using the proposed model are represented in Fig. 6. The dimensionless concentration is the ratio of the experimental element concentration to the initial element concentration in seawater. In this way, both representations start from the same initial point (value of 1) for each element and the curves indicate the change in the element concentration in seawater regarding to the starting point. Laboratory and at external natural conditions experimental results evolution is correctly described with the proposed model, whose parameters are shown in Table 1.

The results of metal release concentration evolution over time obtained at external natural conditions are shown in Fig. S4 of the Supplementary material (black dots), together with the simulated curves (red line) with the proposed model using the kinetic constants indicated in Table 1. In Fig. S5 of the Supplementary material the parity graph of the experimental concentrations versus the simulated ones of the studied elements is represented. Standard deviations and coefficients of variation for each metal are also indicated. Error percentages between the experimental values and those estimated with the model >10 % are only observed in 16 of the 130 values considered. Only one of them shows a variation >20 %. Together with the low CV, which varies between 1.78 for Cd and 10.2 for Fe, it demonstrates the good fit of the model to the outside at external natural conditions experiments carried out.

5. Conclusions

This paper presents a two metal pools kinetic model to interpret and estimate the release pattern, at laboratory and at external natural conditions, and the contribution to Antarctic seawater of dissolved trace metals from Gentoo penguins *(Pygoscelis papua)* guano.

With a growing estimated population of Gentoo penguins south of 60°S of 259.750 breeding pairs we estimate that the Antarctic Gentoo penguin population is recycling annually and in the dissolved fraction, 716 ± 41.9 kg Cu, 188 ± 31.7 kg Mn, 113 kg Fe ± 32.3, 102 ± 6.73 kg Zn, 17.7 ± 1.53 kg Mo, 12.0 ± 0.67 kg Ni, 8.70 ± 0.45 kg Cd, 4.59 ± 0.85 kg Co, 6.27 ± 0.35 kg Pb and 0.790 ± 0.20 kg V. However, elements such as Fe, Zn and V are available in greater than equilibrium

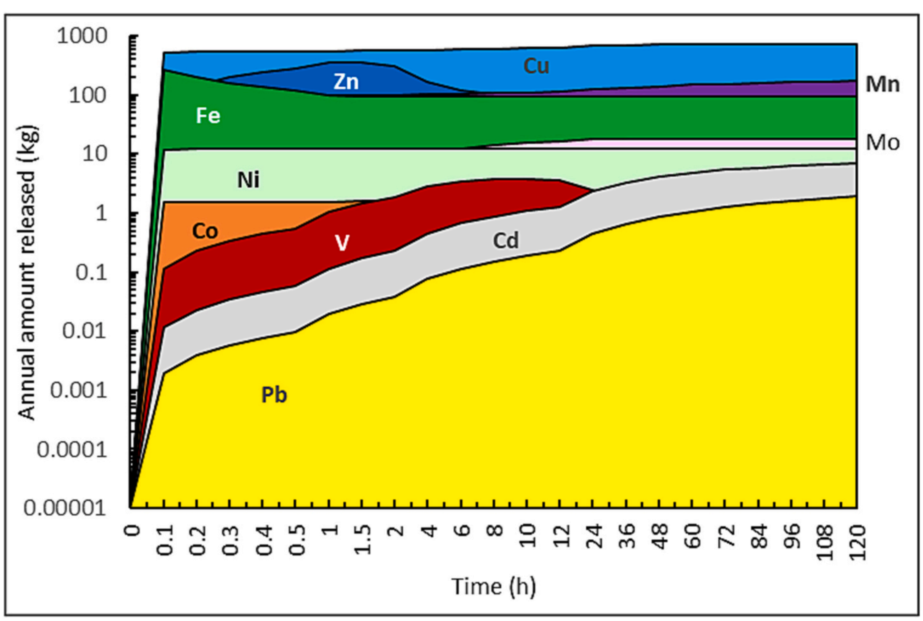

Fig. 5. Temporal evolution of the annual estimated amount of dissolved trace metals released from Gentoo penguin to seawater through the experimental contact period ■ Cu, ■ Zn, ■ Mn, ■ Fe, □ Mo, □ Ni, ■ Co, ■ V, ■ Cd, □ Pb.

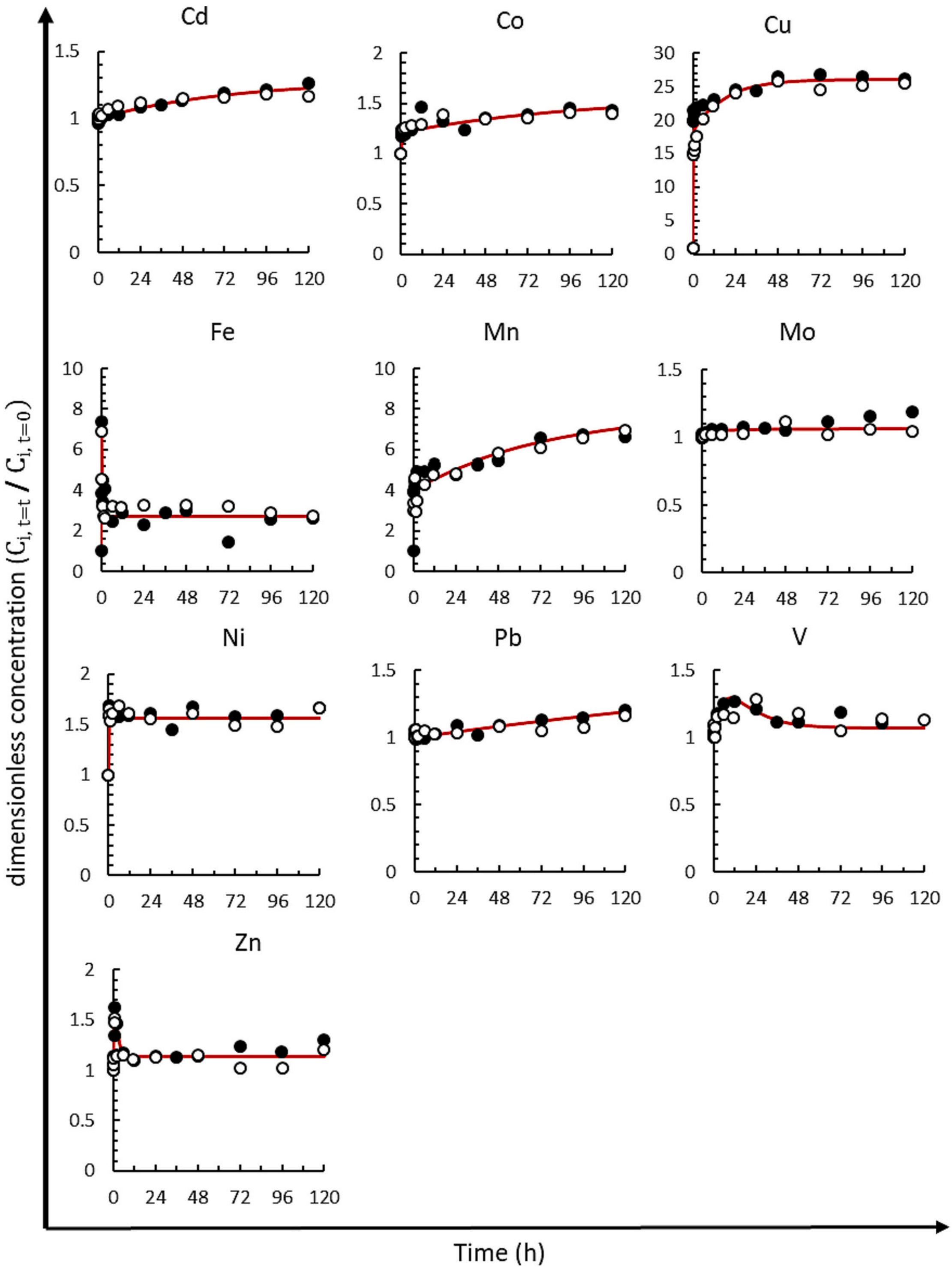

Fig. 6. Evolution of the dimensionless concentration of element release (mg/l) over time. The concentration of the elements studied in the laboratory experiments (●), concentration of the elements studied in the external natural conditions experiments (○) and simulated curves using the proposed model (—) are represented.

amounts in short times before adsorbing.

Different behaviours are observed in the release rate of trace metals. Cadmium and Pb need >48 h to release at least 50 % of their content into water; other elements need 10 min (Ni), 12 h (Cu and Mo), or >72 h (Mn and Co) to release >90 % of the initial content in guano. Under equilibrium conditions, final releases >90 % are obtained for Mo and Cd, between 46 % and 65 % for Co, Ni, Pb and Mn, but only 0.88 for Fe, 1.67 % for V and 5.4 % for Zn. These values are crucial to estimate the guano contribution of potentially toxic soluble metals and metals that are precursors to phytoplankton growth. The results obtained confirm the fundamental role of gentoo penguins in the Southern Ocean through the recycling of trace metals both critical for marine life and those that can negatively affect the health of the ecosystem, mainly for the increasingly large Antarctic areas influenced by gentoo penguin breeding grounds.

CRediT authorship contribution statement

Gema Ruiz Gutierrez: Formal analysis; Software; Methodology;

Writing -original draft. **Berta Galan Corta:** Validation; Writing - review & editing. **Erica Sparaventi:** Methodology; Formal analysis; Chemical analysis. **Antonio Tovar Sanchez:** Visualization; Funding acquisition; Resources; Writing - review & editing. **Javier R. Viguri Fuente**: Conceptualization; Supervision; Writing - original draft; Writing - review & editing.

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.

Data availability

I have shared the data in the manuscript and Supportin Information e

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166448.

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