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Seabird colonies as relevant sources of pollutants in Antarctic ecosystems: Part 1 - Trace elements

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Highlights

- Seabird colonies are verified as possible secondary sources of trace elements.
- Soil and vegetation samples in/around colonies are compared to controls away from them.
- Results suggest colonies act as sources of Cd, Hg and likely As, Se and Zn.
- Isotopes confirm the animal-derived organic matter influence on colony sites.
- Other local Hg sources, likely research stations, could also be inferred.

Abstract: Global distillation is classically pointed as the biggest responsible for contaminant inputs in Polar ecosystems. Mercury (Hg) and other trace elements (TEs) also present natural sources, whereas the biologically mediated input is typically ignored. However, bioaccumulation and biomagnification combined with the fact that seabirds gather in large numbers into large colonies and excrete on land might represent an important local TEs input. A previous work suggested these colonies as sources of not only nutrients, but also organic contaminants. To evaluate a similar hypothesis for TEs, samples of lichen (n = 55), mosses (n = 58) and soil (n = 37) were collected in 13 locations within the South Shetlands Archipelago during the austral summers of 2013-14 and 2014-15. They were divided in: "colony" (within the colony itself for soil and bordering it for vegetation) and "control" (at least 50m away from colony interference), analysed for TEs (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) and stable isotopes (C and N). In most cases, soil seems the best matrix to assess colonies as TEs sources, as it presented more differences between control/colony sites than vegetation. Colonies are clearly local sources of organic matter, Cd, Hg and likely of As, Se and Zn. Conversely, Co, Cr, Ni and Pb come presumably from other sources, natural or anthropogenic. In general, isotopes were more useful for interpreting vegetation data due to fractionation of absorbed animal-derived organic matter. Other local Hg sources could be inferred from high levels in control sites, location and wind patterns.

Keywords: Antarctica, secondary sources, stable isotopes, heavy metals, inorganic contaminants, trace elements

Introduction

"Global distillation", the process by which volatile contaminants evaporate in warmer regions, undergo long-range transport and condensate in colder regions, is classically pointed as the biggest responsible for contaminant inputs in Polar ecosystems. Besides this process, mercury (Hg), a toxic non-essential element (Tan et al., 2009) and other trace elements present natural sources as well, in a lesser scale, which could have local significance (e.g. Mão de Ferro et al., 2013). The biologically mediated transport, however, is typically ignored (Blais et al., 2007, 2005), but might represent an important input at the local (Huang et al., 2014) or regional (Blais et al., 2007) scales, not only in polar environments but also in semi-arid ones for instance (Garcia et al., 2002). This transfer from the sea to the land is reported not only for trace elements, but to other contaminants as well (Negoita et al., 2003; Roosens et al., 2007). Nevertheless, the concentration of non-essential trace elements along the life of organisms (bioaccumulation), and also along the trophic webs (biomagnification), combined with the fact that individuals gather in large numbers into large colonies for some species and excrete on land, are particularly characteristic for seabirds during the reproductive season, resulting in considerable input in these locations.

In maritime Antarctica, seabird colonies represent the major source of nutrients for terrestrial ecosystems (Smykla et al., 2007). Both extant and abandoned colonies (Liu et al., 2006; Outridge et al., 2016; Tatur et al., 1997) play this role due to not only the guano, but also to eggs (Brasso et al., 2012), bird tissues and prey present in these structures (Emslie et al., 2014). Nutrients distribution and availability typically induce a zonation pattern of ornithogenic exposed soil within the colony followed by green algae/cyanobacteria, Antarctic hair-grass, mosses and lichens, in this order, as the distance to the colony increases (Smykla et al., 2007). However, this input of nutrients given by bird colonies comes along with an input of contaminants as well, by a more direct influence of the contaminants present in the excretion itself (Espejo et al., 2014; Nie et al., 2012; Rudolph et al., 2016) or of the transport of trace elements during biogeochemical cycles (e.g. Malandrino et al., 2009; Zheng et al., 2015). In an analogous manner, runoff water is known to have a key influence in marine primary production (Anderson and Polis, 1999) and in carrying trace elements (Bettinelli et al., 2000) to be absorbed by marine life (Cipro et al., 2018)..

Several studies pointed the suitability of both soil (Prus et al., 2015; Sysalová et al., 2013) and vegetation as good indicator matrixes for both organic and inorganic contaminants (e.g. Lim et al., 2009; Wojtuń et al., 2013; Zvěřina et al., 2014) as well as some others that

compare soil, lichens and/or mosses (e.g. Park et al., 2010; Tarcau et al., 2013). However, only a few publications proposed such colonies (e.g. Choy et al., 2010; Cipro et al., 2011; Roosens et al., 2007) or carcasses (e.g. Zvěřina et al., 2016) as an important secondary contaminants source for terrestrial or coastal Polar ecosystems. In addition, studies pointing out the concentration of trace elements in seabird faeces (Celis et al., 2015; Espejo et al., 2014; Finger et al., 2017) reinforce the fact that this input should be more deeply explored.

In a previous study using vegetation as indicator (Cipro et al., 2011), we showed that the degradation of animal derived organic matter (seabird colonies for instance) could constitute non-negligible sources of organic contaminants. In this context, the present work constitutes a geographical and ecological enlargement of scope and reach as well as a refinement of our previous work (Cipro et al., 2011). Differently from the studies published in the open literature, our work aims to evaluate the influence of seabird colonies as secondary sources of trace elements using both soil and vegetation as indicators in maritime in several Antarctic terrestrial ecosystems considering geographical variation, distance to the colonies and the use of different seabird species with different feeding ecology strategies. Moreover, the nature of the different matrixes plays a major role in understanding how these contaminants are exposed to these matrixes *via* different pathways.

Material and methods

Sampling

Samples of lichen (n=55), mosses (n=58) and soils (n=37), were collected in 13 locations within the South Shetlands Archipelago in the austral summers of 2013-14 and 2014-15 during cruises of the Brazilian Antarctic Programme and divided in two types: "colony" (within the colony itself for soil and within the colony or bordering it for vegetation) and "control" (at least 50m away from the respectively closest colony. Lichens and soil were collected in higher elevations than the colony, whereas mosses, due to their water-dependency, were lower). Samples were collected with plastic gear (spoons and tweezers) firstly rinsed in an acid bath (35 mL L⁻¹ nitric acid, 50 mL L⁻¹ hydrochloric acid), then further rinsed with Milli-Q water. Soil samples were dug no deeper than 5cm. They were chosen at the field mainly by their availability, avoiding fresh faeces which could mask the results for the formed ornithogenic soil.

In some cases, samples were collected in more than one spot in each area of interest, which was described by the "main location" and "specific location" labels. Thus, the

comparison between control and colony sites was made within the same specific location, when available, otherwise the closest control sample within the same main location was adopted.

Once collected, samples were stored in hermetically sealed plastic bags, frozen aboard (-20°C) and kept frozen until arrival at the Marine Organic Chemistry Laboratory (LabQOM, University of São Paulo/Brazil), where they underwent lyophilisation. Vegetation species were identified by Prof. Jair Putzke (Universidade Federal do Pampa, São Gabriel, Brazil). Samples were then sent to the University of La Rochelle, France, where they were ground to a fine powder in a ceramic mortar before analyses. Soil samples were sifted (1mm mesh) after freeze-drying in order to remove larger rock fragments and debris. Soil samples were not ground nor before nor after drying and sifting.

Analyses

Trace element analyses were performed in the "Plateforme Analyses Élémentaires" of LIENSs and stable isotope analyses (SIA) were performed in the "Plateforme Analyses Isotopiques" of LIENSs, according to their own protocols, further detailed. Hg analyses were carried out with an Automatic Mercury Analyser spectrophotometer, ALTEC AMA 254, which does not require an acid digestion of the samples following Blévin et al. (2013). According to the Hg content, limit of detection and calibration curves, aliquots ranging from 4 to 61 mg of freeze-dried sample were directly analysed after being inserted in the oven of the apparatus. After drying, the samples were heated under an oxygen atmosphere for 3 min, and the Hg liberated and subsequently amalgamated on an Au-net. The net was then heated to liberate the collected Hg, which was measured by Atomic Absorption Spectrometry. Accuracy and reproducibility of the method were tested using dogfish liver (DOLT-2) and muscle (DORM-2) and lobster hepatopancreas (TORT-2) (National Research Council, Canada) reference standards. Standard and blanks were analysed along with each set of samples, and recoveries of the certified values and recoveries of Hg ranged from 87 to 94%. Concentrations are expressed in dry weight in order to compensate eventual moisture loss during freezing and to facilitate comparison between tissues and with other studies. Blanks were analysed at the beginning and end of each set of samples, and the detection limit of the method was 0.005 $\mu\text{g g}^{-1}$ dry weight (dw).

The analyses of the remaining trace elements (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, and Zn) were performed as described by Lucia et al. (2016): analyses were carried out using a Varian Vista-Pro ICP-OES and a Thermo Fisher Scientific XSeries 2 ICP-MS. Aliquots of

the samples (from 90 to 255 mg) were digested with 6 ml 67–70 % HNO₃ and 2 ml 34–37 % HCl (Fisher Scientific, trace element grade quality). This acidic digestion was performed overnight at room temperature, then using a Milestone microwave (30 min with constantly increasing temperature up to 120 °C, then 15 min at this maximal temperature). Each sample was completed to 50 ml with milli-Q water. Three control samples (two Certified Reference Materials, CRMs, and one blank) treated and analysed in the same way as the samples, were included in each analytical batch. CRMs were dogfish liver DOLT-4 (NRCC) and lobster hepatopancreas TORT-3 (NRCC). Mean recovery rates were, in regard to those CRMs, of 101% for As, 99% for Cd, 101% for Co, 94% for Cr, 96% for Cu, 94% for Fe, 91% for Mn, 99% for Ni, 89% for Pb, 99% for Se, 92% for V and 101% for Zn. Trace element concentrations are also expressed in $\mu\text{g g}^{-1}$ dw. Measurements were also validated by IAEA inter-calibration exercises (Coquery et al., 2001).

SIA was performed as described in previous literature (Chouvelon et al., 2012; Cipro et al., 2017), with some modifications, especially the decarbonation of soil samples. Due to the negligible lipid content and influence in the results verified during previous studies (Cipro et al., 2017, 2011), samples have not undergone delipidation. Soil samples, however, have undergone a decarbonation procedure to avoid the interference of carbonates, which are depleted in ¹³C and could represent a bias in data interpretation. The decarbonation was as follows: up to 100mg of ground sample were put in a glass vial, 1mL of HCl 0.1N were added and the vial was placed in a microwave bath, observing bubble formation as an indicator of carbonates digestion. After one minute, 100 μ l of the same acid was added to verify that no more bubble formation was shown. If that was the case, this first step was repeated many times as needed. Next, the vials were put in a 60°C dry bath (Techne) coupled to an evaporation system consisting of tubes gently blowing filtered analytical quality compressed air into the vials overnight in order to evaporate the liquid acidic phase. The following day, samples were recovered with 1mL of Milli-Q water, homogenised in a microwave bath for 1 minute, frozen and finally lyophilised.

SIA themselves were performed as follows: an aliquot of ground prepared sample (0.8 up to 1.5 mg for vegetation, 1.5 to 8 mg for colony soil, 8 to 16 mg for control soil) was encapsulated in tin cups and injected in a Thermo Scientific Delta V Advantage ConFlo IV interface (NoBlank and SmartEA) coupled to a Thermo Scientific Flash EA1112 Elemental Analyser. Pee Dee Belemnite and atmospheric nitrogen were used as standards for calculation

of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Based on replicate measurements of internal laboratory standards, experimental precision is of $\pm 0.15\text{‰}$ and $\pm 0.20\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Statistics

Statistics were performed using Microsoft Excel and Statsoft Statistica 12 and 13. Before analyses, data were checked for normality of distribution and homogeneity of variances using Shapiro–Wilk and Brown–Forsythe tests, respectively. Statistically significant results are set at $\alpha=0.05$ unless stated otherwise. Differences of colony and control datasets were assessed by means of t-tests and Pearson/Spearman correlations are chosen due to their linear/monotonic nature and not necessarily to parametric/non-parametric analyses.

Normal distribution and log-link function generalized linear models (GLM) were built as follows: trace element concentrations as the dependent variable; matrix, main location, specific location, colony species and matrix species as categorical factors; $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %C and %N as continuous predictors. Biologically relevant models were constructed incorporating the different variables and their interactions taking the care of removing continuous variables that were significantly correlated before model building in each dataset and its subsets as well. Model selection was based on Akaike's Information Criteria adjusted for small sample sizes (AICc). The model with the lowest AICc value was considered to be the most accurate. Models with AICc values differing by less than 2 have a similar level of support in the data, and the model including the least number of parameters can be regarded as the most accurate, according to the principle of parsimony (Burnham and Anderson, 2002). Overall model support was assessed using Akaike weights (w_i), following Johnson and Omland (2004). Residual (R^2_{adj}) analyses should be restricted to description and not be used in model selection (Burnham and Anderson, 2002). Only models with AICc values differing by less than 10 are presented.

Results and discussion

Mean results for selected trace elements (As, Cd, Cu, Hg, Pb, Se, and Zn), vegetation species (when suitable), bird colony species, location (both main and specific, which refers to a subpart of the main one when suitable), SIA, C and N content and sampling numbers are given in Table 1. It should be stated by now that more emphasis is to be given to Cd and Hg data interpretation due to their ecotoxicological relevance in not only terrestrial ecosystems, but in marine ones as well (e.g. Tsui and Wang, 2004). For the same reason (see Olech et al., 1998),

Pb is briefly discussed in the proper section. The whole dataset containing all trace elements is presented as supplementary material for editorial reasons. A possible interference of pinnipeds land excretion has not been taken into consideration for two reasons: from field experience, only one of the sampling locations had pinnipeds presence (mostly the Southern Elephant Seal, *Mirounga leonina*, in Livingston Island), which remain close to the water and do not go uphill as most birds do, so the excreta might end up washed away. And secondly, haulout periods for pinnipeds in general and for this species in particular are not as prolonged as for seabirds (Hindell and Burton, 1988; Lake et al., 1997; Slip and Burton, 1999)

In regard to Hg, mean results show some prevailing patterns. For soils, control samples were less contaminated than the corresponding colony ones in all cases (Table 1 and further discussed in Table 3). Mosses, in turn, presented similar levels when compared to soil, with one notable exception for mosses, Ferraz, located next to the Brazilian Scientific Station (EACF). This could be due to several reasons: fossil fuel burning (a notable source of Hg), less year-round ice cover (meaning more exposed vegetation surface and less Hg trapped within snow and ice) and also the fire from February 2012, which consumed the whole station core and roughly 70% of the total facilities, releasing significant amounts of organic and inorganic contaminants (Colabuono et al., 2015; Guerra et al., 2013).

Lichens presented overall levels roughly with a two-fold increase, but still in the same order of magnitude as mosses, except the sampling sites close to research stations (Ferraz and Stenhouse) confirming Ferraz as a Hg source due to prevailing westerly and southwesterly winds (Wielbinska and Skrzypczak, 1988). This role had been previously proposed by another study (dos Santos et al., 2006). Another local source is due to present or ancient volcanic activity. Deception Island is an active volcano (Goyanes et al., 2014) and Penguin Island, where the *Sterna vittata* samples were collected, is an ancient volcano crater, with samples from these sites clearly distinguished from the rest, concerning lichens, but not necessarily mosses and soil. Particularly for Deception Island, literature indicates volcanic activity as the main Hg source, but also exceptional conditions for Hg methylation and low demethylation processes, increasing its bioavailability for aquatic organisms (Mão de Ferro et al., 2014). Such conditions may also affect Hg levels in vegetation as well *via* marine spray for instance. This marine spray is also reported to have significant influence in the geochemical composition of Antarctic soils (Malandrino et al., 2009).

Regarding Cd in a general way, soil concentrations were comparatively higher than mosses', followed by lichens highlighting lesser mobility when compared to Hg, likely due to

lesser atmospheric deposition and re-suspension among other reasons. Moreover, Cd concentrations remained under the limit of detection in all but one of the soil control sites suggesting the input of this element starkly linked to the bird colonies. Overall results seem in agreement with excreta and soil data (Celis et al., 2015; Espejo et al., 2014). The fact that most of the times colony soil samples present higher values when compared to excreta emphasizes the role of the former as a sink of trace elements. The input notably surpasses the eventual remobilisation processes when the respective control sites present much lower concentrations. This is apparently the case for most of the studied variables, as further discussed.

Correlations

Spearman correlation results are presented in Table 2. PCA is presented as supplementary material (Fig. S1).

Hg in lichens presents no significant correlation in control sites, probably due to the fact that they lack a true root system and absorb contaminants directly from the atmosphere (Park et al., 2010; Villeneuve et al., 1988; Yogui and Sericano, 2008), with no interaction with the elements present in the substrata (Kappen, 2000; Olech, 1994). However, in colony samples, Hg presented significant correlations (+Pb, +Se, -Zn, - $\delta^{15}\text{N}$, -%N) indicating not only the re-suspension/re-emission (see Jiskra et al., 2015) from the colonies as a common source for Hg and these other trace elements but also with nitrogen. In a previous work (Cipro et al., 2011), negative correlation with $\delta^{15}\text{N}$ was associated with more volatile or re-suspended contaminants whereas positive correlation was associated with the less volatile ones and/or more water-soluble as the ammonium. This is due to the fact that the kinetic fractionations during the decomposition of deposited urea and uric acid lead to the formation of in starkly ^{15}N -depleted highly volatile ammonia whereas the remaining ammonium, much less volatile, is conversely ^{15}N -enriched (Heaton, 1986). Therefore it results that plants around the excrement zone are more exposed to ^{15}N -enriched inorganic nitrogen whereas upland sites are more exposed to ^{15}N -depleted nitrogen (Cipro et al., 2011). Consequently, animal-derived nitrogen uptake is associated with large $\delta^{15}\text{N}$ ranges, with typical values ranging between 6 and 26‰ (e.g. Cipro et al., 2011; Cocks et al., 1998; Erskine et al., 1998; Mizutani and Wada, 1988). So, it could be inferred that the ammonia source correlates to the Hg source. Cd in lichens, on the other hand, is clearly related to the organic matter content, since it correlates to %N in both control and colony sites and with $\delta^{15}\text{N}$ in colony sites as well.

Lower $\delta^{13}\text{C}$, in turn, are related to moister habitats and marine influence, as well as high $\delta^{15}\text{N}$ (Cipro et al., 2011; Lee et al., 2009), which comes in agreement with our results both inter and intra-groups: mosses presented, in a general way, lower $\delta^{13}\text{C}$ than lichens and coastal locations presented lower $\delta^{13}\text{C}$ than the more inland ones for both mosses and lichens, in a much analogous way to what was previously described in literature, both qualitatively and quantitatively (Huiskes et al., 2006).

Taking mosses into consideration, a significant positive correlation with Pb could indicate a common source for control sites. The same conclusion could be taken in regard to Co, Cr and Ni presenting significant negative correlations with Hg in colony sites: these trace elements would most likely come from natural sources rather than a colony input. This is reinforced by the fact that these three elements presented significant negative correlations with an indicator of organic matter (%C and/or %N). Cd, in turn, presented significant positive correlation with %N and negative with %C in colony sites, which apparently points in two opposite directions, but could be related to the ornithogenic soil composition and decaying processes as previously described (see Emslie et al., 2013).

In respect to soil samples, the influence of colonies in Hg concentrations has been reported by several studies (Choy et al., 2010; Huang et al., 2014; Nie et al., 2012) and confirmed by significant positive correlations for both %C and %N. The same pattern is repeated for Cd as well as the same significant positive correlation with As, Se and Zn detected in mosses colony samples as well.

The results of the PCA (see supplementary material Fig. S1) corroborate the previous discussion on correlations found for each of the subset of samples. However, some interesting trends appear when the dataset is taken as a whole since opposing behaviours have been detected for the same variables in different matrixes. Considering all matrixes, %C and $\delta^{13}\text{C}$ were positively correlated with each other and negatively correlated to most other trace elements, likely due to the previously discussed decaying processes of organic matter. Conversely, %N and Hg were positively correlated with each other (due to the influence of organic matter previously discussed) and negatively correlated with some trace elements, particularly Mn and Pb. This is likely due to the natural origin of these trace elements, contrary to Hg. All these behaviours are repeated for the Colony subset when all matrixes are taken. However, when only Control samples are taken, %C and Hg were clearly associated and opposed to almost all other variables with two clear exceptions: %N and $\delta^{13}\text{C}$. This is likely

due to the differences in origin (natural/colony and atmospheric/bedrock) for these and the remaining variables.

Factors influencing Hg and Cd concentrations

In order to evaluate the relative importance of the categorical variables (main location, specific location, colony species and matrix species), GLM models were built and model selection was based upon AICc as previously explained.

Regarding Hg, the most accurate model for lichen control samples presented $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %N, Specific Location and Matrix Species as factors ($w_i=0.51$) and Specific Location and Matrix Species for colony samples ($w_i=0.33$). For mosses, the most accurate model for control samples was composed of $\delta^{15}\text{N}$, %N and Specific Location ($w_i=0.31$), whereas for colony samples it was composed of Specific Location, Colony Species and Matrix Species ($w_i=0.17$). Finally, the most accurate model for soil control samples presented %N and Main Location as factors ($w_i=0.30$) and Main Location and Colony Species for colony samples ($w_i=0.26$).

Regarding Cd, the most accurate model for lichen control samples presented $\delta^{13}\text{C}$, %C, Specific Location and Matrix Species as factors ($w_i=0.38$) and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, %N, Main Location and Matrix Species for colony samples ($w_i=0.34$). For mosses, the most accurate model for control samples was composed of $\delta^{13}\text{C}$ and Matrix Species ($w_i=0.31$), whereas for colony samples it was composed of $\delta^{15}\text{N}$, Specific Location, Colony Species and Matrix Species ($w_i=0.31$). Finally, the most accurate model for soil control samples presented $\delta^{13}\text{C}$ and Specific Location as factors, tied to %N, Main Location and Specific Location ($w_i=0.50$ each) and $\delta^{15}\text{N}$, Specific Location and Colony Species for colony samples ($w_i=0.56$).

Thanks to the GLM, it is possible to notice some patterns. Firstly, the factors are quite similar between Hg and Cd, especially for colony samples of mosses and soil. Moreover, for colony samples, there seems to be some effect of the matrix species but the colony species seems to be more present and in models with higher Akaike weights, suggesting colony species as the categorical variable with the most prominent role in explaining both Hg and Cd concentrations. This is likely due to different feeding ecology among species, and therefore, to Hg levels in bird droppings, but also to some variables that could not yet have been explored, such as colony size and/or density, directly linked to the local total Hg input. Moreover, moult

may also play a major role, since they might constitute an important pathway of Hg excretion (Carravieri et al., 2014) towards soils.

Control sites versus colony sites

Firstly, some Tukey HSD/ANOVA tests were performed in order to understand whether some collection sites with apparently abnormal concentrations were really not grouped with their counterparts, confirming some hypothesised local source other than the colonies.

In respect to Hg, in lichens, the colony samples from *S. vittata* ($p < 0.001$) in Penguin Island (an ancient volcano crater) and from *Larus dominicanus* ($p = 0.01$) in Stenhouse Point (subject to winds previously passing by 3 close research stations) were set apart of all the other lichen samples. For mosses, the control sample from Ferraz (the Brazilian research station) was set apart all of the other samples ($p < 0.001$). Finally, for soil samples, *Pygoscelis adeliae* from Penguin Island ($p < 0.001$) and *Pygoscelis antarcticus* from Half Moon ($p < 0.001$) can be set apart of all the other soil samples. Regarding Cd, there was no particular separation other than lichens from *P. antarcticus* from Half Moon ($p < 0.001$). Taking all of that into account, it is possible to infer three local Hg sources: research stations in King George Island, the ancient volcano crater in Penguin Island and another source of both Hg and Cd in Half Moon Island yet to be identified. The Island is home for several reproducing birds and hauling-out pinniped species during the summer, an important ship traffic and tourist site, paleovolcanic activity and also has a summer-only research station, therefore a lot of potential sources. Curiously, even human disturbance might play a role in trace element concentrations in penguins, even if indirectly (Barbosa et al., 2013).

Following, the results for the t-tests of colony sites compared to the closest control site are presented in Table 3.

Results show some interesting patterns. Lichens presented comparatively less difference between colony and control sites than mosses, which presented comparatively less difference between colony and control sites than soil samples. Lichens presented markedly differences in $\delta^{13}\text{C}$ likely due to the previously discussed influence of moisture and marine influence and also %N. For this latter, it is noticeable that in most of the cases when this variable was significantly different between colony and control sites, it was mostly with higher trophic level species, such as *Macronectes giganteus*, that normally form smaller and less dense colonies

when compared to penguins or shags for instance. Mosses, in turn, presented significant difference for most of the stable isotopes, %C and %N, and moreover, among trace elements, particularly for Hg, likely due to the influence of runoff water, as they are more moisture-dependent than lichens, that occur mostly in higher, more exposed spots in this area (Kim et al., 2007). This has already been suggested by other studies for organic contaminants (Cipro et al., 2011; Cunha et al., 2015).

Finally, soil samples presented differences between control and colony sites for almost all of the cases, with two notable exceptions: Cd, which remained under the method detection limit in all control sites, confirming the fact that the input of this trace element in this matrix is closely related to bird colonies (i.e. the difference exists but cannot be tested due to the lack of valid numbers); and $\delta^{15}\text{N}$, which presented significant difference only in *Phalacrocorax atriceps* colony sites, all of them. From field experience (*authors personal observations*), the colonies of this species presented the thickest layer of ornithogenic soil, in most cases located in higher and drier spots when compared to penguins. These locations would favour the accumulation of guano and impair the lixiviation of the guano-derived ammonium, enriching the $\delta^{15}\text{N}$ signature as previously discussed.

A mass balance perspective

At first, the fact that terrestrial ecosystems adjacent to seabird colonies present significantly higher concentrations when compared to locations further from such colonies does not necessarily mean that the referred ecosystem becomes enriched in pollutants as a whole: it could be a mere matter of remobilisation from one compartment to another. It does mean though that there is local enrichment and that assumption in no way excludes the previous hypothesis.

In order to verify the role of colonies as a source from a mass balance perspective, one must recur to the literature. It is estimated that global seabirds inputs of N and P are respectively of 591 Gg y⁻¹ and 99 Gg y⁻¹, with the Antarctic and Southern Ocean coasts receiving the highest amounts, roughly 80% whereas these areas 26% of the global seabird population (Otero et al., 2018). Moreover, various authors (Ellis et al., 2006; Lorrain et al., 2017; Otero et al., 2015; Sutton et al., 2013) suggested that seabird colonies represent a positive geochemical anomaly (i.e., above background values) regarding the concentrations of N and P present in soils, sediments, and water, hence the term ornithoeutrophication (Otero et al., 2018). It is, therefore,

reasonable to assume that seabird colonies enrich their ecosystems as a whole, in a mass balance perspective. Given that previous literature indicates that seabird faeces contain not only organic matter, but various of the concerned trace elements in the present work (Celis et al., 2015, 2014; Espejo et al., 2014; Finger et al., 2017), one can assume that the same affirmation made for organic matter is also valid for trace elements. To corroborate this assumption, literature brings also positive inputs for some trace elements in the Southern Ocean, for instance Hg (Cossa et al., 2011).

Taking into account the estimates for seabird excretion, the composition of excreta and colony size, it would be possible to estimate the net influx of some trace elements in a given colony or set of colonies. Cross-checking the literature for available data that could fit our sampling, a common case appeared: the Chinstrap Penguin (*Pygoscelis antarcticus*). Its total and breeding population are estimated at 12,800,000 and 8,000,000 individuals that would input 55.551 Gg N y⁻¹ and 9.529 Gg P y⁻¹ (Otero et al., 2018), resulting in an individual average of 4.339 Kg N y⁻¹ and 0.744 Kg P y⁻¹. The total N for excrements in this area averages 2.89% dw (Zhu et al., 2009). Using N as a proxy, the trace elements data for faeces from the closest site to Admiralty Bay (Narebski Point) in literature (Espejo et al., 2014) and the breeding population in Admiralty Bay being in average 1436 pairs (Petry et al., 2015), resulting in 4595 individuals (keeping the proportion between total and breeding individuals from Otero et al., 2018), one would have a total annual input of approximately 904 g of Pb, 2160 g of Cd, 460 g of As, 179 Kg of Cu and 157 Kg of Zn, only from the *P. antarcticus* individuals within the ecosystem of Admiralty Bay, King George Island.

Unfortunately, to the best of our knowledge there are no estimates of biomass for lichens and mosses in none of the areas comprehended in this study. Therefore, no direct estimation on the inventories of trace elements in these compartments can be performed nor the transfer from the colonies to these organisms. Estimation for soil inventories (Hawke, 2003; Hawke et al., 1999) is not possible either because of the need for core sampling due to previous colonies, percolation and so on (Liu et al., 2006; Tatur et al., 1997).

However, there is a mean estimate of 62.13 g m⁻² dry mass for *Usnea* lichen biomass from a volcanic mesa (Bohuslavová et al., 2012) a densely covered area that could be used as a starting point to estimate colony sites. The total *P. antarcticus* colony area in King George Island is estimated to be 0.14 ha (Petry et al., 2015) and 10% of the cover is estimated to be *Usnea* spp. (Kim et al., 2007) in another location in King George Island. A conservative estimation of lichen biomass would consider a 50m ring around these colonies, since we

assumed a 50m distance to the colony to classify a sampling site as such. Moreover, since colonies are often ashore, we will consider only half of the resulting surface, since the other half would fall over the water. Yet conservatively we will consider only one colony for this whole 0.14 ha area, therefore a circle of 21.11m radius. This will result in a 7243m² area of *Usnea* under the influence of the colony. Taking all previous data into account, this would result in approximately 45 Kg of *Usnea* in the whole surface under the influence of *P. antarcticus*. With the data from the present work for lichens (unfortunately the only site available was in Half Moon and with *Ramalina terebrata*) and *P. antarcticus* averages, one could deduce an inventory of 27 mg As, 20 mg Cd, 10 mg Hg and 2mg Pb only in the lichen compartment under the influence of *P. antarcticus*. Inventories for mosses will likely overcome those for lichens since mosses presented higher concentrations in a general way and also, from field experience, they should present considerably higher biomass. The same reasoning can be repeated concerning soil samples, which should represent the overall highest inventory among the matrixes sampled in this study.

Conclusions

The use of different matrixes in several different locations allowed the drawing of broader conclusions that otherwise would not be possible. In most cases, soil seems to be the best indicator to assess bird colonies as trace elements sources in these particular ecosystems as this matrix presented comparatively more differences between control and colony sites than vegetation. Bird colonies are clearly local sources of organic matter, Cd and Hg and likely of some other trace elements (As, Se and Zn). Conversely, Co, Cr, Ni and Pb come most likely from other sources than the colonies themselves, since when differences between colony and control sites were detected, in most cases the control ones presented higher concentrations. Both natural and anthropogenic sources for these elements have been reported previously (e.g. Bollhöfer and Rosman, 2001) and further research would be needed to separate them. In a general way, isotopes were more useful for vegetation (particularly mosses) as indicators of colonies influence whereas elemental composition was more useful for soil samples. Local Hg sources could be inferred from high levels in control sites and also from location and wind patterns. The mass balance approach provided fruitful insight, however more accurate field measurements of biomass are needed to refine its results and conclusions. Finally further studies could assess the influence of population status and dynamics on the results hereby found.

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Table 1 - Mean results for trace elements concentration (in $\mu\text{g g}^{-1}$ dw), SIA (‰) and C and N content (%) separated by location, colony species and matrix species

Lichen															
Main Location	Specific Location	Colony species	Matrix Species	n	Hg	As	Cd	Cu	Pb	Se	Zn	$\delta^{13}\text{C}$	%C	$\delta^{15}\text{N}$	%N
Deception Island	Deception Island	Control	<i>Usnea antarctica</i>	3	0.232 ± 0.072	0.787 ± 0.063	0.068 ± 0.024	3.93 ± 0.394	0.325 ± 0.020	1.08 ± 0.158	13.1 ± 2.24	-22.175 ± 3.69	30.8 ± 16.7	-0.680 ± 11.0	2.13 ± 1.22
Half Moon	Half Moon	Control	<i>Usnea aurantiaco-ater</i>	3	0.121 ± 0.007	0.178 ± 0.027	0.077 ± 0.004	2.66 ± 0.533	0.359 ± 0.160	1.57 ± 0.358	13.2 ± 1.15	-22.300 ± 0.136	41.7 ± 0.264	-5.730 ± 0.947	0.960 ± 0.060
		<i>Pygoscelis antarcticus</i>	<i>Ramalina terebrata</i>	3	0.223 ± 0.020	0.610 ± 0.246	0.445 ± 0.244	6.51 ± 1.11	0.045 ± 0.011	1.08 ± 0.291	16.0 ± 8.00	-23.940 ± 0.840	41.3 ± 0.326	-0.343 ± 1.53	2.21 ± 0.466
KGI	Cape Vauréal	<i>Macronectes giganteus</i>	<i>Usnea antarctica</i>	3	0.190 ± 0.065	0.606 ± 0.081	0.111 ± 0.026	3.66 ± 0.668	0.230 ± 0.018	1.75 ± 0.166	15.2 ± 5.48	-21.960 ± 0.701	40.1 ± 0.895	-0.028 ± 0.793	0.668 ± 0.025
	Ferraz	Control	<i>Usnea aurantiaco-ater</i>	3	0.307 ± 0.083	0.217 ± 0.068	0.021 ± 0.002	1.08 ± 0.107	0.766 ± 0.122	1.35 ± 0.286	7.16 ± 1.09	-20.750 ± 0.125	39.4 ± 1.67	-6.612 ± 4.83	0.341 ± 0.046
	Punta Hennequin	<i>Catharacta sp*</i>	<i>Usnea antarctica</i>	3	0.079 ± 0.001	0.493 ± 0.142	0.052 ± 0.022	3.41 ± 2.11	0.115 ± 0.038	0.697 ± 0.108	18.2 ± 4.40	-23.175 ± 0.403	40.4 ± 1.28	0.250 ± 1.35	1.64 ± 0.464
	Stenhouse	<i>Larus dominicanus*</i>	<i>Usnea antarctica</i>	3	0.526 ± 0.160	0.458 ± 0.174	0.017 ± 0.004	3.20 ± 1.42	0.802 ± 0.257	2.14 ± 0.636	10.5 ± 3.28	-22.395 ± 0.864	40.6 ± 0.886	-5.904 ± 0.814	0.526 ± 0.065
	Turret Point	Control	<i>Ramalina terebrata</i>	3	0.187 ± 0.056	0.455 ± 0.120	0.093 ± 0.035	4.71 ± 0.788	0.093 ± 0.007	0.942 ± 0.134	13.1 ± 3.58	-24.366 ± 0.191	40.5 ± 0.774	0.852 ± 0.922	1.67 ± 0.161
			<i>Usnea antarctica</i>	3	0.247 ± 0.056	0.302 ± 0.064	0.039 ± 0.002	3.41 ± 0.846	0.186 ± 0.032	1.01 ± 0.283	11.2 ± 0.421	-22.702 ± 0.223	40.3 ± 1.21	-3.155 ± 0.555	1.40 ± 0.311
			<i>Usnea aurantiaco-ater</i>	3	0.245 ± 0.037	0.453 ± 0.083	0.074 ± 0.008	3.39 ± 0.458	0.247 ± 0.026	1.20 ± 0.277	16.2 ± 2.54	-21.486 ± 0.297	39.9 ± 0.520	-5.875 ± 0.599	1.44 ± 0.076
	Turret Point	<i>Phalacrocorax atriceps</i>	<i>Ramalina terebrata</i>	3	0.123 ± 0.021	0.447 ± 0.109	0.027 ± 0.002	4.14 ± 0.361	0.058 ± 0.005	0.462 ± -	16.0 ± 1.36	-22.880 ± 0.152	38.5 ± 0.522	1.25 ± 0.420	1.50 ± 0.205
		<i>Pygoscelis adeliae</i>	<i>Usnea antarctica</i>	3	0.178 ± 0.073	0.527 ± 0.022	0.076 ± 0.042	11.2 ± 7.10	0.246 ± 0.030	1.48 ± 0.218	25.8 ± 8.95	-23.339 ± 0.102	38.4 ± 1.39	-1.594 ± 1.83	1.42 ± 0.478
	Yellow Point	Control	<i>Usnea aurantiaco-ater</i>	1	0.154	2.40	0.105	7.97	1.75	1.64	18.1	-22.066	39.0	-1.949	1.34
Livingston	Hannah Point	<i>Macronectes giganteus</i>	<i>Ramalina terebrata</i>	2	0.179 ± 0.062	0.691 ± 0.071	0.087 ± 0.034	5.65 ± 0.543	0.121 ± 0.007	1.37 ± 0.806	15.3 ± 1.77	-24.050 ± 0.137	42.1 ± 3.29	-1.693 ± 7.07	1.85 ± 0.662
			<i>Turgidiusculum complicatulum</i>	1	0.087	1.46	0.206	18.5	1.22	2.70	49.9	-16.170	33.7	9.78	4.18
		<i>Pygoscelis papua</i>	<i>Ramalina terebrata</i>	3	0.172 ± 0.018	0.462 ± 0.060	0.075 ± 0.016	5.52 ± 0.369	0.049 ± 0.002	1.47 ± 0.243	19.4 ± 4.19	-22.731 ± 0.333	40.2 ± 1.04	11.2 ± 0.588	2.02 ± 0.071
Nelson Island	Nelson Island	<i>Daption capense**</i>	<i>Usnea aurantiaco-ater</i>	3	0.213 ± 0.022	0.603 ± 0.082	0.104 ± 0.018	2.56 ± 0.608	0.102 ± 0.013	1.41 ± 0.355	13.2 ± 1.91	-22.837 ± 0.525	41.5 ± 2.18	5.04 ± 6.30	1.12 ± 0.171
Penguin Island	Penguin Island	Control	<i>Usnea aurantiaco-ater</i>	3	0.104 ± 0.015	0.218 ± 0.028	0.032 ± 0.003	4.08 ± 1.53	0.184 ± 0.009	0.802 ± 0.077	12.3 ± 1.18	-22.184 ± 0.745	41.3 ± 0.328	-5.393 ± 0.864	0.869 ± 0.151
		<i>Sterna vittata</i>	<i>Ramalina terebrata</i>	3	0.691 ± 0.018	0.201 ± 0.037	0.012	9.93 ± 3.95	0.361 ± 0.032	2.25 ± 0.080	10.6 ± 1.61	-24.832 ± 0.335	34.1 ± 2.38	-2.575 ± 0.336	0.923 ± 0.022
			<i>Usnea aurantiaco-ater</i>	3	0.405 ± 0.039	0.220 ± 0.015	0.019 ± 0.001	5.12 ± 0.325	0.460 ± 0.034	1.77 ± 0.098	9.25 ± 0.450	-23.389 ± 0.332	39.2 ± 0.299	-7.417 ± 0.159	0.724 ± 0.054

Moss															
Main Location	Specific Location	Colony species	Matrix Species	n	Hg	As	Cd	Cu	Pb	Se	Zn	$\delta^{13}\text{C}$	%C	$\delta^{15}\text{N}$	%N
Deception Island	Deception Island	Control	<i>Bryum pseudotrichetrum</i>	4	0.077 ± 0.092	0.737 ± 0.534	0.045 ± 0.012	11.3 ± 5.41	0.172 ± 0.100	1.16 ± 0.692	29.2 ± 12.9	-25.235 ± 1.34	34.2 ± 12.6	1.60 ± 6.59	1.80 ± 0.777
Half Moon	Half Moon	Control	<i>Sanionia uncinata</i>	3	0.039 ± 0.002	0.146 ± 0.033	0.331 ± 0.029	14.4 ± 1.54	0.127 ± 0.035	1.62 ± 0.218	20.9 ± 1.97	-24.298 ± 0.213	43.6 ± 0.392	13.1 ± 0.735	2.27 ± 0.315
		<i>Pygoscelis antarcticus</i>	<i>Prasiola crisa</i>	3	0.084 ± 0.007	1.25 ± 0.331	0.459 ± 0.078	23.6 ± 10.1	0.215 ± 0.074	4.41 ± 0.998	38.9 ± 8.65	-21.493 ± 1.08	42.2 ± 1.57	0.426 ± 0.988	6.53 ± 0.130
	Cape Vauréal	<i>Macronectes giganteus</i>	<i>Colobanthus quitensis</i>	3	0.021 ± 0.004	1.26 ± 0.359	0.568 ± 0.381	32.7 ± 8.82	0.767 ± 0.247	1.50 ± 0.388	45.6 ± 6.63	-26.233 ± 0.172	28.0 ± 6.61	8.79 ± 1.18	1.28 ± 0.235
			<i>Polytrichastrum alpinum</i>	3	0.075 ± 0.006	1.37 ± 0.431	0.072 ± 0.006	65.8 ± 18.4	0.436 ± 0.144	1.93 ± 0.310	26.4 ± 5.51	-26.085 ± 0.395	37.9 ± 3.96	12.2 ± 0.593	1.06 ± 0.071
	Chabrier Rock	<i>Pygoscelis antarcticus</i>	<i>Prasiola crisa</i>	3	0.066 ± 0.004	3.19 ± 0.251	0.206 ± 0.023	44.4 ± 3.00	0.142 ± 0.023	6.40 ± 0.606	56.7 ± 1.93	-20.553 ± 0.187	35.9 ± 2.59	12.8 ± 0.793	5.61 ± 0.360
	Ferraz	Control	<i>Sanionia uncinata</i>	3	0.336 ± 0.060	0.793 ± 0.310	0.132 ± 0.081	10.9 ± 3.05	0.921 ± 0.447	1.33 ± 0.380	23.1 ± 5.69	-27.509 ± 0.401	41.6 ± 1.53	6.27 ± 0.901	1.11 ± 0.276
KGI	Punta Hennequin	<i>Catharacta sp*</i>	<i>Sanionia uncinata</i>	3	0.081 ± 0.001	0.559 ± 0.114	0.192 ± 0.069	4.56 ± 0.237	0.192 ± 0.116	0.904 ± 0.241	21.6 ± 0.945	-25.882 ± 0.473	43.7 ± 0.924	9.51 ± 0.406	2.04 ± 0.094
	Punta Ullmann	<i>Larus dominicanus*</i>	<i>Sanionia uncinata</i>	3	0.107 ± 0.016	1.80 ± 0.134	0.091 ± 0.007	26.5 ± 3.03	0.883 ± 0.124	1.71 ± 0.223	29.5 ± 2.99	-25.439 ± 0.424	35.4 ± 1.27	2.93 ± 0.742	1.65 ± 0.168
	Stenhouse	<i>Larus dominicanus*</i>	<i>Sanionia uncinata</i>	3	0.078 ± 0.015	3.60 ± 1.01	0.790 ± 0.397	55.6 ± 8.61	2.27 ± 0.745	2.72 ± 0.313	35.6 ± 8.30	-25.374 ± 0.599	31.4 ± 6.35	2.33 ± 0.241	1.52 ± 0.410
	Turret Point	Control	<i>Sanionia uncinata</i>	3	0.123 ± 0.006	1.56 ± 0.101	0.215 ± 0.010	16.0 ± 5.94	0.447 ± 0.047	3.19 ± 0.213	27.1 ± 11.3	-25.629 ± 0.172	33.0 ± 1.05	11.9 ± 0.113	2.62 ± 0.034
		<i>Pygoscelis adeliae</i>	<i>Sanionia uncinata</i>	3	0.046 ± 0.002	0.544 ± 0.047	0.180 ± 0.007	15.9 ± 1.45	0.170 ± 0.024	1.28 ± 0.122	37.1 ± 1.87	-26.593 ± 0.342	39.5 ± 0.939	11.5 ± 0.140	1.86 ± 0.062
	Yellow Point	Control	<i>Syntrichia sp</i>	1	0.069	7.09	0.176	49.5	3.47	2.29	28.2	-25.562	31.9	7.08	1.17
Livingston	Hannah Point	<i>Macronectes giganteus</i>	<i>Prasiola crisa</i>	3	0.059 ± 0.007	3.02 ± 0.436	0.537 ± 0.036	14.8 ± 0.943	0.525 ± 0.204	3.43 ± 0.378	33.9 ± 4.19	-28.007 ± 0.166	35.4 ± 2.28	12.7 ± 0.832	3.85 ± 0.256
		<i>Phalacrocorax atriceps</i>	<i>Prasiola crisa</i>	3	0.177 ± 0.019	3.47 ± 0.869	1.46 ± 0.548	59.3 ± 50.4	0.356 ± 0.023	5.13 ± 1.43	209 ± 39.6	-21.806 ± 0.267	20.2 ± 4.53	21.0 ± 2.81	3.53 ± 0.621
		<i>Pygoscelis antarcticus</i>	<i>Prasiola crisa</i>	2	0.077 ± 0.001	3.93 ± 0.265	1.37 ± 0.193	53.6 ± 16.8	0.629 ± 0.201	9.44 ± 1.16	88.9 ± 22.5	-24.147 ± 0.114	25.6 ± 3.45	16.5 ± 0.727	3.85 ± 0.461
		<i>Pygoscelis papua</i>	<i>Prasiola crisa</i>	3	0.030 ± 0.006	3.80 ± 0.459	0.157 ± 0.024	17.3 ± 2.97	0.649 ± 0.063	3.03 ± 0.177	32.1 ± 5.02	-20.161 ± 0.400	26.6 ± 3.32	15.1 ± 0.352	4.06 ± 0.588
Nelson Island	Nelson Island	<i>Daption capense**</i>	<i>Sanionia uncinata</i>	3	0.167 ± 0.013	1.28 ± 0.172	0.263 ± 0.031	15.3 ± 0.712	0.198 ± 0.034	1.78 ± 0.102	19.8 ± 1.58	-25.513 ± 0.282	43.0 ± 0.890	19.5 ± 0.581	3.01 ± 0.219
Penguin Island	Penguin Island	Control	<i>Polytrichastrum alpinum</i>	3	0.037 ± 0.003	0.221	0.134 ± 0.017	14.1 ± 7.11	0.146 ± 0.099	0.481	19.7 ± 1.94	-27.178 ± 0.117	40.0 ± 6.86	5.43 ± 0.205	1.12 ± 0.255
		<i>Pygoscelis antarcticus</i>	<i>Prasiola crisa</i>	3	0.086 ± 0.013	3.09 ± 0.575	0.987 ± 0.488	73.6 ± 18.9	0.186 ± 0.022	10.1 ± 4.29	138 ± 49.6	-21.733 ± 0.448	37.6 ± 1.85	5.80 ± 1.08	6.83 ± 0.404

Soil														
Main Location	Specific Location	Colony species	n	Hg	As	Cd	Cu	Pb	Se	Zn	$\delta^{13}\text{C}$	%C	$\delta^{15}\text{N}$	%N
Deception Island	Deception Island	Control	3	0.006 ± 0.000	0.711 ± 0.086		7.66 ± 0.715	0.268 ± 0.020	0.693 ± 0.073	16.2 ± 0.875	-25.752 ± 0.234	0.095 ± 0.046	29.6 ± 3.12	0.053 ± 0.008
Half Moon	Half Moon	<i>Pygoscelis antarcticus</i>	3	0.247 ± 0.044	6.37 ± 1.22	4.92 ± 0.586	419 ± 39.4	0.249 ± 0.093	30.9 ± 1.53	388 ± 60.3	-28.470 ± 0.529	17.2 ± 2.12	20.1 ± 0.942	4.42 ± 0.262
	Chabrier Rock	<i>Pygoscelis antarcticus</i>	3	0.192 ± 0.020	8.09 ± 1.29	2.93 ± 1.26	316 ± 115	0.055 ± 0.003	27.4 ± 8.62	260 ± 84.6	-28.079 ± 0.924	21.6 ± 1.98	13.5 ± 5.19	9.58 ± 4.48
KGI	Shag Island	<i>Phalacrocorax atriceps</i>	3	0.194 ± 0.012	3.89 ± 0.236	1.46 ± 0.043	59.5 ± 4.35	0.115 ± 0.018	6.43 ± 0.705	255 ± 1.09	-24.745 ± 0.409	9.87 ± 1.40	30.9 ± 0.802	2.76 ± 0.408
	Turret Point	Control	3	0.013 ± 0.004	1.53 ± 0.134		22.0 ± 1.83	0.519 ± 0.089	1.03 ± 0.141	39.5 ± 2.73	-23.414 ± 0.294	0.540 ± 0.335	13.0 ± 7.42	0.156 ± 0.073
		<i>Phalacrocorax atriceps</i>	3	0.197 ± 0.025	6.08 ± 1.33	2.34 ± 0.246	50.1 ± 4.13	0.190 ± 0.045	9.14 ± 1.10	467 ± 11.9	-24.508 ± 0.401	8.66 ± 1.11	30.7 ± 0.718	2.52 ± 0.359
	Yellow Point	<i>Pygoscelis adeliae</i>	3	0.091 ± 0.006	4.23 ± 0.224	2.60 ± 0.375	264 ± 28.3	0.507 ± 0.041	22.7 ± 3.52	385 ± 42.8	-26.813 ± 0.267	9.65 ± 2.24	16.8 ± 0.746	2.03 ± 0.458
		Control	1	0.009	19.3	0.328	80.6	4.45	3.69	81.7	-22.081	0.814	9.12	0.097
Livingston	Hannah Point	<i>Phalacrocorax atriceps</i>	3	0.168 ± 0.002	4.32 ± 0.693	1.42 ± 0.401	30.9 ± 6.01	0.208 ± 0.109	4.92 ± 0.300	262 ± 50.7	-24.423 ± 0.330	7.54 ± 1.42	25.7 ± 2.06	1.94 ± 0.626
		<i>Pygoscelis antarcticus</i>	3	0.133 ± 0.025	5.05 ± 0.158	1.60 ± 0.204	157 ± 14.9	0.843 ± 0.053	14.1 ± 1.63	177 ± 15.1	-28.139 ± 0.317	8.84 ± 0.938	18.8 ± 0.137	1.95 ± 0.186
		<i>Pygoscelis papua</i>	3	0.028 ± 0.006	4.36 ± 0.198	0.132 ± 0.003	49.1 ± 1.71	1.45 ± 0.038	4.54 ± 0.047	64.0 ± 1.41	-27.402 ± 0.560	1.55 ± 0.127	22.4 ± 1.78	0.523 ± 0.051
Penguin Island	Penguin Island	Control	3	0.007 ± 0.001	0.470 ± 0.019		47.8 ± 3.54	0.460 ± 0.043	0.549 ± 0.026	29.9 ± 1.73	-22.317 ± 0.232	1.23 ± 0.392	18.8 ± 0.441	0.228 ± 0.076
		<i>Pygoscelis adeliae</i>	3	0.290 ± 0.014	8.39 ± 0.878	4.94 ± 0.636	416 ± 80.6	0.088 ± 0.034	34.8 ± 5.55	444 ± 13.2	-28.461 ± 0.462	24.6 ± 2.22	17.4 ± 1.53	5.72 ± 0.637

Table 2 - Significant Spearman correlations among the studied variables

	Control sites	Colony sites
Lichen	Hg	+Pb, +Se, -Zn, $-\delta^{15}\text{N}$, -%N
	As	-Co, -Ni, +Zn, +%C, $+\delta^{15}\text{N}$, +%N
	Cd	+Cu, $+\delta^{15}\text{N}$, +%N
	Co	-As, +Cr, +Cu, +Fe, +Mn, +Ni, +Pb, +Se, -V, -%C, $-\delta^{15}\text{N}$
	Cr	+Co, +Cu, +Fe, +Mn, +Ni, +Pb, +Se, -%C, $-\delta^{15}\text{N}$
	Cu	+Cd, +Co, +Cr, +Fe, +Mn, +Ni, +Zn, $-\delta^{13}\text{C}$, -%C, +%N
	Fe	+Co, +Cr, +Cu, +Mn, +Ni, +Pb, +Se, -V, -%C, $-\delta^{15}\text{N}$
	Mn	+Co, +Cr, +Cu, +Fe, +Ni, +Pb, -V, +Zn, $-\delta^{13}\text{C}$, -%C
	Ni	-As, +Co, +Cr, +Cu, +Fe, +Mn, +Pb, +Se, -V, -%C
	Pb	+Hg, +Co, +Cr, +Fe, +Mn, +Ni, +Se, $-\delta^{15}\text{N}$, -%N
	Se	+Hg, +Co, +Cr, +Fe, +Ni, +Pb, -%C, $-\delta^{15}\text{N}$, -%N
	V	-Co, -Fe, -Mn, -Ni, +%C, $+\delta^{15}\text{N}$
	Zn	-Hg, +As, +Cu, +Mn, $+\delta^{15}\text{N}$, +%N
	$\delta^{13}\text{C}$	-Cu, -Mn
	%C	+As, -Co, -Cr, -Cu, -Fe, -Mn, -Ni, -Se, +V
	$\delta^{15}\text{N}$	-Hg, +As, +Cd, -Co, -Cr, -Fe, -Pb, -Se, +V, +Zn, +%N
	N	+As, +Cd, +Co, +Cr, +Cu, +Fe, +Mn, +Ni, -V, +Zn, $+\delta^{15}\text{N}$
%N	-Hg, +As, +Cd, +Cu, -Pb, -Se, +Zn, $+\delta^{15}\text{N}$	
Moss	Hg	-Co, -Cr, -Ni
	As	+Cd, +Cu, +Fe, +Mn, +Pb, +Se, +Zn, $+\delta^{13}\text{C}$, -%C, +%N
	Cd	+As, +Se, +Zn, -%C, +%N
	Co	-Hg, +Cr, +Fe, +Mn, +Ni, +Pb, +V, $-\delta^{13}\text{C}$, -%C, -%N
	Cr	-Hg, +Co, +Cu, +Fe, +Mn, +Ni, +Zn, -%C
	Cu	+As, +Cr, +Fe, +Mn, +Ni, +Se, -%C
	Fe	+As, +Co, +Cr, +Cu, +Mn, +Ni, +Pb, +V, +Zn, -%C, -%N
	Mn	+As, +Co, +Cr, +Cu, +Fe, +Ni, +Pb, +V, +Zn, -%C, -%N
	Ni	-Hg, +Co, +Cr, +Cu, +Fe, +Mn, +Zn, $-\delta^{13}\text{C}$, -%C, -%N
	Pb	+As, +Co, +Fe, +Mn, +V, -%C, -%N
	Se	+As, +Cd, +Cu, +Zn, $+\delta^{13}\text{C}$, -%C, +%N
	V	+Co, +Fe, +Mn, +Pb, -%C, -%N
	Zn	+As, +Cd, +Cr, +Cu, +Fe, +Mn, +Ni, +Se, $+\delta^{13}\text{C}$, -%C, +%N
	$\delta^{13}\text{C}$	+As, -Co, -Ni, +Se, +Zn, +%N
	%C	-As, -Cd, -Co, -Cr, -Cu, -Fe, -Mn, -Ni, -Pb, -Se, -V, -Zn
	$\delta^{15}\text{N}$	+Cd, +Cu, +V
	N	+As, +Cd, -Co, -Fe, -Mn, -Ni, -Pb, +Se, -V, +Zn, $+\delta^{13}\text{C}$
%N	+As, +Cd, -Co, -Fe, -Mn, -Ni, -Pb, +Se, -V, +Zn, $+\delta^{13}\text{C}$	
Soil	Hg	+As, +Cd, -Co, +Cu, -Fe, -Mn, -Pb, +Se, -V, +Zn, +%C, +%N
	As	+Hg, +Cd, -Co, +Cu, -Mn, -Pb, +Se, -V, $-\delta^{13}\text{C}$, +%C, $-\delta^{15}\text{N}$, +%N
	Cd	+Hg, +As, -Co, +Cu, -Mn, -Pb, +Se, -V, +Zn, $-\delta^{13}\text{C}$, +%C, $-\delta^{15}\text{N}$, +%N
	Co	-Hg, -As, -Cd, +Cr, -Cu, +Fe, +Mn, +Pb, -Se, +V, -%C, -%N
	Cr	+Co, +Fe, +Ni, +Pb, +V, +Zn
	Cu	+Hg, +As, +Cd, -Co, -Mn, +Se, +Zn, $-\delta^{13}\text{C}$, +%C, $-\delta^{15}\text{N}$, +%N
	Fe	-Hg, +Co, +Cr, +Mn, +Pb, +V, -%C, -%N
	Mn	-Hg, -As, -Cd, +Co, -Cu, +Fe, +Pb, -Se, +V, -Zn, -%C, -%N
	Ni	+Cr, +Zn, $+\delta^{13}\text{C}$
	Pb	-Hg, -As, -Cd, +Co, +Cr, +Fe, +Mn, -Se, +V, -%C, -%N
	Se	+Hg, +As, +Cd, -Co, +Cu, -Mn, -Pb, -V, +Zn, $-\delta^{13}\text{C}$, +%C, $-\delta^{15}\text{N}$, +%N
	V	-Hg, -As, -Cd, +Co, +Cr, +Fe, +Mn, +Pb, -Se, -Zn, -%C, -%N
	Zn	+Hg, +Cd, +Cr, +Cu, -Mn, +Ni, +Se, -V, +%C, +%N
	$\delta^{13}\text{C}$	-As, -Cd, -Cu, +Ni, -Se, -%C, $+\delta^{15}\text{N}$
	%C	+Hg, +As, +Cd, -Co, +Cu, -Fe, -Mn, -Pb, +Se, -V, +Zn, $-\delta^{13}\text{C}$, $-\delta^{15}\text{N}$, +%N
	$\delta^{15}\text{N}$	-As, -Cd, -Cu, -Se, $+\delta^{13}\text{C}$, -%C
	N	-Hg, -Cu, -Fe, -Mn, -Pb, -V, -Zn, $-\delta^{13}\text{C}$, -%C
%N	+Hg, +As, +Cd, -Co, +Cu, -Fe, -Mn, -Pb, +Se, -V, +Zn, +%C	

Table 3 - Results for the t-tests of colony sites compared to the closest control site, separated by matrix. The simple, double and triple * marks stand for p=0.05, 0.01 and 0.001, respectively

Lichen					Hg	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	$\delta^{13}\text{C}$ (‰)	%C	$\delta^{15}\text{N}$ (‰)	%N	
Main Location	Specific Location	Colony species	Matrix Species	n																		
Half Moon	Half Moon	<i>Pygoscelis antarcticus</i>	<i>Ramalina terebrata</i>	3	**	*				**		***		*				*		**	*	
KGI	Cape Vauréal	<i>Macronectes giganteus</i>	<i>Usnea antarctica</i>	3		**	**	*		**	*	**	*	**				*			***	
	Punta Hennequin	<i>Catharacta sp*</i>	<i>Usnea antarctica</i>	3	**	*						*		***	*		*	***			**	
	Stenhouse	<i>Larus dominicanus*</i>	<i>Usnea antarctica</i>	3														*			*	
	Turret Point		<i>Phalacrocorax atriceps</i>	<i>Ramalina terebrata</i>	3			*	***			***	**	**	**				***	*		
			<i>Pygoscelis adeliae</i>	<i>Usnea antarctica</i>	3		**			*								*	*	*		
Livingston	Hannah Point	<i>Macronectes giganteus</i>	<i>Ramalina terebrata</i>	2		**		*		**	**	***						***				
			<i>Turgidiusculum complicatulum</i>	1		***	**	***	**	**	***	***		*				**	***	**	**	***
		<i>Pygoscelis papua</i>	<i>Ramalina terebrata</i>	3	*	**		*		**	*	***		*							***	***
Nelson Island	Nelson Island	<i>Daption capense**</i>	<i>Usnea aurantiaco-ater</i>	3		**	**		*	*	*		***				**	**		**		
Penguin Island	Penguin Island	<i>Sterna vittata</i>	<i>Usnea aurantiaco-ater</i>	3	***		**		*				*	***	***	*	*		**		*	

Moss

Main Location	Specific Location	Colony species	Matrix Species	n	Hg	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	$\delta^{13}\text{C}$ (‰)	%C	$\delta^{15}\text{N}$ (‰)	%N	
Half Moon	Half Moon	<i>Pygoscelis antarcticus</i>	<i>Prasiola crispa</i>	3	***	**			*						**	***	*	*		***	***	
KGI	Cape Vauréal	<i>Macronectes giganteus</i>	<i>Colobanthus quitensis</i>	3	***			*	**	*	*	**	***			*	*	**	*	*	*	
			<i>Polytrichastrum alpinum</i>	3	**				*	**				*					*		*	***
	Chabrier Rock	<i>Pygoscelis antarcticus</i>	<i>Prasiola crispa</i>	3	**	***			**	***			*	*	***		***	***	*	***	***	
	Punta Hennequin	<i>Catharacta sp*</i>	<i>Sanionia uncinata</i>	3	**			*		*	*								*	**	**	
	Punta Ullmann	<i>Larus dominicanus*</i>	<i>Sanionia uncinata</i>	3	**	**		**	**	**	*	**	**	**		**		**	**	**	**	*
	Stenhouse	<i>Larus dominicanus*</i>	<i>Sanionia uncinata</i>	3	**	*	*	*	*	**	*	**	**			**		**	**	**	**	*
Turret Point	<i>Pygoscelis adeliae</i>	<i>Sanionia uncinata</i>	3	***	***	**								***	***		*	**	*	*	***	
Livingston	Hannah Point	<i>Macronectes giganteus</i>	<i>Prasiola crispa</i>	3	*	***	**	**	*		**	***	**	*	**		**	***	**	***	**	
		<i>Phalacrocorax atriceps</i>	<i>Prasiola crispa</i>	3	***	**	*	***	***		***	***	**	***	*	*		**	***	***	**	*
		<i>Pygoscelis antarcticus</i>	<i>Prasiola crispa</i>	2	***	***	**	*	*	*	*	**	**	**	*	**		*	**	**	*	*
		<i>Pygoscelis papua</i>	<i>Prasiola crispa</i>	3		***	**	**	**		**	***	**	**	***	***		*	***	***	*	*
Nelson Island	Nelson Island	<i>Daption capense**</i>	<i>Sanionia uncinata</i>	3	**									*				**		***	***	
Penguin Island	Penguin Island	<i>Pygoscelis antarcticus</i>	<i>Prasiola crispa</i>	3	**	*	*			**							*	***			***	

Soil				n	Hg	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	$\delta^{13}\text{C}$ (‰)	%C	$\delta^{15}\text{N}$ (‰)	%N	
Half Moon	Half Moon	<i>Pygoscelis antarcticus</i>		3	***	**		**	**	***	***	***	**	*	***	**	***	***	***		***	
	Chabrier Rock	<i>Pygoscelis antarcticus</i>		3	***	***		**	**	*	***	***	**	***	**	***	*	**	***		*	
KGI	Shag Island	<i>Phalacrocorax atriceps</i>		3	***	***		**	**	***	***	***	**	**	***	***	***	*	***	*	***	
	Turret Point	<i>Phalacrocorax atriceps</i>		3	***	**		**	**	***	***	**	**	**	***	**	***	*	***	*	***	
		<i>Pygoscelis adeliae</i>		3	***	***		**		***	**	**	**		***		***	***	**		**	
Livingston	Hannah Point	<i>Phalacrocorax atriceps</i>		3	***	**		**	**		***		**	*	***	**	**	*	**	*	**	
		<i>Pygoscelis antarcticus</i>		3	**	***		**	**	***	**		**	**	***		***	***	***	***		***
		<i>Pygoscelis papua</i>		3	*	***		**	**	***	*		**	***	***	**	***	***	***	**		**
Penguin Island	Penguin Island	<i>Pygoscelis adeliae</i>		3	***	***		***	*	**	***	***	**	***	***	***	***	***	***		***	