

Storm petrels as indicators of pelagic seabird exposure to chemical elements

in the Antarctic marine ecosystem

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Abstract

Data on trace element bioavailability in the south-polar marine ecosystem is still scarce, compared to that relating to temperate zones. Seabirds can be used as indicators of ecosystem health and sentinels of environmental pollution, constituting a link between marine and terrestrial environments. Here, we analysed the concentration of 17 elements (with special emphasis on mercury, Hg) in feathers of adults and chicks of two pelagic seabirds – the Wilson’s storm petrel *Oceanites oceanicus* and the black-bellied storm petrel *Fregetta tropica* – breeding sympatrically in the maritime Antarctic. Since adult feathers are formed during the non-breeding period away from the breeding grounds, but down and body feathers of chicks

25 grow at the breeding sites, we were able to evaluate the birds' exposure to contaminants at
26 various stages of their annual life cycle and in various marine zones. We found that of the two
27 studied species, adult black-bellied storm petrels had significantly higher mercury, selenium
28 and copper levels (5.47 ± 1.61 ; 5.19 ± 1.18 ; $8.20\pm 0.56 \mu\text{g g}^{-1} \text{ dw}$, respectively) than Wilson's
29 storm petrels (2.38 ± 1.47 ; 1.81 ± 0.98 ; $2.52\pm 2.35 \mu\text{g g}^{-1} \text{ dw}$, respectively). We found that
30 Wilson's storm petrel chicks had a significantly different contaminant profile than adults.
31 Arsenic, bismuth and antimony were detected exclusively in the chick feathers, and the Se:Hg
32 molar ratio was higher in chicks than in adults. Our study also suggests considerable maternal
33 transfer of Hg (to down feathers) in both species. As global contaminant emissions are
34 expected to increase, birds inhabiting remote areas with sparse anthropogenic pollution can
35 indicate the temporal trends in global contamination.

36 **Keywords:** contamination, feather, toxic metals, ICP-MS, mercury, Procellariiformes

37

38 1. Introduction

39 Organisms living in Antarctica are exposed to a number of environmental factors that may
40 affect their health and survival. Of those, the most influential are harsh climate conditions,
41 competition for food, and predation, but pollutants may also play an important role (Santos et
42 al. 2006, Metcheva et al. 2006). Contaminants in the polar zone may originate from natural
43 processes [i.e. volcanic activity, the input of sea-spray, mechanical and chemical rock
44 weathering (Malandrino et al. 2009)] and biota [e.g. mammals or bird colonies can be a
45 source of nutrients/organic matter (N, F) and several elements such as Cd, Hg, As, Se and Zn
46 to the terrestrial and coastal ecosystem (Cipro et al. 2018)]. Anthropogenic sources of
47 Antarctic ecosystem contamination are often located outside the region, e.g. lead from
48 industrial emissions is transported from South America (Sañudo-Wilhelmy et al. 2002, Gaiero

49 et al. 2003, Bargagli 2008). However, local sources, too, may contribute due to increasing
50 research and tourism activities resulting in fuel combustion, accidental oil spills, waste
51 disposal sites, sewage and paint residues (Bargagli 2008, Jerez et al. 2011, Mão de Ferro et al.
52 2013).

53 Pelagic seabirds living in the southern polar zone can be used in ecotoxicological studies to
54 assess trace element pollution and marine ecosystem health (Carravieri et al. 2014). They
55 constitute a valuable link between terrestrial and marine zones of the Antarctic (Santos et al.
56 2006). As they often cover vast distances in search of suitable foraging areas, they are
57 exposed to pollutants in various geographical locations. They may also carry these
58 contaminants between wintering and/or stop-over staging and breeding, due to migratory
59 connectivity (Webster et al. 2002).

60 Seabirds' feathers are often used to evaluate their exposure to contaminants (e.g. Jerez
61 et al. 2011, Bustamante et al. 2016, Philpot et al. 2019), providing a record of contaminant
62 uptake at the time of feather growth and development (Bearhop et al. 2002, Jaspers et al.
63 2004). High metals affinity to sulfhydryl groups of the feather structural proteins are making
64 them a suitable biomonitoring tool (Thompson et al. 1998). Elemental deposition in feather
65 tissue is species-specific and depends on multiple factors, including diet, age, detoxification
66 abilities and moulting pattern (Burger and Gochfeld 1997, Evers et al. 2008, Cipro et al. 2014,
67 Pacyna et al. 2017). Knowledge of avian moulting sequences is essential to the reconstruction
68 of the contamination period (Bustamante et al. 2016, Cherel et al. 2018). Adult feathers may
69 provide a wider perspective on metal exposure over the annual cycle, but as seabirds may
70 cover a vast area during the moulting period it is challenging to properly interpret their
71 exposure over time. Also seasonal shifts in element concentrations can occur (Øverjordet et
72 al. 2015). By contrast, chick feathers may provide information over a shorter period of
73 exposure for a more defined area (Evers et al. 2005). Chick down is formed in the egg from

74 maternal nutrients and as such represents female contamination during the pre-laying period
75 (Ackermann et al. 2016). Thus, analysis of feathers collected at various life stages allows the
76 elemental concentrations of various areas to be reconstructed, indicating temporal and spatial
77 trends in pollution in the ecosystems being occupied at the time (Becker, 2003).

78 Despite the growing number of studies on Antarctic and sub-Antarctic food web
79 contamination, still little is known about elemental concentrations in seabirds feeding at low
80 trophic levels (i.e. preying on zooplankton and krill), likely due to their relatively lower
81 exposure to contaminants compared to top predators. For instance, petrels (i.e. species from
82 three families of Procellariiformes: Procellariidae, Oceanitidae, and Hydrobatidae) are still
83 one of the most poorly examined seabird groups, mostly due to their small body size, their
84 nesting predominantly on isolated and inaccessible islands, and their high mobility at sea
85 (Rodríguez et al. 2019). However, even this group is exposed to a multitude of contaminants
86 (e.g., Anderson et al. 2000, Bocher et al. 2003, Cipro et al. 2014, Fromant et al. 2016, Philpot
87 et al. 2019).

88 In this study we determined levels of elements in feathers of two storm-petrel species
89 breeding in the maritime Antarctica, the Wilson's storm petrel (*Oceanites oceanicus*, hereafter
90 WSP) and the black-bellied storm petrel (*Fregetta tropica*, hereafter BBSP). We focused both
91 on elements of wider ecotoxicological interest (i.e. arsenic [As], cadmium [Cd], chromium
92 [Cr], copper [Cu], lead [Pb], mercury [Hg], selenium [Se], and zinc [Zn]) and on those rarely
93 studied in avian tissues (i.e. antimony [Sb], bismuth [Bi], calcium [Ca], cobalt [Co], iron [Fe],
94 nickel [Ni], magnesium [Mg], molybdenum [Mo], and strontium [Sr]). Gathering data about
95 the concentration of various elements in tissues of living animals is crucial in order to
96 properly assess ecosystem health and to comprehend pollutants' abilities for potential
97 bioaccumulation and biomagnification. By studying rarely analysed elements, the results



98 should provide background data for research detecting future inputs of elements in remote
99 polar regions (Santos et al. 2006).

100 We aimed to:

101 1) present reference values for the concentrations of 17 elements that can be used in the future
102 for monitoring contamination level in Antarctic marine predators;

103 2) compare elemental concentrations between feathers collected from different age groups
104 representing various life-history stages (i.e. chick feathers representing the chick-growth
105 period, chick down representing maternal input, and adult feathers representing part of the
106 non-breeding period, when the feathers grew); by considering the spatial and temporal
107 differences in feather growth between these groups, we expected to detect differences in
108 elemental concentrations between the various types of feathers;

109 3) compare elemental concentrations in feathers grown during the non-breeding season
110 between adults of the two species, with special emphasis on Hg and Se:Hg molar ratio (linked
111 to protective action against Hg bioaccumulation and toxicity by creation of Hg-Se compounds
112 [Nigro and Leonzio 1996, Khan and Wang 2009]). Considering inter-specific differences in
113 trophic level (see Materials and Methods) and in the location of non-breeding areas (Fig. 1),
114 we expected to detect differences in elemental concentrations between the species;

115 4) identify patterns in concentrations of elements, and thus identify possible common sources
116 of contamination.

117

118 **2. Materials and Methods**

119 *2.1. Studied species*

120 The two study species – the Wilson’s storm petrel and the black-bellied storm petrel – are
121 small pelagic seabirds, with circumpolar breeding distributions including sub-Antarctic
122 islands and the maritime Antarctic. Both species breed sympatrically in the study area (see
123 below) during the austral summer (from December to March), with similar breeding biology:
124 single-egg clutch, incubation lasting 38–44 days, and chick rearing up to 71 days. Although
125 both species are among the smallest endotherms living in the Antarctic, they play an
126 important role as predators preying on Antarctic krill, myctophid fish and amphipods (Hahn
127 1998, Quillfeldt 2002, Quillfeldt et al. 2005, Wasilewski 1986). Preying on fish and
128 crustaceans in equal proportions (Hahn 1998), BBSP feeds at a higher trophic level than
129 WSP, which eats mainly crustaceans (80–90% of meals) (Quillfeldt 2002, Quillfeldt et al.
130 2017). After breeding, both species migrate northwards, where they spend the non-breeding
131 period at open sea and complete their moult (Beck and Brown 1972). They moult in the
132 Atlantic Ocean in a wide range of habitats: WSP from sub-Antarctic to subtropical waters and
133 BBSP primarily either in sub-Antarctic–subtropical waters or at the continental shelf (Phillips
134 et al. 2009) (Fig. 1).

135

136 *2.2. Sample collection*

137 We studied the two storm-petrel species in the breeding colonies located in the vicinity of
138 Henryk Arctowski Station in Admiralty Bay, King Gorge Island, South Shetlands, Antarctica
139 (62°02’S 58°21’W, Fig. 1) in 2017. King George Island is the largest island in the South
140 Shetlands Archipelago, 90% ice-covered, with rocks mainly formed by andesitic and basaltic
141 magma (Santos et al. 2006). We captured adult birds in the breeding colony (in their nests,
142 using mist-nets spread in the colony area) during the incubation period and collected 4–5
143 body feathers from the back. Back body feathers represent mostly trace element input from
144 food and water intake during part of the non-breeding period when the feathers grew, which

145 takes place outside the colony in the Atlantic Ocean (Fig. 1). To sample chicks we caught
146 them by hand in the nest and collected down (at the time they were starting to lose it, i.e.
147 when their body feathers were well grown, thus minimising the risk of affecting
148 thermoregulation), and 4–5 body feathers from the back (when the nestlings were about to
149 fledge). Down feathers represent trace elements passed on by the female to the embryo,
150 reflecting their input during the pre-laying period, probably from areas around the breeding
151 colony (and likely predominantly reflecting the food intake). Chick body feathers represent
152 the nesting period and input from marine environments (as food and water intake). We stored
153 all the samples in individual plastic zip-lock bags until chemical analysis.

154

155 *2.3. Analytical Procedure*

156 Prior to chemical analysis, we cleaned all feather samples to remove external contamination,
157 firstly with acetone (Sigma-Aldrich, USA) and then two times with deionised water (Mili-Q
158 Gradient A10, Milipore, France) (procedure of Jaspers et al. 2004, modified). We air-dried the
159 washed feathers for 24 h. If the total mass of the sample permitted, we used an aliquot of the
160 collected material for the analysis of all trace elements, using Inductively Coupled Plasma
161 Mass Spectrometry (ICP-MS).

162 We determined concentrations of 17 trace elements by ICP-MS analytical technique in
163 the following feather samples types: adult WSP (n=12), chick body WSP (n=4), chick down
164 WSP (n=4) and adult BBSP (n=4). Mean feather mass was: for adults 10 mg (4–18 mg), for
165 chick body feathers 6 mg (5–8 mg) and for chick down feathers 16 mg (6–39 mg). Due to
166 insufficient amount of chick body BBSP and down BBSP feathers, we measured only Hg
167 content in these samples using the cold vapour technique. In total, we determined Hg
168 concentration in the following types of feather samples: adult WSP (n=35), chick body WSP

169 (n=10), down WSP (n=16), adult BBSP (n=11), chick body BBSP (n=6) and down BBSP
170 (n=6).

171

172 2.3.1. Trace element concentration

173 We homogenised dry feathers by cutting them up, then weighed them to the nearest 0.01 mg,
174 and placed them in a clean Teflon vessel with 7 ml 65% HNO₃ (Merck, Suprapur). We carried
175 out digestion using a high-pressure microwave emitter (Microwave Digestion System, Anton
176 Paar). We increased the temperature from the ambient value to 90°C (approximately 6–
177 8°C/min). We maintained these conditions for 25 minutes, after which we gradually lowered
178 the temperature. Subsequently, we diluted the fully mineralised samples with deionised water
179 to 25 ml in clean plastic flasks. To ensure quality control and check background
180 contamination, we ran blank samples with every batch. To ensure accuracy of obtained results
181 we ran certified reference material (CRM, Human hair ERM-DB001) in triplicate. We
182 analysed samples using an ICP-MS 2030 (Shimadzu, Japan) (for measurement conditions and
183 parameters see Table 1, Supplementary material)

184 2.3.2. Mercury concentration (cold vapour technique)

185 We weighed the dry samples to the nearest 0.01 mg in a ceramic boat, then we covered them
186 with activated Al₂O₃ and analysed them using the thermal vaporisation atomic absorption
187 method (MA-3000 Nippon Instruments Corporation). We analysed at least two feather
188 aliquots (1–10 mg dry weight) for each individual. The details of the program used and the
189 equipment specification are described in Pacyna et al. (2018). We determined total Hg
190 concentration in duplicates or triplicates when possible, taking sub-samples of the
191 homogenised feathers. We calculated the coefficient of variation (CV) based on these. If the
192 CV was above 15%, we excluded samples from the analyses, deeming the estimation of Hg

193 concentration unreliable. Thus, for statistical analysis we used: adult WSP (n=25), chick body
194 WSP (n=5), down WSP (n=16), adult BBSP (n=8), chick body BBSP (n=5) and down BBSP
195 (n=6). Mean CV was $8.64 \pm 4.65\%$ for adults, $6.32 \pm 5.40\%$ and $2.92 \pm 2.64\%$ for chick body
196 feathers and down, respectively. To check background contamination, we performed a quality
197 control including blank samples every 5–6 subsamples. We analysed CRM every 10th
198 subsample run.

199

200 2.4. *Quality control*

201 We found that results for CRM analysis were in agreement with the certified values (mass
202 used for analysis: for trace elements 200 mg, for mercury 14-28 mg). Recoveries were high:
203 As 98%, Cd 106%, Cu 104%, Hg 92%, Pb 96%, Se 92%, Zn 97%. To check accuracy and
204 recoveries of other elements absent in this CRM we applied a treatment used before by
205 Pacyna et al. (2019). We blank-corrected samples analysed on the ICP-MS (by a mean value
206 of all blank samples). For Hg analysis, we found that background contamination was
207 negligible and we did not perform blank correction. The limit of detection (LOD) and
208 quantification (LOQ) values were calculated as the concentrations corresponding to signals
209 equal to three and ten times the standard deviation of blank solution signal, respectively. For
210 Hg LOD/LOQ were calculated based on the standard deviation of the response (s), and the
211 slope of the calibration curve (b) according to the following formulas: $LOD = 3.3(s/b)$, $LOQ =$
212 $10(s/b)$. Method LOD/LOQ were in range of 0.004-0.92 and 0.013-3.07 ng/g respectively. We
213 reported our results as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight (dw). For statistical analysis results below the LOD
214 we assigned half of the LOD value.

215 For calibration of the ICP-MS we used the ICP IV multi-element standard (Merck,
216 USA) and As, Sb, Se, Mo and V (Sigma-Aldrich, USA), Hg (Merck, USA) as single
217 standards. As internal standards we used: Sc, Rh, Tb and Ge in supra pure 1% HNO₃ (Merck,

218 USA). For sample pre-treatment and sample dilution we used deionised water obtained from
219 the Milli-Q Direct 8 Water Purification System.

220 2.5. Statistical analyses

221 To investigate variation in the qualitative and quantitative composition of trace elements in
222 feathers, we firstly performed multivariate analyses for all elements to find general patterns
223 and then we did univariate analyses for particular elements.

224 To compare the qualitative and quantitative compositions of all trace elements in
225 feathers among the life-history stages and species, we applied the following multivariate
226 methods:

227 1) a multivariate (for all trace elements together) PERMANOVA (non-parametric MANOVA
228 based on the Bray–Curtis measure; Anderson 2001)) with concentrations of all elements as a
229 response variable and birds' age (adult WSP, adult BBSP, chick down WSP, chick body
230 WSP) as the explanatory variable;

231 2) A similarity percentage breakdown procedure (SIMPER) to assess the average percentage
232 contribution of individual elements to the dissimilarity in all elements concentrations between
233 age groups in a Bray–Curtis dissimilarity matrix (Clarke 1993).

234 To compare the qualitative and quantitative compositions of particular trace elements
235 in feathers between the life-history stages and species, we used an unimodal Kruskal–Wallis
236 test with a *U* Mann–Whitney test as a *post-hoc* test for all group pairs, excluding adult BBSP
237 vs chick down WSP and adult BBSP vs chick body WSP. In a separate analysis, we compared
238 Hg concentration among all categories for a larger sample size.

239 Then, to find the groups of elements with high degrees of association in feather
240 elemental concentrations, we performed a Hierarchical Cluster Analysis (HCA). A high
241 degree of association between element concentrations, expressed by clustering in one group,
242 can be used to identify common sources of elements (e.g. Hashmi et al. 2013), but it does not

243 require the formulation of any *a priori* hypothesis considering the nature of the relationships
244 (Bianchi et al. 2008). We performed HCA with Euclidean distance as a distance measure, and
245 the paired group method as the linkage method. For each cluster obtained, we calculated
246 Bootstrap Probability (BP) using multiscale bootstrap resampling. BP of a cluster may take a
247 value between 0 and 100, indicating how well the data supported the cluster, with a higher
248 value indicating a better fit (Hammer et al. 2001). We only considered clusters with $BP \geq 95$.
249 To determine how well the generated clusters represented dissimilarities between objects, we
250 calculated the cophenetic correlation coefficient. Values close to 0 indicate poor clustering,
251 and values close to 1 show strong clustering.

252 We performed PERMANOVA, SIMPER and HCA analyses on $\log(x+1)$ transformed
253 data. We classified the strength of the correlation according to Hinkle et al. (2003): strong
254 correlation with $r = |0.90-1.00|$, high correlation with $r = |0.70-0.90|$, moderate correlation with
255 $r = |0.50-0.70|$, and low correlation with $r = |0.30-0.50|$.

256 We performed separate SIMPER and PERMANOVA analyses for three groups of
257 elements:

- 258 1. all elements;
- 259 2. essential elements, i.e. As, Ca, Cr, Cu, Fe, Mg, Mo, Se and Zn; and
- 260 3. non-essential elements, i.e. Bi, Cd, Hg, Pb, Sb, and Sr.

261 We calculated Se:Hg molar ratios based on the mean Hg values and the mean Se
262 values from our study. The Se:Hg molar ratio was obtained using the molecular weight
263 (200.59 and 78.9 for Hg and Se, respectively) (Burger et al. 2013). We compared Se:Hg
264 molar ratios between the studied age categories in both species using a chi-squared test.

265 We performed PERMANOVA, SIMPER, and HCA analyses in PAST software
266 (Hammer et al. 2001) and the Kruskal–Wallis and Mann U Whitney test in R software (R
267 Core Team 2018), using the ggpubr package (Kassambara 2018).

268

269 3. Results

270 3.1. Element concentrations

271 Of all the metals examined, Ni and Co were below the limit of detection in all samples, and
272 were thus excluded from further analysis. As, Bi and Sb were detected exclusively in chick
273 feathers, both body and down. Concentrations of all elements (mean±SD) are presented in
274 Table 1 and Table 4 (for Hg). Concentration chain for particular groups are:

275 a) For adult WSP Mg>Zn>Ca>Fe>Sr>Cu>Mo>Hg>Se>Cr>Pb

276 b) For adult BBSP Mg>Zn>Ca>Fe>Cu>Hg>Se>Sr>Cr>Mo>Pb

277 c) For chicks body feathers Mg>Ca>Fe>Zn>Bi>Sr>Mo>Cu>As>Se>Cr>Pb>Sb>Hg>Cd

278 d) For chicks down feathers Mg>Ca>Fe>Zn>Sr>Se>Mo>Pb=Hg=As>Bi=Cr=Cu>Cd>Sb

279

280 3.2. Inter-group differences in all elements concentration

281 **All elements.** The concentrations of all combined studied elements differed significantly
282 between adult WSP and all other categories (PERMANOVA, Bonferroni-corrected $p<0.04$)
283 (Table 2). SIMPER analysis showed that the overall average dissimilarity was 18.5%. Fe and
284 Bi contributed most (14% and 11%, respectively) to the pattern of overall dissimilarity (Table
285 3). Bi, As and Fe contributed most (19%, 14% and 14%, respectively) to the pattern of
286 dissimilarity in elemental concentrations observed between adult WSP and chick WSP body
287 feathers. Fe and Se contributed most (14% and 12%, respectively) to the pattern of
288 dissimilarity in elemental concentrations observed between adult WSP and chick WSP down.
289 Cu, Fe, Hg and Se contributed most (16%, 15%, 14% and 12%, respectively) to the pattern of
290 dissimilarity in elemental concentrations observed between adult WSP and BBSP.



291 **Essential elements.** The concentrations of all combined studied elements differed
292 significantly between adult WSP and all WSP chick categories (PERMANOVA, Bonferroni-
293 corrected $p < 0.006$). We found no differences between adult WSP and BBSP ($p = 0.136$) (Table
294 2). The SIMPER analysis showed that the overall average dissimilarity was 14.2%. More than
295 half of the pattern of overall dissimilarity observed in elemental concentrations was explained
296 by Fe, As, Cu and Mo (22%, 15%, 13% and 13%, respectively) (Table 3). As, Fe and Mo
297 together contributed over 50% (23%, 22% and 15%, respectively) to the pattern of
298 dissimilarity observed in elemental concentrations between adult WSP and chick WSP body
299 feathers. Fe, Ca, As, Zn and Mo together contributed over 50% (21%, 13%, 12%, 11% and
300 11%, respectively) to the pattern of dissimilarity in elemental concentrations observed
301 between adult WSP and chick WSP down (Table 3). Cu, Fe, Se and Mo together contributed
302 over 50% (23%, 21%, 18% and 13%, respectively) to the pattern of dissimilarity in elemental
303 concentrations observed between adult WSP and BBSP (Table 3).

304 **Non-essential elements.** The concentrations of all combined studied elements differed
305 significantly between adult WSP and all other categories (PERMANOVA, Bonferroni-
306 corrected $p < 0.05$; Table 2). The SIMPER analysis showed that the overall average
307 dissimilarity was 39.3%. Bi, Sr, Hg and Pb together contributed over 50% (29%, 25%, 18%
308 and 14%, respectively) to the pattern of overall dissimilarity observed in elemental
309 concentrations (Table 3). Bi, Sb, Sr and Pb were responsible for 50%, 14%, 13% and 11%,
310 respectively, of the dissimilarity pattern in elemental concentrations observed between adult
311 WSP and chick WSP body feathers. Sr, Pb, Bi and Hg together produced the majority of
312 dissimilarity in elemental concentrations observed between adult WSP and chick WSP down
313 (36%, 19%, 19% and 14%, respectively). Hg, Sr and Pb together contributed over 50% (49%,
314 30% and 16%, respectively) to the pattern of dissimilarity in elemental concentrations
315 observed between adult WSP and BBSP (Table 3).

3.3. Intergroup differences for particular elements

Kruskal–Wallis inter-group tests comparing the concentration of particular elements revealed significant differences for all elements ($p < 0.05$) (Supplementary Materials2, Fig. ES1–ES7) except Mg ($p = 0.44$) and Mo ($p = 0.12$). *Post-hoc* tests revealed the following pattern of significant inter-group differences (Supplementary Materials2, Fig. ES1– ES7):

1. lower concentration of Cu, Hg and Se in adult WSP compared to adult BBSP;
 2. lower concentration of As, Bi, Ca, Cr, Fe, Sb and Se in adult WSP compared to chick WSP body feathers
 3. higher concentration of Zn in adult WSP compared to chick WSP down
 4. higher concentration of As, Bi, Ca, Cd, Fe, Hg, Pb, Sb, Se and Sr in chick WSP down compared to adult WSP
 5. higher concentration of As, Bi, Cr, Cu, Sb and Zn in chick WSP body feathers compared to chick WSP down
 6. lower concentration of Ca and Sr in chick WSP body feathers compared to WSP down
- Other studied inter-group differences were not significant ($p > 0.05$).

3.4. Inter-group differences for Hg concentration determined by cold vapour technique

The Kruskal–Wallis test revealed significant inter-group differences ($p < 0.05$) in the concentration of Hg determined by cold vapour technique. *Post-hoc* tests results and pattern of significant inter-group differences are presented in Fig. 2.

3.5. Grouping of elements

The Hierarchical Cluster Analysis for all studied groups combined (cophenetic correlation 0.902) recognised four main significant clusters grouping the trace elements (Fig. 3). The first cluster included Ca and Zn (BP=100), while the second cluster contained Cd-Sb (BP=99).

341 Then, the third was Bi-As (BP=96) and the fourth was Cu-Hg-Se (BP=96), with a subcluster
342 of Hg-Se (BP=98).

343

344 **4. Discussion**

345 Our study provides reference values for concentration of 17 elements in feathers of two
346 pelagic seabird species from the maritime Antarctic. We revealed several differences in
347 elemental concentrations between the two species, as well as differences in exposure between
348 life-cycle stages. We also identified some patterns in concentrations of particular elements.

349

350 **4.1. Contaminant patterns of selected elements and comparisons with other seabirds** 351 **from south polar areas**

352 Although reference values of 17 elements are provided in our study, below, we discuss only
353 the those considered most relevant in terms of possible effects on birds' health and survival.

354 *4.1.1. Mercury*

355 Hg is an endocrine disruptor associated with several adverse effects, including decreased body
356 condition, immune responses and hormonal secretion (Wolfe et al. 1998, Scheuhammer et al.
357 2007, Tartu et al. 2014, 2015). As such, it affects birds reproduction and survival, and so may
358 impact birds' population dynamics (Tartu et al. 2013, Goutte et al. 2014). Bird feathers are
359 perceived as the main route for Hg excretion (Monteiro and Furness 1995, Santos et al. 2006),
360 but its level would depend on multiple factors, including diet, excretion capacities in the
361 feathers and moulting pattern (Becker et al. 2016, Bustamante et al. 2016). The Hg
362 concentration reported in our results for BBSP adults ($5.47 \pm 1.61 \mu\text{g g}^{-1} \text{ dw}$) are in a range of
363 values reported previously by Carravieri et al. (2014; $4.22 \pm 2.53 \mu\text{g g}^{-1} \text{ dw}$). However, for

364 adult WSP, our values ($2.38 \pm 1.47 \mu\text{g g}^{-1} \text{ dw}$) were much higher compared to other studies
365 ($0.42 \pm 0.13 \mu\text{g g}^{-1} \text{ dw}$; Carravieri et al. 2014). Nevertheless, Hg levels in adult WSP from our
366 study were comparable to mean levels reported for another low-trophic-level seabird, the
367 Antarctic prion *Pachyptila desolata* ($1.73\text{--}2.80 \mu\text{g g}^{-1} \text{ dw}$). In general, there is a high
368 variability between petrel species ($0.42\text{--}12.43 \mu\text{g g}^{-1} \text{ dw}$; Table 4). Here, the inter-species
369 difference in Hg concentration is most likely associated with diet (Thompson and Furness
370 1989a, Bustamante et al. 2016, Blévin et al. 2013) as BBSP feeds at a higher trophic level
371 than WSP (Quillfeldt et al. 2017). Such a dietary explanation was suggested in the study of
372 Blévin et al. (2013), where chicks of 21 various species breeding in the Southern Ocean were
373 been found to vary greatly in terms of Hg concentration (from $0.05 \pm 0.01 \mu\text{g g}^{-1}$ in the South
374 Georgian diving petrel *Pelecanoides georgicus* to $5.31 \pm 1.12 \mu\text{g g}^{-1}$ in the northern giant petrel
375 *Macronectes halli*).

376 Examining Hg concentrations in age groups, in both species we found that it was
377 significantly higher in adults than in chicks of the same species (excluding WSP down; Fig. 2)
378 probably due to the longer exposure time of adults. This is similar to white-chinned petrels
379 *Procellaria aequinoctialis* (Carvalho et al. 2013), for which the same explanation has been
380 suggested. In contrast, in the wandering albatross *Diomedea exulans*, Hg contamination was
381 higher in immatures than adults, which may be associated with moulting intensity and
382 detoxification capacities varying between adults and immatures (Bustamante et al. 2016).

383

384 4.1.2 Selenium and its interaction with mercury

385 Se is an essential trace element for proper organism functioning, including thyroid function
386 (Burger et al. 2013), and it is known for its protective action against Hg bioaccumulation and

387 toxicity through the creation of Hg-Se compounds (Nigro and Leonzio 1996, Khan and Wang
388 2009). However, excess Se may as well have toxic effects on vertebrates (Burger et al. 2013).

389 We found that Se levels significantly differ between the two studied species, with
390 almost three times higher values found in adult BBSP compared to adult WSP ($5.19 \pm 1.18 \mu\text{g}$
391 $\text{g}^{-1} \text{dw}$ vs $1.81 \pm 0.98 \mu\text{g} \text{g}^{-1} \text{dw}$). These values add to a wide range reported so far from other
392 seabirds of the Southern Ocean ($3.40\text{--}19.40 \mu\text{g} \text{g}^{-1} \text{dw}$; Anderson et al. 2010, Fromant et al.
393 2016, Philpot et al. 2019). Interestingly, Se levels in *Pygoscelis* sp. penguins living in King
394 George Island were similar to values found in our study ($2.46\text{--}6.37 \mu\text{g} \text{g}^{-1} \text{dw}$; Jerez et al.
395 2011), while for penguin *Pygoscelis* from other area Hg levels were lower, from <0.80 to 2.0
396 $\mu\text{g} \text{g}^{-1}$ (Metcheva et al. 2006).

397 The studied WSP chicks had higher Se levels compared to adults (Table 1). This trend
398 was not observed in gadfly petrels *Pterodroma spp*, where Se levels were significantly lower
399 in chicks than in adults (Philpot et al. 2019). These differences are difficult to explain given
400 the currently limited knowledge about Se distribution and metabolism.

401 Worldwide studies quantifying Hg-Se co-exposure and interaction in seabirds are still
402 rare, but have increased in recent years, and show that seabirds' ability to deal with high
403 mercury and selenium levels is still not fully understood, and may depend on age and species
404 (e.g., Carvalho et al. 2013, Cipro et al. 2014, González-Solís et al. 2002, Carravieri et al.
405 2017, Philpot et al. 2019). Se-Hg molar ratios in our study differed between chicks and adults,
406 being highest in WSP chick body feathers (Table 1). However, all ratios reported here were
407 >1 , suggesting activation of a defence mechanism against high Hg concentrations and a health
408 impact associated with potential Se toxicity (Lucia et al. 2016).

409

410 *4.1.3. Cadmium*



411 Cd is another toxic element that readily bioaccumulates in food webs (Cipro et al. 2014), and
412 Cd has been reported at even higher levels in Antarctic species including plankton, marine
413 benthic invertebrates, fishes, seabirds and marine mammals (see references in Jerez et al.
414 2011), than in their counterparts sampled in polluted coastal areas (Petri and Zauke 1993). In
415 our study, Cd levels in adults were mostly below the quantification limit (Table 1), but it is
416 not exceptional (e.g. penguin feathers were also generally low ($<LOD-0.10 \mu\text{g g}^{-1} \text{ dw}$, Jerez
417 et al. [2011]; $<0.15-0.21 \mu\text{g g}^{-1} \text{ dw}$ [Metcheva et al. 2006]) and may be related to relatively
418 low deposition of Cd in feathers (Lucia et al. 2010, Cipro et al. 2014). Indeed, in Antarctic
419 prions, Cd levels in internal tissues (kidney $105\pm37 \mu\text{g g}^{-1} \text{ dw}$) were considerably higher than
420 in feathers (mean $0.06\pm0.03 \mu\text{g g}^{-1} \text{ dw}$) (Fromant et al. 2016). Thus, feathers would give only
421 partial information of bird exposure; i.e. only when it reaches high levels.

422

423 4.1.4. Lead

424 After Hg, Pb is another major contaminant of toxicological concern (Burger and Gochfeld
425 2009), affecting breeding success, migratory behaviour and survival of animals at various
426 trophic levels (Burger, 1995). It may affect food web dynamics e.g. by decreasing the
427 abundance and availability of food prey, or by interfering with its natural hiding or escape
428 behaviour (Burger, 1995). Pb is accumulated in feathers at higher rate compared to Cd (Jerez
429 et al. 2011), but can be elevated due to exogenous contamination (Jaspers et al. 2004).
430 Adverse effects from lead toxicity might occur at levels of $4 \mu\text{g g}^{-1}$ in feathers (Burger and
431 Gochfeld 2000) but levels in adult seabirds are usually lower ($0.51-1.68 \mu\text{g g}^{-1} \text{ dw}$, Mendes et
432 al. 2008, Burger and Gochfeld 2009). In our study the Pb concentration was generally low in
433 adults ($<1.17 \mu\text{g g}^{-1} \text{ dw}$, with one outlier reaching $5.06 \mu\text{g g}^{-1} \text{ dw}$), and higher in chicks ($0.36-$
434 $3.67 \mu\text{g g}^{-1} \text{ dw}$). Similarly low values of Pb concentration have been reported for other
435 seabirds from the Southern Ocean (Metcheva et al. 2006, Anderson et al. 2010, Jerez et al.

436 2011, Fromant et al. 2016). However, for penguins breeding on King George Island, high Pb
437 values have been also reported, which has been explained by local human activities (many
438 scientific bases and a small airport in the study area; Jerez et al. 2011).

439

440 4.1.5. Zinc

441 Zn can be bioaccumulated in polar organisms, but most likely does not biomagnify (Santos et
442 al. 2006). Variation of this element concentration in adult storm petrels was relatively low
443 (WSP $109.20 \pm 18.50 \mu\text{g g}^{-1} \text{ dw}$, BBSP $99.95 \pm 13.01 \mu\text{g g}^{-1} \text{ dw}$), and in chicks was even lower,
444 and with small inter-individual variability (WSP down and body feathers 48.30 ± 7.08 and
445 $93.00 \pm 11.3 \mu\text{g g}^{-1} \text{ dw}$, respectively). The reported Zn concentration falls well within the range
446 reported from other seabirds ($6.95\text{--}301 \mu\text{g g}^{-1} \text{ dw}$) (Anderson et al. 2010, Cipro et al. 2014,
447 Fromant et al. 2016, Philpot et al. 2019, Metcheva et al. 2006, Jerez et al. 2011, Santos et al.
448 2006).

449 4.1.6. Copper

450 Cu, like Zn, is also an essential element, with concentrations in seabird tissues controlled
451 mostly in homeostasis processes (Bocher et al. 2003). Variation in the concentration variation
452 of Cu in the two species was much larger than for Zn (adults, WSP: $2.52 \pm 2.35 \mu\text{g g}^{-1} \text{ dw}$,
453 BBSP: 8.12 ± 0.56 ; chick body, WSP: 6.68 ± 3.15 , chick down WSP: $1.52 \pm 0.60 \mu\text{g g}^{-1} \text{ dw}$).
454 These values also seem to fall well within the range reported so far for other seabirds (6.0--
455 $12.7 \mu\text{g g}^{-1} \text{ dw}$; Metcheva et al. 2006, Jerez et al. 2011).

456

457 4.2. Potential sources of elements

458 The contamination of Antarctic biota may have both natural and anthropogenic sources (Jerez
459 et al. 2011, Lu et al. 2012, Deheyn et al. 2005). Our cluster analysis revealed some interesting
460 groupings of elements that suggested common source of contamination.

461 The Bi-As cluster suggests a volcanic origin of the two elements. Worldwide
462 emissions from volcanoes are deemed a considerable source of atmospheric Bi and As
463 (Candelone et al. 1995, Kabata-Pendias and Szteke 2015), and the soils on King George
464 Island are mostly composed of mineral and rock fragments with some volcanic ashes (Lee et
465 al. 2004). The ashes were blown from Deception Island, a volcanic island located ~130 km
466 south-west of King George Island (Jeong and Yoon 2001), where the most recent eruption
467 occurred in the late 1960s (Orheim 1972). Storm petrels may additionally gain As from food
468 sources, as low-trophic organisms (as petrels diet items) easily assimilate this element
469 (Rahman et al. 2012). Mão de Ferro et al. (2013) found As enrichment in several Antarctic
470 abiotic and biotic samples to probably be a result of past volcanic activity and sediment
471 petrologic characteristic, as well as As leaching processes. They also indicated that during a
472 high tide, leaching processes of As can occur to shore and semi-submerged areas, thus being
473 available to aquatic organisms (Mão de Ferro et al. 2013). All feathers were cleaned by the
474 exact same procedure, but we cannot exclude the possibility of external contamination by soil
475 particles, as both As and Bi were only detected in chicks feathers.

476 Both elements of the Ca-Zn cluster are necessary components in the synthesis of the
477 feather pigment melanin (McGraw et al. 2003). They also play an essential role in multiple
478 physiological body functions (Bogden & Klevay 2000). Thus, this cluster may reflect both co-
479 exposure from diet and the similar co-regulation mechanisms responsible for element
480 deposition. Both Ca and Zn accumulation in feathers may depend on melanin type and
481 content, as shown by element enrichment in pigmented feather parts (Niecke et al. 1999
482 2003).

483 The Cd-Sb cluster may represent common food and/or water input. Cd may originate
484 from anthropogenic pollution but also from rock weathering and/or natural sources, as it is
485 more mobile in seawater than in other water bodies and is easily absorbed by aquatic biota
486 (Kabata-Pendias and Szeke 2015). Natural sources (diffusive fluxes, upwelling and
487 continental weathering) can be responsible for higher abundance of Cd in Antarctic water
488 samples (Sañudo-Wilhelmy et al. 2002). High Cd concentrations were found in Antarctic
489 krill *Euphasia superba*, which is the main dietary component for adults and chicks of both
490 storm-petrel species (Wasilewski 1986, Petri and Zauke 1993, Hahn 1998, Nygård et al. 2001,
491 Quillfeldt 2002). The natural sources of Sb and its compounds are volcanic eruptions, sea
492 spray, forest fires and wind-blown dust, suggesting a non-anthropogenic source (Kabata-
493 Pendias and Szeke 2015). Considering its clustering with Cd, we would suggest a natural
494 source of both elements in the feathers of the studied birds.

495 The common clustering of Cu-Hg-Se may be explained by the properties of Se and the
496 high concentration of all these elements in aquatic organisms, including fish. Marine aerosols
497 are enriched in Se resulting from the formation of volatile Se-organic compounds. Volcanic
498 emissions were suggested as a prevalent source of Hg in Deception Island (Mão de Ferro et al.
499 2014). Also, summer input from the Southern Ocean may be a net source for the gaseous
500 element Hg in the marine boundary layer (Wang et al. 2017).

501

502 **4.3. Species and age differences in elemental concentrations**

503 Significant differences in concentration of various elements (i.e. Cu, Hg, and Se) between the
504 WSP and BBSP found in our study are most likely to be associated with inter-species
505 differences in foraging (different trophic levels with a different contribution of fish in their
506 diet [Quillfeldt et al. 2017]).

507 Significant differences in concentrations of various elements (Supplementary
508 Materials2, Fig. ES1–ES7) between WSP age groups are also likely to be associated with diet,
509 although here not with the difference in diet composition but more with the location of food
510 resources exploited during the period of growth of relevant feathers. Chick feathers are more
511 suitable for local exposure assessment, as levels are not affected by moulting patterns,
512 because chicks receive food collected by parents in the vicinity of the breeding colony, and
513 have a shorter exposure time. Thus, in cases when adult and offspring diet do not differ
514 significantly, chick feathers may also be used to reconstruct adults' foraging ecology and
515 adults' exposure to several pollutants during the chick-rearing period (Blévin et al. 2013).

516 Down feathers have been successfully used to estimate Hg concentrations in eggs
517 (Santos et al. 2017), suggesting its potential as a suitable proxy for contaminant
518 determination. A strong correlation between the levels of both Hg and Se in eggs and the liver
519 of incubating females has been found in *Charadriiformes* (Ackermann et al. 2016). In our
520 study, all examined elements except Ni and Co were detected in down feathers, enabling
521 exposure assessment at the earliest phase of life. The highest Ca level was found in WSP
522 down, probably because the developing embryo absorbs Ca and other elements, initially from
523 the yolk and subsequently from the eggshell (Castilla et al. 2010). We found the lowest Zn
524 level in down ($48.30 \pm 7.08 \mu\text{g/g dw}$), at almost two times lower than the level in chick body
525 feathers ($93.00 \pm 11.30 \mu\text{g/g dw}$). Other elements, such as Pb, Hg, Se, Mg and Sr, were higher
526 in down than in chick body feathers, suggesting that exposure changes over time. Maternal
527 transfer of contaminants may be a reason for the increased levels of several metals in chick
528 down feathers, as the maternal transfer is species- and element-specific (Ackermann et al.
529 2016).

530

531 **5. Conclusions**

532 Our study provides a reference values for concentration of 17 elements in feathers of two
533 pelagic seabird species from the maritime Antarctic. Such data may serve to monitor
534 contaminant levels in marine systems and to evaluate variability in contaminant levels in
535 tissues throughout birds' annual cycle (Rodríguez et al. 2019). We also revealed several
536 differences in elemental concentrations between the two species, as well as differences in
537 exposure between life-cycle stages. These inter-species and inter-age differences are
538 attributed to the various diet compositions and geographic areas of feather growth. Finally, we
539 identified some patterns in concentration of particular elements that suggest a primarily
540 natural origin of most elements. We believe that our study contributes to understanding spatial
541 and temporal patterns of contaminant accumulation in the Maritime Antarctic ecosystem. As
542 emissions and global transport of elements such as Hg, Pb and Cd are expected to increase in
543 the future, monitoring studies on seabirds breeding in the Southern Hemisphere may be a
544 warning system for global changes and the consequences of elevated emissions into the
545 marine food web. Despite the limitations of our study, such as: the relatively small sample
546 size; sample collection being restricted to one site and one season; and the lack of data on
547 elemental concentrations in prey items, our study delivered important reference values for
548 elemental concentrations in various age groups of the two study species. The Antarctic Treaty
549 members urge long-term monitoring and sustained observations of the Antarctic environment
550 and the associated data management, to detect, understand and forecast the impacts of
551 climate-change-driven environmental variability (ATCM 2007).

552
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560

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Table 1. Elemental concentrations of the studied elements in feathers of storm-petrels, mean \pm SD (min–max) $\mu\text{g g}^{-1}$ dw, N = the number of individuals sampled, LOD = detection limit, LOQ= quantification limit, Se:Hg – Se:Hg molar ratio

Element	Adult WSP (N=12)	Adult BBSP (N=4)	Chick down WSP (N=4)	Chick body WSP (N=4)
As	75% <LOD	100%<LOQ	1.76 \pm 1.10 (0.45–3.16)	6.11 \pm 1.43 (4.77–8.38)
Bi	92%<LOD	100%<LOD	1.57 \pm 1.78 (<LOD–3.92)	14.16 \pm 5.92 (6.50–20.50)
Ca	96.0 \pm 21.0 (77.7–156.0)	80.0 \pm 11.2 (72.8– 99.3)	242.8 \pm 60.4 (165.0–326.0)	136.50 \pm 11.79 (124.9– 156.0)
Cd	<LOD (<LOD– 0.45)	<LOQ	0.45 \pm 0.22 (<LOQ–0.68)	0.51 \pm 0.24 (<LOD– 0.70)
Cr	0.67 \pm 0.45 (0.11–4.36)*	0.71 \pm 0.45 (0.09– 1.22)	1.54 \pm 0.48 (0.88–2.08)	3.46 \pm 0.78 (2.73–4.78)
Cu	2.52 \pm 2.35* (<LOD–13.9)	8.12 \pm 0.56 (7.52– 9.06)	1.52 \pm 0.60 (0.67–2.26)	6.68 \pm 3.15 (2.49–10.96)
Fe	20.40 \pm 18.00 (<LOD–263.0)*	10.73 \pm 5.04 (<LOD–16.16)	74.30 \pm 19.30 (50.80–102.4)	131.7 \pm 81.8 (63.1–270.0)
Mg	478 \pm 130 (315– 773)	429 \pm 101 (306– 529)	538 \pm 354 (316– 1152)	378 \pm 91 (285–513)
Mo	2.41 \pm 4.09 (<LOD–14.6)	0.56 \pm 0.27 (0.16– 0.84)	1.92 \pm 1.77 (0.36–4.90)	7.13 \pm 7.42 (1.52–19.79)
Pb	0.33 \pm 0.37* (<LOD–5.06)	0.36 \pm 0.24 (0.11– 0.74)	1.77 \pm 0.91 (0.75–2.76)	1.43 \pm 1.32 (0.36–3.67)
Sb	100%<LOD	75% <LOD	0.22 \pm 0.15 (<LOD–0.43)	1.17 \pm 0.53 (0.58–1.92)

Element	Adult WSP (N=12)	Adult BBSP (N=4)	Chick down WSP (N=4)	Chick body WSP (N=4)
Se	1.81 ± 0.98 (<LOD–4.65)	5.19 ± 1.18 (3.74– 6.62)	4.06 ± 0.50 (3.29–4.69)	3.63 ± 1.01 (2.39–5.13)
Sr	5.77 ± 3.62 (2.47–13.53)	2.79 ± 1.21 (1.35– 4.58)	23.19 ± 8.75 (13.4–37.1)	9.57 ± 1.020 (8.63–11.27)
Zn	109.20 ± 18.50 (70.3–141)	99.95 ± 13.01 (85.5–120)	48.30 ± 7.08 (40.30–59.60)	93.00 ± 11.30 (79.4–110.8)
Se:Hg	1.92	2.75	6.00	13.90

* for Cr, Cu, Fe and Pb one outlier was excluded from the mean calculation, but it is shown as the maximal value

Table 2 Intergroup differences (one-way PERMANOVA, Bonferroni-corrected p values) of elemental concentration, $\log(x + 1)$ transformed, in the feathers of the four studied groups of storm-petrels: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's storm-petrel chicks (WSP_down) and body feathers from Wilson's storm petrel fledglings (WSP_CHF)

PERMANOVA, F = 11.48, p = 0.0001				
All elements	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	-	0.182	0.176	0.007
WSP_down		-	0.157	0.004
BBSP_Ad			-	0.037
WSP_Ad				-
PERMANOVA, F = 9.26, p = 0.0001				
Essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	-	0.160	0.181	0.003
WSP_down		-	0.166	0.005
BBSP_Ad			-	0.136
WSP_Ad				-
PERMANOVA, F = 12.93, p = 0.0001				
Non-essential	WSP_CHF	WSP_down	BBSP_Ad	WSP_Ad
WSP_CHF	-	0.160	0.181	0.003
WSP_down		-	0.166	0.012
BBSP_Ad			-	0.043
WSP_Ad				-

Table 3 Sources of variability (average percentage dissimilarity) in the elemental concentrations (log(x+1) transformed) in: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's storm-petrel chicks (WSP_down) and body feathers from Wilson's storm petrel fledglings (WSP_CHF), according to the SIMPER analysis. Only elements with a contribution > 10% are shown. ADis - Average Dissimilarity, Contr. (%) – percentage contribution, Overall - overall average similarity

Overall		WSP_Ad vs				WSP_Ad vs				WSP_Ad vs					
dissimilarity		WSP_CHF				WSP_down				BBSP_Ad					
ADis		Contr.		ADis		Contr.		ADis		Contr.		ADis		Contr.	
All elements															
Fe	2.57	13.9	Bi	4.18	19.1	Fe	2.67	14.0	Cu	2.12	16.1				
Bi	2.07	11.2	As	3.06	14.0	Sr	2.36	12.4	Fe	1.94	14.7				
			Fe	3.02	13.8				Hg	1.89	14.3				
									Se	1.63	12.3				
Overall	18.46			21.87			19.30			13.23					
Essential															
Fe	3.10	21.9	As	3.72	22.6	Fe	3.24	21.5	Cu	2.43	22.8				
As	2.06	14.5	Fe	3.68	22.4	Ca	1.94	12.9	Fe	2.22	20.8				
Cu	1.83	12.9	Mo	2.40	14.6	As	1.85	12.3	Se	1.87	17.6				
Mo	1.83	12.9	Cr	1.87	11.3	Zn	1.71	11.3	Mo	1.41	13.3				
			Cu	1.82	11.0	Mo	1.66	11.0							
Overall	14.16			16.46			15.07			10.65					
Non-Essential															
Bi	11.47	29.2	Bi	23.67	50.3	Sr	13.96	36.4	Hg	14.42	49.1				

	Overall		WSP_Ad vs				WSP_Ad vs				
	dissimilarity		WSP_CHF				WSP_down				
	ADis	Contr.	ADis	Contr.	ADiss	Contr.	ADis	Contr.			
Sr	10.01	25.5	Sb	6.70	14.2	Pb	7.43	19.4	Sr	8.68	29.5
Hg	7.02	17.9	Sr	6.26	13.3	Bi	7.27	19.0	Pb	4.74	16.1
Pb	5.59	14.2	Pb	5.38	11.4	Hg	5.25	13.7			
Overall	39.31			47.09			38.33			29.41	

Table 4 Variability of mercury (Hg) levels in feathers of *Procellariiformes*. N – number of individuals

Species	Study area	Tissue	N	Age	Concentrat. mean± SD µg g ⁻¹ dw	Reference
Antarctic prion (<i>Pachyptila desolata</i>)	Kerguelen	body feathers	10	unknown	2.8±1.2	Fromant et al. 2016
	archipelago		10	adult	1.73±0.50	
White-headed petrel (<i>Pterodroma lessonii</i>)	Kerguelen	body feathers	10	adult	12.43 ± 2.01	Carravieri et al. 2014
	archipelago		10	chicks	1.54 ± 0.34	
spectacled petrel (<i>Procellaria conspicillata</i>)	southwestern Atlantic	contour	38	unknown	11.17 ± 3.78	Carvalho et al. 2013
	Ocean off the Brazilian coast	feathers				
white-chinned petrel (<i>Procellaria aequinoctialis</i>)	Kerguelen	body feathers	22	unknown	7.63 ± 3.87	Cipro et al. 2014
	archipelago					
	southwestern Atlantic	contour	9	adults	3.45 ± 2.84	Carvalho et al. 2013
	Ocean off the Brazilian coast	feathers	21	juveniles	1.14 ± 2	
	Kerguelen	body feathers	14	chicks	1.82 ± 0.51	Blévin et al. 2013
	archipelago					
Leach's storm-petrel	Machias Seal Island,	Breast	15	adult	7.01*	Bond and Diamond,
	New Brunswick,	feathers	20	chicks	1.42*	

<i>(Oceanodroma leucorhoa)</i>	Canada						2009
Wilson's storm-petrel	Kerguelen Islands	body feathers	12	adult		0.42 ± 0.13	Carravieri et al. 2014
<i>(Oceanites oceanicus)</i>	King Gorge Island,	body feathers	25	adult		2.38 ± 1.47	present
	South Shetlands,	body feathers	5	chick		0.67 ± 0.27	study
	Antarctica	down	16	chick		1.72 ± 0.65	
Black-bellied storm-petrel	Kerguelen Islands	body feathers	10	adult		4.22 ± 2.53	Carravieri et al. 2014
<i>(Fregatta tropica)</i>	King Gorge Island,	body feathers	8	adult		5.47 ± 1.61	present
	South Shetlands,	body feathers	5	chick		1.87 ± 0.29	study
	Antarctica	down	6	chick		3.99 ± 1.07	

* *Estimated marginal mean*

Figure

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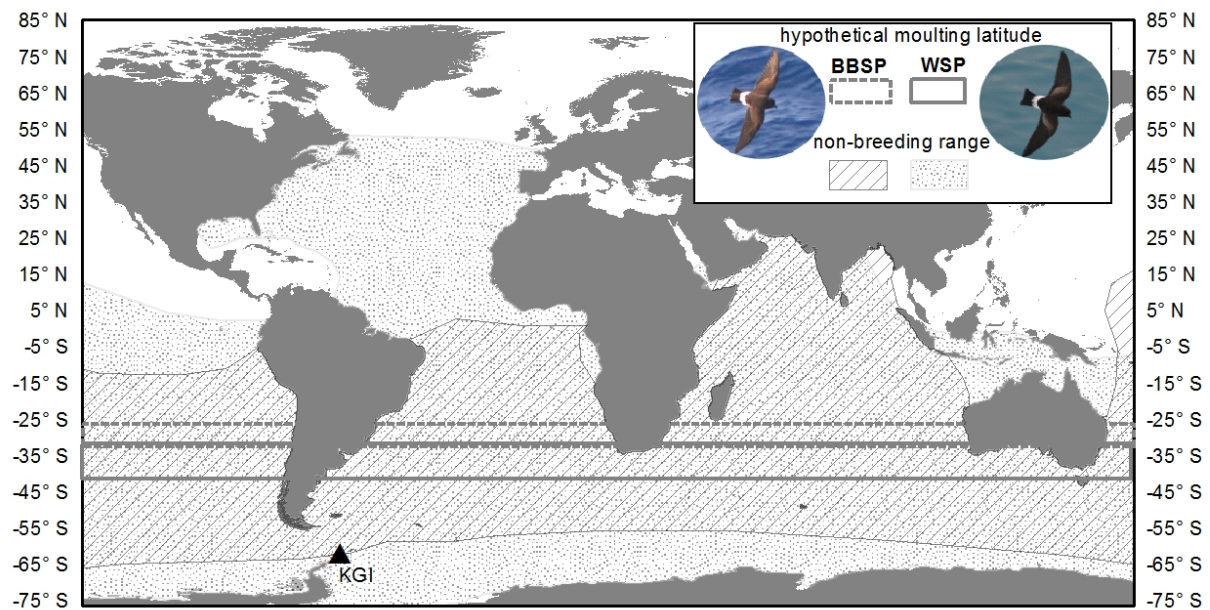


Fig. 1 Range of the studied species and possible areas of elemental input: triangle – study area, King George Island (KGI), grey rectangles – moulting latitudes for adult storm-petrels (dotted for black-bellied storm-petrel (BBSP) and solid for Wilson's storm-petrel (WSP); according to isotopic data from Quillfeldt et al. 2005 and Phillips et al. 2009 calculated based on equation proposed by Quillfeldt et al. 2005). Storm-petrels non-breeding range map source: BirdLife International and Handbook of the Birds of the World 2018. Photos by DJ

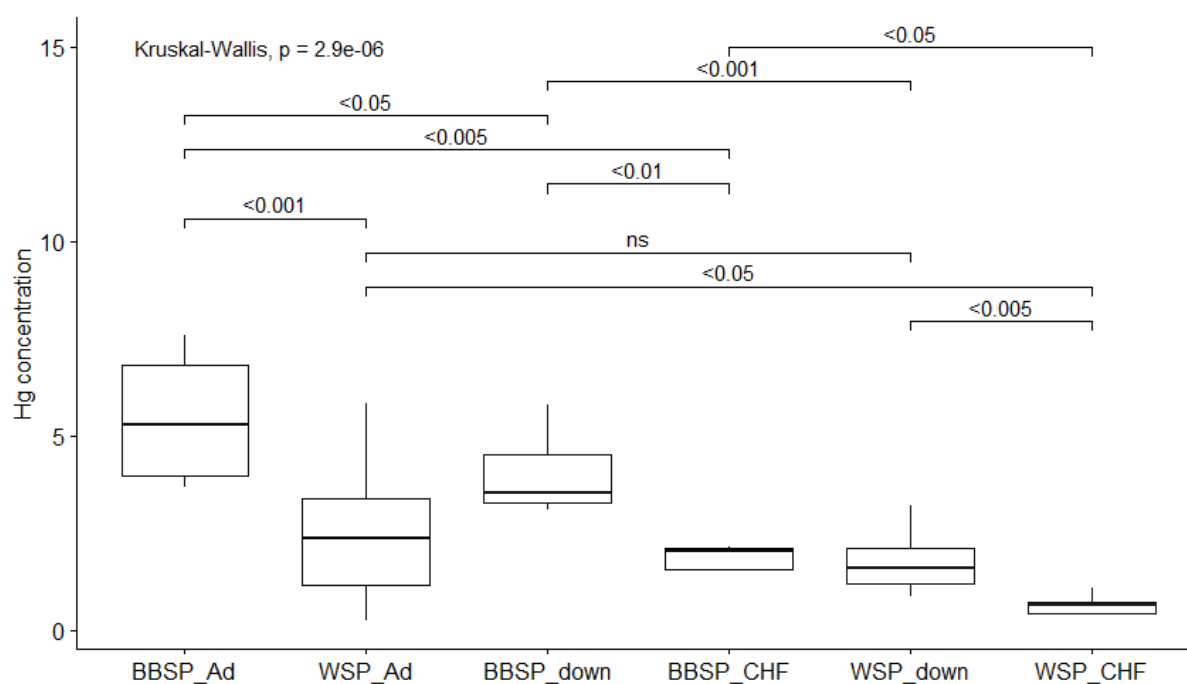


Fig. 2 Concentration of Hg ($\mu\text{g}\cdot\text{g}^{-1}$ dw) in feathers of the six studied groups of storm-petrels: body feathers of adult Wilson's (WSP_Ad) and back-bellied (BBSP_Ad) storm-petrels, down from Wilson's (WSP_down) and black-bellied (BBSP_down) storm-petrel chicks and body feathers from Wilson's (WSP_CHF) and black-bellied (BBSP_CHF) storm-petrel chicks. Boxplots show the median (band inside the box), the first (25%) and third (75%) quartile (box), and the lowest and the highest values within 1.5 interquartile range (whiskers)

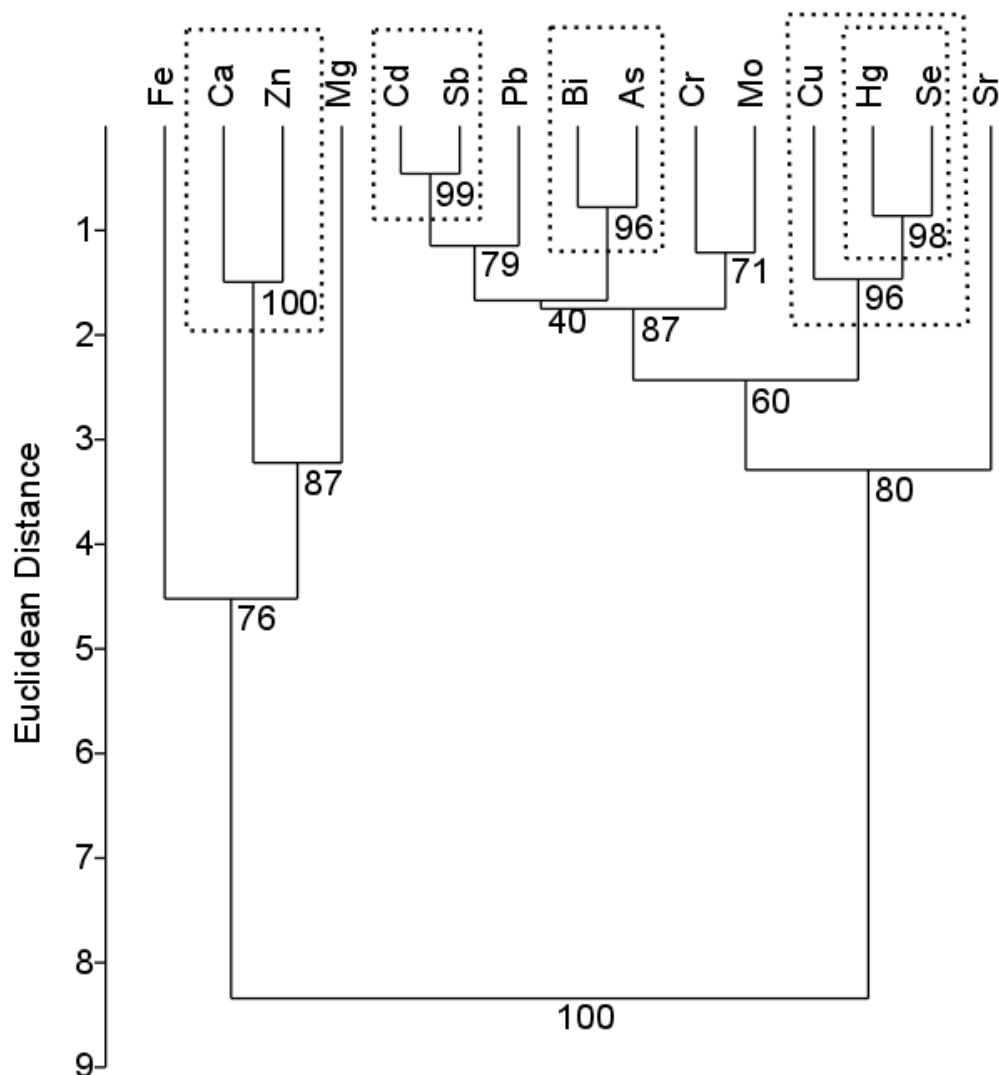


Fig. 3 Hierarchical dendrogram of the studied elements in the feathers of the studied storm-petrels (all age and feather type groups combined), obtained using a paired group method and Euclidean distance matrix (the distance reflects degree of association between different elements). Numbers below branches indicate bootstrap probability values (bootstrap $n = 1000$). Clusters with bootstrap support ≥ 95 denoted with a dotted rectangle.

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