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Biogenic and toxic elements in feathers, eggs, and excreta of Gentoo penguin (*Pygoscelis papua ellsworthii*) in the Antarctic

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Abstract Feathers, eggs, and excreta of Gentoo penguin (*Pygoscelis papua ellsworthii*), adults, from Livingston Island (South Shetlands), chosen as bioindicators, were used to test the quality of the Antarctic environment. Sex was not examined. The bioaccumulations of toxic trace elements (Cd, Pb, Al, and As), essential trace elements (Fe, Cu, Zn, Mn, Cr, V, Ni, and Sr), and major essential elements (Na, K, Mg, Ca, P, and S) were established. For the first time data about the element contents in Gentoo eggs is provided. Two hypotheses were tested: (1) there are differences in the metal levels among eggs and feathers; and (2) the element concentrations are highest in the excreta. The hypotheses were confirmed at 0.01–0.05 confidence levels. The concentrations of almost all trace elements were significantly higher in the feathers compared to those in the eggs. The following values of the concentrations ratio Fe/Zn were obtained: in the embryo, Fe/Zn = 1.5, and in the feathers, Fe/Zn = 0.5. The concentration of Pb in the embryo and excreta was below 0.4 μg/g, and Cd and As in eggs were below 0.05 and 0.3 μg/g, respectively. This indicates that there is no toxic risk for penguin offspring. Arsenic could be considered as a potential pollutant for Antarctic soil due to its relative high concentration in excreta, 5.13 μg/g. The present data (year 2007) were compared to the data for years 2002 and 2003. No trend of toxic element contamination was established. The concentrations of Pb, Cd, and As in representatives from the top of the food chain in the Antarctic (the present study) and Arctic (literature data) were compared. The data supports the hypothesis that there is an abnormality in cadmium levels in polar marine areas. Regarding Pb, the South Shetlands displayed 3-fold lower level compared to the Aleutians.

Keywords Heavy metals · Essential elements · Gentoo penguin · Feathers · Eggs · Excreta · Antarctic

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Introduction

The Antarctic is believed to be an unpolluted region because of its remoteness from other land mass and traffic. Nevertheless, a permanent monitoring of the Antarctic environment will help to establish whether this situation is stable. One of the main aspects of ecomonitoring is the quantification of toxic elements (especially heavy metals and arsenic) in tissues and waste products of the biota. The levels of bioaccumulation of these elements are important indicator of environmental quality and animal health. This problem is a subject of long-term programs for biomonitoring in the Antarctic.

Polar animals are less studied in comparison to animals from other continents and hence Antarctic research should be also focused on investigations of the biological peculiarities of the Antarctic organisms. In the exploration of the content of biogenic elements, both major and trace elements in the Antarctic fauna will be of importance. Biomonitoring could provide useful information about the natural levels of the biogenic elements in animal diets as well as some physiological and metabolic characteristics, specific for the polar conditions (Metcheva et al. 2006).

Seabirds are excellent test subjects for presence of contaminants, and therefore, for assessment of the marine ecosystem health, because they are wide-ranging, large, conspicuous, abundant, long-living, feeding at a wide range of trophic levels, and easily monitored (Walsh 1990; Thompson and Hamer 2000; Burger and Gochfeld 2004a). Some seabirds species are at the top of the food chain where they are susceptible to accumulation of pollutants in their prey. Thus, such species are potentially exposed to highest concentrations of certain chemicals due to their biomagnification in the food chain. In carnivora, bioaccumulation of contaminants progresses with age (Lewis and Furness 1991; Monteiro and Furness 1995; Burger and Gochfeld 2002). Therefore, it is reasonable to study some pollutants in top-level predators such as raptors and fish-eating birds (Hunter and Johnson 1982; Burger 2002; Burger and Gochfeld 2009).

In this context, penguins, and mainly fish-eating ones, that already have permanent ecological niche and dominate the Antarctic aviafauna, could be considered as most suitable species for biomonitoring. Gentoo penguin as fish- and krill-eating species can be considered as a top predator.

Information not only about contaminants but also about the contents of the major essential elements such as Na, K, Mg, Ca, P, and S could be useful because these elements are rarely investigated especially in polar sea birds (Metcheva et al. 2006).

All species in the Antarctic are protected under the Antarctic treaty (1959) that provides that animals cannot be killed. This fact limits to a great degree the determination of element concentrations in the body and different organs and tissues of Antarctic species. Reliable statistically significant results could be obtained only in explorations of the bird's end-"pools": molting feathers, addle eggs, abandoned or broken eggs, and excreta. Birds can eliminate heavy metals through excrements, feathers, and in eggs (Fimreite et al. 1974).

Penguin's feathers are a perfect indicator of elements due to the annual molting of the birds. One could assess whether contamination trend exist if the element concentrations in molting feathers were determined every year (Metcheva et al. 2006). The element contents in embryo could give some information about the initial element levels in the offspring. The levels of heavy metals and toxic elements in feathers, eggs, and excreta are an important criterion for the environmental and animal health.

Two hypotheses were tested here: (1) there are differences in the metal levels among eggs and feathers; and (2) the concentrations of the elements are highest in the excreta.

Both feathers and eggs potentially represent different periods of exposure to contaminants. However, it may appear that significant differences between the levels of toxic elements in the feathers and eggs should not exist due to the similar mechanism of bioaccumulation: (1) recent exposure to food and water, and (2) mobilization of stored metals in body from past intake (Fimreite et al. 1982; Lewis and Furness 1991; Monteiro and Furness 1995; Burger and Gochfeld 1996). However, the metal accumulation in feathers has some specifics. During the feather growth (2–3 weeks), metals incorporate in the keratin structure. The bird feathers are composed of proteins rich in sulfur-containing amino acids. The disulfide bonds readily reduce to sulfhydryl groups, for which metals have high affinity (Altmeyer et al. 1991). When the blood supply atrophies, the metals remain sequestered in the feathers (Burger 1993; Thompson and Furness 1998; Burger et al. 2009). Thus, feathers reflect the metal levels in blood during feather formation. The characteristic incorporation renders profiles inert and stable, and in feathers, occur a relatively higher body burden proportion of certain metals compared to internal tissues (Burger 1993). Arsenic compounds have an affinity for the thiol groups in keratin (Murphy et al. 1990). In this context, one can expect that the metal and As levels in the penguin feathers should be higher compared to those in eggs. One could expect that the concentrations of major essential elements (except sulfur) would be higher in penguin eggs, and especially in embryo, compared to those in feathers. These suppositions were confirmed by our analysis of the samples collected.

Due to the physiological mechanisms of organism self-purification and homeostatic mechanisms modulating body content of some elements, it is reasonable to suppose that excreta would exhibit the highest element contents.

A number of authors have investigated heavy metal levels in feathers. High amount of absorbed metals are eliminated with feathers during molting and the high concentrations found there suggest that is a very important route of metal elimination (Furness et al. 1986; Honda et al. 1986; Lewis and Furness 1991). Goede and De Bruin (1986), Burger (1993, 1996), Burger and Gochfeld (2000a), and Golden et al. (2003) tested bird feathers as an indicator of heavy metals pollution. Relationships between Hg content and molt were studied by Furness et al. (1986). Braune and Gaskin (1987) reported that birds reduce their Hg body burdens by excreting methylmercury in the growing feathers during ontogeny and molt. Gochfeld et al. (1996) revealed age, gender, and tissue differences in heavy metals bioaccumulation and hypothesized that feathers could be used as a biomonitoring tool for assessing levels of metals in other tissues. Burger and Gochfeld (2000b) found correlations between species and age, on the one hand, and heavy metal levels, on the other hand. Burger et al. (2007) established that the levels of most metals are below the known effect levels, but Hg and Se appeared high enough to potentially pose a risk to pigeon guillemots and to their predators. Smith et al. (2008) used X-ray absorption spectroscopy to distinguish the arsenic from exogenous (from the environment) and endogenous (from the body) sources. Investigations of heavy metals and As content in feathers of polar birds also were carried out in order to examine the environmental quality and animal health in these remote areas (Honda et al. 1986; Bargagli et al. 1998; Schiefler et al. 2005; Metcheva et al. 2006; Burger and Gochfeld 2009).

Bird eggs have been similarly analyzed for heavy metals and As. Fimreite et al. (1982) and Becker (1992) reported that females can sequester metals in their eggs. Burger and Gochfeld (1991, 1996) explored the correlations in Cd and Pb levels between parents and eggs. Gochfeld (1997) tested the locational differences in heavy metals and Se concentrations in eggs. Kubota et al. (2002) studied the maternal transfer of As species to eggs of seabirds. Burger (2002) investigating seven metals in five marine bird species found that metal concentrations in eggs mainly represent food chain differences. Burger and Gochfeld (2004b) and Braune (2007) established temporal trends in contaminant levels in eggs of seabirds. Burger et al. (2009) compared heavy metals concentrations in feathers and eggs of a seabird from the Aleutians.

For assessment of the environmental quality, animal excreta, being a direct source for environment contamination, are also suitable substrates for biomonitoring. They could be a sensitive indicator of heavy metals and As pollution in continental as well as in polar regions (Fitzner et al. 1995; Dauwe et al. 2000; Sun et al. 2000; Sun and Xie 2001; Ancora et al. 2002; Xie and Sun 2008; Yin et al. 2008; Leonzio et al. 2009).

In this work, the concentrations of the toxic elements Cd, Pb, Al, and As as well as trace essential elements Fe, Cu, Zn, Mn, Cr, V, Ni, and Sr and major essential elements Na, K, Mg, Ca, P, and S found in samples collected from feathers, eggs, and excreta of Gentoo penguin are discussed. The hypotheses were tested, and some findings explained. The study was carried out on Livingston Island, South Shetlands (Antarctic).

Materials and methods

Feathers, eggs, and excreta samples were collected from Gentoo penguins (*Pygoscelis papua ellsworthii*) breeding at Livingston Island (Fig. 1) 62°38′29″ S (longitude) and 60°24′53″ W (latitude) during the Antarctic summer of 2006–2007.

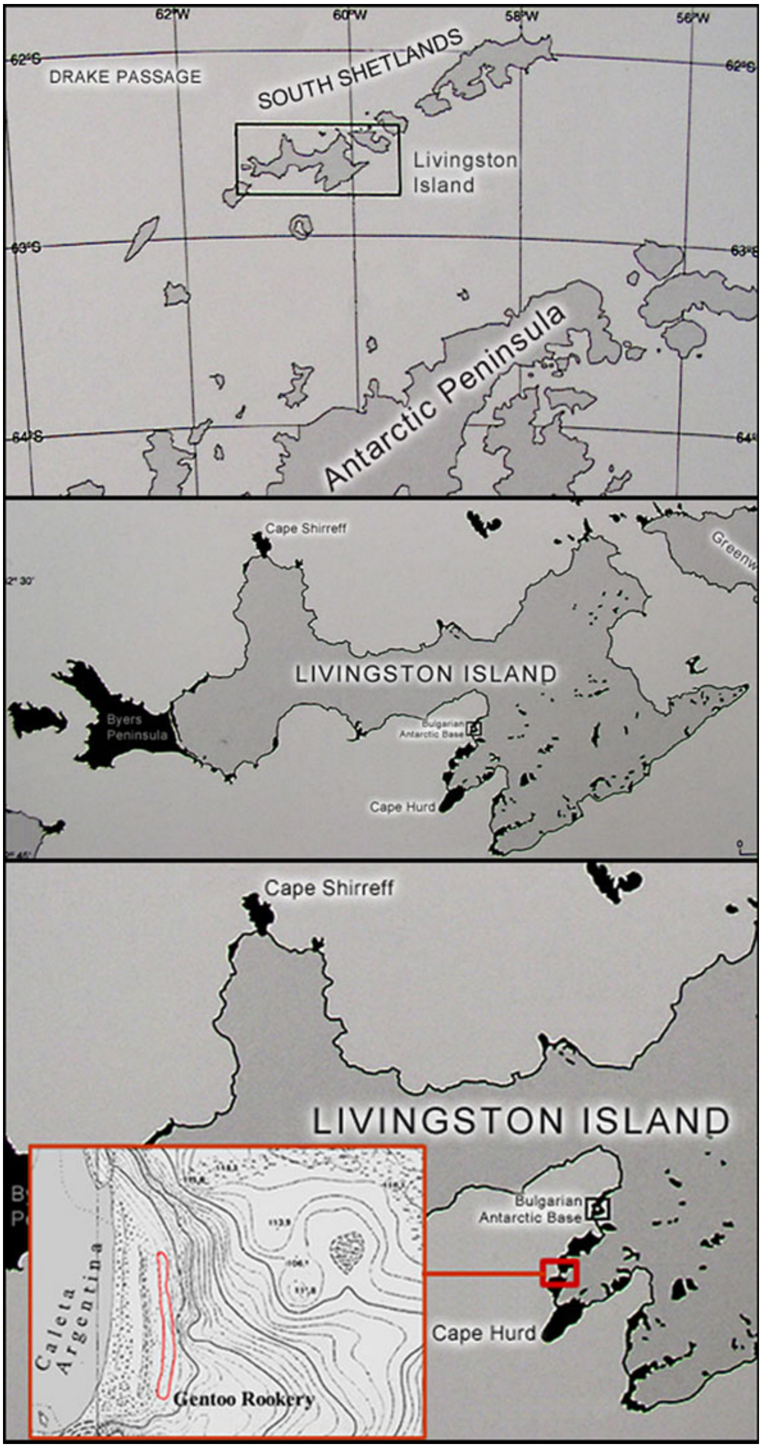

Fig. 1 Map showing the location of collections of feathers, eggs, and excreta of adult Gentoo penguins (*Pygoscelis papua ellsworthii*) on Livingston Island, South Shetlands, Sub-Antarctic

Feather samples were collected from 14 individuals. Old feathers were taken in time of molt. This is easy and without trouble for the penguin. Gentoo molt once in a year regularly. Old feathers begin to fall out on day 12 and it takes until day 21 for this process to be complete (Cherel et al. 2000). Only adult Gentoo were sampled during the post-breeding period. The state of molt was defined as middle. The molting feathers were carefully removed from the skin at the neck and the upper part of the back of the birds. The feathers in these parts of the body are cleanest and relatively easy to collect. In large seabirds, breast feathers were usually used for analyses because they are considered more representative (compared to other feathers) of the exposure to metals especially to mercury (Furness et al. 1986; Burger 1993). In case of penguins, it is necessary to mention that they have a uniform covering of feathers over their bodies unlike most other birds, which have alternating feathered and bare tracts. This cover is interrupted only during the breeding. Feathers were placed in individual polyethylene envelopes and labeled for later identification. The samples were washed in deionized water to eliminate adsorbed external contaminations and then air-dried.

Twelve Gentoo addle eggs were collected. Eggshell with eggmembrane was separated from the embryo. Both embryo and eggshell–eggmembrane complex were dried to constant weight at 60°C. Egg contents were digested individually. Fresh droppings of Gentoo penguin were collected using plastic containers and stored. The excrements were kept at 4°C before being dried and wet mineralized.

All samples were stored deep-frozen (at −18°C) until further analytical treatment. Before analysis, the samples were dried at 40°C until constant weight and then wet-ashed. About 2 g material was treated with 15 ml nitric acid (9.67 M) overnight. The wet-ashed procedure was continued with heating on a water bath, followed by addition of 2 ml portions of hydrogen peroxide. This treatment was repeated until full digestion. The filtrate was diluted with double-distilled water (0.06 μS cm^{-1}) to 25 ml. All solutions were stored in plastic flasks. Triplicates of each sample were prepared independently.

Elements Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, V, and Zn were determined in a certified laboratory by atomic emission spectrometry with inductively coupled plasma on a VARIAN VISTA-PRO instrument. The detection limits (DL), calculated as 3 SD of the lowest instrumental measurements of the blanks (which are stock standard solutions), were 0.002 mg/l for Mn and Sr; 0.004 mg/l for Cd, Cr, Cu, and Ni; 0.005 mg/l for Zn; 0.02 mg/l for As and V; 0.03 mg/l for Pb; 0.04 mg/l for Al and Fe; and 0.5 mg/l for Ca, K, Mg, Na, P, and S.

Triplicates of each sample were prepared independently. Triplicates of blanks—all reagents and all analytical procedures, but without biological material were also analyzed. Three measurements for each solution of the digested sample and blanks for the series were done. Finally, standard deviation in all cases was below 5%. The last concentrations were calculated according to all measurements, dilution of digested solutions, weight of each sample, and blanks. Triplicates of two reference materials were analyzed and the concentrations were compared to the certified values published by Steinnes et al. (1997).

Methodological limit of determination (three measurements for each solution of the digested sample, calculated according to dilution and weight of each sample and corresponding blank) were found under the limits only for: Cd and Ni < 0.05 μg/g, As and V < 0.3 μg/g, and Pb $<$ 0.4 μg/g.

For the statistical analysis, levels of the investigated elements in feather, egg, and excreta, with total number 900, were compared using one-way ANOVA—SPSS 10.0. The single measurement for each sample, taken from feathers, embryo, eggshell–eggmembrane complex, and excreta, was included as the dependent variable. The element concentrations are presented in the results as means ± SD in micrograms per gram on a dry mass basis. The differences were considered significant when p values were less than 0.05.

The following symbols will be used further to identify the sources of the analyzed samples: feathers (F), embryo (E), eggshell + eggmembrane = eggshell–eggmembrane complex (SM), and excreta (X).

Results

The data for the concentrations (mean ± SD) of Na, K, Mg, Ca, P, S, Fe, Cu, Zn, Mn, Cr, V, Ni, Sr, Al, Pb, Cd, and As in feathers, eggs (embryo and eggshell–eggmembrane complex), and excreta of *P. papua ellsworthii* for year 2007 are listed in Table 1. The elements which minimum levels were below the detection limits (Cd and As) are presented by their maximum and minimum concentrations. The detection limits of Cd and As were 0.05 and 0.3 μg/g, respectively. The distribution of element levels was close to normal. In Table 1, the ANOVA results are also displayed.

Comparison between egg components (E and SM)

There were significant differences between E and SM in the concentrations of all analyzed elements (Table 1). Less difference was observed for Mn. The embryo showed higher concentrations of Na, K, P, S, Fe, Cu, Zn, and Cr compared to SM. Ca in SM was 29-fold higher than in E. V and Ni were below DL. The toxic elements in E were at lower concentrations compared to SM. Al was 2-fold and Sr was 25-fold higher in SM than in E. Pb concentration in E was below DL. Cd and As concentrations were below DL in both E and SM.

Comparison between feathers and eggs

There were significant differences among eggs and feathers in *P. papua ellsworthii* for almost all elements (Table 1). The concentrations of major essential elements Na and K were 1.13-fold and 12-fold higher in E than in F, respectively. Ca in SM was 81-fold and 29-fold higher than that in F and E, respectively. P in E was 3-fold higher than in F. S in F was 5-fold and 13.5-fold higher than that in E and SM, respectively. The trace elements Cu and Zn in F were 6-fold and 3.6-fold higher than in E. Sr level in SM was 5-fold and 25-fold higher compared to that in F and E, respectively. Al concentration in F was 2.5-fold and 1.3-fold higher compared to E and SM, respectively. The toxic elements Pb, Cd, and As were significantly higher in feathers compared to eggs.

Comparison between feathers and excreta

The concentrations of all elements exhibited statistically significant differences between feathers and excreta (Table 1). Feather S was 3.7-fold higher compared to that in excreta. Pb concentration in excreta was below DL. All other elements displayed rather higher concentrations in excreta than in feathers. The greatest differences were observed concerning P, Ca, and K; their concentrations in X were 36.8-fold, 21.7-fold, and 21.3-fold higher than in F. The trace elements Cr and Sr were 12-fold and 11.3-fold higher in X compared to F. V in X was 19-fold higher towards the concentration corresponding to DL in F. Cd in X was 2.5-fold higher compared to the maximum concentration in F and 21-fold higher compared to DL in eggs. As in X was 7.4-fold higher than its maximum level in F.

Comparison between feathers, eggs, and excreta

Statistically significant differences were established between F, E, SM, and X regarding the concentrations of all elements analyzed (Table 1). Among the major essential elements the most Fisher factor was obtained for Ca (39,163). Among the trace essential elements the most Fisher factor was calculated for Cr (6,695). The elements K, Mg, P, Fe, Cu, Zn, Mn, Cr, V, Ni, Sr, Al, Cd, and As had highest concentrations in X. Fe level in X was 4.7-fold and 13-fold higher than in E and SM, respectively. Cu level in X was 37-fold and 84-fold higher than in E and SM. Zn level in X was 5.7-fold and 35.6-fold higher than in E and SM. Sr and Al showed 53-fold and 22-fold higher concentrations in X compared to E, and 2-fold and 11-fold higher concentrations in X compared to SM. The major essential elements Na and S and the toxic element Pb had their highest concentration in F.

The concentrations (micrograms per gram of dry weight) of the determined trace elements, essential and toxic, in the several samples

Table 1 Concentrations of biogenic and toxic elements (micrograms per gram of dry weight) and comparison between the biomarkers used (feathers, eggs, and excreta) in adult Gentoo penguins (*Pygoscelis papua ellsworthii*) from Livingston Island

Biomarker's number	1	2	3	4	Comparison between		Comparison between		Comparison between		Comparison between	
Elements	Feather (14)	Embryo (12)	Eggshell–egg membrane (12)	Excreta (10)	2 and 3 *F*, *p*		1–3 *F*, *p*		1 and 4 *F*, *p*		1 and 4 *F*, *p*	
Na	3,834 ± 191	4,350 ± 386	1,046 ± 52	2,485 ± 124	336.4	<0.0001	74.5	<0.0001	7.4	0.015	54.5	<0.0001
K	256 ± 12.7	2,673 ± 134	401 ± 20	5,446 ± 545	318.3	<0.0001	331.5	<0.0001	18,618	<0.0001	601.1	<0.0001
Mg	523 ± 10.2	401 ± 8	818 ± 16	10,554 ± 2,111	269.9	<0.0001	121.1	<0.0001	42,993	<0.0001	22,309	<0.0001
Ca	2,115 ± 53	5,804 ± 116	170,444 ± 47,450	45,806 ± 9,161	65,331	<0.0001	54,129	<0.0001	4,298	<0.0001	39,163	<0.0001
P	1,104 ± 22	3,445 ± 111	1,617 ± 32	40,642 ± 2,032	30.01	<0.0001	23.2	<0.0001	7,125	<0.0001	2,588	<0.0001
S	23,045 ± 3,457	4,246 ± 134	1,702 ± 34	6,276 ± 314	161.8	<0.0001	4,652	<0.0001	1,510	<0.0001	3,403	<0.0001
Fe	47 ± 9.2	38.17 ± 4.5	13.95 ± 2.1	185 ± 9.3	228.7	<0.0001	160.6	<0.0001	1,276	<0.0001	1,118	<0.0001
Cu	17 ± 3.1	2.82 ± 0.7	1.24 ± 0.4	104 ± 2.1	47.9	<0.0001	247.8	<0.0001	2,120	<0.0001	2,666	<0.0001
Zn	92 ± 4.6	25.27 ± 2.5	4.07 ± 0.6	145 ± 2.9	3,018.7	<0.0001	1,173	<0.0001	145.6	<0.0001	1,307	<0.0001
Mn	1.7 ± 0.2	0.67 ± 0.06	0.82 ± 0.08	12.3 ± 1.2	4.559	0.043	139.7	<0.0001	15,337	<0.0001	5,532	<0.0001
Cr	0.17 ± 0.05	0.176 ± 0.06	0.08 ± 003	2.06 ± 0.7	73.8	<0.0001	55.4	<0.0001	15,991	<0.0001	6,695	<0.0001
V	<0.3	<0.3	<0.3	5.74 ± 1.3								
Ni	<0.05	<0.05	<0.05	0.63 ± 0.2								
Sr	49 ± 7.3	10.41 ± 3.1	256 ± 14.7	556 ± 55.5	1,468	<0.0001	1,236	<0.0001	16,876	<0.0001	2,224	<0.0001
Al	37 ± 6.9	14.56 ± 2.4	28.96 ± 4.3	316 ± 47.5	37.7	<0.0001	30.1	<0.0001	2,981	<0.0001	1,897	<0.0001
Pb	1.52 ± 0.5	<0.4	0.68 ± 0.3	<0.4								
Cd	Max 0.41 Min <0.05	<0.05	<0.05	1.03 ± 0.36								
As	Max 0.69 Min <0.3	<0.3	<0.3	5.13 ± 1.79								

Antarctic sample sizes (n) in brackets, mean values ± SD, and ANOVA results (F and p) are given. For the elements below DL, max and min are displayed could be presented in the following descending order:

Feathers:

$$Zn(92) > Sr(49) > Fe(47) > Al(37) > Cu(17) > Mn(1.7) > Pb(1.5) > As(max\ 0.58) > Cd(max\ 0.41) > Cr(0.17) > V(<0.3) > Ni\ (<0.05)$$

Embryo:

$$Fe(38) > Zn(25) > Al(14.6) > Sr(10.4) > Cu(2.8) > Mn(0.7) > Pb(<0.4) > Cr(0.18) > V(<0.3) = As(<0.3) > Ni(<0.05) = Cd(<0.05)$$

Shell–membrane complex:

$$Sr(256) > Al(29) > Fe(14) > Zn(4) > Cu(1.2) > Mn(0.82) > Pb(<0.68) > V(<0.3) = As(<0.3) > Cr(0.08) > Ni(<0.05) = Cd(<0.05)$$

Excreta:

$$Sr\ (556) > Al\ (316) > Fe\ (185) > Zn\ (145) > Cu\ (104) > Mn\ (12) > V\ (5.7) > As\ (5) > Cr\ (2) > Cd\ (1) > Ni\ (0.63) > Pb\ (<0.4)$$

The concentrations of the major essential elements in the several samples, calculated in percentages, could be presented in the following descending order:

Feathers:

$$S\ (2.3\%) > Na\ (0.38\%) > Ca\ (0.2\%) > P\ (0.12\%) > Mg\ (0.05\%) > K\ (0.026\%)$$

Embryo:

$$Ca\ (0.58\%) > Na\ (0.44\%) > S\ (0.42\%) > P\ (0.34\%) > K\ (0.27\%) > Mg\ (0.04\%)$$

Shell–membrane complex:

$$Ca\ (17\%) > S\ (0.17\%) > P\ (0.16\%) > Na\ (0.1\%) > Mg\ (0.08\%) > K\ (0.04\%)$$

Excreta:

$$Ca\ (4.6\%) > P\ (4\%) > Mg\ (1\%) > S\ (0.63\%) > K\ (0.54\%) > Na\ (0.25\%).$$

These relations regarding the feathers are very close to those for years 2002–2003 and the order is the same (Metcheva et al. 2006).

Temporal trends in element concentration in Gentoo feathers

The element concentrations in penguin feathers were compared to those published by us for years 2002 and 2003 (Metcheva et al. 2006). The trace elements only are presented in Table 2.

In year 2007, the same descending order, as in previous years, was observed regarding the major essential elements, calculated in percentages (with small differences in some of percentages):

$$S\ (2.3\%) > Na\ (0.4\%) > Ca\ (0.21\%) > P\ (0.11\%) > Mg\ (0.05\%) > K\ (0.026\%).$$

The concentrations (micrograms per gram of dry weight) of the trace elements analyzed in 2007 formed the following descending order:

$$Zn\ (92) > Sr\ (49) > Fe\ (47) > Al\ (37) > Cu\ (17) > Mn\ (1.7) > Pb\ (1.52) > Cr\ (0.16) > Ni\ (<0.05)$$

This order was the same as that averaged on years 2002–2003 for Gentoo penguin (Metcheva et al. 2006). The concentrations of Fe, Zn, and Sr compared over years 2002, 2003, and 2007 showed statistically significant differences (Table 2). The statistical analysis regarding Cu, Mn, Al, and Pb indicated insignificant differences. The concentration of Ni in year 2007 was below DL (0.05 μg/g). The max concentration of Cd (0.41 μg/g) was

Table 2 Comparison of trace elements (micrograms per gram of dry weight) in feathers of adult Gentoo penguins (*Pygoscelis papua ellsworthii*) from Livingston Island

Elements	2002 (14) (Metcheva et al. 2006)	2003 (28) (Metcheva et al. 2006)	2007 (14)	Comparison between years (F, p)	
Fe	56 ± 20	46 ± 18	47 ± 9.2	11.3	<0.0001
Cu	17 ± 4	16 ± 2	17 ± 3.1	1.1	0.342
Zn	106 ± 8	89 ± 7	92 ± 4.6	24.8	<0.0001
Mn	1.5 ± 0.7	2.6 ± 1	1.7 ± 0.2	2.8	<0.076
Ni	0.84 ± 0.4	2.2 ± 0.9	<0.05		
Sr	47 ± 7.5	59 ± 8	49 ± 7.3	9.8	<0.0001
Al	40 ± 10	46 ± 22	37 ± 6.9	1.6	0.212
Pb	1.7 ± 1.3	1.57 ± 1.1	1.52 ± 0.5	1.3	0.287
Cd	0.21 ± 0.1	Max 0.43	Max 0.41		
		Min < 0.15	Min < 0.05		
As	0.88 ± 0.3	Max 4.0	Max 0.69		
		Min < 0.6	Min < 0.3		

Antarctic in years 2002, 2003, and 2007. Sample sizes (n) in brackets, mean values ± SD, and ANOVA results (F and p) are given. For the elements below DL, max and min are displayed

almost equal to that in 2003. The maximum concentration of As in 2007 was 5.8-fold lower compared to that in 2003.

Discussion

The following questions will be discussed below:

(1) What is the reason for the established disposition of the major essential elements and trace elements in the explored "compartments"? (2) Are the levels of heavy metals and arsenic toxic to penguins? (3) Is there a correlation between the element contents in these "compartments" and the penguin diet? (4) Is there a temporal trend of pollution in the Antarctic environment? (5) Are there some differences in the levels of the most toxic elements between Antarctic and Arctic? (6) The toxic metals Cd, Pb, and Al as well as the toxic element As in eggs are at less concentrations compared to those in feathers. (7) Eggs and feathers exhibit opposite Fe/Zn ratios.

Major essential elements

The major essential elements Na, K, Ca, and P were considerably higher in embryo then in feathers (Table 1) and this is an expected result due to the primary role of these elements for the developing organism. The much higher concentration of S in the feathers compared to eggs and excreta is determined by the very structure of the feather. The epidermal feather proteins are notably rich in the sulfur-containing amino acid cystine. Feather proteins contain proportionately more cystine than most other tissue proteins and most food proteins (Murphy et al. 1990). The main element building up SM complex is Ca. Further examination could establish why Mg concentration was higher in feather than in embryo. Perhaps there is some relationship between Mg and S? No data about feathers were found. However, England et al. (2007) reported that a proportion of Mg content is associated with the sulfated fraction of the organic matrix in the shell of *Terebratulina retusa*.

The high contents of P in all samples are due to the specific diet of Gentoo penguin. It contains about 15–40% fish, especially benthopelagic fish and small squids. The rest is krill and different crustaceans (Del Hoyo et al. 1992; McArthur et al. 2003). It is well known that fish is rich in phosphorus (National Research Council 1989; Food and Nutrition Board 1997). We have established that the level of P in Gentoo penguin was 6-fold higher compared to chinstrap penguin eating exclusively krill and crustaceans (Metcheva et al. 2006).

Metals and arsenic

The concentrations of Fe, Cu, Zn, Mn, Al, Pb, and Cd in the feathers were significantly higher than in both embryo and SM complex (Table 1). Regarding Cu and Pb, our data support those of Honda et al. (1986) who have found these elements in feathers of Adelie penguin in higher concentrations compared to other tissues. The maximum concentration of Cd in the feathers was 3.7-fold lower than that of Pb (Table 1). Relatively low Cd level in seabirds feathers was established also by Burger et al. (1992). The high concentration of Sr in the SM complex was an expected result because of the significant similarity in biogeochemical peculiarities of Sr and Ca, which causes their similar role in processes of mater circle (Kabata-Pendias and Pendias 1979). The concentrations of Cr in feathers and embryo were almost equal. The metals V and Ni are essential elements but at low concentrations. Vanadium plays a role in the bone formation and normal reproduction of organism, especially in birds (Hopkins and Mohr 1974). Nickel, participating in some enzyme systems, has regulatory functions (Nielsen et al. 1975). However, these metals could be toxic at increased levels. Our analysis showed that both V and Ni were below DL in eggs and feathers. In the feathers, the toxic element As displayed a maximum value of 0.69 μg/g.

The concentrations of the most important essential trace elements Fe, Cu, and Zn were considerably higher in the embryo compared to SM complex. This is an expected result. The levels of the toxic metals Pb and Al were almost two times higher in SM complex than in embryo. Burger (1994) notes that eggshells provide another method of excretion of metals for female birds. Moreover, Pb is a bone-seeking (or more generally, a calcium-formations-seeking) element (O'Flaherty 1991; Teodorova et al. 2003) and the elevated Pb content in eggshell is expected. Lead and Cd in embryo were below DL. The level of Cd was below DL also in the SM complex. Kubota et al. (2002) reported that in black-tailed gull relatively high concentration of As were observed in the liver, kidney, pancreas, muscle, and gonad, while in the eggs As level was below DL. Nygard et al. (2001) established that the transport of Cd from the female to the egg in the uterus is very low. They quantified a concentration of 0.02 μg/g Cd in eggs of Antarctic petrel. (We determined Cd concentration <0.05 μg/g in Gentoo eggs). This fact suggests that a probable protection mechanism exists in bird organism regarding the maternal transfer of contaminants to eggs.

Thus, our first hypothesis was confirmed by the analyses of the samples collected.

In excreta, the levels of As and all studied metals with the exception of Pb were much higher compared to feathers and eggs. The maximum ratio excreta/feathers (X/F) was established for V: $V_X/V_F = 19$. The concentrations of Mn, Cr, Ni, and Sr in excreta were about 12-fold higher, compared to feathers. For Al, As, Cu, and Fe the following relation were calculated: $Al_X/Al_F = 8.5$; $As_X/As_F = 7.4$; $Cu_X/Cu_F = 6$; $Fe_X/Fe_F = 4$. (Concerning As the relation was determined taking into account the maximum value in feathers.) The minimum ratio was established for Zn: $Zn_X/Zn_F = 1.6$. For Cd we had: $Cd_X/Cd_F = 2.5$ when calculated regarding the maximum value in feathers and $Cd_X/Cd_F = 21$ when calculated regarding the DL. Ancora et al. (2002) recorded 20-fold higher Cd level in excreta compared to feathers of Adelie penguins from Antarctica. Cadmium in excreta we found (1.03 ppm) was not very high. Lead concentration in excreta was below DL.

It is clear that the results of the element analyses confirm our second hypothesis. The element concentrations in excreta vary depending on the diet and internal needs of organism. Diet stands out as a major factor of element level variation. During the breeding season of the Antarctic summer, Gentoo penguins feed inshore, 68% crustaceans and 32% fish and foraging areas may also be involved (Croxall et al. 1997). Physiological mechanisms of organism self-purification ensure that excreta appear the "compartment" with the highest concentrations of the elements. The low concentration of Pb in excreta can be explained by the strong affinity of Pb to feathers. Bones and epidermal compartments (hair, nails, feathers, and claws) readily accumulate Pb (Lucky and Venugopal 1977; O'Flaherty 1991; Hahn et al. 1993).

The content of the elements in excreta indicates the entering of these elements in the environment. Penguin guano has been considered as the main source of As accumulation in Antarctic soil. The droppings of Gentoo penguin contain far more As than those of the other species—nearly twice as much as the droppings of the southern giant petrel and up to three times more than the local seals (Xie and Sun 2008). Arsenic is in the water, which is absorbed by krill and then accumulates in the food chain, passing to predators such as penguins. In surface waters at five lagoon near the Southern Shetlands the following ranges in total concentrations for Cd, Pb, and As were quantified: <0.04–3.15, <0.1–4.5, and <0.1–5.2 μg/L, respectively (Préndez and Carrasco 2003). The fact that As is excreted more by penguins than by other top predators, such as seals, probably could be explained assuming specific features of As metabolism in penguins. The findings of Sun et al. (2000) and Xie and Sun (2008) suggest that deposition of penguin droppings have had a significant effect on the geochemical composition of the sediment core and that Gentoo penguin populations can be used as a reliable indicator of As levels in Antarctic soil. Based on exploration of lake sediments in Antarctic, Sun and Xie (2001) concluded that Pb concentration in penguin droppings has increased significantly during the last 50 years as compared to the Pb level prior to the Industrial Revolution. Sediments reflect a long-time accumulation. Our data showed that Pb concentration in the excreta of Gentoo penguin was below DL.

The comparison of the As and metals concentrations in Gentoo feathers between year 2007 (the present study) and years 2002 and 2003 (Table 2) showed significant differences only regarding Fe, Zn, and Sr. Regarding Cu, Mn, Al, and Pb these differences were insignificant. Thus, one could consider that further studying of the levels of these elements in penguin feathers might allow determining a range of referent values. Cadmium exhibited a drop in its minimum value. Though insignificantly, Pb level slightly decreased. Arsenic also displayed a reduction in its level. These data give assurance that Antarctic persists as a relatively unpolluted continent.

In blood and organs of vertebrates Fe levels are usually higher than those of Zn. This fact is supported by the above relations regarding the egg components. In the penguin feathers, however, the opposite situation was observed. The level of Zn was two times higher than Fe level ($p < 0.001$). Honda et al. (1986) have established 3.4-fold higher concentration of Zn towards Fe in Adelie penguin feathers. It is known that homeostatic mechanisms, acting principally on absorption and liver disposition, modulate Zn body content (Bertholf 1986; Walshe et al. 1994). Bearing in mind that birds rid their bodies of heavy metals through both excretion and disposition in feathers (Burger 1994), we can conclude that in birds part of Zn is forwarded to the feathers. Thus, females also eliminate toxic substances in the content of their eggs. In addition, another mechanism could be assumed, that is linked to the elevated concentrations of Cd in the feathers. Zinc and selenium are known to reduce the toxic effect of cadmium and mercury, respectively (Underwood et al. 1977; Magos and Webb 1980; Goyer 1997). Thus, the elevated level of Cd leads to increase of Zn level as a protection mechanism (Norheim 1987). This may be an adaptive reaction of birds to the Antarctic conditions, characterizing with the elevated cadmium levels, due to upwelling of Cd-rich waters and local volcanism (Sanchez-Hernandez 2000) and thus, the relatively high Cd concentrations in crustaceans (Petri and Zauke 1993; Szefer et al. 1993) providing food for fish and penguins.

In SM complex and excreta, Sr occupies the leading position. Strontium in small quantities is a necessary element for bone formation in juveniles. In adults, Sr accumulates in bones. However, there is a barrier in mammals and birds against the over-accumulation of Sr (degree of Sr discrimination) as an organism protective mechanism (Kabata-Pendias and Pendias 1979).

It is interesting to compare the bioaccumulation of the most toxic elements Cd, Pb, and As in seabirds occupying highest position in the food chain in both Polar Regions Antarctic and Arctic. Among birds, raptors and fish-eating seabirds are at the top of the food chain. Fish often is polluted, contaminated through the consecutive trophic levels (Bustamante et al. 1998). Gentoo penguin eating fish, squids, and krill is among the seabirds at the top of the Antarctic food chain. Burger and Gochfeld (2009) consider bald eagle as clearly being at the top of the food chain of the Aleutians (Alaska), because it eats primarily fish that are larger than other species eat. The comparison between bird feathers is presented in Table 3. The concentration of Pb in Alaska is 3-fold higher, compared to that in South Shetlands ($p < 0.001$). The mean value of Cd level in bald eagle was a half of the maximum level established in Gentoo penguin in 2003 and 2007 and it was almost the same as the mean Cd level determined by us in year 2002 (Table 2) and as that reported by Honda et al. (1986) for *Pygoscelis adeliae*. The maximum As levels in Gentoo penguin was 1.25 higher than the mean As value in bald eagle ($p < 0.02$). Close values of Cd concentrations (micrograms per gram of dry weight) have been reported for muscle of some Antarctic and Arctic fish: 0.03 in pelagic fish (Bustamante et al. 2003) from Southern Ocean and <0.1 averaged on muscle of 15 marine fish from Barents Sea (Zauke et al. 1999). About liver, there was significant difference: 10 in pelagic fish and a range of 2.4–4.8 in Arctic fish species.

Table 3 Comparison between Cd, Pb, and As concentrations (micrograms per gram of dry weight) in feathers of Gentoo penguin (*Pygoscelis papua ellsworthii*) from South Shetland Islands (Antarctic) and Bald eagle (*Haliaeetus leucocephalus*) from Aleutian Chain of Alaska (Arctic)

Toxic elements	Seabird species	
	Gentoo penguin (14) (means ± SD)	Bald eagle (23) (means ± SE) (Burger and Gochfeld 2009)
Lead	1.52 ± 0.49	4.57 ± 0.8
Cadmium	Max 0.41 Min <0.05	0.25 ± 0.04
Arsenic	Max 0.69 Min <0.3	0.55 ± 0.076

Sample sizes (n) are given in brackets. For the elements below DL, max and min are given

Zauke et al. (1999) talk about a general “cadmium anomaly” in polar marine waters. The elevated level of Pb in Alaska compared to South Shetlands may be due to the fact that Aleutians are a built-up area. Besides, Aleutian Chain, situated between Bering Sea and North Pacific Ocean, is a region extremely important commercially, especially to the fishing industry (Burger and Gochfeld 2009) and therefore, it is close to the traffic and potential Pb pollution.

Gochfeld et al. (1996) have tested heavy metal levels in laughing gulls collected at John F. Kennedy International Airport, New York. The following concentrations (parts per million) of Pb and Cd in the gull feathers were found: 2.82 ± 0.8 and 0.2 ± 0.03, respectively. When comparing continental (America) and Antarctic data, it is seen that Cd levels determined in New York and Livingston Island differ insignificantly. However, the Pb level in gull feathers is almost two times higher ($p < 0.001$) than that in Gentoo penguin feathers (Table 1).

In conclusion, the results showed that there were significant differences in the metal levels among eggs and feathers. The concentrations of the trace elements, including toxic ones, were higher in the feathers. In addition, the lead, cadmium, aluminum, and arsenic concentrations in eggs and feathers of Gentoo penguin were below those known to cause adverse effects on bird organism. The element concentrations, except of lead, were highest in the excreta. Arsenic in penguin excreta could be a local weak contaminant for the soil. In embryo, Pb, Cd, and As were below DL, and Al was 2.5-fold lower in feather. This fact suggests positive expectations concerning offspring health. In this context, although the contaminants (heavy metals, arsenic etc.) are transported around the globe and move rapidly particularly in water, Antarctic as a remote polar region can still be considered as an unpolluted area.

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