

## A preliminary study of trace metals and porphyrins in excreta of Gentoo penguins (*Pygoscelis papua*) at two locations of the Antarctic Peninsula<sup>#</sup>

Estudio preliminar de metales traza y porfirinas en heces de pingüinos Papua (*Pygoscelis papua*) en dos localidades de la península Antártica

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### RESUMEN

Aunque las concentraciones de la mayoría de los elementos químicos en la Antártica son bajas en comparación con otras áreas del planeta, éstas se han ido incrementando con el tiempo debido al gran crecimiento de la población y desarrollo industrial de los países del Hemisferio Sur, lo cual podría estar afectando a especies vulnerables como los pingüinos. Se determinaron las concentraciones (peso seco) de arsénico (As), cadmio (Cd), mercurio (Hg) y plomo (Pb) y porfirinas (copro-, uro- y proto-) en heces de colonias de pingüinos Papua (*Pygoscelis papua*), colectadas en dos lugares de la península Antártica (O'Higgins y Videla). Se encontraron mayores ( $P < 0,05$ ) niveles de Hg ( $7,55 \pm 1,28 \mu\text{g g}^{-1}$ ) en las heces de colonias de pingüinos en O'Higgins, mientras que las colonias de pingüinos en Videla exhibieron mayores concentraciones de Cd ( $1,68 \pm 0,71 \mu\text{g g}^{-1}$ ). Los pingüinos Papua de O'Higgins mostraron mayores ( $P < 0,05$ ) niveles de copro- ( $1,81 \pm 0,61 \text{ nmol g}^{-1}$ ), uro- ( $1,74 \pm 0,72 \text{ nmol g}^{-1}$ ) y protoporfirinas ( $1,24 \pm 0,46 \text{ nmol g}^{-1}$ ), en directa relación con los mayores niveles de Hg y Pb medidos. Esto podría implicar que algunas colonias de pingüinos Papua de la península Antártica están propensas a desarrollar algunos efectos bioquímicos asociados a contaminación por metales traza. Estos resultados preliminares levantan inquietantes interrogantes acerca de la contaminación de origen antropogénico que estaría afectando áreas tan remotas como la Antártica.

*Key words:* heavy metals, excrement, bio-monitoring, seabirds, Antarctic, polar region.

*Palabras clave:* metales pesados, excremento, bio-monitoreo, aves marinas, Antártica, región polar.

### INTRODUCTION

Heavy metal contamination is wide spread globally as a result of mining and other industrial processes. Human activities such as oil spills, sewage, hazardous wastes, pesticides, mining, smelting or forest fires could contribute to increase Hg, As and Cd levels, whereas electrical devices, mining or explosives could increase Pb levels in marine ecosystems (Boersma 2008). However, heavy metals are difficult to estimate as pollutants because these chemicals occur naturally in the environment (Ancora *et al* 2002). In any case, there are evidences showing that remaining pristine regions of the planet are being affected, and environmental contamination researches on these ecosystems attracted great interest from the scientific community (Smichowski *et al* 2006).

Antarctica is a remote, polar region surrounded by oceans, and seems far away from industrial and other high impact anthropogenic activities. However, recent studies

have shown pollution affecting Antarctic fauna, which could be linked to a raising tourist activity and research activities or some local environmental accidents (Lohan *et al* 2001, Sanchez-Hernandez 2000, Negri *et al* 2006).

Because seabirds are top predators, long-lived, often feed on a long distance from land and exhibit different trophic levels, they are sentinels of environmental changes in ecosystems (Boersma 2008). Heavy metals are toxic for mammals and birds, and can cause severe damage to kidneys, liver and the central nervous system (Szefer *et al* 1993, Kim *et al* 1998, Horai *et al* 2007).

A useful technique to determine the risk of chemical exposure in wildlife and humans is the use porphyrins profile alterations as a marker of biochemical effects (De Matteis and Lim 1994). Porphyrins comprise a large, diverse and universal group of organic compounds. They are a group of metabolites that participate in the biosynthesis of haemoglobin and cytochroms. They are produced and accumulate in trace amounts in erythropoietic tissues such as liver and kidneys and are excreted via urine or faeces (Lim 1991). Porphyrins are capable of binding metals and can be detected in different biological materials, even at very low concentrations (De Matteis and Lim 1994). This characteristic makes it possible for porphyrins to be used as biomarkers for measuring the level of exposure to heavy metals such as Pb, Hg and As in seabirds and

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mammals (Casini *et al* 2003). Also, some environmental contaminants such as heavy metals, can interfere with heme biosynthesis causing alterations in the level of porphyrins that are accumulated or excreted (Casini *et al* 2001). The resulting oxide by-products as copro-, uro- and protoporphyrins are not toxic at normal levels, but when accumulated in excess can affect liver and bony marrow (Lim 1991). There is evidence showing a direct correlation between metal pollution and accumulation of porphyrins in liver and excreta (Leonzio *et al* 1996).

Even though concentrations of most chemical elements in Antarctic ecosystems appear to be very low, a continuous level of contamination due to population's growth and industrial development in countries of the Southern Hemisphere could be affecting some endemic species with unique ecophysiological characteristics, like penguins (Bargagli 2008). Biomaterials such as plants, lichens, mosses, algae, tissues and organs have been usually used as environmental bioindicators. However, they can be difficult to obtain due to strict regulations for wildlife protection, or just because living organisms are generally difficult to monitor in an ecosystem (Yin *et al* 2008). For that reason, the aim of this research was to determine the concentrations of some trace metals and porphyrins in excrements from colonies of Gentoo penguins (*Pygoscelis papua*) at two locations of the Antarctic Peninsula.

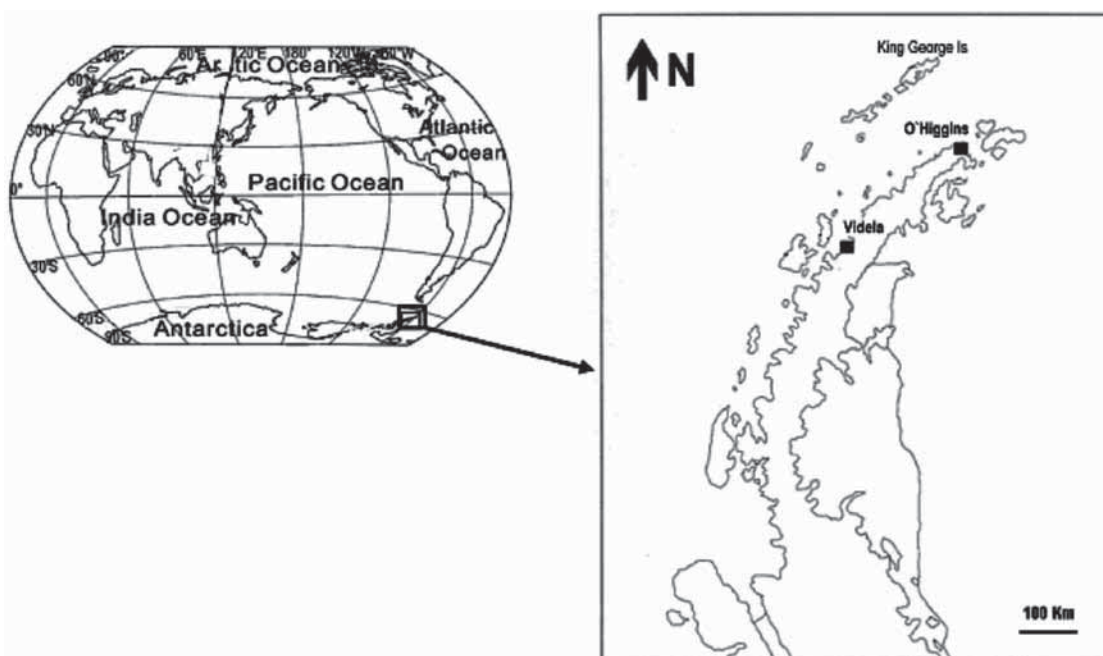
## MATERIAL AND METHODS

Fresh samples of excreta from Gentoo penguin colonies were collected during February of 2011 at two locations of

the Antarctic Peninsula (figure 1). Twenty excreta samples (4 g) were collected from Gentoo penguin colonies breeding at Videla (63°28'S, 56°17'W) and other twenty samples from Gentoo penguin colonies breeding at O'Higgins (63°19'S, 57°53'W). Fresh excrements were produced by penguins in the past couple of days, and are generally brown with white spots (Yin *et al* 2008). Both stations are usually occupied by a small crew of maintenance personnel and are currently visited by scientific staff and tourists from September to March, particularly O'Higgins, located 150 km from King George Island.

Samples were put into polyethylene bags, stored at -4 °C and freeze-dried before analysis. Samples were washed with distilled water, dried at room temperature and then ground and screened (24 mesh dm<sup>-2</sup>). Then samples were digested with nitric acid HNO<sub>3</sub> (Ancora *et al* 2002). The levels of elements in the samples were determined by atomic fluorescence for Hg, whereas atomic absorption spectrometry (for As) with graphite furnace (for Cd and Pb) was used. In order to ensure quality control a certified reference material, human hair, was used as an internal standard in a proportion of 10% each batch of samples.

Porphyrins were determined as described by Lockwood *et al* (1985). Briefly, 1 ml hydrochloric (HCl) 5 N solution was placed in a graduated centrifuge tube containing 100 mg excreta and vortex-mixed. Diethyl ether (3 ml) was added and mixed to obtain an emulsion; then 3 ml water were added and the solution was mixed again and then centrifuged at 700 g for 10 min. The supernatant was decanted and the precipitant containing the porphyrins was exposed to fluorimetric analysis (Casini *et al* 2001).



**Figure 1.** Location of sampling sites at the Antarctic Peninsula.  
Ubicación de los sitios de muestreos en la península Antártica.

Means and standard deviations were calculated for all the groups using descriptive statistics. Differences among colonies in metals and porphyrins concentrations in Videla and O'Higgins were analysed by using one-way ANOVAs, although a nonparametric test (Mann-Whitney) was used when the assumptions of normality and homocedasticity were not met (Jerez *et al* 2011). Concentrations were log-transformed to meet the normality assumptions of the statistical analyses, which were conducted in Statistica software (Statsoft). Pearson's correlation coefficients were calculated for trace metals and porphyrins to study the existence of any relations among data collected. The level of significance was set at  $P \leq 0.05$ . The detected levels are presented as mean  $\pm$  standard deviation in  $\mu\text{g g}^{-1}$  dry weight.

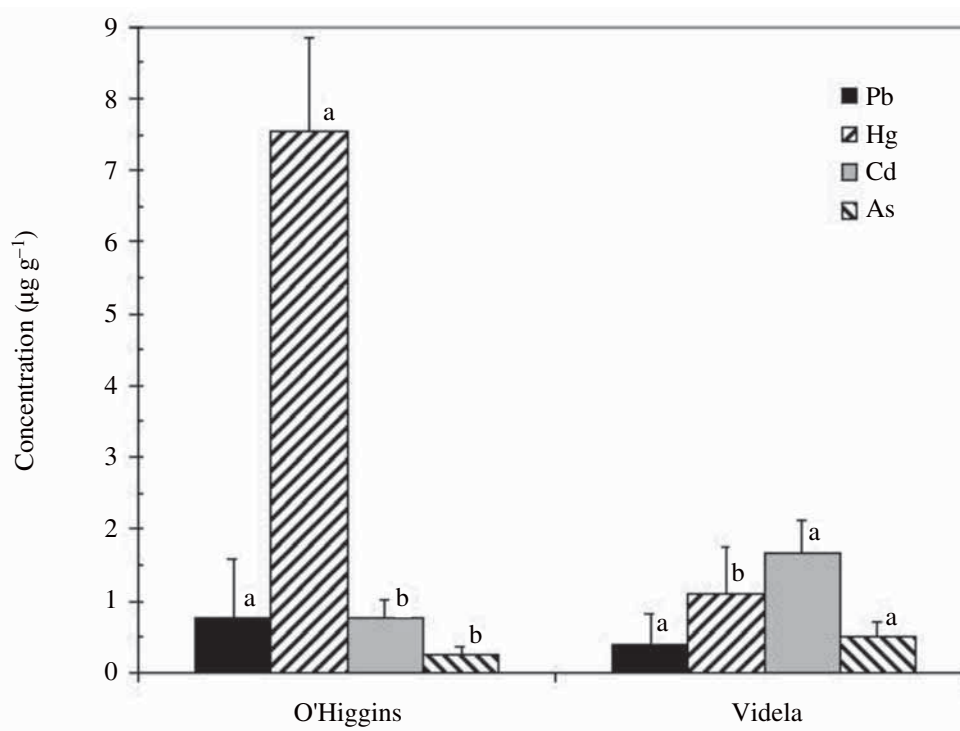
## RESULTS AND DISCUSSION

Figure 2 shows the heavy metal concentration in excrements of Gentoo penguins collected at both locations. The Hg concentration in the excrements of Gentoo penguins collected at O'Higgins station was significantly 7 times higher ( $7.55 \pm 1.28 \mu\text{g g}^{-1}$ ) than that of penguins at Videla station and Pb levels were twice higher ( $0.75 \pm 0.84 \mu\text{g g}^{-1}$ ) than that of penguins at Videla station. The Cd ( $1.68 \pm 0.71 \mu\text{g g}^{-1}$ ) and As ( $0.50 \pm 0.40 \mu\text{g g}^{-1}$ ) concentrations measured

at Videla were twice higher than those of penguins at O'Higgins location. The following relationship among trace elements was observed in penguins at Videla:  $\text{Cd} > \text{Hg} > \text{As} > \text{Pb}$ , compared to penguins at O'Higgins where it was  $\text{Hg} > \text{Cd} > \text{Pb} > \text{As}$ .

The Hg levels we detected were 70 times higher than those reported in excrements of Gentoo penguins and 40 times higher than those of Chinstrap penguins (*P. antarctica*) at King George Island (Yin *et al* 2008). Moreover, Hg levels were 30 times higher than those reported in Adélie penguins (*P. adeliae*) at Terra Nova Bay, Ross Sea (Ancora *et al* 2002), a remote area highly influenced by volcanic emissions (Bargagli *et al* 1998). As a result, penguin colonies in O'Higgins Station have been exposed to higher levels of Hg.

The Pb levels we found were 25% lower than those reported in excrements of Gentoo penguins and 57% lower than those in Chinstrap penguins at King George Island (Yin *et al* 2008). We detected As concentrations one order of magnitude higher than those reported by Jerez *et al* (2011) in soft tissues of Adélie penguin chicks from King George Island, and two orders of magnitude of those levels measured by Smichowski *et al* (2006). Our results for As and Cd are higher than those of Jerez *et al* (2011) who reported levels under  $0.20 \mu\text{g g}^{-1}$  for As and  $0.1 \mu\text{g g}^{-1}$  for Cd in feathers of Adélie penguins and Chinstrap penguins, respectively. The



**Figure 2.** Mean concentrations of trace metals from samples of excrements of Gentoo penguin colonies measured at O'Higgins and Videla, Antarctic Peninsula. Colonies with the same letter for a given element are not significantly different ( $P < 0.05$ ).

Concentraciones medias de metales traza en muestras de excrementos de colonias de pingüinos Papua medidas en O'Higgins y Videla, península Antártica. Colonias con la misma letra para un elemento dado no son significativamente diferentes ( $P < 0,05$ ).

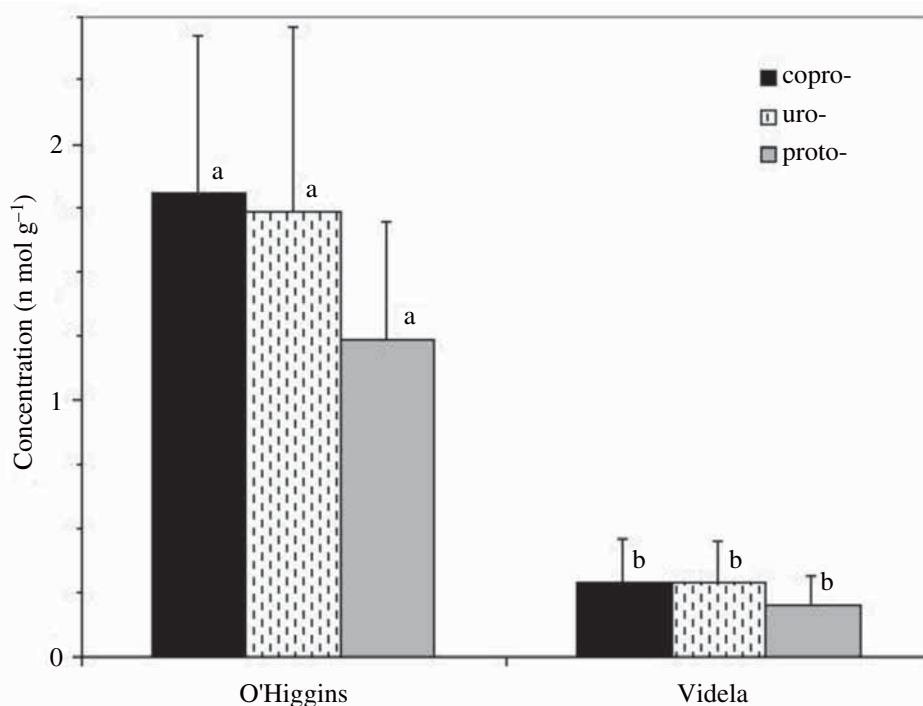
Cd levels we found are 70% lower than those reported by Ancora *et al* (2002) in excreta of Adélie penguins breeding at Edmonson Point (Terra Nova bay, Antarctica). It may indicate that the excrements of penguins are more useful for monitoring traces of heavy metals than feathers, as also noted by Jerez *et al* (2011), a point that should be more deeply investigated.

Various human activities are known to be Hg and Pb sources, such as charcoal combustion, waste incineration, sewage disposal, paint or oil spills (Bargagli 2008, Santos *et al* 2005). In our case, Hg and Pb detected at O'Higgins location seem to be related to a higher concentration of human activities that exists in the Antarctic Peninsula, where shipping, boating, and loading and unloading of fuel and goods are more concentrated, when compared to other Antarctic areas. In fact, there is a small airport at King George Island, where there is heavy traffic of vessels, planes, and helicopters to transport tourists, scientists and support personal (Tin *et al* 2009). Furthermore, people from all over the world must stop over there just before they reach the Antarctic Peninsula. There is some evidence of Pb contamination caused by Antarctic scientific stations, as previously reported by Boutron and Patterson (1987). Additionally, some evidence indicates there is a global metal transport resulting in the deposition of airborne metals to the marine ecosystem (Yin *et al* 2008).

Significantly twice Cd and As levels were detected in Gentoo penguins at Videla, a location more distant from King George Island than O'Higgins, seem strongly related to local volcanism in the area under study (Deheyn *et al* 2005, Jerez *et al* 2011), even though anthropogenic activities can not be neglected. Cd is one of the most biotoxic elements; it binds strongly to metallothioneins, proteins in the membranes of cell organelles, in the kidney of marine vertebrates, and levels increase with age in some marine mammals (Smichowski *et al* 2006).

Although regional volcanic emissions are a possible natural source of Hg in Antarctica (Bargagli *et al* 1998), the higher Hg levels found at O'Higgins when compared to Videla (a more remote location) suggest a possible human source. The traffic of vehicles by sea and air around the coast of O'Higgins may be an important factor for Hg bioaccumulation.

Figure 3 shows the levels of porphyrins found in excreta from penguins at O'Higgins and Videla locations. Higher levels ( $P < 0.05$ ) of proto- ( $1.24 \pm 0.46 \text{ nmol g}^{-1}$ ), uro- ( $1.74 \pm 0.72 \text{ nmol g}^{-1}$ ), and coproporphyrins ( $1.81 \pm 0.62 \text{ nmol g}^{-1}$ ) corresponded at O'Higgins, where coincidentally we found higher Hg and Pb concentrations in the excrements. As previously noted, O'Higgins constitutes an area with currently heavier anthropogenic activity than Videla. Despite the lack of studies on porphyrins



**Figure 3.** Mean concentrations of porphyrins from samples of excrements of Gentoo penguin colonies measured at O'Higgins and Videla, Antarctic Peninsula. Colonies with the same letter for a given porphyrin are not significantly different ( $P < 0.05$ ).

Concentraciones medias de porfirinas en muestras de excrementos de colonias de pingüinos Papua medidas en O'Higgins y Videla, península Antártica. Colonias con la misma letra para una porfirina dada no son significativamente diferentes ( $P < 0,05$ ).

**Table 1.** Pearson correlation among trace metals (As, Cd, Hg, Pb) and porphyrins (copro-, uro-, proto-) in excrements of Gentoo penguins (\*significant at  $P \leq 0.05$ , 2-tailed,  $n = 40$ ).Correlación Pearson entre metales traza (As, Cd, Hg, Pb) y porfirinas (copro-, uro-, proto-) en excrementos de pingüinos Papua (\*significativo a  $P \leq 0,05$ , 2 colas,  $n = 40$ ).

	As	Cd	Hg	Pb	Copro-	Uro-	Proto-
As	1	0.303	-0.249	-0.288	-0.198	-0.201	-0.219
Cd	0.303	1	-0.238	-0.130	-0.219	-0.223	-0.246
Hg	-0.249	-0.238	1	0.286	0.707*	0.709*	0.731*
Pb	-0.288	-0.130	0.286	1	0.736*	0.742*	0.729*
Copro-	-0.198	-0.219	0.707*	0.736*	1	0.995*	0.999*
Uro-	-0.201	-0.223	0.709*	0.742*	0.995*	1	0.997*
Proto-	-0.219	-0.246	0.731*	0.729*	0.999*	0.997*	1

in penguin excrements to which we could compare our results, a Pearson analysis showed that higher levels of copro-, uro- and protoporphyrins in penguins are directly related to higher levels of Hg and Pb (table 1). The Hg and Pb concentrations, as well as Cd and As, show a positive relationship that may indicate that the source of pollution could be the same or closely related for each pair of metals.

Although metal concentrations appear to be lower than  $10 \mu\text{g g}^{-1}$  (dry weight) as described in faeces from other bird's species from non-contaminated areas (Beyer *et al* 1997, Mateo *et al* 2006, Martinez-Haro *et al* 2010), our results suggest that adverse physiological effects in Gentoo penguins could be triggering even at low exposure levels.

The results indicate that Hg and Pb contamination can be assessed by using porphyrins as a nondestructive biomarker in penguins. It is consistent with findings of Leonzio *et al* (1996), who showed that birds treated with Hg led to accumulation of porphyrins in liver and excreta. Also, a local study suggested the use of porphyrins in excreta of sea birds as a biomarker of exposure of contaminants (Casini *et al* 2001). Hg and Pb concentrate porphyrins in the kidney and pancreas, affecting their functions (Lim 1991, Casini *et al* 2003). Gentoo penguins prey mainly on coastal fishes and krill (*Euphausia superba*) of the Antarctic Ocean (Berón *et al* 2002). Trace metals detected in penguins are strongly related to high metal levels found in the Antarctic krill (Barbante *et al* 2000) and usually found in fish (Szefer *et al* 1993, Beltcheva *et al* 2011). This may explain why Gentoo penguins are reflecting metal pollution.

These preliminary findings reveal that Gentoo penguins living around O'Higgins are potentially likely to suffer some illness for Hg and Pb pollution. Our results suggest that Gentoo penguins are a sentinel for monitoring porphyrins in excreta, as previously observed in other birds, such as gulls and cormorants (Casini *et al* 2001), and also geese (Mateo *et al* 2006). However, further research in the area is extremely needed to examine potential local sources of contamination.

## SUMMARY

Even though concentrations of most chemical elements in Antarctic ecosystems are very low as compared to other world's areas, their increasing time trends, as a consequence of the strong population growth and industrial development in countries of the Southern Hemisphere, could be affecting some vulnerable endemic species such as penguins. Concentrations (dry weight) of arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) and porphyrins (copro-, uro- and proto-) were determined in feces of Gentoo penguins (*Pygoscelis papua*), collected at two locations of the Antarctic Peninsula (O'Higgins and Videla). We found higher ( $p < 0.05$ ) levels of Hg in excreta of penguin colonies at O'Higgins ( $7.55 \pm 1.28 \mu\text{g g}^{-1}$ ), whereas penguins at Videla showed the highest concentrations of Cd ( $1.68 \pm 0.71 \mu\text{g g}^{-1}$ ). Gentoo penguins from O'Higgins site showed higher levels of copro- ( $1.81 \pm 0.61 \text{ nmol g}^{-1}$ ), uro- ( $1.74 \pm 0.72 \text{ nmol g}^{-1}$ ) and protoporphyrins ( $1.24 \pm 0.46 \text{ nmol g}^{-1}$ ), directly related to higher levels of Hg and Pb measured. These findings imply that some Gentoo penguin colonies of the Antarctic Peninsula are likely to develop some biochemical effects through trace metal contamination at some level. These preliminary results raise concerns about anthropogenic pollutants in Antarctica.

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