TRACE METALS (HG, PB, AND CD) IN FEATHERS OF FOUR GALAPAGOS WATERBIRD SPECIES

E. SANTIAGO JIMÉNEZ[1], GUSTAVO JIMÉNEZ-UZCÁTEGUI[2*], DAVID A. EGAS[1], NATALIA SOLIS[3], PATRICIO CARRERA-JÁTIVA[2,4], ROMMEL L. VINUEZA[5], JAVIER COTÍN[2], AINOA NIETO[2], CAROLINA GARCÍA[2], CHRISTIAN SEVILLA[6], DANNY RUEDA[6]

[1]Departamento de Ingeniería Química, Colegio de Ciencias e Ingenierías – El Politécnico, Universidad San Francisco de Quito, Quito, Ecuador
*[2]Charles Darwin Research Station, Charles Darwin Foundation, Puerto Ayora, Galapagos, Ecuador *(gustavo.jimenez@fcdarwin.org.ec)*
[3]Instituto de Biomedicina, Universidad San Francisco de Quito, Quito, Ecuador
[4]Carrera de Medicina Veterinaria – Universidad Nacional de Loja (UNL). Loja, Ecuador
[5]Escuela de Medicina Veterinaria, Universidad San Francisco de Quito, Quito, Ecuador
[6]Dirección Parque Nacional Galapagos, Islas Galapagos, Ecuador

Received 30 May 2019, accepted 12 September 2019

ABSTRACT

JIMÉNEZ, E.S., JIMÉNEZ-UZCÁTEGUI, G., EGAS, D.A., SOLIS, N., CARRERA-JÁTIVA, P., VINUEZA, R.L., COTÍN, J., NIETO, A., GARCÍA, C., SEVILLA, C. & RUEDA, D. 2020. Trace metals (Hg, Pb, and Cd) in feathers of four Galapagos waterbird species. *Marine Ornithology* 48: 85–89.

Contamination by anthropogenic heavy metals can produce significant concentration-dependent damage to ecosystems. Therefore, we sought to determine levels of heavy metals and their possible origins by analyzing the feathers of four endangered Galapagos species: Galapagos Penguin *Spheniscus mendiculus*, Flightless Cormorant *Phalacrocorax harrisi*, Waved Albatross *Phoebastria irrorata*, and American Flamingo *Phoenicopterus ruber* from the Galapagos Archipelago. Feathers were collected using non-invasive procedures, and calibration curves were used to measure heavy metals via electrochemical methods for mercury (Hg) and spectroscopic methods for lead (Pb) and cadmium (Cd). Pb and Cd were detected in flamingo feathers with no attributable anthropogenic or near-island origin. Hg was not found in any of the analyzed species. It is important to continue monitoring the presence of heavy metals in these endangered species, with a minimum frequency of five years, to facilitate their long-term conservation on the Galapagos Islands.

Key words: Flightless Cormorant, Galapagos Penguin, Galapagos Flamingo, Waved Albatross, heavy metals, pollution, wildlife surveillance

INTRODUCTION

It is important to investigate levels of trace metals, especially in pristine areas such as the Galapagos Islands, to know if pollution is adversely affecting species or habitats. This type of pollution is a threat that could be managed in most cases (Jiménez-Uzcátegui *et al.* 2019). However, marine currents and atmospheric deposition carry the heavy metals from natural or anthropogenic sources to other areas of the planet (Duce *et al.* 1991, Sun & Xie 2001, Jiménez-Uzcátegui *et al.* 2017).

Increased heavy metal contamination in seawater is an ongoing problem that has many short-term effects on the health of entire ecosystems (Burger & Gochfeld 2004). Anthropogenic heavy metal pollution is mainly related to the mismanagement of biological and industrial waste, while pollution of natural origin is mainly due to volcanic activity (Furness & Greenwood 1993, Burger & Gochfeld 2004). Birds can be used as environmental indicators of pollution (Furness & Greenwood 1993, Furness & Camphuysen 1997, Burger & Gochfeld 2001, Burger & Gochfeld 2004, Jiménez-Uzcátegui *et al.* 2019); estimating heavy metal pollution using feathers or bird organs is one of the most common methods to establish contaminant levels within ecosystems (Burger 1993). Therefore, native Galapagos avian species are good indicators of the flow of pollution in and out of the marine, terrestrial, and lagoon ecosystems of the archipelago (Jiménez-Uzcátegui & Huyvaert 2018, Jiménez-Uzcátegui *et al.* 2018).

There are numerous cases of pollutants reported in marine and wetland birds. For example, mercury (Hg) has been detected in flamingos in the western Mediterranean Sea (Borghesi *et al.* 2011) and in Southern Ocean penguins on the Kerguelen Islands (Carravieri *et al.* 2013), and lead (Pb) poisoning was recorded in flamingos in Spain, resulting in the death of 22 flamingos (Mateo *et al.* 1997). Previous studies on Galapagos Penguins *Spheniscus mendiculus* and Flightless Cormorants *Phalacrocorax harrisi* from the Galapagos Islands have reported high levels of Pb, while cadmium (Cd) levels were below the limit of detection (LOD) (Table 1) (Jiménez-Uzcátegui *et al.* 2017).

Currently, the only data available regarding concentrations of heavy metals in Galapagos bird species is from Jiménez-Uzcátegui *et al.* (2017), who used feathers of the Galapagos Penguin, Flightless Cormorant, and Waved Albatross *Phoebastria irrorata* to investigate levels of Pb and Cd. Our aim for the current study is to strengthen heavy metal surveillance within the Galapagos Islands by using key species such as the penguin, cormorant, albatross, as well as the American Flamingo *Phoenicopterus ruber*. By assessing these species for Hg, Pb, and Cd, we hope to better understand the extent of heavy metal contamination in Galapagos environments.

METHODS

Study area

The Galapagos Archipelago is located on the Equator approximately 960 km from the west coast of mainland Ecuador, and it consists of 13 large islands and more than 100 islets and rocks (Snell *et al.* 1996). We collected feather samples from various nesting areas (Fig. 1) as follows:

For Galapagos Penguins, we collected samples on Isla Isabela at Caleta Iguana (00°58.6′S, 091°26.7′W), Puerto Pajas (00°45.3′S, 091°22.5′W), and Islotes Marielas (00°35.8′S, 091°05.4′W).

TABLE 1
Mercury (Hg), lead (Pb), and cadmium (Cd) concentrations in feathers of Galapagos Penguins (GAPE), Flightless Cormorants (FLCO), Waved Albatross (WAAL), and American Flamingos (AMFL)

Species	Location	Feathers Weight (g)	Mean ± SD (ppm)		
			Hg	Pb	Cd
GAPE	Caleta Iguana	0.154	LOD	55.5 ± 27.80*	LOD*
	Puerto Pajas	0.235	LOD	71.3 ± 35.60*	LOD*
	Islotes Marielas	0.075	LOD	193.0 ± 48.30*	LOD*
FLCO	Punta Albemarle	0.244	LOD	17.00 ± 8.51*	LOD*
	Playa Escondida	0.170	LOD	-	-
	Carlos Valle	0.136	LOD	42.60 ± 8.52*	LOD*
WAAL	Plot A	0.324	LOD	LOD*	LOD*
	Plot B	0.248	LOD	LOD*	LOD*
AMFL	Laguna Roja	0.004	LOD	7.085 ± 0.178	0.479 ± 0.016
	El Sartén	0.971	LOD	10.253 ± 0.122	0.445 ±0.006
	Islote Bainbridge	2.780	LOD	8.649 ± 0.122	0.215 ± 0.012
	Tortuga Bay	1.170	LOD	10.663 ± 0.101	0.175 ± 0.025
	Bahía Borrero	0.160	LOD	7.573 ± 0.212	0.468 ± 0.023
	La Torta	2.133	LOD	11.161 ± 0.078	0.160 ± 0.031
	Montura	0.570	LOD	9.017 ± 0.187	0.455 ± 0.074
	Quinta Playa	0.469	LOD	9.678 ± 0.078	0.229 ± 0.092
	Las Salinas	0.301	LOD	10.326 ± 0.078	0.383 ± 0.016

*Data from Jiménez-Uzcátegui *et al.* (2017); LOD = below the limit of detection.

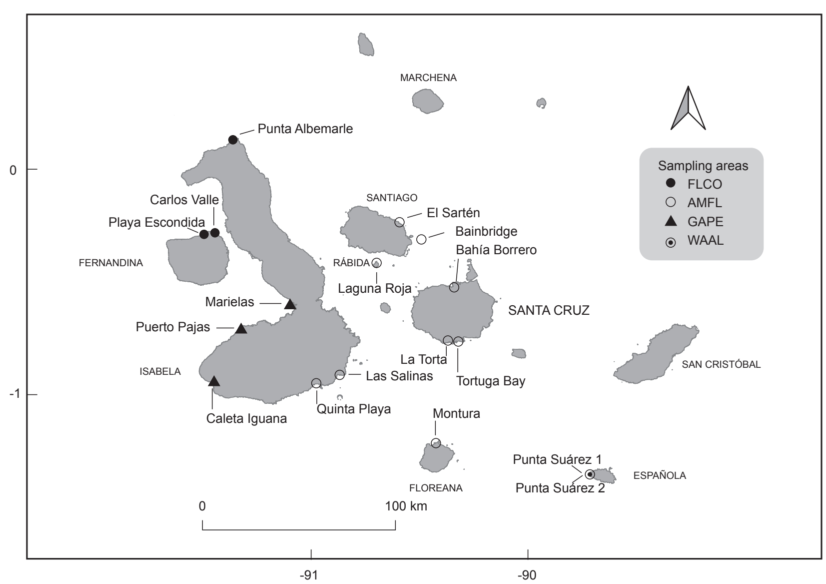

Fig. 1. Galapagos Penguins (GAPE), Flightless Cormorants (FLCO), Waved Albatross (WAAL), and American Flamingos (AMFL) breeding colonies sampled in 2018 in the Galapagos Archipelago.

For Flightless Cormorants, we collected samples on Isla Isabela at Punta Albemarle (00°09.7′N, 091°21.6′W) and on Isla Fernandina at Playa Escondida (00°15.7′S, 091°28.1′W) and Carlos Valle (00°15.6′S, 091°27.5′W).

For Waved Albatross, we collected samples on Isla Española at Punta Suárez (Plot A: 01°22.3′S, 089°44.4′W and Plot B: 01°22.5′S, 089°44.1′W).

For the Galapagos population of American Flamingo, we collected samples on Isla Rábida at Laguna Roja (00°24.0′S, 090°42.4′W); on Isla Santiago at El Sartén (00°13.0′S, 090°36.8′W); on Isla Santa Cruz at Tortuga Bay (00°45.8′S, 090°21.2′W), Bahía Borrero (00°31.0′S, 090°21.2′W), and La Torta (00°46.2′S, 090°23.6′W); on Isla Floreana at Montura (01°13.6′S, 090°25.7′W); on Isla Isabela at Quinta Playa (01°00.2′S, 091°04.9′W) and Las Salinas (00°57.3′S, 090°58.1′W); and on Islote Bainbridge (00°21.1′S, 090°34.0′W).

Sampling

Feather collection is the most common and non-invasive sampling method to assess the presence of heavy metals in birds (Burger 1993). Samples collected from penguins (Jul 2018), cormorants (Jul 2018), and albatross (May 2018) were taken from live and apparently healthy individuals prior to marking and releasing. Secondary feathers were collected from the wings of cormorants and albatross, while tail feathers were taken from penguins. Clean, recently shed feather samples from flamingos were collected from the lagoons (Jan 2018). Each sample collected was treated individually and labeled (date, place, species). Samples were stored at room temperature or frozen at −20 °C.

Laboratory analyses

Analyses were performed at the Chemistry Laboratory of the Department of Chemical Engineering at Universidad San Francisco de Quito. The feathers were cut into 1-cm^2 portions. Sets of feather samples were weighed separately and placed in independent Erlenmeyer flasks. They were then labeled with species, location, weight, and collection date before being digested under a fume hood using the USEPA 7000B method (USEPA 2007, Fonseca *et al.* 2017, Jiménez-Uzcátegui *et al.* 2017).

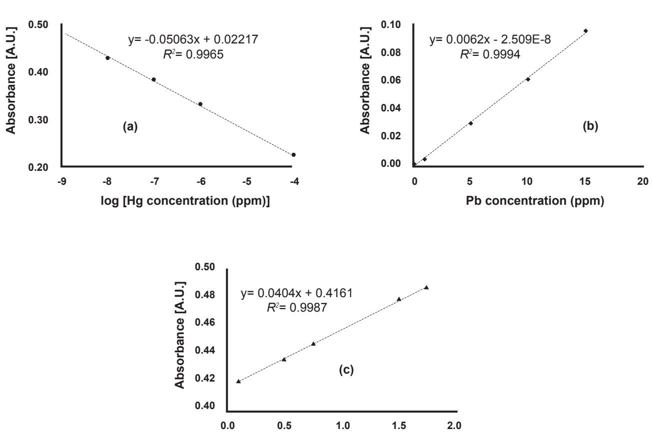

Fig. 2. Calibration curves for (a) Hg, (b) Pb, and (c) Cd used during this study.

To determine Hg, Pb, and Cd concentrations in the samples, calibration curves were used as references. The calibration equations worked well when sample concentrations were within the working range, and samples were concentrated or diluted as necessary.

Mercury concentrations were determined using an electrochemical method, due to its greater versatility and its relatively wide detection range. We used a μStat400 Potentiostat (Dropsens) in Potentiometric Stripping Analysis mode with the following conditions: starting potential of −0.7 V swept to a final potential of 0.8 V in 10 s, with an equilibration time of 3 s. In addition, we used a Ag/AgCl reference electrode, a glassy-carbon working electrode, and a platinum-wire counter electrode (BAS Inc., Japan) (Jagner & Graneli 1976, Jagner 1979, Jaya & Prasada Rao 1982). For the Hg calibration curve, five specific concentrations were measured (ranging from 1E−9 M to 1E−5 M), which produced an equation of $y = -0.05063x + 0.02217$ with $R^2 = 0.9965$ (Fig. 2a).

For Pb and Cd, the procedure was close to that described by Jiménez-Uzcátegui *et al.* (2017). A Flame Atomic Absorption Spectrophotometer (Buck Scientific, model 210 VGP, Norwalk, CT) was used to determine the concentrations. Then, a calibration curve was made for each element. The wavelengths of Cd and Pb were 228.9 nm and 283.3 nm, respectively. The detection range of this equipment is 0.08–20.00 ppm for Pb and 0.01–2.00 ppm for Cd. After choosing strategic concentrations for the standards, we proceeded to create the calibration curve, with triplicate measurements for each standard. A linear regression with $R^2 = 0.9994$ was obtained for Pb, and the equation was $y = 0.0062x + 2.509E{-}08$ (Fig. 2b). For Cd, a linear regression was obtained with $R^2 = 0.9987$, and the equation was $y = 0.0404x + 0.4161$ (Fig. 2c).

RESULTS AND DISCUSSION

Mercury is the contaminant that is monitored most in seabirds because it bioaccumulates in the upper food web, has generally increased over time, and is known to affect bird health (Wobeser 2006, Frederich & Jayasena 2011, Lamborg *et al.* 2014, Scheuhammer *et al.* 2015). Mercury concentration was too low to be detected in our four study species (Table 1, Fig. 3); there was no signal obtained between 0.4 and 0.7 V, indicating that concentrations were not within detection range (Jagner 1979; Fig. 4). Penguins, cormorants, albatross, and flamingos are biological indicators of the marine-coastal zones and lagoons, from inside and outside of the Galapagos Marine Reserve (Jiménez-Uzcátegui & Huyvaert 2018; Jiménez-Uzcátegui *et al.* 2018, 2019). Therefore, the non-detectable levels of Hg indicate that their food web has very low levels of this substance or that is biologically unavailable to them.

On the other hand, flamingo feathers showed detectable levels of Pb. The highest level of Pb was recorded at La Torta on Isla Santa Cruz (11.161 ± 0.078 ppm), followed by Las Salinas on Isla Isabela (10.326 ± 0.078 ppm). According to Burger (1993), the amounts of heavy metals in bird feathers are caused not only by external agents such as seawater or air, but also by the level of contamination in the tissue of the animals. As an example, in the Ebro Delta in Spain, 24% of flamingos that were illegally hunted contained Pb in their gizzard. The origin of this contamination was from the birds ingesting the shot used in shotgun shells (Friend 1999, Ancora *et al.* 2008, Mateo *et al.* 2013). Hunting of introduced animals (e.g., cattle, goats, pigs) for human consumption often happens near lagoons and areas of freshwater; these areas are, therefore, more

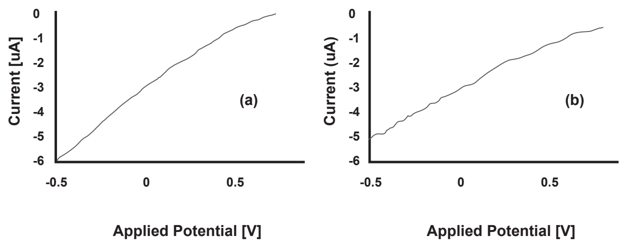

Fig. 3. Mercury (Hg) measurements in (a) American Flamingo feathers and (b) Galapagos Penguin feathers.

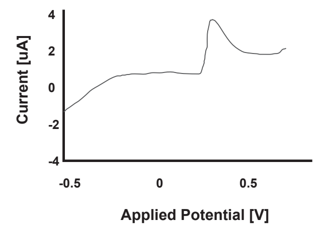

Fig. 4. Mercury (Hg) measurement for one of the standard samples (Jagner 1979).

likely to be polluted by Pb from the associated shotgun pellets (Friend 1999). However, the concentration of Pb in flamingos is lower than in penguins and cormorants in the Galapagos Islands. Coupled with data from Jiménez-Uzcategui *et al.* (2017), this indicates that Pb levels in Galapagos birds continue to be higher than those in other species of penguin and albatross worldwide, such as the Little Penguin *Eudyptula minor* on Phillip Island (Victoria, Australia), Wandering Albatross *Diomedea exulans* on Bird Island (South Georgia), and others (Anderson *et al.* 2010, Seco Pon *et al.* 2011, Finger *et al.* 2015). Given the protection afforded Galapagos wildlife, it should be mentioned that the amount of pollution from shotgun pellets should be minimal. In that case, the presence of Pb on the islands is unlikely to be of local anthropogenic origin but rather of natural origin due to the volcanic nature of the islands.

Flamingo feathers showed detectable levels of Cd. The highest level of Cd was recorded in Laguna Roja on Isla Rábida (0.479 ± 0.016 ppm), followed by Bahía Borrero on Isla Santa Cruz (0.468 ± 0.023 ppm; Table 1). In previous publications (Jiménez-Uzcátegui *et al.* 2017), Cd was not detected in penguins, cormorants, and albatrosses. However, this study detected Cd in flamingo feathers from all lagoon study sites, probably because the presence of Cd in water is generally correlated with high concentrations of nutrients (Boyle *et al.* 1981). Given this apparent relationship between heavy metals and labile nutrients such as phosphates and nitrates (up to 75–160 pmol/kg) (Boyle *et al.* 1981, Linn *et al.* 1990), it is possible that the Cd we detected came from natural sources.

We have shown that Hg levels in four Galapagos seabird species were all below the LOD, which is encouraging for the conservation of species and of the island habitats (Burger & Gochfeld 2004; Table 1). The Pb and Cd detected in flamingo, penguin, and cormorant feathers are probably not of local anthropogenic origin and may have been naturally present in their environment for a long time, as shown here and in Jiménez-Uzcátegui *et al.* (2017).

The concentration of Cd decreases during the El Niño Southern Oscillation (ENSO) in the Galapagos Islands, while Pb is not affected (Linn *et al.* 1990). Our study contributes “normal” baseline values (i.e., measurements taken in the hot season during a non–El Niño year) for future monitoring of heavy metal contamination and examination of the relationship between heavy metals and ENSO events, using seabirds as sentinels of the marine environment (Furness & Camphuysen 1997). The use of standardized techniques with high sensitivity is important to obtain comparable data between studies and to ensure that protocols can be replicated in the future. Long-term surveillance of wildlife and environmental health is critical for the conservation of pristine and isolated ecosystems, such as the Galapagos Archipelago and its unique species.

ACKNOWLEDGEMENTS

We wish to express our appreciation to our donors for financial support of the Galapagos Penguin, Flightless Cormorant, and Waved Albatross Project at the Charles Darwin Foundation: Galapagos Conservation Trust, the Truell Charitable Foundation, the Leona M. and Harry B. Helmsley Charitable Trust, Lindblad – National Geographic, the Penguin Fund of Japan, and Mr. Seishi Sakamoto and Sophie Lenoir of Tunememi Conservancy. We are grateful to our collaborators and to more than 80 assistants and volunteers who helped in the research from 2009, including Manuel Masaquisa, Johannes Ramirez, Franklin Gil, and Wilman Valle. Thanks to Sarah Brooks for editing the grammar in the original manuscript. Thanks to the reviewers, especially D. Ainley and K. Nabeta, for their helpful comments on this manuscript. We thank to the Galapagos National Park Directorate for granting our research permissions (PC-35-15, PC-51-16, PC-10-17, PC-05-18, PC-10-18, PC-33-19, PC-34-19). We thank the Charles Darwin Research Station for their support. This publication is contribution number 2283 of the Charles Darwin Foundation for the Galapagos Islands.

REFERENCES

ANCORA, S., BIANCHI, N., LEONZIO, C. & RENZONI, A. 2008. Heavy metals in flamingos (*Phoenicopterus ruber*) from Italian wetlands: The problem of ingestion of lead shot. *Environmental Research* 107: 229–236.

ANDERSON, O.R.J., PHILLIPS, R.A., SHORE, R.F., MCGILL, R.A.R., MCDONALD, R.A. & BEARHOP, S. 2010. Element patterns in albatrosses and petrels: Influence of trophic position, foraging range, and prey type. *Environmental Pollution* 158: 98–107. doi:10.1016/j.envpol.2009.07.040

BORGHESI, F., ANDREOTTI, A., BACCETTI, N. ET AL. 2011. Flamingo feathers to monitor metal contamination of coastal wetlands: Methods and initial results concerning the presence of mercury at six Mediterranean sites. *Chemistry and Ecology* 27: 137–151.

BOYLE, E.A., HUESTED, S.S. & JONES, S.P. 1981. On the distribution of copper, nickel, and cadmium in the surface waters of the North Atlantic and North Pacific Ocean. *Journal of Geophysical Research* 86: 8048–8066.

BURGER, J. 1993. Metals in avian feathers: Bioindicators of environmental pollution. *Reviews in Environmental Toxicology* 5: 203–311.

BURGER, J. & GOCHFELD, M. 2001. Metal levels in feathers of cormorants, flamingos and gulls from the coast of Namibia in Southern Africa. *Environmental Monitoring and Assessment* 69: 195–203.

BURGER, J. & GOCHFELD, M. 2004. Marine birds as sentinels of environmental pollution. *EcoHealth* 1: 263–274.

CARRAVIERI, A., BUSTAMANTE, P., CHURLAUD, C. & CHEREL, Y. 2013. Penguins as bioindicators of mercury contamination in the Southern Ocean: Birds from the Kerguelen Islands as a case study. *Science of the Total Environment* 454–455: 141–148.

DUCE, R.A., LISS, P.S., MERRILL, J.T. ET AL. 1991. The atmospheric input of trace species to the World Ocean. *Global Biogeochemical Cycles* 5: 193–259.

FINGER, A., LAVERS, J.L., DANN, P. ET AL. 2015. The Little penguin (*Eudypula minor*) as an indicator of coastal trace metal pollution. *Environmental Pollution* 205: 365–377.

FONSECA, M., MARMOLEJO, Y., ZURIA, I., PRIETO, F., PÉREZ, F. & ROMO, C. 2017. Cadmio en tejidos, plumas y egagrópilas de tecolote llanero de Hidalgo, Mexico. *Revista Iberoamericana de Ciencias* 4: 144–153.

FRIEND, M. 1999. Lead. In: FRIEND, M. & FRANSON, J.C. (Eds). *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds.* Information and Technology Report 1999–001. Washington, USA: US Department of the Interior; US Geological Survey, Biological Resources Division, pp 317–334.

FREDERICH, P. & JAYASENA, N. 2011. Altered pairing behavior and reproductive success white ibises exposed to environmentally relevant concentrations of methylmercury. *Proceedings of the Royal Society B: Biological Sciences* 278: 1851–1857.

FURNESS, R.W. & CAMPHUYSEN, C.J. 1997. Seabirds as monitors of the marine environment. *ICES Journal of Marine Science* 54: 726–737.

FURNESS, R.W. & GREENWOOD, J.J.D. (Eds.). 1993. *Birds as Monitors of Environmental Change.* London, UK: Chapman and Hall.

JAGNER, D. 1979. Potentiometric stripping analysis for mercury. *Analytica Chimica Acta* 105: 33–41.

JAGNER, D. & GRANELI, A. 1976. Potentiometric stripping analysis. *Analytica Chimica Acta* 83: 19–26.

JAYA, S. & PRASADA RAO, T. 1982. Potentiometric stripping analysis. *Reviews in Analytical Chemistry* 6: 343–358. doi:10.1515/REVAC.1982.6.4.343

JIMÉNEZ-UZCÁTEGUI, G., BOERSMA, D. & HERNÁN VARGAS, F. 2018. Pingüino de Galapagos. In: FUNDACIÓN CHARLES DARWIN (FCD) & WWF-ECUADOR (WWFE). *Atlas de Galapagos Ecuador: Especies Nativas e Invasoras*. Quito, Ecuador: FCD, WWFE, pp. 118–119.

JIMÉNEZ-UZCÁTEGUI, G. & HUYVAERT, K.P. 2018. Albatros de Galapagos. In: FUNDACIÓN CHARLES DARWIN (FCD) & WWF-ECUADOR (WWFE). *Atlas de Galapagos Ecuador: Especies Nativas e Invasoras.* Quito, Ecuador: FCD, WWFE, pp. 121–122.

JIMÉNEZ-UZCÁTEGUI, G., VINUEZA, R., URBINA, A. ET AL. 2017. Lead and cadmium levels in Galapagos Penguin *Spheniscus mendiculus*, Flightless Cormorant *Phalacrocorax harrisi*, and Waved Albatross *Phoebastria irrorata*. *Marine Ornithology* 45: 159–163.

JIMÉNEZ-UZCÁTEGUI, G., WIEDENFELD, D., VALLE, C.A. ET AL. 2019. Threats and vision for the conservation of Galapagos birds. *The Open Ornithology Journal* 12: 1–15.

LAMBORG, C.H., HAMMERSCHMIDT, C.R., BOWMAN, K.L. ET AL. 2014. A global ocean inventory of anthropogenic mercury based on water column measurements. *Nature* 512: 65–68.

LINN, L.J., DELANEY, M.L. & DRUFFELL, E.R.M. 1990. Trace metals in contemporary and seventeenth-century Galapagos coral: Records of seasonal and annual variations. *Geochimica et Cosmochimica Acta* 54: 387–394.

MATEO, R., DOLZ, J.C., AGUILAR SERRANO, J.M., BELLIURE, J. & GUITART, R. 1997. An epizootic of lead poisoning in Greater Flamingos (*Phoenicopterus ruber roseus*) in Spain. *Journal of Wildlife Diseases* 33: 131–134.

MATEO, R., VALLVERDÚ-COLL, N. & ORTIZ-SANTALIESTRA, M.E. 2013. Intoxicación por munición de plomo en aves silvestres en España y medidas para reducir el riesgo. *Ecosistemas* 22: 61–67.

SECO PON, J.P., BELTRAME, O., MARCOVECCHIO, J., FAVERO, M. & GANDINI, P. 2011. Trace metals (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) in feathers of Black-browed Albatross *Thalassarche melanophrys* attending the Patagonian Shelf. *Marine Environmental Research* 72: 40–45. doi:10.1016/j.marenvres.2011.04.004

SCHEUHAMMER, A.M., BRAUNE. B., CHAN, H.M. ET AL. 2015. Recent progress on our understanding of the biological effects of mercury in fish and wildlife in the Canadian Arctic. *Science of the Total Environment* 509–510: 91–103

SNELL, H.M., STONE, P.A. & SNELL, H.L. 1996. A summary of geographical characteristics of the Galapagos Island. *Journal of Biogeography* 23: 619–624.

SUN, L. & XIE, Z. 2001. Changes in lead concentration in Antarctic penguin droppings during the past 3,000 years. *Environmental Geology* 40: 1205–1208.

US ENVIRONMENTAL PROTECTION AGENCY (USEPA) 2007. *SW-846 Method 7000B: Flame atomic absorption spectrophotometry.* [Accessed online at https://www.epa.gov/hw-sw846/sw-846-test-method-7000b-flame-atomicabsorption-spectrophotometry on 28 Jan 2016].

WOBESER, G.A. 2006. *Essentials of Disease in Wild Animals.* Ames, USA: Blackwell Publishing Professional.