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TITLE: Natural hazards and wildlife health: the effects of a volcanic eruption on the Andean condor

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P.I.P, B.G, W.G, SAL, conceived the idea, design, and experiments. P.I.P, B.G, W.G, L.R.I, H.M.D, D.J.A, H.F, JDLR, SAL, performed the experiments. B.G, W.G, D.J.A, H.F, and SAL collected the samples. P.I.P, and SAL wrote the first draft of the paper, and all authors contributed to the final version of the manuscript.

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No conflict of interest declared.
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ABSTRACT

Volcanic eruptions produce health changes in animals that may be associated with emitted gases and deposited ashes. We evaluated whether the Puyehue–Cordón Caulle volcanic eruption in 2011 produced health changes in the threatened Andean condor (*Vultur gryphus*) living in the area most affected by the eruption, north-western Patagonia. We studied clinical and biochemical parameters of condors examined before and after the eruption. We also examined concentrations of different metals and metalloids in blood of individuals sampled after the eruption. The most common clinical abnormality associated with the eruptive process was irritating pharyngitis. In condors sampled after the eruption, blood concentrations of albumin, calcium, carotenoids and total proteins decreased to levels under the reference values reported for this species. We found different chemical elements in the blood of these condors, such as arsenic and cadmium, with the potential to produce health impacts. Therefore, the health of Andean condors was affected in different ways by the eruption, and thus remaining in the affected area appears to be costly. However, in comparison to other animal species, the health impacts were not as strong and were mainly related to food shortages due to the decrease in availability of livestock carcasses. This suggests that condors dealt relatively well with this massive event. Future research is needed to evaluate if the health changes we found reduce the survival of this species, and if the cost of inhabiting volcanic areas has any ecological or evolutionary influence on the condor’s life history.
KEY WORDS: Ashes, Biochemical parameters, Carotenoids, Pollution, Puyehue volcano, Toxic metals.
1-INTRODUCTION

Natural disasters such as large storms, floods, hurricanes and volcanic eruptions can produce important behavioral and physiological alterations in different species (Wiley and Wunderle 1993, Boyle et al. 2010, Alho and Silva 2012, Ropert-Coudert et al. 2015). In particular, volcanic eruptions impact wild and domestic animals as a result of exposure to gases and ashes emitted during eruption, as well as habitat loss (Robles 2011, Flueck and Smith-Flueck 2013). For instance, the Kasatochi Island eruption in Alaska produced high mortality rates in shore birds and Passeriformes as a consequence of ashes, but also due to the loss of foraging and nesting habitats (Williams et al. 2010). Similarly, volcanic eruptions in Patagonia have caused skin (e.g. dermatitis), digestive (e.g. dental loss, enteritis), reproductive (e.g. abortions) and respiratory alterations (e.g. rhinitis, pneumonia) in both wildlife (e.g. deer) and livestock (Araya et al. 1990, Robles 2011, Flueck and Smith-Flueck 2013). Therefore, the health changes that natural disasters cause in different animal species are a relevant topic of study, particularly in light of possible synergic effects with health impacts caused by anthropogenic activities. This topic is essential when these changes affect species of conservation concern.

The Andes mountains host more volcanoes that have been active in the last 10,000 years than any other volcanic region in the world (Tilling 2009). The Puyehue–Cordón Caulle volcanic complex in Chile erupted in June 2011 and ejected approximately 1500 million m³ of ash, which accumulated mainly in north-western Argentine Patagonia (Neuquén and Rio Negro provinces) (Gaitán et al. 2011). The erupted material was composed mainly of silica oxides but also contained oxides of magnesium, aluminum, potassium, sodium, calcium and sulfurs in lower proportions (Caneiro et al. 2011, Botto et
al. 2013, Buteler et al. 2017). Given the high contents of silica, the ashes were highly irritating to the respiratory tract and potentially harmful to the ocular surface of humans and various terrestrial animals (Caneiro et al. 2011, Robles 2011, Buteler et al. 2017, Tesone et al. 2018). Moreover, the ashes also contained traces of some chemical elements such as arsenic and chromium (Daga et al., 2014), which may be potentially damaging to wild and domestic animals if they are ingested or inhaled in excess.

The distribution of Andean condors (Vultur gryphus) overlaps with the Andean volcanic arc (AVA), an area in the Andean mountains that includes at least 122 volcanoes (Tilling 2009). The largest population known for this species lives in north-western Patagonia (Lambertucci 2010), the area most affected by the Puyehue-Córdon Caulle volcanic eruption. According to a previous study, this condor population remained in the affected area despite the large environmental changes produced by the eruption (e.g. heavy ash fall and increased livestock mortality), possibly to maintain familiar foraging and reproductive areas (Alarcón et al. 2016). Therefore, Andean condors were exposed to ashes during the eruptive process, through inhalation, ingestion, and contact with the ocular mucosa and skin, which may have produced short to long-term health changes that are not well known and with consequences difficult to predict.

We have studied the health status of Andean condors in Patagonia since 2010. Thus, the Puyehue–Cordón Caulle eruption in 2011 offered a unique opportunity to study the health effects of this event on this threatened condor. To do this, we studied clinical and biochemical parameters of condors from the affected area sampled before and after the volcanic eruption. We also studied metal and metalloid blood concentrations in a sub sample of individuals captured after the volcanic eruption. We hypothesized that the
volcanic eruption influenced the health of the Andean condors living in north-western Patagonia. We then predicted that Andean condors would show differences in clinical and biochemical parameters before and after the volcanic eruption, reflecting potential health changes produced by this natural disaster. We also predicted that in Andean condors captured after the eruption, there would be traces of metals and metalloids in their blood associated with the ash composition with the potential to produce harmful effects.

2-METHODS

2.1 Study area

We conducted the study in north-western Argentine Patagonia (Neuquén and Rio Negro provinces; ca. 41°S; 71°W) (Fig. 1). This area is a steppe dominated by grasses and shrubs bordering the Andean forest to the west (Cabrera 1971). This geographical area belongs to the southern volcanic region, which includes around 60 potentially active volcanoes and several minor eruptive centers (Tilling 2009). These volcanoes and eruptive centers have shown variable eruptive activity with low activity over long periods, alternating with high-activity phases and many eruption processes during short time periods (Tilling 2009). The weather of this area is characterized by a mean annual precipitation that declines from approximately 4,000 mm in the western part, to 500 mm in the eastern part and is mainly concentrated in autumn and winter (March to August) (Paruelo et al. 1998). The dominant winds in this area blow from the west (Paruelo et al. 1998).

2.2 Volcanic eruption

The eruption of the Puyehue–Cordón Caulle (2236 m, 40°35′S–72°6′W) began on 4 June 2011 and the eruptive process lasted approximately 8-9 months with a gradual
decrease in intensity. The explosive phase generated a column of ashes that reached
approximately 12-15 km in height and then decreased and stabilized at a height between 2
and 3 km. More than 950 million tons of ashes were emitted into the environment during
the first three months of the eruption (Gaitán et al. 2011). Most of this material drifted
downwind to the east associated with the prevailing winds (Bonadonna et al. 2015, Pistolesi
et al. 2015), and was deposited on 24 million ha of Patagonian land in layers of different
thicknesses and particle size. The first analyses of the ashes through EDS (electron
diffraction spectroscopy) indicated that they were mainly composed of silica (70%) making
it highly irritating to the respiratory and digestive tract and capable of producing different
health problems and mortality in livestock (e.g. ocular, dental and digestive alterations)
(Robles 2011). In addition, while in lower concentrations, the ashes contained traces of
different metals and metalloids (e.g. arsenic, cadmium, cobalt, chromium, magnesium,
manganese, zinc), some of which have the potential to cause health changes in different
species (Botto et al. 2013, Daga et al. 2014).

2.3 Study species

The Andean condor is the largest New World vulture (weight up to 16 kg, wingspan
3 m) (Del Hoyo et al. 1994, Alarcón et al. 2017). It is listed in CITES Appendix I and is
classified as Near Threatened worldwide by the IUCN red list (IUCN 2018), but is
considered Threatened in Argentina (Aves Argentinas, 2017). In the study area, this species
scavenge mainly on livestock (cows and sheep), hares (Lepus europaeus) and red deer
(Cervus elaphus) (Lambertucci et al. 2009, Ballejo et al. 2017), using large areas of steppe
habitats where domestic and wild ungulates concentrate (Lambertucci et al. 2014, 2018,
Pérez-García et al. 2018). In addition, Andean condors exhibit dominance in their access to
carcasses according to sex and age classes, being the adult males the ones at the top of the hierarchy (Donázar et al., 1999). Moreover, the pigmentation present in the bare parts, which could be determined mainly by carotenoids, influences the access to carcasses, the more pigmented individuals being the most dominant (Marinero et al., 2018). The species exhibits a strong sexual dimorphism, with males being up to 30%-50% larger than females (Alarcón et al. 2017). They nest in caves or on vertical walls of the Andes mountains (Lambertucci 2007, Lambertucci and Mastrantuoni 2008). Age classes differ by their different feather colors being brown-grey in immatures and black and white in mature individuals (Del Hoyo et al. 1994, Ferguson-Lees and Christie 2001).

2.4 Andean condors sample collection

We captured 44 Andean condors in the same site of Río Negro province near San Carlos de Bariloche (41° 13´ S -71° 04´ W). Of these, 23 were captured in September-November 2010 (before the volcanic eruption) and the remaining 21 were captured in November-December 2011 (after the volcanic eruption). The distance between the volcano and the capture site was approximately 120 Km, but inside the home range of condors living in that area (Alarcón et al. 2016, Fig. 1). To trap these birds, we used cannon nets baited with sheep and cow carcasses. We performed a complete physical examination to determine the health status of each individual captured. A sample of blood (10 ml) was extracted from the tarsal or brachial vein to evaluate differences in biochemical parameters between individuals captured before and after the volcanic eruption, and in the case of individuals captured after the volcanic eruption to evaluate a set of chemical elements (see next section). Blood samples were transferred to vials containing dry heparin and transported to the laboratory in a portable cooler with ice at approximately 4 °C. On the day
of collection, we separated two aliquots of this blood sample. The first was centrifuged at 1,300 g for 10 min to obtain plasma, which was frozen at -20 °C until analysis. This aliquot was used to study the biochemical parameters. The second (whole blood) was frozen at -20 °C and used to study metals and metalloids. Sampled individuals included immature birds (1-6 year old) and adults (>6 years old) of both sexes. We marked each condor captured with subcutaneous microchips in the right side of the chest (ID100-B, Trovan Electronic Identification Systems).

2.5 Determination of clinical and biochemical parameters

2.5.1 Physical examination

The physical examination of condors included the recording of weight with a portable digital balance (Wei Heng®, Shijiazhuang, China), heart and lung auscultation with a neonatal stethoscope (3M Littmann®, USA), and a comprehensive examination searching for apparent lesions and any signs of disease or poor body condition. In the physical examination of individuals captured after the eruption, we focused on known signs potentially associated with irritating volcanic ashes, such as the presence or absence of ocular (kerato-conjunctivitis or ulcers) and respiratory alterations (nasopharynx irritation, tracheitis or abnormal lung auscultation). Except for weight, we registered all abnormalities detected through physical examination as "present" or "absent" according to the system that was studied (i.e. respiratory, gastrointestinal, etc.).

2.5.2 Biochemical parameters

We studied the following biochemical parameters in individuals captured before and after the volcanic eruption: albumin, bile acid, calcium, calcium/phosphorus ratio,
carotenoid compounds, globulins, magnesium, phosphorus and total proteins. Except for
carotenoids, the rest of the parameters were determined using a Bio Systems A 15 auto-
analyser with Biosystem and Wiener Lab regents. Globulin concentrations were estimated
through the subtraction of the albumins from the total proteins (Cray and Tatum 1998). For
Albumin and globulins estimation in condors captured after the eruption we were able to
obtain based on a sub sample of 13 individuals because the volume of blood extracted
available from those some birds was scarce, so we had to prioritize the measurement of
other parameters studied.

Carotenoids were analyzed as described by Blanco et al. (2013). Briefly, 100 μL of
plasma were lyophilized, and the carotenoid pigments were extracted from the dry residue
with 200 μL of N, N-dimethyl-formamide for 60 min, including sonication for 5 min every
30 min. The resulting extract was subsequently centrifuged at 12,000 g for 5 min and the
upper layer stored at -30 °C until analysis by high performance liquid chromatography
(HPLC). Quantitative analysis of carotenoids by HPLC was carried out according to the
method of Minguez-Mosquera and Hornero-Mendez (1993) with some modifications. The
HPLC system consisted of a Waters e2695 Alliance chromatograph fitted with a Waters
2998 photodiode array detector, and controlled with Empower2 software (Waters
Cromatografía, S.A., Barcelona, Spain). A C18 reversed phase analytical column
(Mediterranea SEA18, 200 mm ×4.6 mm i.d., 3 μm; Teknokroma, Barcelona, Spain) was
used. Separation was achieved by a binary-gradient elution using an initial composition of
75% acetone and 25% deionized water, which was increased linearly to 95% acetone in 10
min, then raised to 100% in 2 min, and maintained constant for 10 min. Initial conditions
were reached in 5 min. An injection volume of 20 μL and a flow rate of 1 mL/min were
used. Detection was performed at 450 nm, and the online spectra were acquired in the 350-700 nm wavelength range. Quantification was carried out using external standard calibration curves prepared with zeaxanthin, lutein, β-cryptoxanthin and β-carotene standards previously isolated (Minguez-Mosquera and Hornero-Mendez 1993). Calibration curves were prepared in the range of 0.5 - 45.0 μg/mL, and constructed by plotting the peak area at 450 nm versus the pigment concentration. The calibration curve of all-trans-lutein was also used to determine the concentration of the cis isomers of lutein. The chromatographic analysis was carried out on the same day as the preparation of the extracts. All operations were carried out under dimmed light to prevent isomerization and photo-degradation of carotenoids. We differentiated two groups of circulating carotenoids: 1) xanthophylls (all-trans-zeaxanthin, all-trans-lutein, cis isomers of lutein and zeaxanthin, all-trans-α-cryptoxanthin, all-trans-β-cryptoxanthin and echinenone), which represent the yellow and red carotenoids used by birds as pigments (Hill and Johnson 2012); and 2) all-trans-β-carotene, which is the main precursor of vitamin A (Hill and Johnson 2012).

2.6 Estimation of blood metals

Given that the eruption was an unexpected and catastrophic event, and that metal concentrations were not part of our aims in previous studies, we do not have information on this for condors captured before the eruption. We measured the following chemical elements in a sub sample of blood taken from condors captured after the volcanic eruption: chromium, manganese, cobalt, nickel, copper, zinc, arsenic, cadmium, lead and iron. To do this, we first weighed 2 grams of the whole blood and then added 1 mL of H₂O₂ and 4 mL of HNO₃. The digestion was completed with a microwave digestion procedure. The
samples were diluted with deionized water (Milli Q water) in a proportion of 1:5 and brought to a total volume of 25 mL, adding 10 ppb of Rh solution as an internal standard.

Trace metal analysis was performed on an Agilent 7700 x ICP-MS system. It was used in a collision cell mode with helium. The synchronization of the ICP-MS was performed with a dilution (1:10) of a solution consisting of 10 ppb of Ce, Co, Li, Mg, Tl, and Y, and we monitored the masses of $^{59}$Co, $^{89}$Y, and $^{205}$Tl obtaining a standard deviation below 5%. The drift and other possible effects derived from the use of the collision cell were corrected with the use of an internal standard (Rh solution), and controlled by the analysis of a monitor standard (the 10 ppb or the 50 ppb, depending on the element and the concentration range) interspersed every 10 samples in the sequence of analysis. The precision of the measurement was calculated from the repetition of 10% of the analyses of the samples, and was around 5-10% for all the elements. The accuracy of the method was determined by the repeated digestion and analysis of "Clin Chek Whole Blood Control" Level I, Level II and Level III and was around 5-10%. The precision of the method was determined by making duplicates of digestions of some of the samples (of 10% of the total samples), and was within the range of 5-10%. The limit of quantification of the method was 0.005 ppm for most elements and in the case of Fe and Zn it was 0.05 ppm.

2.7 Data analysis

To evaluate differences in the physical examination between individuals captured before (2010) and after (2011) the volcanic eruption, we computed the prevalence of the different physical abnormalities according to the time of capture (before and after the eruption). This prevalence was computed as the number of individuals with some physical
... abnormalities over the total of individuals sampled multiplied by 100. To evaluate differences in biochemical parameters and weight before and after the volcanic eruption, we performed a set of linear regression models (Gelman and Hill 2006), using the concentration of each parameter (log-transformed) as a response variable and the individual age, sex and the year (before and after volcanic eruption) as predictors. We used age and sex as predictors because some biochemical parameters could show different values according to these categories (Blanco et al. 2013, Doussang et al. 2018). All statistical analyses were performed with R core team (2015) and we considered p-values < 0.05 as significant.

3-RESULTS

The only physical abnormality found in condors captured after the volcanic eruption compared to condors captured before was irritating pharyngitis. The prevalence of this alteration was 0% in condors captured before, but 100% in condors captured after the eruption (Fig. 2). We found no other physical abnormalities such as kerato-conjunctivitis, eye ulcers or auscultation abnormalities.

The blood concentrations of albumin, calcium, carotenoid compounds (xanthophylls and β-carotene), total proteins and magnesium showed statistical differences between years (before and after the eruption), but not between ages or sexes (Table 1). The only exception was xanthophylls, which showed differences according to age classes with immature condors showing higher concentrations than mature condors (Table 1). After the volcanic eruption, Andean condors presented lower concentrations of albumin, calcium, carotenoid compounds (xanthophylls and β-carotene) and total proteins (Fig. 3, Table 1 and 2). In
addition, Andean condors captured after the volcanic eruption had higher magnesium levels compared to condors captured before (Fig. 3, Table 1 and 2). Weight, bile acid, calcium/phosphorus ratio, globulins, phosphorus and total lipids were similar in individuals captured before and after the volcanic eruption, with some of these parameters showing differences according to age-sex categories (Fig. 4, Table 1 and 2).

Finally, we found metals and metalloids in the blood of condors captured after the volcanic eruption, being some of them potentially detrimental to health (Table 3).

Unfortunately, we do not have metal estimations from condors before the volcanic eruption for comparisons.

4-DISCUSSION

The volcanic eruption produced some health problems in Andean condors. Individuals captured after the volcanic eruption presented abnormalities in the respiratory system (irritating pharyngitis). We also found changes in some biochemical parameters (e.g., albumin, total proteins, calcium and carotenoids) reflecting potential health problems. Finally, we found some metals and metalloids, such as cadmium and arsenic, in the blood of individuals after the volcanic eruption, which could be considered detrimental to their health. However, we were not able to compare these to concentrations before the eruption since that information is lacking and the samples were obtained from a limited number of condors. In summary, the propensity of condors to stay in the area affected by the volcanic eruption (Alarcón et al. 2016) had some costs related to the health problems we found in this population. However, in comparison to other species, such as mammals (Robles 2011, Flueck and Smith-Flueck 2013), reptiles (Boretto et al. 2014), and some insects...
Masciocchi et al. 2013, Buteler et al. 2017), in which the impacts caused by the eruption were severe, the impacts on condors appear to be mild, at least in the short term.

Pharyngitis in birds can be the result of different causes, mainly related to infectious agents such as bacteria, viruses and an array of micro and macro-parasites (Ritchie et al. 1997). However, there is little information about irritating pharyngitis and its potential consequences in birds, especially associated with volcanic ashes. Acute respiratory signs, irritating pharyngitis and laryngeal edema are frequent and common problems in humans associated with volcanic eruptions that can lead to respiratory infections (Merchant et al. 1982, Longo et al. 2010, Monick et al. 2013). In fact, there is a strong association between volcanic ash exposure and respiratory infections in children (Naumova et al. 2007).

Similarly, volcanic ashes produce important alterations in the respiratory mucosa of rodents, which predispose them to infections (Schiff et al. 1981). Therefore, it would be reasonable to think that Andean condors suffering pharyngitis associated with the ashes could have suffered secondary respiratory infections with unknown consequences at the individual and population level. However, we did not find any individual clinically affected (abnormal auscultation) with severe respiratory alterations such as pneumonia, airsacculitis or rhinitis. Therefore, it seems that the ashes produced only mild physical effects on the respiratory system of Andean condors, in contrast to what has been found in livestock species (Robles, 2011).

Individuals captured after the eruption showed lower blood concentrations of albumin, calcium, and total proteins compared to individuals captured before the eruption. In fact, the mean values of albumin, calcium and total proteins in condors after the volcanic eruption were lower than the reference values reported for this species (Gee et al. 1981,
Given that all of these biochemical parameters are strongly related to the nutritional and metabolic state of birds (Ritchie et al. 1997, Samour 2000, Blanco et al. 2013), these changes are probably signaling nutritional problems associated with a food shortage (Ritchie et al. 1997, Blanco et al. 2013). In fact, the eruption of the volcano Puyehue-Cordón Caulle affected food availability for scavengers since it could have acted as a pulse resource, with a massive mortality of livestock in the early stages of the eruption and a lack of food after a few months (Alarcón et al. 2016). Moreover, livestock that survived the eruption was moved to unaffected areas (Robles 2011), in the same way as in other eruptions in Patagonia (Wilson et al. 2012). This produced an abrupt decrease in the resource availability for scavengers. However, it is important to highlight that the birds' weight was similar before and after the eruption. This may be related to the fact that Andean condors were only mild affected by this food shortage, thus maintaining their weight. (Harrison and Lightfoot 2006). Moreover, it is important to note that in the case of albumin, the sample of individuals after the eruption was just 13 of 21, which could bias the mean concentrations we found. In addition, the low levels of calcium should be interpreted with caution because they can be influenced by the lower levels of albumin (Ritchie et al. 1997).

Condors captured after the volcanic eruption showed a large decrease in plasma carotenoid concentrations, both β-carotene and xanthophylls. In birds, carotenoids are only acquired through the diet (Olson and Owens 1998), with vegetal matter possibly acting as an unusual source of these compounds for Andean condors (Olson and Owens 1998, Blanco et al. 2013). Carotenoids are associated with different physiological functions such as vitamin A precursors and immune system enhancers (Faivre et al. 2003, Hill and
Johnson 2012, Simons et al. 2012). In fact, among the different carotenoid compounds, the xanthophylls fraction (oxygenated carotenoids) seem to be the fraction most related to the immune system function in birds and mammals, producing an enhancement of the immune response, innate, cell mediated and humoral immune response, which in turn improves the resistance against infections (Chew 1993; Saino et al. 1999; Kim et al. 2000 a, b; Rajput et al. 2012). Therefore, a decrease in carotenoids (β-carotene and xanthophylls) may lead to reduced immune function, which in turn could increase susceptibility to infection by some opportunistic pathogens (López-Rull et al. 2015). However, we did not find any individuals with serious infections and even the globulin concentrations, acute phase proteins that increase during infections (Cray and Tatum 1998), were similar between individuals before and after the volcanic eruption. Therefore, given that carotenoids are a limited and costly resource only acquired from the diet (Olson and Owens 1998), these results support the idea that Andean condors suffered an important food shortage after the eruption, or a lack of access to the plants they may typically consume.

We found similar concentrations of bile acids, total lipids, phosphorus and Ca/P ratios between condors captured before and after the eruption. In birds, bile acids are an important indicator of liver function (Ritchie et al. 1997, Samour 2000); thus, the fact that their concentrations were similar in individuals before and after the eruption reflect that the liver function would not be seriously affected. However, this should be interpreted with caution because we were not able to obtain other parameters such as hepatic enzymes (Aspartate aminotransferase and Alanine aminotransferase) to address the potential effect on hepatic function more in depth (Ritchie et al. 1997, Samour 2000). In addition, similar concentrations of phosphorus and the Ca/P ratio between individuals suggest a stable
kidney function (Ritchie et al. 1997, Samour 2000). Similarly, the lack of changes in total lipids between individuals discards the possibility of some endocrine and digestive disorders such as pancreatitis (Samour 2000, Harrison and Lightfoot 2006).

Similarly, the lack of changes in total lipids between individuals discards the possibility of some endocrine and digestive disorders such as pancreatitis (Samour 2000, Harrison and Lightfoot 2006).

Individuals captured after the volcanic eruption had higher concentrations of magnesium than individuals captured before the eruption, which is probably associated with the presence of this element in the ashes (Botto et al. 2013). This result is important because high magnesium levels could produce bone mineralization alterations and the death of young birds (Samour, 2008). In this sense, further research is needed to evaluate the potential of volcanic eruptions to influence bone mineralization in this species. Moreover, these high magnesium levels in condors after the eruption could have influenced the low calcium concentrations in blood found in these individuals as was reported in other bird species (Hess and Britton 1997). Some biochemical parameters such as phosphorus, Ca/P ratio, bile acids and xanthophylls differed between age-sex classes. This may obey to the wide array of physiological variations between these categories, which are known to influence biochemical parameters in similar species (Gee et al. 1981, Dujowich et al. 2005, Hernández and Margalida 2010, Blanco et al. 2013, Doussang et al. 2018).

We found different trace metals and metalloids in a sub sample of blood of Andean condors after the volcanic eruption, some of them potentially harmful to health, probably associated with ash composition (Daga et al. 2014). Although threshold levels in different tissues have been established for some metals and some species (Burger and Gochfeld 1997), there is little information available for vulture species, except for lead, which is an important environmental problem mainly associated with hunting activities (Plaza and Lambertucci 2018). In fact, excluding lead and iron, the concentrations we found in
condors were lower than the concentrations reported as non-toxic for the African white
backed vultures (Gyps africanus) in a mildly contaminated area in South Africa (Van Wyk et al. 2001), lower than the concentrations reported for mallards (Anas platyrhynchos) in
Poland (Binkowski and Meissner 2013) and lower than other bird species from
contaminated (Benito et al. 1999) and non-contaminated areas (García-Fernández et al.,
1995; Burger and Gochfeld 1997) (Table S1). However, it is known that even at low
concentrations, heavy metals can induce hidden health alterations in bird species (Blanco et
al., 2004; Espín et al., 2014). Therefore, their presence in the blood of condors captured
after the eruption could have led to hidden health problems, especially over a long term
period as has occurred in other bird species (Baos Sendarrubias 2016). However, we cannot
assume that those contaminants came only from the volcano, since there are no previous
estimations of all of those metals in the blood of this species. In the case of lead, the main
source of this toxic metal in the study area previous to the eruption for condors was is
ammunition (Lambertucci et al. 2011). This source of lead has produced high
concentrations in many individuals in the study area (Lambertucci et al. 2011; Plaza et al.
in press) with consequences at population level which are difficult to predict. Therefore, it
is probably responsible for the lead concentrations over the threshold levels (150 ppb ww)
proposed in Espín et al. (2015) and Plaza and Lambertucci (2018) we found in this study. In
the case of iron, the levels we found were higher than the levels reported in African white
backed vultures, and this is probably associated with the physiological differences between
species (Ritchie et al. 1997).

Volcanic eruptions can be an important source of metals for the environment
(Gauthier and Le Cloarec 1998). Given that north-western Patagonia may be considered a
pristine site, it is reasonable to assume that the concentrations of different metals and metalloids we found in the blood of condors captured after the eruption were probably related to the ash composition (Daga et al. 2014). However, it is difficult to differentiate this source of metals from other sources, such as the pollution generated by human settlements in this area (Guevara et al. 2002, Rizzo et al. 2010), because we were not able to measure these metals and metalloids in the blood of condors captured before the volcanic eruption. Moreover, there is almost no data on this topic for the study area. Therefore, this last result must be interpreted with caution. Further research is needed to evaluate the potential sources of metal and metalloid pollution in this area.

5-CONCLUSIONS

We found that Andean condors living in the area affected by the eruption suffered irritating pharyngitis and changes in some biochemical parameters. In addition, condors showed some chemical elements in blood potentially harmful to their health. In sum, our results suggest that the propensity of Andean condors to stay in the eruption area has some health costs. However, in comparison to other species, the impacts on condors appear to be low, at least in the short term. This suggests that this species dealt fairly well with this massive event, as could be expected for a species that has evolved in a volcanic area (Balmford 1996). Nonetheless, long-term effects of exposure to volcanic ashes, the reduction in food sources, and the increase in some toxic metals deserve special attention in areas with more recurrent volcanic eruptions. Detrimental effects of heavy metals and other non-essential compounds from volcanic eruptions may be exacerbated by anthropic contamination on threatened species, which deserves further research.
6-ACKNOWLEDGMENTS

7-FUNDING STATEMENT:

8-ETHICS STATEMENT:

9-AUTHOR CONTRIBUTION:

10-REFERENCES


from birds feeding in the area around Doñana National Park affected by the toxic spill from the Aznalcóllar mine. Science of the Total Environment 242:309–323.


Figure 1: Map of the southern Andean Mountains in the Northwestern of Patagonia showing (i) the Puyehue-Cordón Caulle Volcano location (black triangle), (ii) the daily ash-plume trajectories three months after the eruption (white lines), (iii) breeding sites of 23 adult Andean condors sampled (red dots), and (iv) GPS locations collected between July and December 2010 and 2011 (dark grey areas) from the same 23 individual condors. Breeding areas and GPS locations obtained from Alarcón et al. (2016). Note that the ash plume mostly crossed the area used by condors for breeding and foraging.

Figure 2: Oropharynx of Andean condors. A) Before the volcanic eruption (2010) B) After volcanic the eruption (2011). Red arrow shows the irritating pharyngitis. In the right corner is shown a silicon crystal present in the ashes (photo under microscope 10X).

Figure 3: Biochemical parameters showing statistically significant differences in Andean condors captured before (2010) and after (2011) the Puyehue–Cordon Caulle eruption.

Figure 4: Biochemical parameters showing non-statistically significant differences in Andean condors captured before (2010) and after (2011) the Puyehue–Cordon Caulle eruption.
Dear Dr. Catherine A Lindell

Editor in chief-The Condor: Ornithological Applications

Thank you for the comments on our article CONDOR-19-102 entitled “Natural hazards and wildlife health: the effects of a volcanic eruption on the Andean condor”. We really appreciate your comments, as well as those from the associate editor. Here, we are re-submitting our article following your suggestions, after revising it in line with the reviewers’ comments. In this point-by-point response letter, we indicate how we have incorporated such comments and provide a marked-up copy with the changes we have made in the MS, as requested (the line numbers correspond to the marked-up copy). Please, do not hesitate to contact us if you require any further information.

Sincerely,

Pablo Plaza and co-authors

EDITOR-IN-CHIEF COMMENTS:

1) Lines 173-175...It’s not clear what this sentence means. Please rewrite.

Response: We rewrote this sentence to explain that the blood volume obtained from some condors after the eruption was scarce and we prioritized the measurement of the other parameters studied. Please see lines 165-169.

2) Line 264...delete “being”.

Response: Done, please see line 257.

3) Grey dots in figure 1 are not really dots (like the red dots, for example). Do you mean the grey areas? So there were many, many GPS locations? Please find another way to explain this than “grey dots”.

Response: We changed the word “dots” by the word “areas” and we changed “grey” by “dark grey”, thanks.

AE COMMENTS:

1) The revised manuscript entitled "Natural hazards and wildlife health: the effects of a volcanic eruption on the Andean condor" has been improved from the original submission. Thank you for your work. There are a few issues in need of further work, however. These issues are as follows.

Response: Thank you for the comment. We really appreciate all the time and work that the editorial team has devoted to our manuscript. We hope you find our review satisfactory.

2) Lines 394-395: I would adjust the tense of the statement such that what is described
was found in the past rather than inferring that something is currently happening. The Lambertucci et al 2011 study identified a condition that occurred during their study.

Response: We changed the tense of this sentence as suggested. Please, see line 376-380.

3) Lines 332-335: The effect of carotenoids on immunity requires further consideration. For example, which arm of immunity is "enhanced" by the carotenoids in question? All arms (innate, adaptive, cell-mediated, humoral, etc) or just a subset of immune traits? And if only a subset of immune traits/functions, which type of infections might be more effectively defended by a bird with carotenoid-enhanced immunity? Staying general as currently is in the manuscript is inappropriate if more detailed information is available in the literature. Too much immunity just like not enough immunity is also a problem (e.g., auto-immunity, allergenicity, destruction of cell membranes by reactive nitrogen species, etc).

Response: We changed this sentence as suggested by the associate editor to clarify that xanthophylls enhance the innate, cell mediated and humoral response in birds and mammals, and that this improves the resistance against infections. Please see lines 319-324. However, there is no information available in wild birds about the relationship between carotenoids and immunity disorders such as auto-immunity, allergenicity, and this is an interesting topic that merits future research.

4) Figure 1: please include country names on the map

Response: We changed the figure 1 as suggested by the associate editor.

5) Table S1: It would be very helpful to provide the current findings alongside values from the other studies. Additionally, the term "reference values" in toxicology refers to values that can be used to interpret toxicant concentrations in terms of potential health effects on the tested individual. The authors use 150 ppb Pb in this manner. It would be extremely helpful to investigate the available toxicological literature for an assemblage of health reference values that can be compared against for your study. You might start with Beyer and Meador 2011 (Environmental Contaminants in Biota: Interpreting Tissue Concentrations, Second Edition 2nd Edition). Government agencies (US EPA, e.g.) also provide these types of reference values for site clean-up activities.

Response: Thanks for this comment and the suggested references. We changed the word “reference values” for “reported values” in Table S1 in order to clarify that the values presented correspond to the values reported in the articles cited, but are not necessarily "reference values". Regrettably, there is little information available about threshold levels in blood for most of the metals studied in birds, especially in birds of prey (see for instance, Samour 2000, Donázar et al. 2002, Bildstein and Bird 2007). The only threshold level widely stablished in bird species is for lead. In fact, most studies on metals in birds blood do not specify threshold levels or reference values and only compare their result with studies in similar species or similar landscape (see for instance, Benito et al. 1999, Van Wyk et al. 2001, Binkowski and Meissner 2013). In our study, we showed that the values we found are below the levels reported for species living in contaminated and uncontaminated areas. However, we highlight that even at
low concentrations the metals we found in blood could be detrimental for condors health according to Espín et al. (2014). Therefore, as the information available is poor and incomplete we prefer to maintain it in the way presented in the Table S1, to avoid misinterpretations, or biased conclusions based on partial data of dissimilar species. However, in the Table S1, we highlighted potential threshold levels accepted for wild birds for lead, cadmium and arsenic that are the best known according to the available literature. We also clarified that for the rest of the metals studied, there is no commonly accepted toxicity threshold levels for birds. In the case of cadmium, we used the threshold levels proposed in Benito et al. (1999) to be conservative because these levels are lower than the levels proposed in Beyer and Meador (2011).

REFERENCES


Dear Dr. Catherine A Lindell

Editor in chief-The Condor: Ornithological Applications

Thank you for the comments on our article CONDOR-19-102 entitled “Natural hazards and wildlife health: the effects of a volcanic eruption on the Andean condor”. We really appreciate your comments, as well as those from the associate editor. Here, we are re-submitting our article following your suggestions, after revising it in line with the reviewers’ comments. In this point-by-point response letter, we indicate how we have incorporated such comments and provide a marked-up copy with the changes we have made in the MS, as requested (the line numbers correspond to the marked-up copy). Please, do not hesitate to contact us if you require any further information.

Sincerely,

Pablo Plaza and co-authors
**Table 1**: Linear models (log normal) evaluating the effect of the year (before and after the eruption), and the age or sex classes on the weight and the different biochemical parameters measured.

<table>
<thead>
<tr>
<th>Clinical and biochemical parameters</th>
<th>Variable</th>
<th>Estimate*</th>
<th>Std error</th>
<th>T value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Intercept</td>
<td>2.365</td>
<td>0.016</td>
<td>144.354</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age [mature-immature]</td>
<td>-0.072</td>
<td>0.019</td>
<td>-3.805</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Sex [female-male]</td>
<td>0.209</td>
<td>0.020</td>
<td>10.495</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Year [2010-2011]</td>
<td>0.011</td>
<td>0.019</td>
<td>0.620</td>
<td>0.538</td>
</tr>
<tr>
<td>Albumin</td>
<td>Intercept</td>
<td>0.234</td>
<td>0.114</td>
<td>2.067</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>Age [mature-immature]</td>
<td>0.008</td>
<td>0.138</td>
<td>0.058</td>
<td>0.954</td>
</tr>
<tr>
<td></td>
<td>Sex [female-male]</td>
<td>0.201</td>
<td>0.141</td>
<td>1.421</td>
<td>0.166</td>
</tr>
<tr>
<td></td>
<td>Year [2010-2011]</td>
<td>-0.412</td>
<td>0.139</td>
<td>-2.959</td>
<td>0.006</td>
</tr>
<tr>
<td>Globulins</td>
<td>Intercept</td>
<td>0.974</td>
<td>0.097</td>
<td>10.049</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age [mature-immature]</td>
<td>0.061</td>
<td>0.118</td>
<td>0.516</td>
<td>0.609</td>
</tr>
<tr>
<td></td>
<td>Sex [female-male]</td>
<td>0.176</td>
<td>0.121</td>
<td>1.454</td>
<td>0.157</td>
</tr>
<tr>
<td></td>
<td>Year [2010-2011]</td>
<td>-0.210</td>
<td>0.119</td>
<td>-1.763</td>
<td>0.088</td>
</tr>
<tr>
<td>Total proteins</td>
<td>Intercept</td>
<td>1.351</td>
<td>0.079</td>
<td>17.023</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age [mature-immature]</td>
<td>0.010</td>
<td>0.092</td>
<td>0.117</td>
<td>0.907</td>
</tr>
<tr>
<td></td>
<td>Sex [female-male]</td>
<td>0.169</td>
<td>0.096</td>
<td>1.745</td>
<td>0.088</td>
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<tr>
<td></td>
<td>Year [2010-2011]</td>
<td>-0.213</td>
<td>0.092</td>
<td>-2.318</td>
<td>0.025</td>
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<td>Calcium</td>
<td>Intercept</td>
<td>2.322</td>
<td>0.044</td>
<td>52.149</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age [mature-immature]</td>
<td>-0.059</td>
<td>0.052</td>
<td>-1.137</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>Sex [female-male]</td>
<td>-0.017</td>
<td>0.054</td>
<td>-0.324</td>
<td>0.747</td>
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<tr>
<td></td>
<td>Year [2010-2011]</td>
<td>-0.182</td>
<td>0.051</td>
<td>-3.530</td>
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<tr>
<td>Phosphorus</td>
<td>Intercept</td>
<td>1.016</td>
<td>0.108</td>
<td>9.351</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Age [mature-immature]</td>
<td>-0.417</td>
<td>0.127</td>
<td>-3.283</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------</td>
<td>------------</td>
<td>------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>Ca/P ratio</td>
<td>1.308</td>
<td>0.104</td>
<td>12.573</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Age [mature-immature]</td>
<td>0.357</td>
<td>0.121</td>
<td>2.942</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Sex [female-male]</td>
<td>-0.096</td>
<td>0.127</td>
<td>-0.761</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td>Year [2010-2011]</td>
<td>-0.212</td>
<td>0.120</td>
<td>-1.760</td>
<td>0.086</td>
<td></td>
</tr>
</tbody>
</table>

| Magnesium       | Intercept | 3.595      | 0.155      | 23.103   | <0.001   |
| Age [mature-immature] | 0.174     | 0.181      | 0.958      | 0.344    |
| Sex [female-male] | 0.139     | 0.190      | 0.735      | 0.467    |
| Year [2010-2011] | 0.823     | 0.180      | 4.556      | <0.001   |

| Total lipid     | Intercept | 1.101      | 0.048      | 22.680   | <0.001   |
| Age [mature-immature] | -0.079    | 0.056      | -1.399     | 0.170    |
| Sex [female-male] | 0.024     | 0.059      | 0.414      | 0.681    |
| Year [2010-2011] | 0.021     | 0.056      | 0.388      | 0.700    |

| Bile acid       | Intercept | 1.890      | 0.346      | 5.454    | <0.001   |
| Age [mature-immature] | 1.358     | 0.411      | 3.301      | 0.002    |
| Sex [female-male] | 0.654     | 0.422      | 1.550      | 0.129    |
| Year [2010-2011] | 0.503     | 0.408      | 1.231      | 0.225    |

| β-Carotene      | Intercept | -0.023     | 0.152      | -0.152   | 0.880    |
| Age [mature-immature] | 0.157     | 0.178      | 0.885      | 0.381    |
| Sex [female-male] | 0.204     | 0.186      | 1.098      | 0.279    |
| Year [2010-2011] | -1.595    | 0.177      | -9.014     | <0.001   |

| Xanthophylls    | Intercept | 1.237      | 0.144      | 8.548    | <0.001   |
| Age [mature-immature] | 0.380     | 0.169      | 2.249      | 0.030    |
| Sex [female-male] | -0.221    | 0.176      | -1.255     | 0.216    |
| Year [2010-2011] | -1.640    | 0.168      | -9.754     | <0.001   |

* The values of estimates correspond to immature male condors and year 2011.
**Table 2:** Mean, SD and range of weight and the different biochemical parameters measured in Andean condors captured before (2010) and after (2011) the volcanic eruption.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>n</th>
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<td><strong>2010</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>11.09</td>
<td>1.29</td>
<td>9.45-14.00</td>
<td>23</td>
<td>11.37</td>
<td>1.63</td>
<td>8.80-15.67</td>
<td>21</td>
</tr>
<tr>
<td>Albumin (g/dL)*</td>
<td>1.49</td>
<td>0.68</td>
<td>0.57-3.02</td>
<td>19</td>
<td>0.93</td>
<td>0.16</td>
<td>0.62-1.10</td>
<td>13</td>
</tr>
<tr>
<td>Globulins (g/dL)</td>
<td>3.08</td>
<td>1.24</td>
<td>1.31-6.15</td>
<td>19</td>
<td>2.43</td>
<td>0.44</td>
<td>1.96-3.35</td>
<td>13</td>
</tr>
<tr>
<td>Total proteins (g/dL)*</td>
<td>4.41</td>
<td>1.82</td>
<td>2.00-9.00</td>
<td>23</td>
<td>3.37</td>
<td>0.44</td>
<td>2.69-4.45</td>
<td>21</td>
</tr>
<tr>
<td>Calcium (mg/dL)*</td>
<td>10.06</td>
<td>1.61</td>
<td>7.30-12.70</td>
<td>23</td>
<td>8.35</td>
<td>1.47</td>
<td>6.10-12.00</td>
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<tr>
<td>Phosphorus (mg/dL)</td>
<td>2.60</td>
<td>1.11</td>
<td>1.2-5.7</td>
<td>23</td>
<td>2.74</td>
<td>1.50</td>
<td>0.80-7.30</td>
<td>21</td>
</tr>
<tr>
<td>Ca/P ratio</td>
<td>4.41</td>
<td>1.61</td>
<td>2.12-7.46</td>
<td>23</td>
<td>3.84</td>
<td>2.06</td>
<td>1.42-10.62</td>
<td>21</td>
</tr>
<tr>
<td>Magnesium (mg/L)*</td>
<td>47.69</td>
<td>21.76</td>
<td>3-90</td>
<td>23</td>
<td>104.0</td>
<td>48.16</td>
<td>48-240</td>
<td>21</td>
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<tr>
<td>Total lipid (g/L)</td>
<td>3.00</td>
<td>0.66</td>
<td>2.14-4.90</td>
<td>23</td>
<td>3.02</td>
<td>0.47</td>
<td>2.07-4.01</td>
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</tr>
<tr>
<td>Bile acid (mg/dL)</td>
<td>45.81</td>
<td>67.49</td>
<td>0.94-226.0</td>
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<td>45.19</td>
<td>47.10</td>
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<tr>
<td><strong>2011</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>11.37</td>
<td>1.63</td>
<td>8.80-15.67</td>
<td>23</td>
<td>11.37</td>
<td>1.63</td>
<td>8.80-15.67</td>
<td>21</td>
</tr>
<tr>
<td>Albumin (g/dL)*</td>
<td>0.93</td>
<td>0.16</td>
<td>0.62-1.10</td>
<td>13</td>
<td>0.93</td>
<td>0.16</td>
<td>0.62-1.10</td>
<td>13</td>
</tr>
<tr>
<td>Globulins (g/dL)</td>
<td>2.43</td>
<td>0.44</td>
<td>1.96-3.35</td>
<td>13</td>
<td>2.43</td>
<td>0.44</td>
<td>1.96-3.35</td>
<td>13</td>
</tr>
<tr>
<td>Total proteins (g/dL)*</td>
<td>3.37</td>
<td>0.44</td>
<td>2.69-4.45</td>
<td>21</td>
<td>3.37</td>
<td>0.44</td>
<td>2.69-4.45</td>
<td>21</td>
</tr>
<tr>
<td>Calcium (mg/dL)*</td>
<td>8.35</td>
<td>1.47</td>
<td>6.10-12.00</td>
<td>21</td>
<td>8.35</td>
<td>1.47</td>
<td>6.10-12.00</td>
<td>21</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>2.74</td>
<td>1.50</td>
<td>0.80-7.30</td>
<td>21</td>
<td>2.74</td>
<td>1.50</td>
<td>0.80-7.30</td>
<td>21</td>
</tr>
<tr>
<td>Ca/P ratio</td>
<td>3.84</td>
<td>2.06</td>
<td>1.42-10.62</td>
<td>21</td>
<td>3.84</td>
<td>2.06</td>
<td>1.42-10.62</td>
<td>21</td>
</tr>
<tr>
<td>Magnesium (mg/L)*</td>
<td>104.0</td>
<td>48.16</td>
<td>48-240</td>
<td>21</td>
<td>104.0</td>
<td>48.16</td>
<td>48-240</td>
<td>21</td>
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<tr>
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<td>0.47</td>
<td>2.07-4.01</td>
<td>21</td>
<td>3.02</td>
<td>0.47</td>
<td>2.07-4.01</td>
<td>21</td>
</tr>
<tr>
<td>Bile acid (mg/dL)</td>
<td>47.10</td>
<td>47.10</td>
<td>3.84-207.69</td>
<td>20</td>
<td>47.10</td>
<td>47.10</td>
<td>3.84-207.69</td>
<td>20</td>
</tr>
<tr>
<td>β-Carotene (µg/mL)*</td>
<td>0.29</td>
<td>0.20</td>
<td>0.04-0.75</td>
<td>21</td>
<td>0.29</td>
<td>0.20</td>
<td>0.04-0.75</td>
<td>21</td>
</tr>
<tr>
<td>Xanthophylls (µg/mL)*</td>
<td>0.94</td>
<td>0.73</td>
<td>0.25-3.03</td>
<td>21</td>
<td>0.94</td>
<td>0.73</td>
<td>0.25-3.03</td>
<td>21</td>
</tr>
</tbody>
</table>

* Variable showing differences statistically significant between years.
Table 3: Concentrations of chemical elements found in the blood of Andean condors captured after the volcanic eruption. Units are expressed in ppb ww (wet weight).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
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<td>BDL*- 6.23</td>
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<td>8.49</td>
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<td>5.05</td>
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<td>Copper</td>
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<td>250.30-596.73</td>
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<tr>
<td>Iron</td>
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<td>Lead</td>
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<td>Nickel</td>
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<td>11.61</td>
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<td>Zinc</td>
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<td>2856.19-5301.49</td>
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*BDL: Below detection levels
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