Cadmium and Lead content in Liver and Kidney tissues of Wild Turkey Vulture *Cathartes aura* (Linneo, 1758) from Chañaral, Atacama desert, Chile

Contenido de Cadmio y Plomo en tejido de Hígado y Riñón en el Jote de Cabeza Colorada *Cathartes aura* (Linneo, 1758) de Chañaral, Desierto de Atacama, Chile

PABLO VALLADARES F.1*, SERGIO ALVARADO2, CAROLINA URRA R.3, JORGE ABARCA1, JORGE INOSTROZA4, JUANA CODOCEO4 & MANUEL RUIZ4

1Departamento de Biología, Facultad de Ciencias, Universidad de Tarapacá, Casilla 7-D, Arica, Chile.
2Programa de Salud Ambiental. Escuela de Salud Pública - Facultad de Medicina, Universidad de Chile. Av. Independencia 1027, Santiago, Chile.
3Departamento de Ecología y Biodiversidad, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. Avenida República 237, Santiago, Chile.
4Departamento de Nutrición, Facultad de Medicina, Universidad de Chile. Av. Independencia 1027, Santiago, Chile.
*Email: pvalladares@uta.cl

ABSTRACT

The Atacama region, Chile, presents one of the highest levels of mining exploitation of the country, which leads to high levels of contamination from mine tailings and other related environmental liabilities. One of the most complex situations occurred in the Chañaral city, north of Chile, where for over 50 years mine tailings were dumped on the coast, causing severe damage in the ecosystem. To evaluate the effects on terrestrial biota, we analyzed the concentration of cadmium and lead in tissues of Turkey Vulture (*Cathartes aura*). The results indicate that accumulation of cadmium in kidney was 10.31 μg/g (SD 8.00, range 0.27 to 20.73 μg/g) while in the liver was 5.24 μg/g (SD 8.00, range 0.49 to 19.70). There values are very high when compared to data for other birds with similar ecological role. In relation to the lead, concentration in liver was 0.86 μg/g (SD 2.03, range 0.15 – 7.90), while in kidney was 1.05 μg/g (DS 2.54, range 0.044 to 9.86), values considered within the normal range. It is interesting to analyze from the perspective of the availability of these metals in the mining tailings, where lead (1.57 to 21.2 μg/g) presented higher levels than cadmium (0.061 to 1.085 μg/g). The difference between organs may be related to the role of metallothionein. We discuss the role of Turkey Vulture as a bioindicator of environmental liabilities.

KEYWORDS: Ecotoxicology, Heavy Metals, *Cathartes aura*.

RESUMEN

La Región de Atacama, Chile, presenta uno de los mayores niveles de explotación minera del país, el cual a su vez genera altos niveles de contaminación por relaves mineros y otros pasivos ambientales relacionados. Una de las más complejas situaciones ocurrió en la ciudad de Chañaral, norte de Chile, donde fueron liberados relaves mineros a sus costas por sobre 50 años, causando graves daños en el ecosistema. Para evaluar los efectos en la biota terrestre, nosotros analizamos la concentración de Cadmio y Plomo en tejidos del Jote de Cabeza Colorada (*Cathartes aura*). Los resultados indican que la acumulación de cadmio en el Riñón fue 10.31 μg/g (DS 8.00, rango 0.27 a 20.73 μg/g) mientras en el Hígado fue 5.24 μg/g (DS 8.00, rango 0.49 a 19.70). Estos valores son muy altos si se compara con datos de otras aves de similar rol ecológico. En relación al Plomo, la concentración en Hígado fue de 0.86 μg/g (DS 2.03, rango 0.15 a 7.90), mientras que en Riñón fue de 1.05 μg/g (DS 2.54, rango 0.044 a 9.86), valores considerados dentro del rango normal. Estos resultados son interesantes de analizar desde la perspectiva de la disponibilidad de estos metales en el relave minero, donde el Plomo (1.57 a 21.2 μg/g) presentó mayores niveles que el Cadmio (0.061 a 1.085 μg/g). La diferencia entre órganos puede ser relacionado al rol de las metalot/inen. Discutimos el rol del Jote de Cabeza Colorada como bioindicador de contaminaciones ambientales.

PALABRAS CLAVES: Ecotoxicología, Metales Pesados, *Cathartes aura*.
INTRODUCTION

The Atacama region, Chile, is characterized by high levels of mining extraction, main income source of the Country. This production releases a number of residues affecting significantly the environmental sustainability, including heavy metals such as copper, zinc, lead, cadmium and mercury, among others. These particulates are critical for public health. In high concentration they can cause nausea, vomiting, encephalopathy, multiple organ dysfunction syndrome, diabetes, various cancer, headaches, ataxia, anemia, nephropathy, nephrotoxicity, hepatotoxicity, osteomalacy, hypertension (Lauwerys 1982, Nriagu & Kabata Pendias 2004, Anning et al. 2013, Amer et al. 2013), however it is important to assess the incorporation of these elements in the primary consumers, and the rest of the trophic networks. For example, high levels of heavy metals and other chemicals have been found in several species, such as waterfowl (Oxyura leucocephala) (Taggart et al. 2009), cormorants and gannets (Martin et al. 2008) and several species of raptors (Mendoza et al. 2006, Pérez-López et al. 2008, Garcia-Fernandez et al. 2008) and vultures (van Wyk et al. 2001, Hermoso de Mendoza et al. 2006, Nam & Lee 2009). These studies are highly efficient for ecosystemic biomonitoring, especially in regions with high mining exploitation.

Furthermore, some plants can incorporate these elements (Kabata-Pendas 2004, Anning et al. 2013, Amer et al. 2013), however it is important to assess the incorporation of these elements in the primary consumers, and the rest of the trophic networks. For example, high levels of heavy metals and other chemicals have been found in several species, such as waterfowl (Oxyura leucocephala) (Taggart et al. 2009), cormorants and gannets (Martin et al. 2008) and several species of raptors (Mendoza et al. 2006, Pérez-López et al. 2008, Garcia-Fernandez et al. 2008) and vultures (van Wyk et al. 2001, Hermoso de Mendoza et al. 2006, Nam & Lee 2009). These studies are highly efficient for ecosystemic biomonitoring, especially in regions with high mining exploitation.

The best bioindicator species are the top of the trophic network, for example the secondary consumers and scavengers (Jager et al. 1996, Hunt et al. 2006, Carbonel-Martin et al. 2007). Vultures, for example, have been repeatedly analyzed as bioindicators of environmental pollution because they are highly sensitive to the bioaccumulation of heavy metals (Mendoza et al. 2006, Pérez-López et al. 2008), and other types of contaminants such as strychnine (Tarruella et al. 2008), organophosphate pesticides, anticoagulants rodenticides of second generation, organochlorate pesticides (Gil & de Frutos 2008, Hernández & Margalida 2008, Sánchez - Barbudo et al. 2008), antibiotics (Blanco & Lemus 2008), diclofenac (Lindsay et al. 2004), anticholinesterase poisoning (Hill & Fleming 1982, Martínez - Haro et al. 2006) and antiparasitic (Lemus & Blanco 2008).

The toxic effects of tailings on marine communities of Chañaral was the total elimination of algae, benthic herbivorous and carnivorous (Castilla & Nealler 1978, Castilla 1983, Paskoff & Petiot 1990, Vermeer & Castilla 1991, Correa et al. 1995, Castilla 1996, Correa et al. 1996, Castilla & Correa 1997, Riquelme et al. 1997, Correa et al. 1999, Correa et al. 2000, Lee et al. 2002, Lee & Correa 2004). For this reason, and taking into account the level of contamination in Chañaral, Chile, for a historical mining exploitation (Ramírez et al. 2005), we hypothesized that the specimens of Cathartes aura (Linneo, 1758) will present high concentrations of cadmium and lead fulfilling the role as bioindicators.

MATERIALS AND METHODS

SPECIMEN COLLECTIONS

We used only dead birds picked up from a landfill in June 2010. The location was checked every day for a week. All specimens exhibited signs of having been hunted by dogs. We collected only intact and recently dead. The landfill is located 15 km northwest of Chañaral, Atacama Region; Chile (26°20'10.35"S, 70°33'57.09"O), at an altitude of 120 m. Specimens were transported to the laboratory where the organs were extracted.

SAMPLE PROCESSING

Samples were sectioned into small chunks, stored in cryotubes and stored at -20°C before being processed. Analysis of cadmium and lead were performed using the same procedure both biological samples and the reference material used (NIST Bovine Liver 1577b). We used an atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 100) with a graphite furnace incorporated (Perkin Elmer HGA 800) and autosampler (Perkin Elmer AS-72), for the quantitative determination of Cd and Pb. The equipment has a graphite tube for atomization and transverse longitudinal effect Zeemann as a background checker. All glass material was washed with nonionic detergent and treated with HNO₃, 30% (Suprapur ®, Merck, Darmstadt, Germany) for 48 hours. The metal used (tweezers, scissors, etc.) is stainless steel and plastic material used (tubes, tips, etc.) free of trace elements and disposable.

The stock standard reference solutions used were Cd (Titrisol 9960, Merck, Darmstadt, Germany) and Pb (Titrisol 9969, Merck, Darmstadt, Germany), 2mg/ml. Working solutions were prepared by dilution in 0.2% nitric acid, from the stock solution and standards intermediates in obtaining the requisite concentrations. We used high purity deionized water obtained with Biosearch NANOpure deionization system, Barnstead / Thermolyne Co. Dubuque, IA, USA.

Each piece of liver and kidney was homogenized to obtain a representative sample. Subsequently these samples
were subjected to acid digestion process in an Anton Paar Multiwave 3000, it was weighed to approximately 0.5 g of tissue in Teflon tubing. Was added 5 ml of concentrated HNO₃ and 2 ml of H₂O₂ (Merck, Darmstadt, Germany) and applied the recommended program for biological samples. Finally, the samples were reconstituted to 10 ml with 0.2% HNO₃ and analyzed. Depending on the level of Cd and Pb, dilutions were applied to obtain reading within the prescribed range in the calibration curves. The calibration curve was prepared using both white and standard solutions (Pb 5 to 10 - 20 and 40 ug / L and Cd 0.5 to 1 - 2 and 4 ug / L). The final concentration was expressed as ug / g tissue and the calculation was applied according to the dilution factor and weight of each aliquot. For comparison of the magnitude of heavy metals in C. aura from Chañaral, we used bibliographic information about other similar species from other latitudes.

**RESULTS**

Fourteen specimens were collected. Morphometric parameters and heavy metal concentrations are shown in Table 1.

In relation to the morphometric measurements, wing length ranged from 460 mm to 500 mm, while the length of the tail was from specimens without tail to 262 mm. The range of the wingspan was between 1478 mm and 1635 mm. The length of the beak was 22 mm to 26 mm, and the post-orbital width was 38 mm to 42 mm. No significant correlations are found between these measurements and heavy metals.

Cadmium concentrations in livers averaged 5.24 μg/g d/w with a minimum of 0.49 and maximum 19.7 μg/g d/w, while in the kidney, concentrations varied from 0.27 to 20.73 μg/g d/w, averaged 10.31 μg/g d/w. Lead concentrations in liver ranged from 0.15 to 7.9 μg/g d/w and averaged 0.86 μg/g d/w. In kidney, ranged to 0.04 to 9.86 μg/g d/w, averaged 1.05 μg/g d/w. There were significant differences among liver and kidney for a cadmium concentrations (Z = -2.229, p = 0.026), where kidney contains higher concentration but not significant differences in lead content (Z = -1.664, p = 0.096).

### Table 1. Concentrations of heavy metals and morphometric traits in Turkey Vulture analyzed. ID: Number of each Individual, CdL: Concentration of Cadmium in Liver, CdK: Concentration of Cadmium in Kidney, PbL: Concentration of Lead in Liver, PbK: Concentration of Lead in Kidney, WL: Wing Length, TL: Tail Length, WP: Wingspan, BL: Beak Length, PW: Postorbital Width and W: Weight. Concentration in ug/g dw, measures in mm, weight in gr.

| ID | CdL | CdK | PbL | PbK | WL  | TL  | WP  | BL  | PW  | W  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| 1  | 19.70 | 2.02 | 0.19 | 0.15 | 490 | 250 | 1540 | 26  | 40  | 1530 |
| 2  | 2.62  | 0.27 | 0.18 | 0.17 | 469 | 255 | 1600 | 26  | 41  | 1720 |
| 3  | 5.28  | 18.84 | 0.15 | 0.31 | 460 | 245 | 1530 | 23  | 40  | 1540 |
| 4  | 2.68  | 16.82 | 7.90 | 9.86 | 482 | 258 | 1612 | 26  | 39.5 | 1670 |
| 5  | 2.49  | 5.87 | 0.22 | 0.34 | 497 | 242 | 1630 | 24  | 39  | 1550 |
| 6  | 6.26  | 20.34 | 0.33 | 0.04 | 492 | 256 | 1575 | 24.6 | 42  | 1530 |
| 7  | 0.49  | 1.19 | 0.35 | 0.53 | 496 | 262 | 1618 | 25  | 39  | 1460 |
| 8  | 4.72  | 20.73 | 0.29 | 0.54 | 491 | 243 | 1620 | 25  | 41  | 1710 |
| 9  | 1.41  | 5.85 | 0.60 | 0.63 | 461 | 253 | 1616 | 23  | 39  | 1470 |
| 10 | 5.38  | 18.73 | 0.41 | 0.51 | 500 | 235 | 1626 | 23  | 39  | 1580 |
| 11 | 0.72  | 3.67 | 0.30 | 0.43 | 479 | 247 | 1575 | 22  | 38  | 1330 |
| 12 | 13.50 | 17.67 | 0.59 | 0.46 | 481 | 249 | 1538 | 25  | 41  | 1540 |
| 13 | 4.28  | 8.63 | 0.29 | 0.42 | 469 | 240 | 1610 | 23  | 39  | 1650 |
| 14 | 3.74  | 3.78 | 0.26 | 0.33 | 483 | 236 | 1604 | 23  | 40  | 1600 |
Finally we performed a Spearman correlation between each heavy metal and body weight of individuals. Correlated variables were Cd - Liver; Cd - Kidney, Pb - Liver and Pb - Kidney versus the weight of each individual of C. aura. There were no significant correlations between the weight of individuals and Cadmium concentrations in both liver ($p = 0.875$) and kidney ($p = 0.352$). In the case of Lead, there was no significant correlation with liver ($p = 0.344$) and kidney ($p = 0.329$).

**DISCUSSION**

This work corresponds to the first study in Chile about heavy metal accumulation in vultures. This study was conducted in one of the places with the highest contamination indices from mining activities, as is the city of Chañaral, Atacama region, Chile.

Cadmium concentration in the unpolluted sediment is 0.17 μg/g dw, while the tailings area is up to 1.085 μg/g. Lead in the unpolluted sediment concentration is 19 μg/g dw, while the tailings is up to 21.2 μg/g dw (Ramirez et al. 2005), but in the tissues of birds accumulation of cadmium is greater than accumulation of lead, although the environmental availability of the latter is higher. One possible explanation is that lead is deposited in the bones rather than soft tissue replacing calcium of bone tissue (Hoogesteijn et al. 2003). On the other hand, lead has a very short half-life in the blood, just a few days, and while in liver and kidney is from weeks to months, but on bone it remains for years, reflecting a lifelong exposure (Pain 1996).

A high exposure to cadmium causes significant accumulation of this metal in the soft tissues, because a small proportion is excreted, primarily remaining retained in the kidney and liver (Webb & Cain 1982, Torra et al. 1994). When cadmium reaches the blood it is retained in the liver, where it stimulates the synthesis of metallothioneins, forming a complex cadmium-metallothionein (Cd-MT), which is directed to the kidney for excretion (Cherian & Goyer 1978, García-Rico et al. 1999). However, the high bioaccumulation of Cd-MT in the kidney causes nephrotoxic effects, affecting the proximal tubules (Torra et al. 1994). Likewise, concentration of 6 μg/g dw in kidney may cause a blockage in the absorption of Zn and Cu, causing various nephropathies (Onosaka et al. 1984, Gallant & Cherian 1987, Ohta et al. 1989, Kaji et al. 1995, López-Alonso et al. 2012).

Lead concentrations found in Turkey Vultures from Chañaral are low compared to those reported in the literature for other vultures (Table 2). In fact, there have been high levels of lead in livers of other species, such Torgos tracheliotos, with 21.54 μg/g dw and Pseudogyps africanus with 14.81 μg/g dw (van Wyk et al. 2001), Aegypius monachus with 10.5 μg/g dw (Nam & Lee 2009), and in the case of diurnal raptors, Falco tinnunculus with 6.64 μg/g dw (Pérez-López et al. 2008) or 42.6 Alectoris chukar with μg/g dw (Bingham et al. 2006) values considered as risk of death according to Franson (1996). In the kidney, reported lead concentrations are also very high, up to 34.1 μg/g dw in Aegypius monachus (Nam & Lee 2009), 17.93 μg/g dw in Torgos tracheliotos (van Wyk et al. 2001) and 16.72 μg/g dw in C. aura from Canada (Martina et al. 2008). In our study, C. aura had 9.86 μg/g dw as a higher concentration and correspond to one male individuals, consistent with abnormal lead exposure approaching those associated with poisoning ($\geq 6.0 \mu g/g dw$) (Franson 1996). With this exceptions, all other individuals presents low levels of lead (0.04 – 0.63 μg/g dw) contrasting with the high levels found in the environment.

In relation to cadmium, particularly in liver, has been reported a maximum concentration of 15.91 μg/g dw for Buteo buteo (Hermoso de Mendoza et al. 2006, Pérez-López et al. 2008), while in nocturnal raptors maximum concentrations are 39.89 μg/g dw in Tyto alba, 29.24 μg/g dw in Strix aluco and 18.28 μg/g dw in Asio otus (Pérez-López et al. 2008). In the case of catartids, it was reported on average 13.93 g/dL in feces and 7.25 g/dL in plasma of Coragyps atratus (Bravo et al. 2005), the remaining records are very low (Table 2). Levels of cadmium in the kidney are higher, where we recorded an average 10.31 μg/g dw, with a maximum value of 20.73 μg/g dw. However, it is not possible to compare because there are few records of cadmium in kidneys for other species of vultures.

The higher levels of cadmium in the kidneys of Turkey vultures is a consequence of higher levels of contamination in Chañaral, and would be interesting evaluate if this species present an efficient detoxification system or have serious renal damage. On other hand, the low levels of lead contras with the high levels found in the environment. This can be explained by assuming that the lead could have been bioaccumulated in bone, where its half-life is higher. Our results support the use of C. aura as a bioindicator of high concentrations of cadmium and lead in environmental conditions.
| Species                  | Country            | n | Tissue | Cd  | Pb  | Reference                      |
|-------------------------|--------------------|---|--------|-----|-----|-------------------------------|
| **raptors**             |                    |   |        |     |     |                               |
| *Circus pygargus*       | Spain (Extremadura)| 8 | Liver  | ND  | 0,11 | 0,15  | 0,33 | 1,69 | 3,11 | 5,06 | Hermoso de Mendoza et al 2006* |
| *Milvus milvus*         | Spain (Extremadura)| 5 | Liver  | ND  | 0,27 | 0,85  | ND   | 2,54 | 5,44 | Hermoso de Mendoza et al 2006* |
| *Buteo buteo*           | Spain (Extremadura)| 3 | Liver  | 0,43 | 0,56 | 0,68  | 3,28 | 5,57 | 7,84 | Hermoso de Mendoza et al 2006* |
| *Accipiter gentiles*    | Spain (Galicia)    | 44| Liver  | ND  | 1,39 | 15,9  | ND   | 4,17 | 18,08 | Hermoso de Mendoza et al 2006* |
| *Falco tinnunculus*     | Spain (Galicia)    | 3 | Liver  | ND  | 1,24 | 3,72  | 1,3  | 6,64 | 11,79 | Hermoso de Mendoza et al 2006* |
| *Accipiter nisus*       | Spain (Galicia)    | 8 | Liver  | ND  | 0,60 | 1,46  | ND   | 3,03 | 8,88 | Hermoso de Mendoza et al 2006* |
| **Nocturnal**           |                    |   |        |     |     |                               |
| *Asio otus*             | Spain (Galicia)    | 4 | Liver  | 0,16 | 4,87 | 18,3  | 1,31 | 4,09 | 7,02 | Pérez-López et al 2008*       |
| *Strix aluco*           | Spain (Galicia)    | 17| Liver  | ND  | 5,52 | 29,2  | ND   | 2,75 | 7,20 | Pérez-López et al 2008*       |
| *Tyto alba*             | Spain (Galicia)    | 16| Liver  | ND  | 3,40 | 39,9  | ND   | 3,12 | 7,20 | Pérez-López et al 2008*       |
| *Athene noctua*         | Spain (Galicia)    | 3 | Liver  | ND  | 3,38 | 9,55  | 3,7  | 4,00 | 4,17 | Pérez-López et al 2008*       |
| **vulturs**             |                    |   |        |     |     |                               |
| *Gyps fulvus*           | Spain (Extremadura)| 5 | Liver  | 0,14 | 0,27 | 0,55  | 2,4  | 3,83 | 5,22 | Hermoso de Mendoza et al 2006* |
| *Aegypius monachus*     | Spain (Extremadura)| 6 | Liver  | 0,22 | 0,54 | 0,99  | 3,22 | 4,44 | 6,62 | Hermoso de Mendoza et al 2006* |
| *Aegypius monachus*     | South Korea        | 20| Liver  | ND  | 0,60 | 4,6   | 1,3  | 10,50| 19,70| Nam & Lee 2009*               |
| *Aegypius monachus*     | South Korea        | 20| Kidney | ND  | 0,40 | 1,1   | 2,2  | 10,60| 34,10| Nam & Lee 2009*               |
| *Pseudogyps africanus*  | South Africa       | 28| Kidney | -   | -    | -     | -    | 12,30| -    | van Wyk et al 2001*           |
| *Pseudogyps africanus*  | South Africa       | 28| Liver  | -   | -    | -     | -    | 14,81| -    | van Wyk et al 2001*           |
| *Gyps coprotheres*      | South Africa       | 1 | Kidney | -   | -    | -     | -    | 7,84 | -    | van Wyk et al 2001*           |
| *Gyps coprotheres*      | South Africa       | 1 | Liver  | -   | -    | -     | -    | 9,86 | -    | van Wyk et al 2001*           |
| *Torgos tracheliotos*   | South Africa       | 1 | Kidney | -   | -    | -     | -    | 17,93| -    | van Wyk et al 2001*           |
| *Torgos tracheliotos*   | South Africa       | 1 | Liver  | -   | -    | -     | -    | 21,54| -    | van Wyk et al 2001*           |
| *Coragyps atratus*      | Venezuela          | 10| Plasmatic | 2  | 7,25 | 12    | 0,3  | 6,85 | 15,00| Bravo et al 2005**           |
| *Coragyps atratus*      | Venezuela          | 5 | Feces  | -   | 13,93| -     | -    | -    | -    | Bravo et al 2005**           |
| *Gyps fulvus*           | Spain (Cazorla)    | 23| Blood  | -   | -    | -     | 17,4 | 43,07| 144,8| García-Fernández et al 2005** |
| *Cathartes aura*        | Canadá             | 6 | Bone   | -   | -    | -     | 3,3  | 7,63 | 11,34| Martina et al 2008*          |
| *Cathartes aura*        | Canadá             | 8 | Liver  | -   | -    | -     | 0,02 | 2,31 | 11,57| Martina et al 2008*          |
| *Cathartes aura*        | Canadá             | 3 | Kidney | -   | -    | -     | 0,56 | 6,79 | 16,72| Martina et al 2008*          |
| *Cathartes aura*        | Atacama, Chile     | 14| Liver  | 0,49 | 5,24 | 19,7  | 0,15 | 0,86 | 7,90 | this study*                   |
| *Cathartes aura*        | Atacama, Chile     | 14| Kidney | 0,27 | 10,31| 20,7  | 0,04 | 1,05 | 9,86 | this study*                   |
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