# 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.<sup>1\*</sup>, Sergio Alvarado<sup>2</sup>, Carolina Urra R.<sup>3</sup>, Jorge Abarca<sup>1</sup>, Jorge Inostroza<sup>4</sup>, Juana Codoceo<sup>4</sup> & Manuel Ruz<sup>4</sup>

<sup>1</sup>Departamento de Biología, Facultad de Ciencias, Universidad de Tarapacá, Casilla 7-D, Arica, Chile.

<sup>2</sup>Programa de Salud Ambiental. Escuela de Salud Pública - Facultad de Medicina, Universidad de Chile. Av. Independencia 1027, Santiago, Chile.

<sup>3</sup>Departamento de Ecología y Biodiversidad, Facultad de Ecología y Recursos Naturales, Universidad Andrés Bello. Avenida República 237, Santiago, Chile.

<sup>4</sup>Departamento 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 metalotioneinas. 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 & Simons 1994, Salgado-Garcia et al. 2006, Vasallo et al. 2011, Kim & Lee 2012, Mendy et al. 2012), carotid atherosclerosis (Lind et al. 2012) and polycystic ovary syndrome (Kurdoglu et al. 2012), kidney failure and heart disease, abnormal metabolism of vitamin C and acute gastrointestinal disorders, affect myometrial activity altering the menstrual cycle, causing dysmenorrhea and even can cause infertility, spontaneous abortions and stillbirths (Tchernitchin et al. 2008).

Furthermore, some plants can incorporate these elements (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.* 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 algaes, 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 HNO<sub>3</sub> 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 Bioresearch 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 HNO<sub>3</sub> and 2 ml of H<sub>2</sub>O<sub>2</sub> (Merck, Darmstadt, Germany) and applied the recommended program for biological samples. Finally, the samples were reconstituted to 10 ml with 0.2% HNO, 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.

# $S_{\rm TATISTICAL\ ANALYSIS}$

Statistical analysis was performed in STATA 11.2. We performed a nonparametric statistical analysis to compare the concentrations of heavy metals between liver and kidneys. We also used the Wilcoxon test for paired comparison to related variables. Significance levels will be p < 0.05. We performed a Spearman correlation to assess concentrations of heavy metals and various morphological measures. Morphological traits measured were Wing Lenght (WL), Wingspan (WP), Tail Length (TL), Beak

Length (BL) and Postorbital Width (PW).

#### 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 this 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.

TABLA 1. Concentraciones de metales pesados y medidas morfométricas en especímenes analizados de Jote de Cabeza Colorada. ID: Número de cada individuo, CdL: Concentración de Cadmio en Hígado, CdK: Concentración de Cadmio en Riñón, PbL: Concentración de Plomo en Hígado, PbK: Concentración de Plomo en Riñón, WL: Largo ala, TL: Largo Cola, WP: Envergadura alar, BL: Largo pico, PW: Ancho Postorbital and W: Peso. Concentraciones en ug/g dw, medidas en mm, peso en 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  $\mu g/g \, dw$ , while the tailings area is up to 1.085  $\mu g/g$ . Lead in the unpolluted sediment concentration is 19  $\mu g/g \, dw$ , while the tailings is up to 21.2  $\mu g/g \, dw$  (Ramírez *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 & Caín 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  $\mu$ g/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  $\mu$ g/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  $\mu g/g dw$ (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  $\mu$ g/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  $(0.04 - 0.63 \mu g/g dw)$  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.

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TABLA 2. Concentraciones de metales pesados en especies de rapaces diurnas, nocturnas y otros buitres. n: número de especímenes analizados, Min: valores mínimos, Av: promedio, Max: valores máximos, Cd: Cadmio, Pb: Plomo. \*Concentraciones en ug/g dw, \*\* en ug/dL, ND: No Detectado.

							M	etal			
						Cd			Pb		
	Species	Country	u	Tissue	Min	Av	Max	Min	Av	Max	Reference
raptors	Circus pygargus	Spain (Extremadura)	8	Liver	ND	0,11	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)	Э	Liver	0,43	0,56	0,68	3,28	5,57	7,84	Hermoso de Mendoza et al 2006*
	Buteo buteo	Spain (Galicia)	44	Liver	ND	1,39	15,9	ND	4,17	18,08	Hermoso de Mendoza et al 2006*
	Accipiter gentiles	Spain (Galicia)	б	Liver	0,14	1,46	4,07	1,16	3,92	6,03	Hermoso de Mendoza et al 2006*
	Falco tinnunculus	Spain (Galicia)	б	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)	б	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)	9	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	ı	ı	ı	·	12,30	ı	van Wyk et al 2001*
	Pseudogyps africanus	South Africa	28	Liver	ı		ı	·	14,81	ı	van Wyk et al 2001*
	Gyps coprotheres	South Africa		Kidney	ı	ı	ı	ı	7,84	ı	van Wyk et al 2001*
	Gyps coprotheres	South Africa	1	Liver	ı	I	ı	ı	9,86	ı	van Wyk et al 2001*
	Torgos tracheliotos	South Africa	-	Kidney	ı	I	ı	ı	17,93	ı	van Wyk et al 2001*
	Torgos tracheliotos	South Africa		Liver	ı	ı	ı	,	21,54	·	van Wyk et al 2001*
	Coragyps atratus	Venezuela	10	Plasmatic	7	7,25	12	0,3	6,85	15,00	Bravo et al 2005**
	Coragyps atratus	Venezuela	5	Feces	ı	13,93	ı	'	·	ı	Bravo et al 2005**
	Gyps fulvus	Spain (Cazorla)	23	Blood	ı		ı	17,4	43,07	144,8	García-Fernández et al 2005**
	Cathartes aura	Canadá	9	Bone	ı	ı	ı	3,3	7,63	11,34	Martina et al 2008*
	Cathartes aura	Canadá	8	Liver	ı		ı	0,02	2,31	11,57	Martina et al 2008*
	Cathartes aura	Canadá	Э	Kidney	ı		ı	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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