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Assessment of environmental contaminants exposure in Argentinean Patagonian scavenger birds using nonlethal samples

Evaluación de la exposición a contaminantes ambientales en aves carroñeras de la Patagonia Argentina mediante el uso de muestras de obtención no cruentas

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Tesis Doctoral Internacional

"Escuela Internacional de Doctorado de la Universidad de Murcia"

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Área de Toxicología

Departamento de Ciencias Sociosanitarias

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Memoria presentada por

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"Escuela Internacional de Doctorado de la Universidad de Murcia"

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A mis padres, Attilio y Gabriella

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Indice

Estructura de la Tesis Doctoral

La presente tesis doctoral sigue una estructura clásica, empezando con una introducción general que trata en manera más profunda las temáticas propuestas en cada capítulo, seguida por material y métodos y la bibliografía de esta primera parte. A continuación, se presentan los objetivos generales de la tesis, así como los objetivos específicos de cada capítulo en español. Siguen los cinco capítulos que forman el cuerpo principal de la tesis, referidos en el texto en números romanos. Cada capítulo es un trabajo científico original. Todos los capítulos están en ingles, y siguen el formato típico de las publicaciones científicas internacionales. Cada capítulo presenta las secciones típicas de una publicación científica (introducción, material y métodos, resultados y discusión, conclusiones y referencias). A continuación, presentamos la discusión general, en español, sobre lo tratado en los capítulos previos, centrando la atención del lector en los puntos más relevantes con su bibliografía. Siguen unas conclusiones generales de la tesis (en ingles) en su conjunto y algunas recomendaciones para el desarrollo de futuras investigaciones sobre estos argumentos. Finalmente presentamos un resumen extendido de la tesis, también en idioma ingles.

Introducción

En 1962, Rachel Carson publica "*Primavera silenciosa*", libro que aborda una forma nueva de entender la toxicología más allá del individuo, extrapolando de los efectos que la contaminación podía tener sobre un individuo (o una especie) a los efectos sobre lo que ella llamó el "*balance natural*". A estas nuevas ideas, en 1969, René Truhaut dio el nombre de *ecotoxicología*, definiendo esta nueva ciencia como "*la rama de la toxicología que se ocupa de los efectos tóxicos, causado por contaminantes naturales o sintéticos, sobre los organismos que constituyen los ecosistemas (ser humano incluido)*" (Truhaut, 1977). Los organismos (enteros o partes de ellos) pueden ser utilizados como bioindicadores o biomonitores, aunque muchas veces las dos palabras son erróneamente consideradas sinónimos. Como indicaba Markert (2007) los bioindicadores proporcionan informaciones sobre la calidad de los ecosistemas; mientras que los biomonitores aportan datos cuantitativos sobre la calidad de los ecosistemas. Otro término que puede inducir a confusión es el de *especie centinela*. García-Fernández y colaboradores (2020) destacan la importancia del estudio de las especies centinela para "*la recopilación de datos para estimar los riesgos para la salud humana, identificar los contaminantes de la cadena alimentaria, determinar los niveles de contaminación ambiental e identificar los efectos adversos en los propios animales*." Pese a esta definición, varios autores han identificado como bioindicadores aquellas especies que proporcionen informaciones pertinentes tanto para la salud humana como para la de los ecosistemas (Burger y Gochfeld, 2004; Di Giulio y Monosson, 1996), para desarrollar planes de evaluación de riesgos ecológicos (Bartell, 2006) o desarrollar reglamentos (Natesan y Slimak, 2006). En el presente trabajo cuando hablamos de "*especie biomonitora*" nos referimos a especies: 1) ubicadas en un nivel trófico elevado, 2) ampliamente distribuidas y numéricamente abundantes en el territorio a monitorizar, 3) sedentarias o con patrones de movimiento bien conocidos, 4) cuyo muestreo resulte factible y de costo aceptable (Hollamby et al., 2006; Holt y Miller, 2011). Un dato que también merece la pena evidenciar es que, en las últimas décadas a nivel global, los estudios de biomonitorización se han centrado principalmente en moluscos y peces (Burger, 2006; Di Marzio et al., 2019). Además de utilizarse especies poco aptas para ser consideradas biomonitoras, este esfuerzo de investigación se centra principalmente en los ecosistemas acuáticos en detrimento de los ecosistemas terrestres (Di Marzio et al., 2019), aunque esté ampliamente demostrada la interconexión de estos dos tipos de ecosistemas (Blázquez et al., 2016; Speir et al., 2014; Xie et al., 2008).

Las aves representan un grupo de animales utilizados para estudios toxicológicos desde los años 30 del siglo pasado, siendo las aves rapaces (incluyendo las aves carroñeras y las rapaces nocturnas) las que presentan todas las características necesarias para ser consideradas buenas especies biomonitoras (García-Fernández, 2014). Además de los puntos enumerados anteriormente, estos animales tienen una longevidad suficiente para que los efectos adversos de la exposición a contaminantes ambientales se manifiesten (Gómez-Ramírez et al., 2014). Debido a la elevada posición que ocupan en la cadena trófica, las rapaces resultan sensibles a números contaminantes ambientales (Di Marzio et al., 2020,2018; Espín et al., 2014a,b; García-Fernández et al., 2008, 2005; Gómez-Ramírez et al., 2012; Martínez-López et al., 2015, 2007; Plaza et al., 2019). Al ser especies cada vez más estudiadas (en 33 de los países de Europa hay actualmente en marcha más de 180 programas de estudios ecotoxicológicos con rapaces) y con una distribución amplia, se han desarrollados protocoles internacionales facilitando la armonización de la metodología de biomonitoreo, la comparación de los resultados y el flujo de información entre países (Espín et al., 2020, 2016). A la hora de desarrollar un estudio de biomonitorización hay que considerar que muchas especies de aves rapaces están protegidas y que en las últimas décadas estamos experimentado a nivel global una preocupante pérdida de biodiversidad (WWF, 2018). Es por estas razones que la elección de la especie y del tipo de muestra necesitará tener en cuenta criterios legales y éticos (Ansara-Ross et al., 2013; Movalli, 2000). Según los datos de Espín y colaboradores (2016), las muestras más empleadas para estudios con rapaces son huevos no eclosionados y plumas, un dato acorde a lo comentado anteriormente. Ambos tipos de muestras permiten llevar a cabo estudios no invasivos y, sobre todo, en el caso de las plumas se trata de un tipo de muestras de rápida y fácil recolección, transporte ligero y resistente, así como de almacenamiento fácil y de bajo costo, a temperatura ambiente (Espín et al., 2016). Además, varios estudios han encontrado correlaciones entre los niveles de contaminantes detectados en plumas y los niveles detectados en sangre y/o tejidos internos (Ansara-Ross et al. 2013; Lodenius y Solonen 2013; Martínez-López et al., 2004,2005; Monteiro y Furness 2001; Solonen y Lodenius 1990) lo que facilita la comparación de resultados entre estudios con distintos tipos de muestras. Lo comentado anteriormente permite entender por qué en los últimos 30 años las plumas han sido ampliamente utilizadas en estudios sobre contaminación, y principalmente por metales (Ansara-Ross et al., 2013; Furness y Greenwood, 1993). Las plumas, como otras estructuras queratinizadas, pueden almacenar contaminantes presentes en el torrente circulatorio durante la fase de crecimiento, actuando como un tejido de depósito y detoxicación (García-Fernández et al., 2013). Este periodo, cuya duración varía en función de la especie, es seguido por una progresiva reducción del suministro de sangre hasta llegar a su interrupción total. En este momento la pluma se convierte en una unidad de almacenamiento estable hasta su muda (Burger, 1993). Diversos factores intraespecíficos (ej. edad, fase reproductiva etc.) e interespecíficos (ej. dieta, hábito migratorio) pueden influenciar en los niveles de contaminantes presentes en las plumas (Dauwe et al., 2005; Zolfaghari et al., 2007). Las plumas pueden proceder de muestreos activos (animales capturados o plumas recogidas de nidos vigilados) o de muestreos pasivos (plumas de cadáveres o plumas mudadas recogidas del suelo). Todos estos factores, muchas veces de difícil control, complican la interpretación de los resultados, a lo que hay que añadir el problema de la contaminación externa de las plumas por contaminación atmosférica y por la liberación de contaminantes a través de las glándulas uropígeas durante el acicalamiento (Jaspers et al., 2011; García-Fernández et al. 2013). El depósito de contaminantes sobre la superficie externa de la pluma, que en algunos casos persisten también después de los procesos de lavado de las plumas (Borghesi et al., 2016), puede dificultar la interpretación de los resultados. Pese a estas desventajas o debilidades, sus fortalezas hacen de las plumas una importante herramienta de estudio para biomonitorización (García-Fernández et al., 2013).

Metales

Metales y metaloides son sustancias ubiquitarias, persistentes en el medio ambiente y con capacidad de bioacumularse (y algunos de biomagnificarse) en los ecosistemas (García-Fernández et al., 2005). Aunque algunos metales puedan estar presentes como oligoelementos esenciales (ej. zinc, selenio, cobre, cromo) en los organismos, favoreciendo los procesos celulares (FAO-WHO, 1996), los organismos que queden expuestos a estas sustancias, en altas dosis y/o por tiempos prolongados, puede experimentar efectos perjudiciales. Dependiendo de la sustancia y de las dosis, los principales efectos adversos para los individuos y las poblaciones serán reproductivos, neurotóxicos, genotóxicos y cancerígenos (Abbasi et al. 2015; Flora et al., 2008; Leonard et al., 2004) con origen, entre otros mecanismos, en procesos de estrés oxidativo celular (Espín et al., 2016, 2014a,b; Flora et al., 2008; García-Fernández et al., 2002). Los metales forman parte de la costra terrestre y por este motivo, entre las principales fuentes de emisión natural encontramos los eventos geotérmicos (ej. erupciones volcánicas) (Nriagu, 1990). Se trata normalmente de una forma puntual de contaminación, que, salvo algunos casos de gran magnitud, como las emisiones de mercurio (Hg) durante las erupciones del volcán Krakatoa de 1883 y del Mt. St. Helens de 1980 (UNEP, 2013), tienen un impacto regional. Por otro lado, la casi totalidad de las actividades industriales, la agricultura intensiva y muchas otras actividades humanas representan algunas de las principales fuentes de emisión de estas sustancias. En la presente tesis el estudio de la contaminación por mercurio (Hg) ha sido abordada en todos los capítulos. En tres capítulos hemos tratado también la contaminación por plomo (Pb). Para el tercer capítulo hemos investigado también la contaminación por silicio (Si), cromo (Cr), cobre (Cu), cinc (Zn), arsénico (As), selenio (Se) y cadmio (Cd) posiblemente generada por la erupción volcánica del Complejo Volcánico Puyehue-Cordón Caulle (CVPCC), (Chile). As, Cd, Cr, Cu, Pb, Hg, Se y Zn están incluidos por USEPA (2015) en la lista de los 129 contaminantes ambientales más peligrosos.

Mercurio (Hg)

El mercurio es un metal pesado ubiquitario, en sus formas orgánica e inorgánica, en los ecosistemas con tendencia a bioacumularse y biomagnificarse en la cadena trófica (Eisler, 1987). La contaminación ambiental por mercurio procede de fuentes naturales y antrópicas. Entre las naturales destacan las erupciones volcánicas y las reliberaciones de mercurio mediante incendios o por efecto aerosol en cuerpos de agua. La movilización del mercurio está muy influenciada por factores climáticos como temperatura, precipitaciones, etc. (Nriagu y Becker, 2003; Pirrone et al., 2003). Entre las causas antrópicas encontramos el uso de combustibles fósiles, la minería artesanal (principalmente de oro), la industria siderúrgica y la quema de residuos (Driscoll et al., 2007; Pacyna et al., 2006). A través de procesos de metilación el mercurio inorgánico es biotransformado en mercurio orgánico. Las formas orgánicas de mercurio, principalmente metilmercurio, son más toxicas de las inorgánicas (Morel et al., 1998). Las bacterias reductoras de sulfatos presentes en los ecosistemas acuáticos son responsables del 95% de los procesos de metilación del mercurio (Broo y Odsjo, 1981; Burger y Gochfeld, 1997; Eisler, 1987; PNUMA, 2002; Rigét et al., 2007). La contaminación por mercurio ha sido investigada principalmente en ecosistemas acuáticos (Di Marzio et al., 2019); aunque varios estudios hayan demostrado que los ambientes acuáticos y los terrestres están muy relacionados y la contaminación por mercurio afecta a ambos (Speir et al., 2014; Xie et al., 2008). La forma en la que se encuentran los compuestos de mercurio influencia la absorción (Gad, 2005). La forma elemental, volátil a temperatura ambiente, es absorbida hasta un 75% por vía inhalatoria, mientras que la absorción del metilmercurio es completa por vía oral (Goyer, 1996). Estas diferencias hacen que también en el organismo las diferentes formas de mercurio tengan afinidad por diferentes órganos dianas. El metilmercurio, por su carácter liposoluble, presenta afinidad por los tejidos adiposos y el encéfalo (Gad, 2005). En las aves, el metilmercurio es excretado entre un 70-90% a través de las plumas y el mercurio contenido en las plumas es en un 90% metilmercurio (Burger, 1993; Rimmer et al., 2005; Renedo et al., 2017). La intoxicación por mercurio puede provocar trastornos reproductivos, neurológicos, hematológicos y celulares (Espín et al., 2014a,b; Nichols et al., 1999; Wolfe et al., 1998; Solonen y Lodenius 1984). Varios estudios indican que la Hg tiene efectos inmunotóxicos (estimulación dependiente de la dosis/supresión de la respuesta de los linfocitos) (Ortega et al., 1997; Fallacara et al., 2011). El mercurio es excretado del organismo con las deyecciones, y puede ser secuestrado en las plumas durante el crecimiento, ligándose a la creatina (García-Fernández et al., 2013).

Plomo (Pb)

El plomo es un metal natural de color gris azulado presente en pequeñas cantidades en la corteza terrestre (Tchounwou et al., 2012). Conocido y empleado desde la antigüedad, su en el Antiguo Egipto el plomo venia utilizado a veces para cometer homicidios (De Michele 1984). En la Antigua Roma era muy apreciado y conocido, tanto que se estima que el gran uso de plomo en este periodo hay incrementado notablemente su nivel basal a nivel ambiental (Eisenreich et al. 1986). Aunque puede aparecer de forma natural en el medio ambiente, la contaminación por plomo deriva de causas antrópicas. La minería, la producción de baterías, la producción de objetos metálicos y la producción de municiones representan algunas de las principales fuentes de emisión (Eisler, 1998a; Tchounwou et al., 2012). Los compuestos organoplomados son más tóxicos que los compuestos inorgánicos de Pb y en ambiente acuático el plomo resulta más toxico. El plomo bioacumula en la cadena trófica (Eisler, 1998a). En la fauna silvestre, y especialmente en las aves, las municiones plomadas representan una importante fuente de contaminación (García-Fernández et al., 2005; Plaza y Lambertucci, 2019). Dada su peligrosidad en ecosistemas acuáticos y terrestres recientemente las municiones plomadas han sido prohibida en la Comunidad Europea (Pain et al., 2019). En las aves, la principal fuente de entrada de plomo es representada por la vía oral, aunque también los perdigones subcutáneos/intramusculares de disparos no mortales pueden causar intoxicaciones (Eisler, 1998a; Fisher et al., 2006). Una vez absorbido, una parte del plomo es rápidamente depositadas en los huesos, los tejidos blandos y las plumas en crecimiento siendo el hueso el órgano de elección (Franson y Pain, 2011). La intoxicación crónica por plomo genera un cuadro de postración del animal, con síntomas gastroentéricos (patognómica la diarrea verde), síntomas neurológicos. La intoxicación aguda es un evento más raro (Eisler, 1998a).

Arsénico (As)

El arsénico es un elemento ubiquitario en los ecosistemas, tanto en sus formas orgánicas como inorgánicas (FAO-WHO, 1996). Después de la forma gaseosa (arsina), las formas inorgánicas resultan las más toxicas, y entre ellas la de arsénico trivalente es entre 2-10 veces más toxica que la pentavalente (Goyer, 2001) Las principales fuentes de emisión antrópicas del arsénico son la industria agroquímica, la industria metalúrgica y la industria farmacéutica, mientras que a nivel natural las principales emisiones proceden de las erupciones volcánicas y los fenómenos de erosión del suelo (ATSDR, 2000; Eisler,1988b). En las aves, las formas inorgánicas son absorbidas por vía oral o inhalatoria; las formas orgánicas, aunque menos estudiadas, parecen presentar una buena absorción por ambas vías (ATSDR, 2007). Los mecanismos de detoxicación se dan principalmente en el hígado y los riñones; y muchos organismos vivos convierten, por metilación, las formas inorgánicas en compuestos orgánicos para excretarlos de esta forma (FAO-WHO, 1996; ATSDR, 2007). Los efectos adversos generados por arsénico son de tipo cancerígeno y genotóxico, debido principalmente a fenómenos de estrés oxidativo (Flora et al., 2008; Koivula y Eeva, 2010; Sánchez-Virosta et al., 2015). El arsénico se excreta rápidamente con las deyecciones (ATSDR, 2007). En las aves, el arsénico puede ser eliminado a través de las plumas durante la fase de crecimiento (Geens et al., 2010; Janssens et al., 2001), puede ser transferido en los huevos durante la temporada de cría (Ruuskanen et al., 2014; Tsipoura et al., 2008) o depositado en la glándula uropigial y en la glándula salina (Burger y Gochfeld, 1985).

Cadmio (Cd)

El cadmio es un elemento muy presente en la costra terrestre, considerado a la par de mercurio y plomo uno de los metales más peligros desde el punto de vista ambiental y toxicológico (García-Fernández et al., 1996). Sus principales fuentes de emisión son de origen antrópico (minería, industria metalúrgica, fabricación de baterías, fertilizantes y estabilizadores plásticos) (Pacyna et al. 1991). Las fuentes de emisión de origen natural incluyen las erupciones volcánicas y los incendios (Burger, 2008). El cadmio sufre fenómenos de bioacumulación y biomagnificación (Burger, 2008); como vimos anteriormente las concentraciones detectadas en diferentes tejidos están influenciadas por factores intra e inter específicos (García-Fernández et al., 1996). En las aves el cadmio es absorbido por vía oral e inhalatoria (Cigankova et al., 2010) y su eliminación, además que, con las deyecciones, se produce con la inclusión del cadmio en las plumas, en las cascaras de los huevos o a través de las glándulas de sal (Burger, 2008, Brasfield et al., 2004). Las intoxicaciones por cadmio pueden ser de tipo agudo (más raras) o crónicas. Los efectos adversos subletales incluyen déficit de crecimiento, anemia y daños a los aparatos reproductores (Eisler, 1985). Martínez-López y colaboradores (2005) han demostrado que las plumas pueden representar una muestra válida para los análisis cadmio.

Cromo (Cr)

El cromo es un metal ubiquitario, presente en la costra terrestre, con estado de oxidación que van desde Cr II hasta Cr VI (Tchounwou et al., 2012). El cromo es un microelemento esencial, con acción potenciadora de la insulina, influenciando los metabolismos de carbohidratos, lípidos y proteínas (FAO-WHO, 1996). Los compuestos de cromo son ampliamente utilizados en la industria (metalúrgica, de curtido de piel, producción de pinturas); por lo que las fuentes antrópicas representan su principal fuente de emisión al medio (Tchounwou et al., 2012). El cromo hexavalente resulta la forma más toxica y peligrosa para el ser humano y los animales (Gomez y Callao, 2006). Las principales vías de absorción del cromo son la inhalatoria y la oral (Tchounwou et al., 2012). El cromo hexavalente puede penetrar las membranas celulares y su toxicidad se debe a los efectos inducidos por procesos de estrés oxidativo. Como otros metales, también el cromo puede ser secuestrado por las plumas durante el crecimiento (Dmowski, 1999).

Cobre (Cu)

El cobre es un oligoelemento esencial, ampliamente distribuido en los tejidos de los seres vivos (FAO-WHO, 1996). Se trata de un metal conocido y muy utilizado por el hombre desde la prehistoria. Las principales fuentes antrópicas son las industrias manufacturera, eléctrica, electrónica y química (Sernageomin, 2015). La ingestión crónica de cobre puede inducir efectos tóxicos a nivel hepático en aves (Chiou et al. 1999; Jackson et al. 1979; Jackson y Stevenson 1981). Como otros metales, puede ser secuestrado en las estructuras queratinizadas de las plumas durante el crecimiento (Dmowski, 1999).

Zinc (Zn)

El zinc es oligoelemento esencial para todos los seres vivos, asegurando estabilidad a moléculas como el ADN y a estructuras biológicas como membranas y ribosomas (Eisler, 1993). El zinc es utilizado para aleaciones de metales (Sernageomin, 2015) y la mayor fuente de intoxicación para las aves es la ingestión de elementos cincados, de pintura a base de zinc, de fertilizantes y hasta de monedas (Eisler, 1993). Los niveles más altos de zinc en las aves se registran en hígado y riñones. El efecto toxico del zinc se debe a fenómenos disruptivos en los múltiples procesos fisiológicos en que está involucrado (Eisler, 1993). Las plumas en crecimiento necesitan zinc (Eisler, 1993).

Selenio (Se)

El selenio es un oligoelemento esencial, presente en la naturaleza en asociación con depósitos de carbón, de fosfatos y de otros minerales. Las actividades humanas como la minería, el regadío (y otras actividades que producen movilización de estos depósitos), así como la industria metalúrgica causan un incremento de los niveles de este elemento en el ambiente (Debruyn y Chapman, 2007), pudiendo sufrir fenómenos de bioacumulación principalmente en ambiente acuático (Spallholz y Hoffman, 2002). En ecosistemas acuáticos el selenio presenta cuatro estados oxidativos estables y varias formas orgánicas e inorgánicas (Mason et al., 2000; Fan et al., 2002). Esta variedad hace difícil identificar el mecanismo exacto de toxicidad del selenio, aunque se puede afirmar que está relacionado con la producción de radicales libres y el estrés oxidativo

(Spallholz y Hoffman, 2002). El selenio, absorbido por vía oral (Debruyn y Chapman, 2007) puede ser excretado a través de las plumas durante la fase de crecimiento o de los huevos durante la temporada de cría (Ohlendorf y Heinz, 2011).

Silicio (Si)

El silicio es un elemento presente en los suelos, muy importante por el crecimiento de las plantas (Jones y Handreck, 1964). El silicio representa uno de los elementos más importantes de algunos tipos de magma volcánico, que junto con la minería representan las principales fuentes de emisión del silicio en el aire (Ehrlich et al., 2006; Lara et al., 2006). Las principales vías de absorción son la oral (para los herbívoros) y la vía respiratoria (Jones y Handreck, 1964). La forma oral es absorbida a nivel intestinal y puede ser excretada con las heces y la orina. En este segundo caso pueden generarse cálculos en las vías urinarias. Por vía respiratoria el silicio causa problemas en las vías aéreas (Plaza et al., 2019). Hasta la fecha se han realizado pocos estudios sobre silicio en plumas de aves.

Área de estudio

Los trabajos de campo realizados para obtener las muestras analizadas en la presente tesis han sido llevados a cabo en el norte de la Patagonia Argentina. Se trata de una extensa área ubicada en la parte centro meridional del Cono Sur de América, dividida geográficamente de la Patagonia Chilena por la cadena de los Andes (Falkner, 2013). Nuestra área de estudio se ubica entre las ciudades de San Carlos de Bariloche (o Bariloche), en la provincia de Rio Negro y la ciudad de Neuquén en la provincia de Neuquén. Dentro de esta área, elegimos 3 zonas de muestreo: “Bariloche”, “El Chocón” y “El Valle”. "Bariloche" incluye los alrededores de los centros de Bariloche, Dina Huapi y Villa La Angostura, situadas entre las provincias de Neuquén y Río Negro, una zona rural con baja densidad humana (Rizzo et al. 2011). La elevación media del área es de 893 metros sobre el nivel medio del mar (m s.n.m.); el clima es continental húmedo (clasificación climática de Köppen), con una temperatura media anual de 8,1 °C y una precipitación total de 782,6 mm. Los ecotonos representados son el bosque subantártico y la estepa (Bustos y Rocchi 2008). El área incluye el Parque Nacional Nahuel Huapi con su lago principal Nahuel Huapi y varios otros lagos. Las principales actividades económicas de la zona son el turismo de naturaleza, la ganadería extensiva y las piscifactorías de truchas, ubicada en varios puntos del río Limay, emisario del lago Nahuel Huapi. En la zona se registra una intensa actividad volcánica asociada a la extensa red de volcanes activos presentes en la Cordillera de los Andes tanto en el lado argentino como principalmente en el chileno. Siguiendo la cuenca hidrográfica del rio Limay (que nos llevará hasta la última área de estudio) nos encontramos con "El Chocón". Esta área de estudio incluye los alrededores de la Villa El Chocón, situada aguas abajo de Bariloche, a 80 km de Neuquén. El poblado surgió alrededor de la construcción de la represa hidroeléctrica sobre el rio Limay, que constituye una de las principales actividades económicas del área. Nos encontramos en el ecotono de la estepa, una región árida y templada con una precipitación media anual de 180 ±36 mm. La temperatura media anual es de 15 °C, con unas minimas media de 3 °C en invierno y una máxima media de 30 °C en verano (Tadey, 2006). Las principales actividades económicas, además de las relacionadas a la represa hidroeléctrica, son la ganadería extensiva, la extracción de petróleo. La tercera área de estudio "El Valle" comprende la confluencia del río Neuquén y el río Limay, entre las localidades de 5 Saltos y la ciudad de General Fernández Oro; una superficie de 100.000 ha (más de 40.000 ha se destinan a la agricultura) (Pozo 2013; Romero Gámez 2013). La elevación media de la zona es de 270 m s.n.m.; el clima es desértico seco y frío (clasificación climática de Köppen), con una temperatura media anual de 14,5 °C y una precipitación anual de 186,9 mm (Bustos y Rocchi 2008). El ecotono representado es la estepa. Es una zona muy urbanizada, Neuquén con sus 232000 habitantes es la ciudad más poblada de esta parte de la Patagonia, donde las principales actividades económicas están representadas por la agricultura (producción de frutas y verduras), la industria y la extracción de petróleo (observaciones personales).

Aves carroñeras de la Patagonia Argentina

Como comentado anteriormente, las aves rapaces y carroñeras son consideradas buenos biomonitores. Para este trabajo de investigación hemos utilizados muestras de: Cóndor andino (*Vultur gryphus*), Jote colorado (*Cathartes aura*) y Jote negro (*Coragys atratus*), de la familia Cathartidae, Carancho (*Caracara plancus*) y Chimango (*Milvago chimango*) de la familia Falconidae. Los Cathartidae, también conocidos como "*Buitres del Nuevo Mundo*", son una familia de aves no completamente estudiada. La estrategia de muda del Condor andino es uno de los aspectos no investigados. Los únicos datos proceden de un estudio en Cóndor de California (*Gymnogyps californianus*) y los resultados, parciales, parecerían indicar que esta especie realizaría una muda altamente estacional, variable y asimétrica de las plumas primarias 2 años (Snyder et al. 1987). En los últimos años varios estudios (Zhang 2015; Hackett et al. 2008) parecen sugerir que la familia Cathartidae está relacionada con la familia Accipitridae, que incluye los "*Buitres del Viejo Mundo*". Gracias a este dato podemos indirectamente estimar la duración de la muda de las plumas primarias del Condor andino, que debería ser 5-7 años, como en los buitres del género *Gyps* (Zuberogoitia et al. 2013). Tampoco existen estudios sobre la estrategia de muda del jote negro. En este caso, considerando que la muda del jote colorado es anual (Chandler et al., 2010), similar a la que se ha registrado en otros Accipitridae de tamaño similar, podemos considerar que también el jote negro tenga muda anual. Estos datos sobre las estrategias de muda serán fundamentales para entender como interpretamos los resultados de nuestros análisis.

El cóndor andino, la mayor ave carroñera del mundo (11-15 kg de peso), habita en todo el continente sudamericano a lo largo de los Andes, de hábitos sedentarios (Del Hoyo et al. 1994). Como todos los Cathartidae se trata de una especie carroñera estricta; su dieta consiste prevalentemente en carroñas de vertebrados de tamaño mediano a grande (Lambertucci et al. 2009a). Es una especie fuertemente jerárquica y los machos adultos tienen un acceso prioritario a fuentes de comida (Donázar et al., 1999). A nivel global la especie está clasificada como Casi Amenazada (*Near Threatened)* (NT), debido a los efectos negativos de varias perturbaciones humanas (Alarcón y Lambertucci 2018; IUCN 2017; Lambertucci et al. 2009b, 2011). Regionalmente, la población norteña (Perú, Ecuador) se considera en peligro crítico. En Chile la especie está clasificado como Vulnerable (Inventario Nacional de Especies de Chile 2019) y en Argentina como amenazada (MAyDS y AA 2017). Localmente, la situación es diferente; la población de Cóndor andino en nuestra área de estudio se estima en al menos 300 especímenes (Lambertucci, 2010).

El jote negro (2 kg, p.c.) es una especie de ave carroñera sedentaria, presente en toda Sudamérica y el sur de los Estados Unidos (Del Hoyo et al. 1994); su predilección por los asentamientos humanos ha permitido su expansión en los últimos años (Barbar et al., 2015). La dieta del buitre negro consiste en cadáveres de mamíferos e insectos (Del Hoyo et al., 1994; Ballejo et al., 2018).

El jote colorado (1,4 kg, p. c.) es una especie de ave carroñera migratoria, ampliamente distribuida en el continente americano, abarcando un territorio que va desde el sur del Canadá hasta Tierra del Fuego (Del Hoyo et al., 1994). De los Cathartidae estudiados es la especie con la dieta más variada, que incluye también restos de reptiles, peces y aves (Ballejo et al., 2018). En jote negro y de forma menor en jote colorado, se ha demostrado la existencia de una jerarquía basada en la edad, con los ejemplares adultos que tienen acceso prioritario a la comida (Wallace and Temple, 1987).

El carancho (2-3 kg, p. c.) es un carroñero oportunista de la familia Falconidae (Del Hoyo et al., 1994), presente en América del Sur (excepto en la Amazonia, Colombia, Perú y los altos Andes), varias islas del Caribe y México, y rara vez se encuentra en el sur de los Estados Unidos. (Del Hoyo et al., 1994). La dieta del carancho consiste principalmente en carroñas, juveniles, aves heridas, aves de movimiento lento, pequeños roedores, reptiles, anfibios, peces y artrópodos (Ferguson-Lees y Christie 2001).

El chimango (0,3-0,4 kg, p. c.) es un carroñero oportunista, de la familia Falconidae, considerado la rapaz más abundante en América del Sur, al sur del bosque amazónico (excluyendo los altos picos andinos) (Del Hoyo et al, 1994). La dieta de la especie incluye insectos, pequeños vertebrados, carroñas y peces (Biondi et al., 2005).

Material y método

En la presente tesis se utilizaron plumas primarias recién mudadas (P1-P10) recogidas en dormideros de las cinco especies estudiadas. El muestreo se llevó a cabo durante la primavera austral (octubre-diciembre) de 2007, 2009, 2011, 2013 y 2017 (Tabla 1). Debido al tipo de muestra y a la metodología de muestreo, se desconoce la edad o el sexo de los individuos. Para reducir la posibilidad de una seudoreplicación, sólo se recogió una pluma por punto de muestreo (o más de una si se trataba de la misma pluma primaria de la misma ala).

	2007	*2009*	*2011*	*2013*	*2017*
Condor andino	**8**	**18**	**-**	**-**	**22**
Jote negro	**-**	**-**	**25**	**9**	**111**
Jote colorado	**-**	**-**	**44**	**13**	**85**
Carancho	**-**	**-**	**21**	**-**	**39**
Chimango	**-**	**-**	**25**		**21**

Tabla 1. Muestras utilizadas en la presente tesis, divididos por especie y año

Cada muestra se mantuvo en una bolsa de papel individual, etiquetada (área de estudio, día y especie) y almacenada a temperatura ambiente en un lugar seco hasta su análisis. Se recogieron un total de 441 plumas primarias.

Como primer paso, antes del análisis, las plumas fueron sometidas a una serie secuencial de baños con agua del grifo, agua destilada y agua Milli-Q® (ISO 3696) para eliminar posibles contaminantes depositados sobre la superficie de las plumas. Las plumas se secaron a temperatura ambiente durante 12 h (Espín et al. 2012). Una vez limpias, las plumas fueron picadas, con la finalidad de obtener muestras uniformes de plumas que incluyeran barbas, raquis y cálamo y fueron almacenadas en recipientes estériles.

Para el análisis del Hg total, utilizamos 0,05 g peso seco (p.s.) de cada pluma. Las muestras fueron posicionadas en navecillas de níquel y analizadas mediante el Milestone DMA-8 Direct Hg Analyze por espectrofotometría de absorción atómica, con un límite de detección de 0,0001 ppm, siguiendo el Método 7473 de la USEPA (sedimentos, suelos y lodos). La aplicabilidad de este método al análisis de muestras bióticas ha sido demostrada anteriormente (Haynes et al. 2006). La curva de calibración se calculó con 11 puntos (por duplicado) de 0 a 1004 ng de Hg. La precisión y exactitud del método se comprobaron utilizando material de referencia certificado (CRM) (n = 11; estándar de Hg para AAS, Fluka, 1000 mg/L Hg en ácido nítrico al 12%). La recuperación del Hg total de siete réplicas del CRM diluido a 1 ppm fue de 98,14 ± 3,52% (media ± desviación estándar). El coeficiente de variación para la repetibilidad fue de 3,58%.

Para el análisis de Si, Cr, Cu, Zn, As, Se, Cd y Pb previsto en el objetivo 3, que veremos a continuación, las muestras fueron colocadas en tubos de ensayo de PEBD (propileno de baja densidad) con la adición de una mezcla de ácidos (nítrico/perclórico/sulfúrico, 8:4:1) para la desintegración de la materia orgánica (1 ml de mezcla de ácidos/100 mg de cada pluma).

Transferimos 1 ml de cada extracto predigerido a un tubo de cuarzo, para secar completamente la muestra con un tratamiento térmico progresivo. Cuando los tubos se enfriaron, añadimos agua purificada, transfiriéndola toda al recipiente de medición, completando el volumen final de 10 ml con un 1% de ácido nítrico. Después de la digestión, la detección y cuantificación se realizaron mediante espectrometría de emisión óptica de plasma de acoplamiento inductivo (Agilent Technologies ICP-MS. Modelo 7900). El Sistema Integrado de Introducción de Muestras (ISIS) se configuró para un muestreo discreto. El sistema de introducción de matriz ultra alta (UHMI) se operó en modo robusto. El sistema de reacción octopolar de cuarta generación (ORS4) se operó en modo helio (He) para reducir las interferencias poliatómicas. Los límites de detección fueron 0,065 ppm (Si), 0,073 ppb (Cr), 0,292 ppb (Cu), 0,871 ppb (Zn), 0,023 ppb (As), 0,816 ppb (Se), 0,061 ppb (Cd), y 0,046 ppb (Pb).

Para el análisis de Pb, Cd, Zn, Cu previsto en el objetivo 5 las muestras fueron digeridas con la misma técnica descrita anteriormente para posteriormente ser analizadas por voltamperometría de redisolución anódica (ASV).

Para el objetivo 1 realizamos una revisión sistemática y extensa usando palabras clave específicas (Contaminación, Metales pesados, Caribe, América Central, América Latina, América del Sur, además de cada país por separado), en Google Scholar, PubMed & Scopus. De esta manera identificamos los estudios sobre la contaminación de metales pesados en América Latina. Incluimos artículos desde 1990 hasta 2017, este rango cronológico se decidió debido a la dificultad de encontrar material en línea publicado antes de 1990. Además, revisamos e incluimos los artículos citados en las referencias de los artículos encontrados que fueran relevantes. Se encontraron un total de 5450 artículos y de ellos 690 fueron considerados pertinentes y por lo tanto se incluyeron en la base de datos. La revisión se hizo en inglés y en español. La zona de estudio fue delimitada al norte por la frontera entre México y los Estados Unidos, al sur por el Canal de Beagle, al oeste por el Océano Pacífico y al este por el Océano Atlántico, incluidas las Islas Galápagos al oeste y las Islas Malvinas (Falkland Islands) al este. Los otros criterios para incluir un documento fueron: 1) que la especie utilizada como biomonitor fuera un animal, y 2) que los metales investigados fueran Hg, Cd, Cr, Cu, Pb, Ni, Zn, Fe, Mn, As y Se. Para cada estudio se recogieron los siguientes datos: 1) el año de publicación, 2) el país donde se realizó el estudio, 3) las especies utilizadas, 4) el tipo de medio ambiente donde se realizó el estudio (terrestre/acuático), 5) los tipos de muestras utilizadas, 6) las técnicas de muestreo utilizadas (animales vivos/muertos/sacrificados). Sólo se seleccionaron los estudios que analizaran los metales en muestras del campo, excluyendo las evaluaciones de toxicidad realizadas en el laboratorio. Además, en Scopus se buscó el número total de estudios realizados por instituciones nacionales (utilizando el filtro de "país de afiliación") de cada país en el área de Ciencias Ambientales (en inglés y español) entre 1990 y 2017. También se realizó una segunda búsqueda manteniendo los mismos parámetros. El filtro "país de afiliación" fue sustituido por "palabras clave, título o resumen". De esta manera se obtuvo el número de estudios en el área de Ciencias Ambientales realizados en cada país por instituciones extranjeras. A fin de cuantificar el interés de cada país en las cuestiones ambientales, comparamos el número de estudios realizados por instituciones nacionales con el número de estudios realizados por instituciones extranjeras.

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Objetivos

El objetivo general de esta tesis es estudiar la contaminación por metales en el norte de la Patagonia Argentina, evaluando las fuentes de emisión naturales y humanas y evidenciando las posibles amenazas para la vida silvestre y los seres humanos. Para esto hemos utilizado cinco especies de aves carroñeras como biomonitoras.

Objetivo 1. (Capítulo I) Recopilar toda la información sobre el uso de las especies de fauna silvestre como biomonitor/bioindicador de la contaminación ambiental en América Latina.

Para lograr este objetivo se realizó una amplia revisión bibliográfica de los estudios de contaminación ambiental por metales en América Latina entre 1990 y 2017. Esta revisión se centró en el uso de especies animales como biomonitores/bioindicadores. Se prestó especial atención en destacar las áreas más estudiadas, así como los principales metales, ecosistemas y tipo de muestras utilizadas.

Objetivo 2. (Capítulo II) Evaluar la influencia de las actividades antropogénicas en la exposición al mercurio de las aves carroñeras del norte de la Patagonia Argentina.

Para lograr este objetivo se analizó, por primera vez, la contaminación ambiental por Hg en los ecosistemas terrestres del norte de la Patagonia Argentina, utilizando plumas mudadas de tres especies de aves carroñeras como unidad de biomonitoreo.

Objetivo 3. (Capítulo III) Evaluar las tendencias temporales y la actividad volcánica sobre la exposición a metales en el cóndor andino.

Para lograr este objetivo se investigó las emisiones de metales durante la erupción del Complejo Volcánico Puyehue-Cordón Caulle (CVPCC) (2011) utilizando plumas primarias mudadas de cóndor andino, muestreadas en los alrededores de Bariloche (Argentina). Además, se realizó el primer screening de los niveles 9 de metales y metaloides (Si, Cr, Cu, Zn, As, Se, Cd, Pb, Hg) en el cóndor andino.

Objetivo 4. (Capítulo IV) Evaluar la potencial utilidad de los cathartidos patagónicos como especie sustituta del cóndor andino y como biomonitores de la contaminación a Hg.

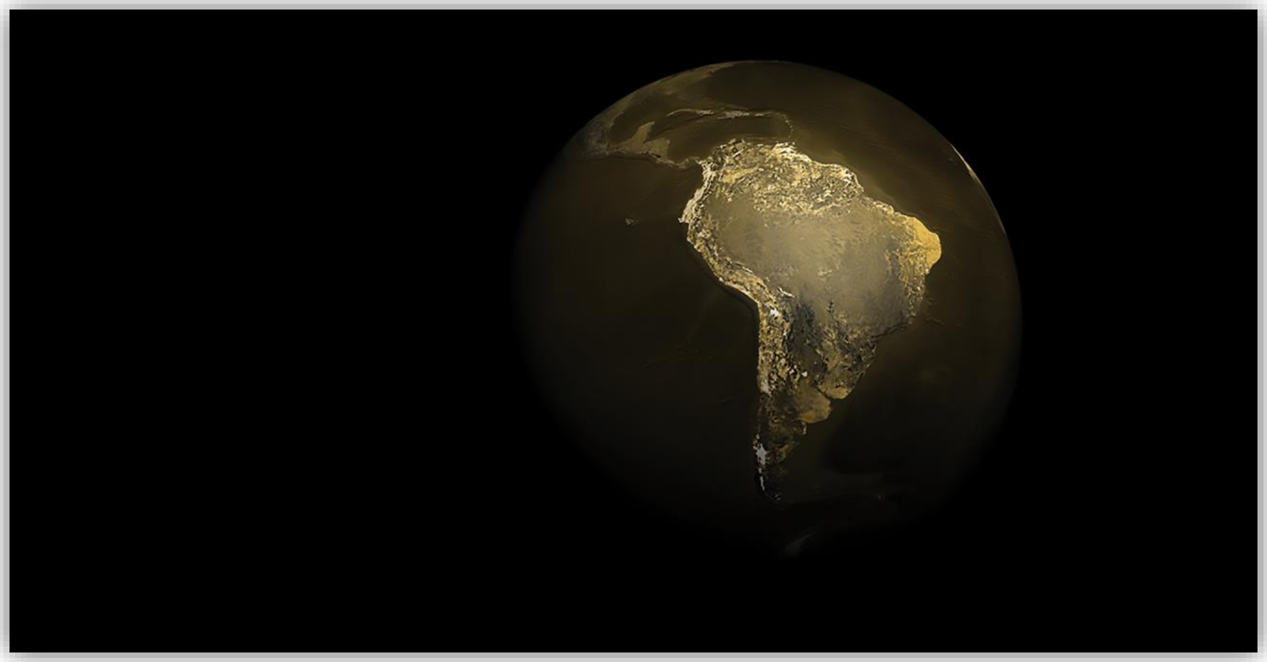
Para lograr este objetivo se compararon los niveles de Hg detectados en las plumas del jote negro y del jote colorado con los niveles de Hg detectados en las plumas del cóndor andino, para comprobar la posible utilización del jote negro y del jote colorado como especie sustituta del cóndor andino. Al mismo tiempo, se comprobó que el jote colorado podría ser un buen biomonitor para los estudios sobre la contaminación por Hg en nuestra zona de estudio.

Objetivo 5. (Capítulo V). Evaluar las tendencias temporales y espaciales de los niveles de Hg, Cd, Pb, Cu y Zn en la cuenca del río Limay en ambientes antropizados y naturales.

Para lograr este objetivo, se compararon por primera vez los niveles de Hg, Cd, Pb, Cu, Zn en muestras de jote negro y jote colorado, muestreadas en tres zonas de muestreo durante varios años. Asimismo, se realizó por primera vez una comparación de los niveles de Cd, Pb, Cu, Zn en la zona de Bariloche entre el cóndor andino, el jote negro y el jote colorado. También se evaluó la contaminación por Hg en el carancho en dos zonas de muestreo y la contaminación por Pb y Hg en el chimango en dos zonas de muestreo. En el caso del chimango fue posible evaluar la contaminación en subáreas de muestreo debido a su reducido home range. Finalmente, se compararon los niveles de Hg detectados en el vertedero de Villa la Angostura entre jote negro, carancho y chimango.

CHAPTER I

From Mexico to the Beagle Channel: A review of metal and metalloid pollution studies on wildlife species in Latin America.



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Resumen

Las emisiones de metales y metaloides (Hg; Cd; Cr; Cu; Pb; Ni; Zn; Fe; Mn; As; Se) generadas por causas naturales (ej., la actividad geotérmica) o antrópicas (ej., la industria o la minería) representan un problema de contaminación mundial, especialmente en los países en desarrollo. La exposición a altas concentraciones de estos elementos es perjudicial para los seres vivos, incluidos los humanos. La información sobre este tipo de contaminación es escasa y fragmentada, lo que limita las investigaciones que podrían beneficiarse de estos datos. Para conocer el estado de la investigación, revisamos los estudios de contaminación ambiental por metales y metaloides realizados en especies animales en América Latina. El uso de animales como biomonitores de la contaminación por metales y metaloides es una práctica en continua expansión que permite la detección temprana de problemas. Con este trabajo se han podido identificar las zonas más estudiadas de América Latina (Amazonas, Golfo de California, zona costera entre Río de Janeiro y Florianópolis y Estuario del Río de la Plata, Norte de la Patagonia Argentina). Además, proporcionamos información sobre los metales más estudiados (Hg, Cd, Cu, Pb, Zn) y las especies silvestres, que evidencian el uso de especies en peligro de extinción. Los datos examinados deberían ayudar a los investigadores a orientar sus esfuerzos hacia zonas poco investigadas y facilitar la consulta bibliográfica de la información científica sobre la exposición a metales y metaloides en América Latina.

Abstract

Emissions of metals and metalloids (Hg; Cd; Cr; Cu; Pb; Ni; Zn; Fe; Mn; As; Se) generated by natural (e.g., geothermal activity) or anthropic causes (eg., industry or mining) represent a worldwide contamination problem, especially in developing countries. Exposure to high concentrations of these elements is harmful to living beings, including humans. Information on this type of contamination is scarce and fragmented, limiting research which could benefit from these data. To know the state of the research, we reviewed the studies of environmental pollution by metals and metalloids carried out on animal species in Latin America. The use of animals as biomonitors of contamination by metals and metalloids is a continuously expanding practice that allows for early detection of problems. With this work, we were able to identify the most studied areas in Latin America (Amazon, Gulf of California, coastal area between Rio de Janeiro and Florianopolis, River Plate Estuary, north of Argentinian Patagonia). Moreover, we provide information on the most studied metals (Hg, Cd, Cu, Pb, Zn) and wild species, which evidence the use of endangered species. The data reviewed should help researchers to direct their efforts towards sparsely researched areas and facilitate bibliographic consultation of scientific information on exposure to metals and metalloids in Latin America.

Introduction

Metals and metalloids are ubiquitous substances used in human activities from antiquity to the present (Järup, 2003). Emission sources may be anthropogenic (Fig. 1) or natural, in the latter case geothermal activity and forest fires represent the main causes (Nriagu, 1990; Pirrone et al., 2010). These substances are very persistent in the environment and bioaccumulate in species and the ecosystems (García-Fernández et al., 2005). Some trace metals may be necessary for the development of normal cellular functions, but exposure to high and/or prolonged doses can lead to neurotoxic, genotoxic and carcinogenic alterations (Flora et al., 2008; Leonard et al., 2004), through mechanisms such as oxidative stress (Espín et al., 2014a, b, 2016a; Flora et al., 2008; García-Fernández et al., 2002). Although the problems related to exposure to heavy metals have long been known, the problem itself remains unsolved. Several authors report that chronic exposition to metals is an increasing problem in those areas of the planet that are currently under development (Järup, 2003; Suresh Kumar et al., 2015).

Heavy metals	Principals anthropic sources
Hg	Production of fuel and energy, mining industrial and pharmaceutical wasts (CRBAS, 2012)
Cr	Metal alloys (85%) (Guy Morrison et al., 2010)
Cd	Mining, usage of phosphate fertilazers, industrial users (batteries, plating, pigments and plastics) (CSEM, 208)
Cu	Manufacturing, electric, electronic and chemical industries (SERNAGEOMIN, 2015)
Pb	Industrial uses (batteries, cables and pine). Atomic industries and production of alloy (SERNAGEOMIN, 2015), production of shot and bullet (Plaza et al., 2018)
Zn	Metal alloy (SERNAGEOMIN, 2015)
Se	Coal burning, mining and smelting of sulphide ores (HHS, 2003)
As	Agricultural insecticides and poisons (Peryea, 1998); metallic arsenic in alloying with lead (eg. car batteries) (Salomone et al., 2005). 2% of arsenic is used in lead alloys for lead shot and bullets (Guruswamy, 1999)
Mn	Industrial and metallurgical applications, batteries and chemicals (Barceloux, 1999)
Ni	Ferronickel for stainless steel (66%), production of non-ferrous alloy, alloy steels, plating, foundry and batteries (Neikov et al., 2008)
Fe	Fe is the most widely used of all the metals, accounting for over 90% of worldwide metal production (Earnshaw et al., 1998)

Fig. 1. Principal human sources of metal and metalloids in Latin America

The anthropic sources of metal and metalloid pollution in Latin America are mainly agriculture, industry, mining, and, in large megalopolises, urban pollution (Da Rocha et al., 2015;

Pereira et al., 2007; Soto-Jiménez and Flegal, 2009). In addition, the U.S. border area is contaminated by North American anthropogenic activities (Soto-Jiménez and Flegal, 2009). The magnitude and importance of mining activity in the vast Latin America is relevant (Lagos and Peters, 2010). The 44.6% of the world's copper is extracted in Latin America. Four of the five countries with the highest mining activity of various minerals are in Latin America: Peru (1st silver, 2nd zinc, 3rd copper and tin, 4th molybdenum and lead, 5th gold), Chile (1st copper, lithium and iodine), Brazil (1st niobium, 2nd iron, 3rd bauxite, 5th tin) and Bolivia (3rd antimony and 4th tin) (Lagos and Peters, 2010). Annual mining reports issued by each country are extremely detailed, and can be used to monitor the mining production and to identify the most affected areas. Other anthropogenic activities that generate metallic pollution such as hunting/fishing, industry, hydrocarbon extraction and intensive agriculture have been previously investigated and result suggest they are threatening wildlife (Ferreyra et al., 2014; Plaza et al., 2018; Ronco et al., 2007; UNEP et al., 2010). However, the information available is incomplete and less structured than the mining related emissions (Burger, 2006; Utmazian and Wenzel, 2006), making it difficult to compare results. Moreover, current situation of environmental pollution studies in Latin America is affected by the scarce development of adequate environmental legislation in many of the countries of the region. Apart from Brazil, where this issue is being addressed since 1980s, for other countries it took decades for activities that could have an impact on the environment to be regulated (Lagos and Peters, 2010). The use of animal species as biomonitors is a technique used for several decades (Espín et al., 2012; García-Fernández, 2014). In recent years there has been an increase in the number of studies using non-lethal samples, for example, using such samples as blood or feathers (Di Marzio et al., 2018a; Espín et al., 2016b; Martínez-López et al., 2004, 2005; Movalli et al., 2017). A series of parameters can be used for the selection of biomonitor species, that may vary depending on the type of research being conducted (Gadzała-Kopciuch et al., 2004). If we consider that one of the purposes of these studies is environmental monitoring in order to detect possible sources of pollution and promote the conservation of biodiversity, the choice of biomonitors should also be guided by ethical and legal aspects (Ansara-Ross et al., 2013; Movalli, 2000).

In this study we made an extensive search for all studies on environmental pollution by metals and metalloids (Hg, Cd, Cr, Cu, Pb, Ni, Zn, Fe, Mn, As, Se) in animals. We aimed at obtaining information on 1) which areas of each Latin-American country have been studied, 2) which metals and metalloids have been studied, and 3) which wild species have been used in the studies. The studies are geographically limited to Latin America (the Caribbean, Central America, and South America), while the time period evaluated varied between 1990 and December 2017. Thus, we compile all the scientific publications available, facilitating access to this material, and evidence the topics where scientific research has been scarce so far. In this way we expect this review will be useful for professionals who want to start a new research in Latin America to know what is known, and where to direct their effort.

Methodology

We performed a systematic and extensive review using specific key words (Contamination, Heavy metals, Caribbean, Central America, Latin America, South America, plus each country separately), in Google Scholar, PubMed & Scopus. In this way we looked for identifying studies on heavy metals contamination in Latin-America. We included articles from 1990 to 2017, this chronological range was decided because of the difficulty to find online material published before 1990. In addition, we reviewed and included those papers cited in the references of the articles found that were relevant. A total of 5450 papers were found and from them 690 were pertinent and thus included in the database. The review was done in English and Spanish.

The study area was delimited in the north by the border between Mexico and the United States, in the south by the Beagle Channel, in the west by the Pacific Ocean and in the east by the Atlantic Ocean, including the Galapagos Islands to the west and the Falkland Islands (Malvinas Islands) to the east. The other criteria to include a paper were: 1) the species used as biomonitor must be an animal, and 2) the metals investigated were Hg, Cd, Cr, Cu, Pb, Ni, Zn, Fe, Mn, As, and Se. The following data were collected for each study: 1) the year of publication, 2) the country where the study was carried out, 3) the species used, 4) the type of environment where the study was carried out (terrestrial/aquatic), 5) the types of samples used, 6) the sampling techniques used (live/dead/sacrificed animals). Only studies analyzing metals in samples from the field were selected, excluding toxicity assessments performed in the laboratory.

In addition, we searched in Scopus to find the total number of studies conducted by national institutions (using the “affiliation country” filter) of each country in the area of Environmental Sciences (in English and Spanish) between 1990 and 2017. By comparing the data obtained with the results of our search on metal contamination studies, we were able to obtain the percentage of metal and metalloid contamination studies using wild species as biomonitors with respect to all environmental science studies for each country.

A second search was also carried out keeping the same parameters. The filter “country of affiliation” was replaced by “keywords, title or summary”. In this way we obtained the number of studies in the area of Environmental Sciences carried out in each country by foreign institutions. In order to quantify each country's interest in environmental issues, we compared the number of studies carried out by national institutions with the number of studies carried out by foreign institutions.

Results and discussions

Temporary distribution

We found 690 scientific papers on metal and metalloid pollution corresponding to our search criteria (more details in supplementary material Table 1sm). The general trend of scientific research on pollution by metals and metalloids in Latin America shows an increase in the last 28 years. Until the year 2008 the number of papers never exceed 30 articles per year, while since 2009 (excluding 2017) this value is always exceeded (Fig. 2).

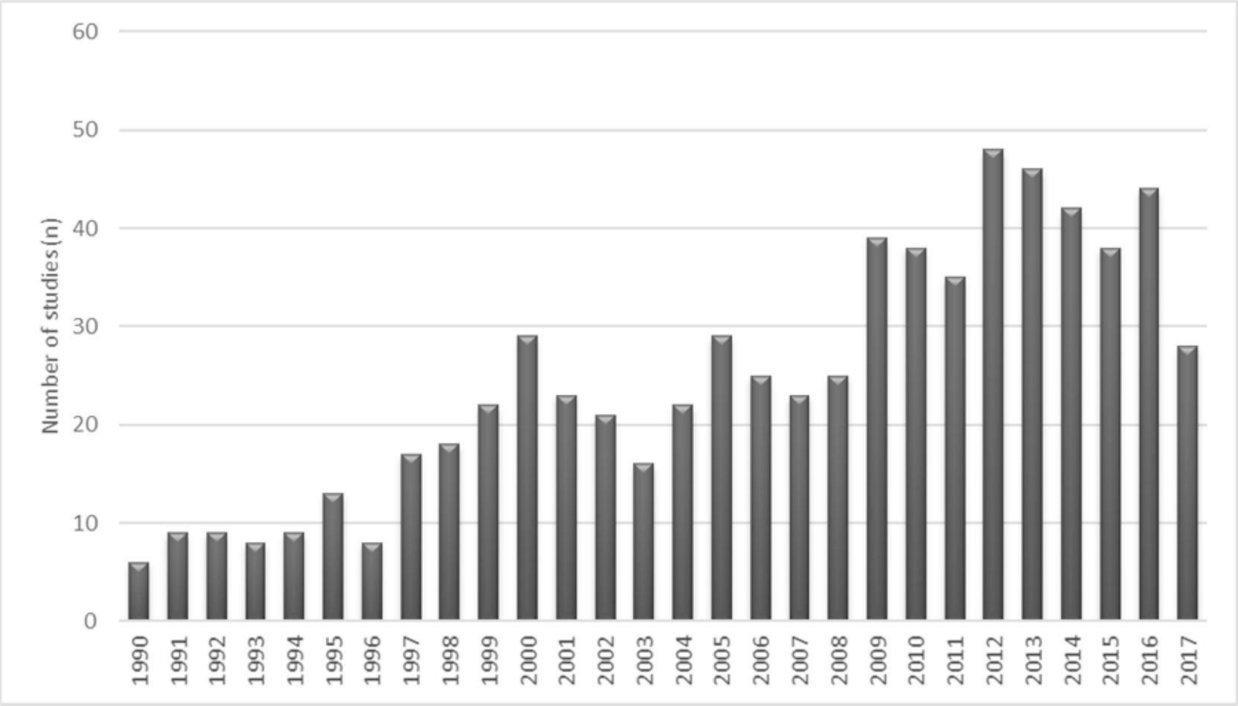


Fig. 2. Studies of metal contamination in animal species in Latin America per year (1990–2017).

Geographical distribution and ecosystems

These data are in line with the trend of scientific research in Latin America evidenced by Santa and Herrero Solana (2010) and with the global trend evidenced by Burger (2006).

The papers we found were distributed in 27 countries, including 1 study in the Malvinas Islands (Falkland Islands) and 1 study in Haiti and the Dominican Republic (it was considered as 1 country). Brazil, Mexico and Argentina were the first countries in terms of number of publications, equivalent to 74% of the total (Fig. 3).

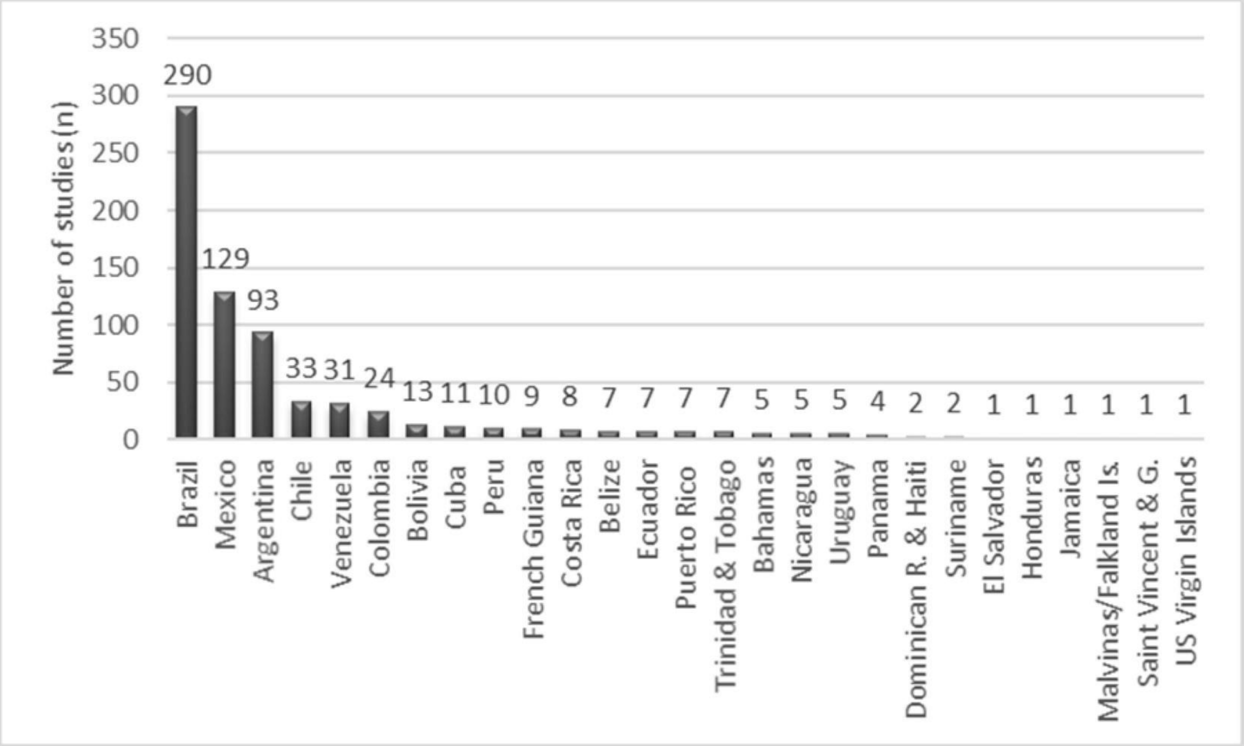


Fig. 3. Studies of metal contamination in animal species by each Latin America country (1990–2017).

Of the first nine countries (those with more than 10 studies) Mexico, Bolivia and Peru are the only countries where scientific research is carried out mainly by foreign research groups (Fig. 4). To try to give an interpretation we have evaluated the average GDP (Gross Domestic Product) (1990–2017) and the average percentage of GDP devoted to scientific research (1996–2017) in each country, using World Bank data (www.worldbank. org). The first country by percentage of GDP devoted to scientific research is Brazil (6409 USD/capita; 1.07% for research) followed by Argentina (8309 USD/capita; 0.48% for research), Cuba (3970 USD/per capita; 0.48% for research), Peru (3382 USD/per capita; 0.44% for research), Chile (8344 USD/capita; 0.36% for research), Bolivia (1550 USD/per capita; 0.28% for research), Colombia (3926 USD/per capita; 0.20% for research), Mexico (7247 USD/per capita; 0.08% for research). In the case of Venezuela, it has not been possible to make a comparison due to the lack of data regarding the percentage of GDP that the country dedicates to research.

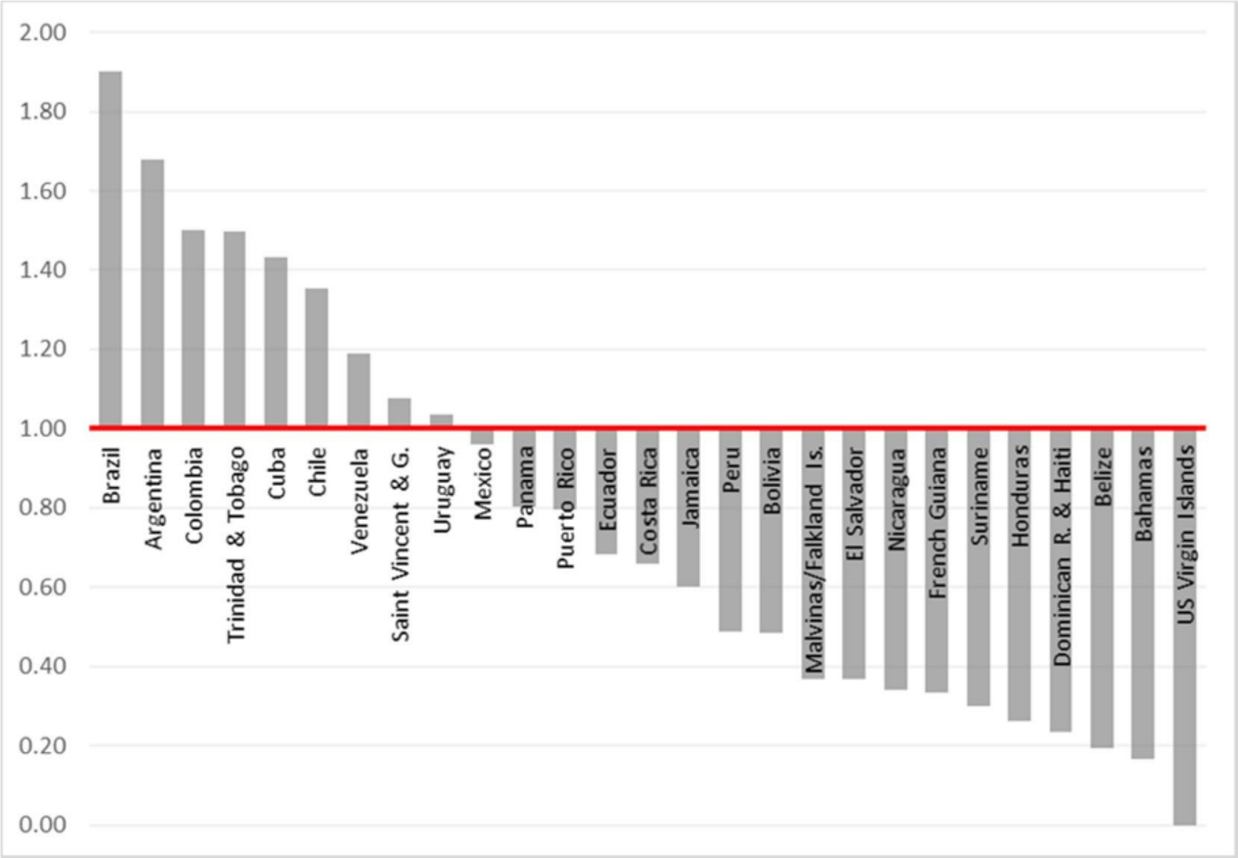
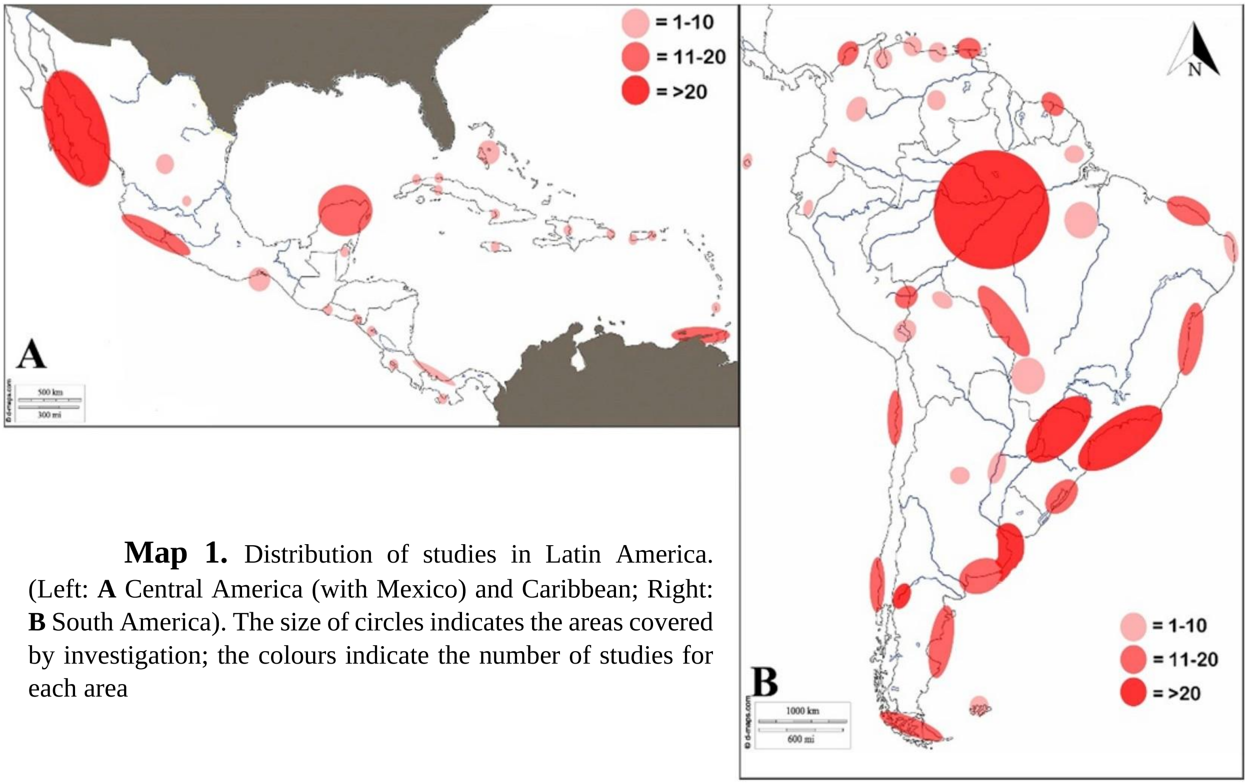


Fig. 4. Relationship between studies carried out in each country and studies carried out by national teams. (> 1 predominance of national research; 1 < predominance of foreign research). The US Virgin Islands have a value of 0 because no studies carried out by national groups have been found.



Map 1. Distribution of studies in Latin America. (Left: **A** Central America (with Mexico) and Caribbean; Right: **B** South America). The size of circles indicates the areas covered by investigation; the colours indicate the number of studies for each area

It is very important to remind that the levels of GDP and the percentage invested in the research represent only one of the multiple factors that influence the scientific output of each country. Our interpretation of the results using this parameter has been only a first approximation to the question. A more in-depth study of these factors would exceed the purposes of this paper.

The studies show a wide and unequal distribution (Map 1 A, B), with large unstudied areas and focal points where research efforts have been concentrated. In the Caribbean area, except for a study on land birds in the Dominican Republic and Haiti (Townsend et al., 2013), all studies have been conducted in aquatic ecosystems. Central America and Mexico follow a similar pattern and the studies are mainly related to marine and freshwater aquatic ecosystems (Map 1 A). The largest number of studies (n > 80) are concentrated in the Gulf of California (Mexico), an area of high ecological and economic importance, according to the Mexican government (Soto-Jiménez and Flegal, 2009). Researchers have been particularly interested in the southern part of Baja California, the Southern Peninsula and the area of Mazatlán Port (State of Sinaloa). The Gulf of

California is one of the most economically developed areas of the country, with a strong presence of mining, industry and agriculture; activities that generate pollution by metals (Sánchez-Rodríguez et al., 2001; Soto-Jiménez and Flegal, 2009). In the interior of the states of Sinaloa and Sonora there is the Sierra Madre Occidental, an important mining area of the country. Due to erosion events, contaminants from this area reach coastal lagoons, which represent an important reservoir of metals and other substances (Soto-Jiménez and Flegal, 2009). The state of Baja California Sur represents another mining area characterized also by the presence of ancient volcanoes, source of metal emissions to the environment (Ruelas-Inzunza et al., 2013; Sánchez-Rodríguez et al., 2001). The presence of Pb in this area is also partly caused by the industrial's emissions from the U.S.A. (Soto-Jiménez and Flegal, 2009). The central mining area of the country (Soto-Jiménez and Flegal, 2009) and the Gulf of Mexico represent two areas that need to be studied due to the risk of contamination by mining as other contaminants coming from the US. In South America we found the same trend as in Central America and the Caribbean, with most of the studies conducted in coastal areas or inland freshwater ecosystems. We found four areas where most studies are concentrated (Fig. 1 B). The first is the Amazon river basin (n = 80). In this vast area of Brazil, the studies are distributed along the river, from the border with Peru to the mouth of the Atlantic Ocean. The Madeira river basin in its stretch within the State of Rondônia, the Tapajos river basin in the State of Pará and the Río Negro basin (including the confluence in Manaus) are the most studied areas of the entire Amazon basin. In these three river basins mercury (Hg) has been used in artisanal gold mining since the XVI century Between 1550 and 1880, nearly 200,000 metric tonnes of mercury were released to the environment and to this day gold mining continues in these areas (Malm, 1998). This type of problem affects the entire north central part of the country (Malm, 1998), including areas surrounding the Amazon basin (Fig. 1B). The problem of mining pollution is amplified by the presence of a vast network of hydroelectric dams and their reservoirs. Several studies have shown how the construction of dams has caused a global change in the renewal of water, increasing it from 20 days to 100. This reduces the capacity for self-purification of water and accelerates consequent decline in water quality, with increases concentrations of metals and other pollutants (Kummu and Varis, 2007; Wang et al., 2012). Brazil has an extensive system of hydroelectric dams and the issue is being investigated in Brazil, where a significant number of studies (n = 26) focus on evaluation of the concentration of heavy metals in biological samples obtained from aquatic animals, many of them part of the diet of local populations (da Silva Rabitto et al., 2011; Kasper et al., 2014; Malm, 1998). The problems of the impact of hydroelectric dams as well as contamination by mining are also studied in French Guiana, Chile, Venezuela, Argentina, Peru (Alcalde and Gil, 2000; Cid et al., 2009; Copaja et al., 2016; Diringer et al., 2015; Durrieu et al., 2005; Rondon and Pérez, 1999). The second area covers the coastal strip from Rio De Janeiro to Curitiba (Map 1B). We have found a high number of studies in this area (n = 83). It is a highly populated area, including the cities of Rio De Janeiro and Sao Paolo. Urban pollution and industrial activities represent the main problems of this area (Avelar et al., 2000; Pereira et al., 2007; Quiterio et al., 2004). Behind this strip we detected another zone where pollution studies are concentrated (Map 1B). This is the area between the states of Mina Gerais and Mato Grosso do Sul (n = 24). The contamination in this area is generated by mining, industry and agriculture (Da Rocha et al., 2015; Jordão et al., 1996; Veado et al., 2006). La Plata River estuary represents another point where a high number of studies (n > 40) of metallic contamination are concentrated (Fig. 1B). The Paraná-La Plata river system is subjected to metal pollution from the intensive agricultural activities upstream (Cr, Cd, Cu, Zn, Ni, Pb) (Carnelo et al., 1997; Salazar et al., 2012) and urban and industrial activities in the delta area, where almost half of the population of Argentina is concentrated (Ronco et al., 2007). Another area investigated, although in a lower level (n = 21) is the area of San Carlos de Bariloche, in the Province of Rio Negro (Map 1B). This area contains numerous lakes and natural parks; thus, this is an area of low environmental pollution (Di Marzio et al., 2018a). However, one of the main sources of metal contamination there could be volcanic eruptions (e.g. As, Cu, Zn, Cr, Si) (Bubach et al., 2015; Conti et al., 2016; Ruggieri et al., 2012), or lead from hunting (Lambertucci et al., 2011; Wiemeyer et al., 2017).

Studies on metal pollution in terrestrial ecosystems represent only 4% (n = 28) (Fig. 5). With 96% of the studies (n = 662) concentrated in aquatic ecosystems (marine and freshwater), Latin America is well above the global trend of approximately 40% (n = 214) (Burger, 2006).

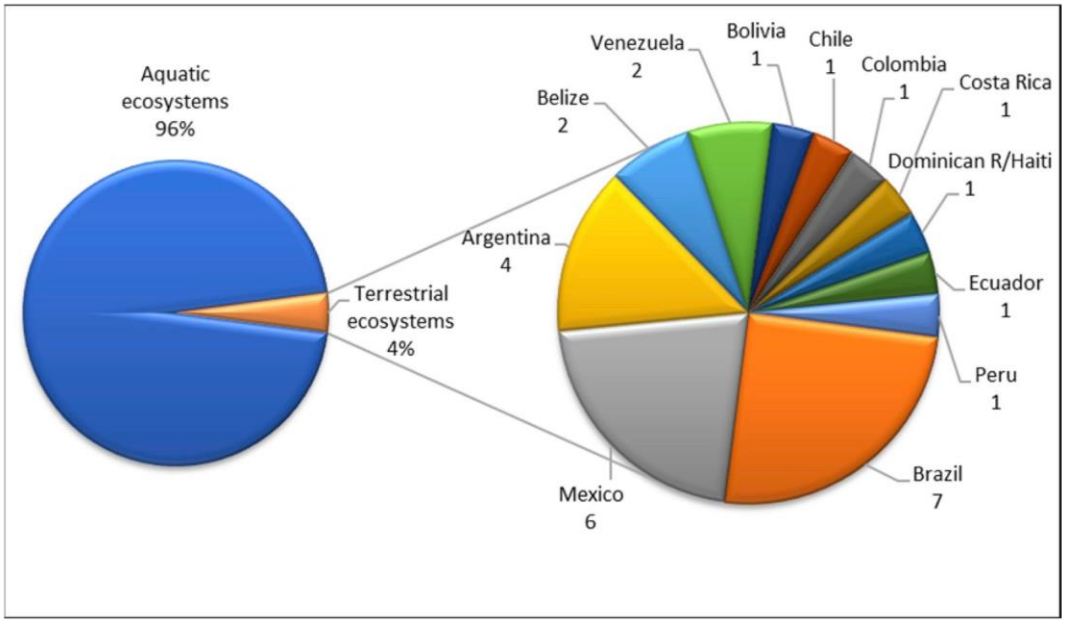


Fig. 5. Left: aquatic versus terrestrial percentage of studies. Right: number of the studies in each country with research on terrestrial environments.

This situation generates a gap of information that makes difficult to interpret data from new research on terrestrial ecosystems, slowing down scientific progress in this field (Di Marzio et al., 2018a; Martínez-López et al., 2015). This is important, especially considering that different ecosystems are closely linked, and several studies have shown how pollution can move from the aquatic to the terrestrial environment (Blázquez et al., 2016) or directly affect terrestrial species (Espín et al., 2014a, 2014b; Zolfaghari et al., 2007).

According to our Scopus search, studies included in our data base represent only 0.9% of studies of environmental sciences in Latin America. The type of ecosystem investigated was not differentiated in this research. We had difficulty in finding a study to compare the results. However, Speziale and collaborators (2012) made a similar bibliographic search in Scopus to find the average number of studies carried out in the South American continent on the presence of invasive species in relation to the total number of ecological studies. The index for the South American continent in this case was 2.64%, considered a low value by researchers, when compared to other continents (Speziale et al., 2012). Our results, much lower than those of Speziale and collaborators (2012), which suggest that the study of environmental pollution by metals and metalloids using animal samples is increasing but it is not still a priority in Latin America.

Heavy metals

In our bibliographic review we found studies related to 11 metals and metalloids (Hg, Cd, Cr, Cu, Pb, Ni, Zn, Fe, Mn, As, Se). The most studied metals in the three geographical areas of Latin America have mainly been the same, with some slight variations between the areas (Caribbean: Hg > Pb > Cu > Cd > Zn; Central America: Cd > Pb > Hg > Cu > Zn; South America: Hg > Cd > Cu > Zn > Pb) (more details in supplementary material Fig. 2smA, B, C). In South America, the most studied metal was Hg (n = 317). The interest is caused by the use of this substance in the extraction of gold in artisanal mining. This technique has been used and is still being used in the Amazon river basin and in all the gold mining areas of the north central part of South America (Malm, 1998). Studies on pollution by other metals (Cd, Cu, Zn, Pb) could be motivated by the presence of other important sources of emissions such as industry, intensive agriculture, among others (Plaza et al., 2018; Ronco et al., 2007). In Central America, the first two metals studied were Cd and Pb. In both cases, this is due to the predominance of studies from Mexico, where there is contamination by Cd in the Gulf of California. Part of that contamination may be originated in the sedimentary and volcanic rocks of Monterrey formation, rich in Cd and used to produce fertilizer. By the action of the wind the Cd may reach the Gulf (Mendez and Paez-Osuna, 1998). Other causes, common to Pb pollution, are the mining areas of the Sierra Madre (Espinosa and Armienta, 2007; 2009; Soto-Jiménez and Flegal, 2009) and harbors drainage (Gutiérrez-Galindo et al., 2010). Hg seems to be an emerging problem, as the increased number of studies in the last 9 years may reflect. In the case of the Caribbean, wastewater inputs, mining (bauxite), agriculture, industry and hydrocarbon extraction are indicated as possible causes of pollution (Fernandez et al., 2007). The predominance of Hg as the most researched pollutant could be explained due to the concern that the elements with the high level of biomagnification in the aquatic environment produce.

Biomonitoring species and samples

Biomonitors are species, or group of species, that indicate environmental quality (Asif et al., 2018; Gadzała-Kopciuch et al., 2004), and can be used in ecological risk assessment programs or to understand the mechanisms of adverse effects of environmentally mediated pollutants, helping to detect problem insurgency (Burger, 2006). We have classified the species used in the studies according to their taxonomical class, except for invertebrates that are grouped together in the subphylum "invertebrates", and the classes Actinopterygii and Chondrichthyes that are grouped under the generic definition of "fish". The most studied groups in the three geographical areas have been the same, with some minor differences (Caribbean: Invertebrates = Fish; Central America: Invertebrates > Fish; South America: Fish > Invertebrates). The fish group presents an important variability in the percentage of studies using Chondrichthyes, depending on the area (Caribbean 3%; Central America 37%; South America 8%). This value is influenced by the tradition of shark fishing in Mexico (de Borhegyi, 1961; McGoodwin, 1976; Sosa-Nishizaki et al., 2008). In the three geographic areas analyzed (Central America, Caribbean, South America) there has been a change of preference with respect to the species used as biomonitors (more details in supplementary material Fig. 1sm A, B, C). The common trend of the last evaluated period (2008–2017) shows an increase of the studies on Mammalia and a decrease of studies using invertebrates in all areas. Several studies consider species that occupy high positions in a trophic pyramid to be good biomonitors (Asif et al., 2018; Burger, 2006; Gómez-Ramírez et al., 2014; Rahman and Ismail, 2017; Wei Zhang and Zhang Ma, 2011). Many papers reviewed, although they use animal species to determine pollution levels, do not consider (nor evaluate) whether these species could be good biomonitors. This disagrees with studies in the rest of the world where the use of biomonitors is widely documented (Beeby, 2001; Burger, 2006; Gadzała-Kopciuch et al., 2004; García-Fernández, 2014; Holt and Miller, 2011).

Only a small percentage of studies (12%) have used live animals as biomonitors, although in recent years there has been much emphasis on the responsible use of species for scientific research (Ansara-Ross et al., 2013). In terms of sample type, soft tissues were the most common choice in the studies (88%). Soft tissues mainly provide indices of recent exposure and therefore provide a more reliable source of information (Ansara-Ross et al., 2013). The use of subrogate species (Di Marzio et al., 2018b; West et al., 2017; Wiemeyer et al., 1986) allows the ecosystem of threatened species to be studied without the need to resort to them directly. The data presented in the “Living Planet Report 2018" (WWF, 2018) throw an important alarm on the loss of biodiversity on the planet. Searching for how many investigations carried out in the last 28 years in Latin America have used endangered species as biomonitors, we found 49 species of various classes (Mammalia, Actinopterygii, Aves, Reptilia, Chondrichthyes) classified as Near Threatened (NT, n = 16), Vulnerable (VU, n = 25), Endangered (EN, n = 7) and Critically Endangered (EN, n = 1) according to the IUCN (International Union for Conservation of Nature) Red List categories (Table 1). No-lethal studies showed percentages for species (n = 49) of 37% (Mammalia = 10%; Actinopterygii = 4%; Reptilia = 8%; Aves = 14%) and in each species group, the specimens (n = 3350) percentages of 20% (Mammalia = 3%; Actinopterygii = 0.2%; Reptilia = 10%; Aves = 7%). The studies that resorted to the sacrifice of biomonitors showed percentages for species/specimens of 39%/29% (Mammalia = 2%/0.3%; Chondrichthyes = 12%/21.6%; Reptilia = 8%/4%; Birds = 16%/3%); of the sacrificed species 4 were NT, 12 VU and 2 EN. It is very striking that specimens of species classified EN have been sacrificed. These are 10 specimens of *Pelecanoides garnotii*, captured for the American Museum of Natural History collection (Ochoa-Acuna et al., 2002) and 570 specimens of *Mustelus schmitti*, captured at 7 sampling stations in the Bahia Blanca estuary (Argentina) during 1985–1986 (Marcovecchio et al., 1991). A high number of samples come from animals fished intentionally or accidentally (and eggs from the reptiles' nests). In this case the percentages of species/ specimens have been 53%/51% (Mammalia = 6%/10.8%; Actinopterygii = 4%/0.2%; Chondrichthyes = 26%/13%; Reptilia = 8%/24%; Aves = 8%/3%). In the case of Reptilia (all species are turtles), the turtles that have been sacrificed come from the Amazon basin, while the marine species were not sacrificed. In the case of mammals, the 306 specimens were from La Plata dolphin (*Pontoporia blainvillei*) accidentally fished. Also, in this case is striking the high number of species and specimens of Chondrichthyes captured (n = 426). All specimens of sharks and rays investigated have died, either by sacrifice or because they were caught accidentally or intentionally.

Species	***n***	***Area***	***Duration of sampling***	***Origin of specimens***	***Condition after sampling***	***Authors***
Mammalia						
Carnivora						
Chrysocyon brachyurus (NT)	10	South America		Captured for the study	Live	*de Almeida Curi et al., 2012*
Pteronura brasiliensis (EN)	/	South America	/	Samples of feces	/	*Gutleb et al., 1997*
	2	South America	1 year	Dead	Dead	*Dias Fonseca et al., 2005*
Lontra longicaudis (NT)	/	South America	13 months	Samples of feces	/	*Ferreira Josef et al., 2008*
	/	Central America	/	Samples of feces	/	*Ramos-Rosas et al., 2013*
Cetacea						
Physeter macrocephalus (VU)	45	Central / South America/Caribbean		Sampled for the study	Live	Savery et al., 2013, 2014a, 2014b
Pontoporia blainvillei (VU)	2	South America	/	Stranded	Dead	*Marcovecchio, 1990*
	18	South America	/	Accidentally fishing	Dead	*Gerpe et al., 2002*
	23	South America	3 years	Accidentally fishing	Dead	*Kunito et al., 2004*
	44	South America	6 years	Accidentally fishing	Dead	*Dorneles et al., 2007*
	7	South America	2 years	Accidentally fishing	Dead	*de Carvalho et al., 2008*
	31	South America	8 years	Accidentally fishing	Dead	Seixa et al., 2008
	59	South America	10 years	Accidentally fishing	Dead	*Panebianco, 2011*
	55	South America	/	Accidentally fishing	Dead	*Polizzi et al., 2013, 2014*
	16	South America	26 months	Stranded/Accidentally fishing	Dead	*Baptista et al., 2016*
	11	South America	/	Accidentally fishing	Dead	*Kehrig et al., 2016*
	42	South America	/	Accidentally fishing	Dead	*Romero et al., 2016*
Chiroptera						
Myotis vivesi (VU)	10	Central America	/	Captured for the study	Killed	Méndez & Alvarez-Castañeda 2000
Sirenia						
Trichechus manatus m. (VU)	19 7	Central America	10 years	Samples of bones Captured for the study	Dead Live	Rojas-Minguer & Morales-Vela, 2002
	16	South America	/	Captured for the study	Live	*Anzolin et al., 2012*
	14	Central America	1 month	Captured for the study	Live	*Siegal-Willott et al., 2013*
	33	Central America	24 years	Samples of bones	Dead	*Romero-Calderón et al., 2016*
Reptiles						
Caretta caretta (VU)	5	Central America	/	Accidentally fishing	Dead	*Gardner et al., 2006*
	35	Central America	6 months	Dead	Dead	*Andreani et America, 2008*
	22	Central America	/	Captured for the study	Live	*Ley-Quiñónez et al., 2011*
	16	Central America	/	Stranded/Accidentally fishing	Dead	*Kampalath et al., 2012*
	29	South America	8 months	Stranded	Dead	*da Silva et al., 2014*

Chelonia mydas (EN)	11	Central America	/	Accidentally fishing	Dead	*Gardner et al., 2006*
	8	Central America	15 months	Accidentally fishing	Dead	*Talavera Saez et al., 2007*
	30	South America	1 year	Dead	Dead	*Barbieri, 2009*
	56	Central America	2 years	Captured for the study	Live	*Labrada-Martagón et al., 2011*
	90	Central America	4 months	Unhatched eggs/Dead hatchlings	Dead	*Roe et al., 2011*
	20	South America		Accidentally fishing	Live	*Bezerra et al., 2012a*
	5	South America		Dead	Dead	*Bezerra et al., 2012b*
	42	Central America	/	Stranded/ Accidentally fishing	Dead	*Kampalath et al., 2012*
	18	South America	33 months	Stranded	Dead	*Bezerra et al., 2013*
	1	South America		Dead	Dead	*Bezerra et al., 2014*
	41	South America	3 years	Stranded	Dead	*Bezerra et al., 2015*
	10	South America	17 months	Stranded	Dead	*de Macêdo et al., 2015*
	27	South America	14 months	Captured for the study	Live	*da Silva et al., 2016*
	31	Central America	12 hours	Eggs samples	/	*Ross et al., 2016*
Dermochelys coriacea (VU)	78 76	South America	1 month	Captured for the study Eggs samples	Live	*Guirlet et al., 2008*
	73	Caribbean	9 hours	Captured for the study	Live	*Perrault et al., 2013*
Eretmochelys imbricata (CR)	1	Central America	/	Accidentally fishing	Dead	*Gardner et al., 2006*
	16	South America	17 months	Stranded	Dead	*de Macêdo et al., 2015*
Lepidochelys olivacea (VU)	6	Central America	/	Accidentally fishing	Dead	*Gardner et al., 2006*
	25 250	Central America	5 days	Captured for the study Eggs samples	Live	*Páez-Osuna et al., 2010a, 2010b, 2011*
	23	Central America	/	Stranded/ Accidentally fishing	Dead	*Kampalath et al., 2012*
	41 13	Central America	2 months	Captured for the study Stranded	Live Dead	*Cortes-Gomez et al., 2014*
	7	Central America	3 months	Stranded	Dead	*Frias-Espericueta et al., 2015*
	30	Central America	12 hours	Eggs samples	/	*Ross et al., 2016*
Peltocephalus dumerilianus. (VU)	(21*)	South America	/	Captured for the study	Killed	*Thomé Souza et al., 2004*
	5	South America	1 month	Captured for the study	Killed	*Burger et al., 2009* *Schneider et al., 2010, 2011*
Podocnemis erythrocephala (VU)	(21*)	South America	/	Captured for the study	Killed	*Thomé Souza et al., 2004*
	39	South America	1 month	Captured for the study	Killed	*Burger et al., 2009* *Schneider et al., 2010, 2011*
Podocnemis sextuberculata (VU)	12	South America	1 month	Captured for the study	Killed	*Burger et al., 2009* *Schneider et al., 2010, 2011*

Podocnemis unifilis (VU)	29	South America	1 month	Captured for the study	Killed	*Souza Araujo et al., 2007*
	2	South America	1 month	Captured for the study	Killed	*Schneider et al., 2010, 2011*
	10	South America	1 month	Captured for the study	Killed	*Schneider et al., 2015*
	28	South America	1 month	Captured for the study	Killed	*Eggins et al., 2015*
Chondrichthyes						
Alopias pelagicus (VU)	13	Central America	1 year	Accidentally fishing	Dead	*Garcia Hernandez et al., 2007*
Carcharhinus falciformis (VU)	15	Central America	5 years	Captured for the study	Killed	*Mas Courrau & López Vera; 2012, 2017*
Carcharhinus leucas (NT)	1	Central America	10 months	Fishing	Dead	*Ruelas-Inzunza & Paez-Osuna, 2005*
Carcharhinus limbatus (NT)	22	Central America	9 months	Fishing	Dead	*Nuñez-Nogueira, 2005*
	19	Central America	5 months	Fishing	Dead	*Mendoza-Díaz et al., 2013*
Carcharinus signatus (VU)	6	South America	1 year	Captured for the study	Killed	*De Pinho et al., 2002*
	38	South America	4 years	Commercial fishing	Dead	*Ferreira et al., 2004*
Carcharhinus obscurus (VU)	4	Central America	1 year	Accidentally fishing	Dead	*Garcia Hernandez et al., 2007*
Isurus oxyrinchus (VU)	4	South America	/	Fishing	Dead	*Marsico et al. 2007*
	20	Central America	1 month	Fishing	Dead	*Vélez Alvarez, 2010*
	24	Central America	5 years	Captured for the study	Killed	*Maz Courrau & López Vera; 2012, 2017*
	69	South America	2 months	Accidentally fishing	Dead	*Lopez et al., 2013*
Mobula japanica (NT)	3	Central America	1 year	Accidentally fishing	Dead	*Escobar Sanchez et al., 2010*
Mobula munkiana (NT)	5	Central America	1 year	Accidentally fishing	Dead	*Escobar Sanchez et al., 2010*
Mobula thurstoni (NT)	15	Central America	1 year	Accidentally fishing	Dead	*Escobar Sanchez et al., 2010*
Mustelus canis (NT)	79	South America	1 year	Captured for the study	Killed	*De Pinho et al., 2002*
Mustelus schmitti (EN)	570	South America	2 years	Captured for the study	Killed	*Marcovecchio et al., 1991*
Rhinobatos productos (NT)	45	Central America	6 months	Fishing	Dead	*Murillo Cisneros, 2014*
Rhinoptera steindachneri (NT)	35	Central America	2 months	Fishing	Dead	*Gutierrez Mejia et al., 2009*
	25	Central America	1 year	Accidentally fishing	Dead	*Escobar Sanchez et al., 2010*
Sphyrna lewini (EN)	12	Central America	7 months	Fishing	Dead	*Hurtado-Banda et al., 2012*
	40	Central America	3 months	Fishing	Dead	*Bergés-Tiznado et al., 2015*
	10	Caribbean	/	Fishing	Dead	*Mohammed & Mohammed, 2017*
Sphyrna zygaena (VU)	4	Central America	1 year	Accidentally fishing	Dead	*Garcia Hernandez et al., 2007*

	5	South America	/	Fishing	Dead	*Marsico et al. 2007*
	37	Central America	1 year	Accidentally fishing	Dead	*Escobar Sanchez et al., 2010*
	31	Central America	5 years	Captured for the study	Killed	*Maz Courrau & López Vera; 2012, 2017*
Aves						
Ardenna creatopus (VU)	28	South America	1 month	Captured for the study	Live	*Becker, 2000*
	19	South America	3 months	Sampling feather ^{B}	Killed	*Ochoa Acuña et al., 2002*
	1	South America	2 years	Fishing/Stranded	Dead	*Gil et al., 2006*
Ardenna grisea (NT)	8	South America	3 months	Sampling feather ^{B}	Killed	*Ochoa Acuña et al., 2002*
Eudyptes chrysocome (VU)	35 32	South America	1 year	Captured for the study	Live Killed	*Keymer et al., 2001*
Larosterna inca (NT)	7	South America	3 months	Sampling feather ^{B}	Killed	*Ochoa Acuña et al., 2002*
Pelecanoides garnotii (EN)	10	South America	3 months	Sampling feather ^{B}	Killed	*Ochoa Acuña et al., 2002*
Phalacrocorax gaimardi (NT)	1	South America	2 years	Fishing/Stranded	Dead	*Gil et al., 2006*
Procellaria aequinoctialis (VU)	30	South America	6 months	Captured for the study	Live	*Carvalho et al., 2013*
Procellaria conspicillata (VU)	38	South America	6 months	Captured for the study	Live	*Carvalho et al., 2013*
Pterodroma externa (VU)	15	South America	3 months	Sampling feather ^{B}	Killed	*Ochoa Acuña et al., 2002*
Pterodroma longirostris (VU)	2	South America	3 months	Sampling feather ^{B}	Killed	*Ochoa Acuña et al., 2002*
Spheniscus humboldti (VU)	/	South America	2 months	Excreta samples	/	*Celis et al., 2014*
Spheniscus magellanicus (NT)	12	South America	1 year	Captured for the study	Killed	*Keymer et al., 2001*
	16	South America	2 years	Fishing/Stranded	Dead	*Gil et al., 2006*
	39	South America	3 months	Captured for the study	Live	*Frias et al., 2012*
	47	South America	5 months	Stranded	Dead	*Vega et al., 2012*
	24	South America	/	Stranded	Dead	*Kehrig et al., 2016*
Vultur gryphus (NT)	/	South America	4 months	Molted feather samples	/	*Lambertucci et al., 2011*
	62	South America	7 years	Animals for rehabilitation	Live	*Wiemeyer et al., 2017*
	14			Animals for necropsy	Dead	
Actinopterygii						
Coeligena prunellei (VU)	1	South America	4 months	Captured for the study	Live	*Gongora et al., 2016*
Eriocnemis cupreoventris (NT)	4	South America	4 months	Captured for the study	Live	*Gongora et al., 2016*
Mycteroperca jordani (EN)	6	Central America	1 year	Accidentally fishing	Dead	*Garcia Hernandez et al., 2007*
Mycteroperca rosácea (EN)	2	Central America	1 year	Accidentally fishing	Dead	*Garcia Hernandez et al., 2007*

Table 1 Endangered species used in the studied (NT= Near Threatened; VU= Vulnerable; EN Endangered; CR=Critically endangered, IUCN State of Conservation Categories) (* = the number comes from 2 species) (B = during sampling of specimens for the American Museum of Natural History).

Conclusions

This paper catalogs studies of environmental pollution by metals and metalloids in wildlife inform Latin America for the first time. We found high levels of metal and metalloid contamination in the three geographical areas from Latin America (Angeli et al., 2013; Barrera-García et al., 2012; Cabrera Páez et al., 2012; Cortés-Gómez et al., 2014; Fielding and Evans, 2014), with industrial activity, intensive agriculture, urban contamination indicated as the main emission sources (Almeida Savassi et al., 2016; Arrieta et al., 2001). In some cases, the contaminant levels detected in certain species have been among the highest globally (Bezerra et al., 2014, 2015). Several studies conducted on species of interest for human consumption show values that exceed safety limits; for instance, Barrera-García et al. (2012), detected 1.69 ± 0.18 μg/g−1 ww Hg level in shark sample from Mexico, while the limit established for human consumption by international agencies, such as US Food and Drug Administration, World Health Organization (WHO) and the Mexican Official Norm (NOM 242-SSA1) is 1.0 μg/g−1 ww. The study by Akagi and Naganuma (2000) detected levels of Hg ranged from 0.08 to 3.82 ppm in fish from the Tapajos River system, Brazil. In this case the levels of mercury exceeded the Brazilian permitted limit of 0.5 ppm. Other studies show that despite detecting levels of pollution below the limits, they can represent risks for health to pregnant women, children and those populations that base their diet almost exclusively on aquatic animals coming from contaminated areas (Bourdineaud et al., 2015; Schneider et al., 2015).

We provide here a useful database for researchers and governments to develop environmental research strategies, promoting the protection of biodiversity, including human beings. One of the gaps observed in Latin Americas research is the lack of a single working protocol that facilitates the comparison of data, unifying the results of the different studies. It would be advisable that the different countries create biomonitoring schemes of contaminants and adopt a common protocol, as it has been done in others regions of the world (eg. García-Fernández et al., 2008; Gómez-Ramírez et al., 2014). The development of research with biomonitors rather than bioindicators, which currently represent most studies, should be a priority. Following the global trend, we suggest researchers should use non-lethal sampling techniques and use non-threatened species as biomonitoring, increasing studies on subrogate species. The use of sentinel species with a high position in the food chain would be advisable; in this way the results could be used to detect possible risks to human's health at an early stage. Considering the possible effects of chronic exposure at doses slightly below the safety limits established for food resources and the needs of the population most sensitive to contaminants, further research should be undertaken to define safety limits more in line with these conditions. To assess their actual pollution status, in addition to aquatic ecosystems, terrestrial ecosystems should also be the subject of further research.

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CHAPTER II

Mercury in the feathers of bird scavengers from two areas of Patagonia (Argentina) under the influence of different anthropogenic activities: a preliminary study

Photo: Alessandro Di Marzio

Resumen

El mercurio (Hg) es un contaminante ubiquitario, con capacidad de bioacumularse y biomagnificarse en la cadena trofica, cuyos efectos adversos se han registrado tanto en los seres humanos como en la fauna silvestre. Muestreamos plumas de aves carroñeras para evaluar los niveles de Hg en dos áreas del norte de la Patagonia Argentina. Analizamos muestras de jote colorado, jote negro y carancho procedentes de dos zonas de muestreo: “Bariloche” y “El Valle”. Se detectó Hg en todas las muestras analizadas; los niveles de Hg detectados pueden considerarse bajos para las tres especies en ambas zonas de muestreo. La concentración media de Hg en “Bariloche” fue de 0,22 ± 0,16 mg/kg de peso seco (p.s.) en jote negro, 0,13 ± 0,06 mg/kg p.s. en jote colorado y 0,13 ± 0,09 mg/kg p.s. en carancho; en “El Valle”, la concentración media de Hg fue de 1.02 ± 0.89 mg/kg p.s. en el jote negro, 0.53 ± 0.82 mg/kg p.s. en jote colorado, y 0.54 ± 0.74 mg/kg p.s. en carancho. Los niveles de Hg detectados se explicaron por la zona de muestreo, pero no por la especie. Los niveles de Hg fueron comparables a los niveles obtenidos en otros estudios de aves rapaces terrestres y acuáticas. Las especies del presente estudio se encuentran en gran parte del continente americano. Podrían ser biomonitores apropiados en toda la zona de distribución de las especies; pudiendo ser utilizadas como especies sustitutas, especialmente en las zonas de distribución compartidas con carroñeros en peligro de extinción como el cóndor de California y el cóndor andino.

Abstract

Mercury (Hg) is a global pollutant that bioaccumulates and biomagnifies in food chains and is associated with adverse effects in both humans and wildlife. We used feather samples from bird scavengers to evaluate Hg concentrations in two different areas of Northern Patagonia. Hg concentrations were analyzed in feathers obtained from turkey vultures, black vultures, and southern crested caracaras from the two areas of Northern Patagonia (Argentina): Bariloche and El Valle. Hg was detected in all the samples analyzed, but the concentrations can be considered low for the three species in both sampling areas. The mean concentration of Hg in Bariloche was 0.22 ± 0.16 mg/kg dry weight (d.w.) in black vulture, 0.13 ± 0.06 mg/kg d.w. in turkey vulture, and 0.13 ± 0.09 mg/kg d.w. in southern crested caracara; in El Valle, the mean concentration of Hg was 1.02 ± 0.89 mg/kg d.w. in black vulture, 0.53 ± 0.82 mg/kg d.w. in turkey vulture, and 0.54 ± 0.74 mg/kg d.w. in southern crested caracara. Hg concentrations in feathers were explained by the sampling area but not by the species. The concentrations of Hg contamination were comparable to those obtained in other studies of terrestrial raptors and aquatic bioindicator raptors. The species of the present study occur throughout much of North and South America. Thus, they may be appropriate bioindicators across the species' range, which is particularly useful as a surrogate, especially in distribution areas shared with endangered scavengers such as the California condor and the Andean condor.

Introduction

Mercury (Hg) is a persistent, toxic heavy metal, with a tendency of bioaccumulation and biomagnification across food chains (Eisler 1985). Primary sources of anthropogenic Hg emissions include the combustion of fossil fuels, mining and reprocessing of ores (gold, copper, lead, and zinc), iron, and steel, and cement production, operation of chlor-alkali plants, and waste incineration and disposal (Pacyna et al. 2006; Driscoll et al. 2007). Hg poisoning can result in reproductive, neurological, hematologic, and cellular disorders (Solonen and Lodenius 1984; Wolfe et al. 1998; Nichols et al. 1999; Espín et al. 2014a, b). Several studies indicate that Hg has immunotoxic effects (dose-dependent stimulation/suppression of lymphocyte response) (Ortega et al. 1997; Fallacara et al. 2011). Inorganic Hg is biotransformed by methylation processes (biotic and abiotic) to organomercury (i.e., methylmercury), becoming more toxic than inorganic Hg (Morel et al. 1998). Both paths have not been fully investigated, but the existing evidence indicates that the biotic pathway is the most common, as it is related to sulfate-reducing bacteria present in aquatic environments, which are responsible for 95% of the biomethylation (Broo and Odsjo 1981; Eisler 1985; Burger and Gochfeld 1997; PNUMA 2002; Rigét et al. 2007).

Birds have been widely used for assessing the levels of certain contaminants, especially metals (Furness and Greenwood 1993; García-Fernández et al. 2008; Lodenius and Solonen 2013; García-Fernández 2014). Due to their feeding habits, waterbirds present higher Hg concentrations compared to other birds as shown in many waterfowl contamination studies (Cahill et al. 1998; Monteiro and Furness 2001; Champoux et al. 2006; Sanpera et al. 2007; Rattner et al. 2008; Ribeiro et al. 2009; Espín et al. 2012). Therefore, aquatic ecosystems have been most frequently studied. However, there is evidence that the methylation process applies to terrestrial ecosystems as well, and some studies have found significant concentrations of this metal in terrestrial birds of prey (Broo and Odsjo 1981; Palma et al. 2005; Zolfaghari et al. 2007; Espín et al. 2014a, b). In this sense, several authors have reported significantly higher metal concentrations in birds inhabiting mining areas than in those from unpolluted or reference sites (Henny et al. 1994; García-Fernández et al. 2005; Gómez-Ramírez et al. 2011). Besides the pollution from mining areas, one of the main sources of anthropogenic Hg in the environment is chlor-alkali plants, and the effects can be observed long after the plants have ceased operation (Parks et al. 1984).

The analyses of Hg in feathers have been widely used, demonstrating that they are very useful as a non-invasive and non-lethal alternative to internal tissues (Martínez-López et al. 2004, 2005; Garitano-Zavala et al. 2010; Espín et al. 2012, 2014a, 2014b). Their collection and storage are fast and low-cost. In addition, Hg can also be analyzed in feathers from museums, which may offer valuable information about the temporal trends of contamination (Furness and Greenwood 1993; Ansara-Ross et al. 2013; García-Fernández et al. 2013). Hg is accumulated in the feathers during their growth by binding to disulphide bonds (Leonzio et al. 2009; Zolfaghari et al. 2009; García-Fernández et al. 2013), and Hg concentration in the feathers is correlated to its concentration in the blood (Solonen and Lodenius 1990; Monteiro and Furness 2001; Ansara-Ross et al. 2013; Lodenius and Solonen 2013). Once the growth process is completed and vascular connections that feed the feather atrophy, Hg concentrations do not vary significantly over time (García-Fernández et al. 2013).

Between 2005 and 2010, global industrial use of Hg cell chlorine has decreased by 30%. Although in Argentina, and other South American countries, regulations on the use of products, substances, and residues that contain Hg have been established in order to protect health and biodiversity, there is no regulation regarding the treatment of Hg waste (CRBAS 2012). Moreover, studies evaluating Hg in sediments and biota of the upper Negro River basin (Northern Patagonia, Argentina; Arribére et al. 2003) showed the probable influence of a chlor-alkali plant long after its closure in 1999. In this study, we use feather samples of scavenger birds in two contrasting areas of Northern Patagonia—one with a history of Hg contamination due to the presence of a chlor-alkali plant and the other with no history of contamination. We then compare our results with several other species of birds of prey with different feeding habits (including scavengers, hunters, and fishers) and discuss their implications for highly threatened scavenger species, which share the same areas with our focal species.

Materials and methods

Study area

The sampling was conducted during the austral spring of 2011 (October to December), as part of a preliminary study to determine the distribution of nests and roosts of the three species of this study in two previously not studied areas of Patagonia (Argentina) (Fig. 1), carried out by an interdisciplinary team formed by researchers from the University of Murcia (Spain) and CONICET-Universidad Nacional del Comahue de Bariloche (Argentina). The first study area (El Valle) includes the Rio Negro Valley (Cipolletti and Allen cities) (Fig. 1), an area of 100,000 ha (over 40,000 ha are used for agriculture) (Pozo 2013; Romero Gámez 2013). The average elevation of the area is 270 m above mean sea level (amsl); the climate is dry and cold desert (climatic classification of Köppen), with an average annual temperature of 14.5 °C and annual rainfall of 186.9 mm (Bustos and Rocchi 2008). The ecotone represented is the steppe. As already mentioned above, there was a chlor-alkali plant, active from 1950 to 1995 (Arribére et al. 2003) in the city of Neuquén (Fig. 1), and contamination by Hg in rivers has been detected more than 100 km downstream from the point of emission (Jackson et al. 2011). Thus, the presence of a gold and silver mining area of Andacollo in the area of Huaracu stream, Neuquén River tributary, between the towns of Andacollo and Huinganco should be taken into account. This area has a long mining tradition, which has been used by the Chilean-Canadian mining company “Andacollo Gold” since 2001. This company has received complaints from the inhabitants of Andacollo who accuse it of polluting the Huaraco stream (Ortiz 2008; Bellotti 2011). The second study area (Bariloche) was nearby San Carlos de Bariloche city, between the provinces of Neuquén and Rio Negro (Fig. 1), a rural area with low human population density (Rizzo et al. 2011). The average elevation of the area is 893 amsl; the climate is humid continental (climatic classification of Köppen), with an average annual temperature of 8.1 °C and total rainfall of 782.6 mm. The ecotone represented is the subantarctic forest (Bustos and Rocchi 2008). The area includes the Nahuel Huapi National Park with its main lake Nahuel Huapi and several other lakes. The main economic activity of the area is represented by nature tourism.

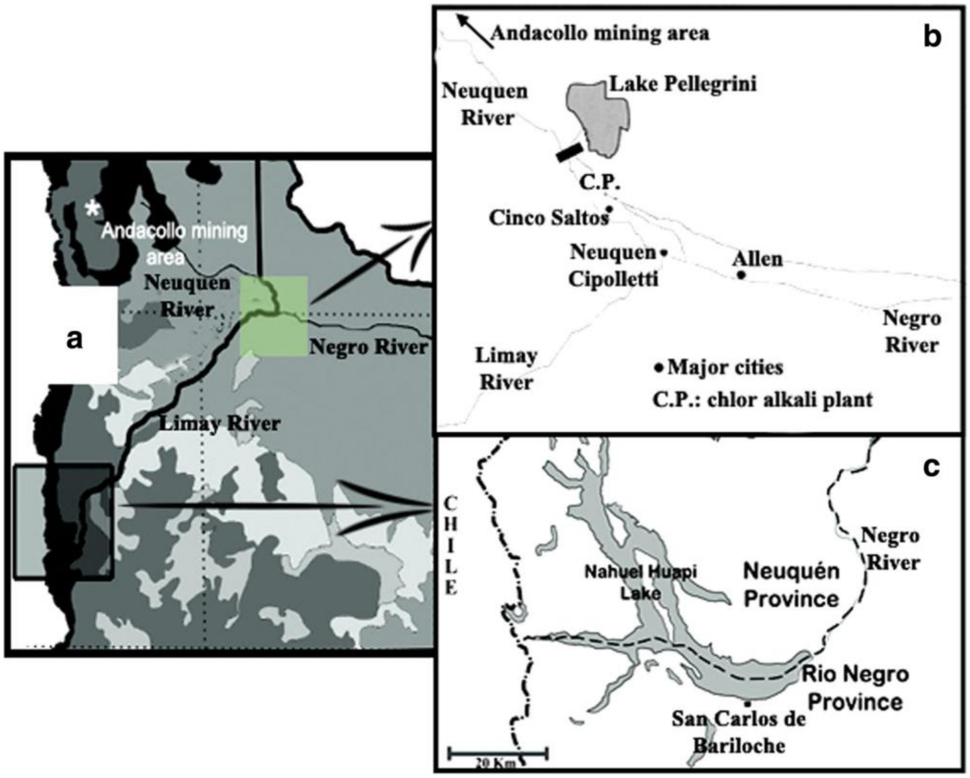

Fig.1

Geographical representation of the study area. a) Sampling areas (* represent the location of Andacollo mining ares, Huarachu stream, and Andacollo village). b) Sampling area “El Valle”, c) Sampling area “Bariloche”

Species

The species studied were the turkey vulture (*Cathartes aura*) and black vulture (*Coragys atratus*), both species belonging to the family Cathartidae, plus another species of facultative scavenger bird of the American continent, the southern crested caracara (*Caracara plancus*). This species, unlike the previous two, belongs to the family Falconidae (Ferguson-Lees and Christie 2001). Turkey vulture is the most widely distributed New World vulture species. Its distribution area ranges from South Canada to Tierra del Fuego (Argentina/Chile) (Ferguson-Lees and Christie 2001). The distribution area of black vulture covers the majority of Central and South America, including the Atlantic states of USA (Ferguson-Lees and Christie 2001). The distribution of this species has been expanding, following the expansion of human settlements (Evans 2013, Novaes and Cintra 2013; Barbar et al. 2015; Ballejo et al. 2017). The distribution area of southern crested caracara includes South America (except Amazonia, Colombia, Peru, and high Andes), several Caribbean islands, Mexico, and is rarely encountered in the southern USA. (Ferguson-Lees and Christie 2001). Black vulture and turkey vulture are scavenger birds, while southern crested caracara is an opportunistic scavenger (Ferguson-Lees and Christie 2001). The diet of black vulture and turkey vulture consists of carcasses of mammals, dead or stranded fish, insects, scraps from waste dumps and seabird colonies, occasionally reptiles, and eggs, and nestlings of herons (Ardeidae) and seabirds (Ferguson-Lees and Christie 2001; Haskins et al. 2013). A recent study conducted in Mexico shows the reliance of turkey vulture on the remains of fish (Blázquez et al. 2016). The diet of southern crested caracara is primarily carcasses, juveniles, injured, slow-moving birds, little rodents, reptiles, amphibians, fish, and arthropods (Ferguson-Lees and Christie 2001). Although we have no complete information on the feeding patterns of the three species in our study area, it has been found that the main food source of black vultures in “Bariloche” are the remains of slaughterhouse and exotic wildlife hunting, followed by arthropods (Ballejo and De Santis 2013). Both species of vultures are scavengers, but while black vultures mainly feed on remains of domestic animals, the diet of turkey vulture includes reptiles, fishes, and small rodents (Ballejo et al. 2017). As the three species also consume aquatic animals, they can be exposed to Hg from the aquatic environment in addition to Hg from terrestrial ecosystems. Another possible way to accumulate Hg from the aquatic environment can be the consumption of arthropods (Cristol et al. 2008). In this part of Patagonia, the black vulture is the most abundant species in urbanized areas, feeding from landfills and fishing areas (Bellati 2000; Ferguson-Lees and Christie 2001; Ballejo and De Santis 2013), while the southern crested caracara and especially the turkey vulture are species that prefer countryside (Bellati 2000; Ferguson-Lees and Christie 2001).

The black vulture and the southern crested caracara are resident birds, while the turkey vulture is migratory (Bildstein 2004). The northwestern Patagonian population of turkey vulture migrates to northern South America in winter (Graña Grilli et al. 2017). Black vulture can be considered site faithful. Several studies show that the home range of this species is approximately 30–40 km. In the case of turkey vulture, this range can reach 60–70 km (Coleman and Fraser 1989; De Vault et al. 2004; Holland et al. 2017). Site faithfulness can also be justified by the strong social bond and the aggressiveness of black vulture community that reduce the number of non-local vultures in the communal roosts (Buckley 1999).

There is scarce information on the molting patterns of the species under study. Southern crested caracara, as other medium-small-sized Falconidae, undergoes a complete molt each year (Ferguson-Lees and Christie 2001). As phylogenetic relationships of Cathartidae to other avian groups remain unresolved, it is difficult to compare with other species, and direct studies are insufficient (Chandler et al. 2010). Thus, the interpretation of molt patterns of Cathartidae is more complex. It is known that the molt of turkey vulture is serial. It starts by molting of the primary feathers (P1–P10). The molt of P1–P4 is completed before the reproductive period, followed by a suspension during the reproductive period, a resumption before the winter migration and suspended again during winter (Chandler et al. 2010). This strategy is similar to the California condor (*Gymnopsys californianus*), which lasts 2 years (Snyder et al. 1987).

Due to the limited number of Hg studies in scavenger birds, a bibliographic search including other birds of prey was done. Thus, both aquatic and terrestrial-based raptor species were chosen, in order to compare Hg concentrations in birds living in the same area, but foraging in different ecosystems. To check whether our Hg contamination results from both sampling areas could be considered contaminated, they were compared with other studies in the same species from other areas (Fig. 2).

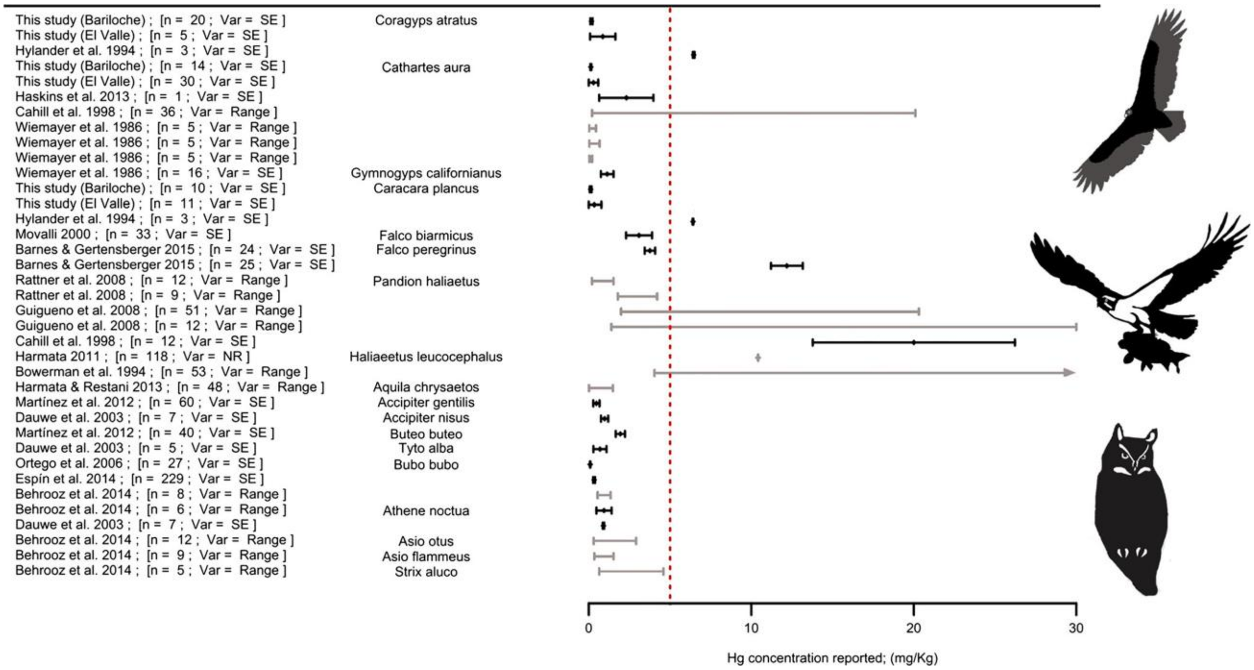

Fig. 2 Summary of Hg studies in feathers from different species, including scavengers around the world

Sample collection

Only fresh-molted primary flight feathers (P1–P10) of adult individuals collected from the roosting areas were used in this study. The sampling was conducted during the austral spring of 2011 (October to December); this time of year, covers the courtship, mating, and breeding periods of the species studied in the sampling region. Due to the type of sample and the sampling methodology, age or sex of the individuals is unknown. To reduce the possibility of pseudo replication, only one feather per sampling point (or more than one if it was the same primary feather from the same wing) was collected. Each sample was kept in an individual plastic bag, labeled (study area, day, and species), and stored at room temperature in a dry place until analysis. A total of 90 primary feathers were collected: 44 in "Bariloche" from black vultures (n =20), turkey vultures (n = 14), and southern crested caracaras (n = 10) and 46 in "El Valle" from black vultures (n = 5), turkey vultures (n = 30), and southern crested caracaras (n =11) (see more details in Martínez-López et al. 2015).

Hg analysis

In order to remove external contamination from the surface of the feathers, a washing process was performed prior to analytical determination sequentially using tap water, distilled water, and Milli-Q® water (ISO 3696). The feathers were subsequently dried at room temperature overnight (Espín et al. 2012). Total Hg was analyzed in a Milestone DMA-8 Direct Hg Analyzer by atomic absorption spectrophotometry, with a detection limit of 0.005 ng. The whole feathers were individually cut, mixed shaft and vane, and stored in sterile bottles. Then the subsamples (0.5 g dry weight for vane and shaft) were loaded in nickel boats and analyzed, following USEPA Method 7473 (sediments, soils, and sludges). The applicability of this method to the analysis of biotic samples has been previously demonstrated (Haynes et al. 2006). The calibration curve was calculated with 11 points (in duplicate) from 0 to 1004 ng of Hg. The precision and accuracy of the method were tested using certified reference material (CRM) (n = 11; Hg standard for AAS, Fluka, 1000 mg/L Hg in 12% nitric acid). Recovery of total Hg from seven replicates of CRM diluted to 1 ppm was 98.14 ± 3.52% (mean ± standard deviation). The coefficient of variation for repeatability was 3.58%.

Statistical analysis

All analyses were carried out using the SPSS v.15.0 statistical package. Reported Hg concentrations represent median, mean ± standard deviation, and range. We used the Mann-Whitney test for the comparison between species. We used generalized linear models (GLM, normal distribution) to analyze the concentrations of Hg in each sample, using Hg concentration in each sample as the response variable. The explanatory variables considered were the study area and species. Four models were compared: (a) the null model, (b) the model with the variable “study area,” (c) the model with the variable “species,” (d) the model with two variables (study area + species), and finally, (e) the model with the interaction of both variables (study area × species). The level of significance for these tests was set at $\alpha = 0.05$. Furthermore, the quality of each model relative to each of the other models was estimated by applying the Akaike information criterion (AIC) on the collection of model data (Burnham and Anderson 2002).

Results and discussion

Hg concentrations in feathers

Hg concentrations were detected in all feather samples. In “Bariloche” area, Hg concentrations were similar between species; but in “El Valle”, the highest mean and median concentrations (1.02 mg/kg and 0.86 mg/kg, respectively) were found in the black vulture. The two highest concentrations of Hg feather concentrations were found in a turkey vulture (4.20 mg/kg) and in a southern crested caracara (2.61 mg/ kg) (Table 1).

Hg (mg/kg)	**Bariloche**	**El Valle**	**All areas**
Black vulture (*Coragyps atratus*)	0.17 0.22 ± 0.16 (SD) (R 0.09–0.65) (n = 20)	0.86 1.02 ± 0.89 (SD) (R 0.23–2.44) (n = 5)	0.18 0.38 ± 0.51 (SD) (R 0.09–2.44) (n = 25)
Turkey vulture (*Cathartes aura*)	0.13 0.13 ± 0.06 (SD) (R 0.06–0.25) (n = 14)	0.29 0.53 ± 0.82 (SD) (R 0.04–4.2) (n = 30)	0.17 0.4 ± 0.7 (SD) (R 0.04–4.2) (n = 44)
Southern crested caracara (*Caracara plancus*)	0.12 0.13 ± 0.09 (SD) (R 0.03–0.36) (n = 10)	0.34 0.54 ± 0.74 (SD) (R 0.09–2.61) (n = 11)	0.15 0.35 ± 0.56 (SD) (R 0.03–2.61) (n = 21)
All species	0.14 0.17 ± 0.12 (SD) (R 0.03–0.65) (n = 44)	0.33 0.59 ± 0.81 (SD) (R 0.04–4.2) (n = 46)	0.17 0.39 ± 0.62 (SD) (R 0.03–4.2) (n = 90)

Table 1 Concentrations of mercury for the three species of the study in the two sampling areas (median (in bold), mean ± standard deviation (SD), between parenthesis range (minimum/ maximum) (*n* = number of samples)

However, no significant differences among species were found. In fact, the application of GLM to study the effect of the sampling area and species on concentrations of Hg in feathers shows that the model including only the variable "study area" was significant (D2 = 72.89, p = 0.005) and with the best Akaike index (AIC = 1225.12). The value was significantly higher in "El Valle" than "Bariloche" for the three species (Table 1). As mentioned above, "El Valle" is considered contaminated by Hg as a result of the activity of a chlor-alkali plant (Arribére et al. 2003). These results are consistent with the data from several Hg and heavy metal contamination studies, carried out using samples of sludge and biota from rivers and lakes from the same area

(Guevara et al. 2002; Arribére et al. 2003; Rizzo et al. 2011). We compared our results with other studies in feathers of different bird species, including scavengers, from different areas around the world (Fig. 2). The study of black vulture and southern crested caracara (Hylander et al. 1994) in Alto Pantanal (Brazil) was used to represent a contaminated area. Alto Pantanal has a large history of Hg pollution due to the gold mining activities (Alho et al. 1988). The mean concentration of Hg in feathers of black vulture and southern crested caracara from Alto Pantanal was 6.2 mg/kg and 6.7 mg/kg, respectively (Hylander et al. 1994). Those concentrations are much higher than the mean concentrations of turkey vulture (0.53 mg/kg; n =30) and southern crested caracara (0.54 mg/kg; n = 11) detected in “El Valle”. We are talking about two areas distant 3000 km, with different ecoregions (Patagonian steppe vs. Tropical wetland) (Olson et al. 2001) and with two different stories of environmental pollution. For these reasons, we may consider "contaminated" the "El Valle" zone locally, even though these concentrations cannot be compared to the levels of other contaminated areas.

Some considerations need to be made regarding the interpretation of the black vulture results. Due to the lack of previous information regarding the area and species, only five samples of black vulture feathers were obtained for our study. In addition, one of these five samples showed a high Hg concentration (2.44 mg/kg). If this outlier was not considered, the mean Hg concentration of this species (0.66 mg/kg of Hg) remained similar to the values of turkey vultures and southern crested caracaras from “El Valle”. Nevertheless, due to a scarce number of samples of black vulture in “El Valle” these data must be considered relative.

Our results could not be compared with those found by Haskins et al. (2013), as primary and secondary flight, and tail feathers from a single specimen of turkey vulture were analyzed, with the aim to determine the element composition of vane and rachis structures. On the other hand, Cahill et al. (1998) found an average of 1.26 mg/kg Hg (n = 36) in turkey vulture feathers, higher than in the same species from “El Valle” (0.53 mg/kg Hg, n = 30), found in our study. In this case, the difference could be due to the different degree of contamination of the two areas. Clear Lake (California) has a history of contamination due to the activities of now abandoned Sulphur Bank Hg Mine that poured 100 tons of Hg into the lake between 1872 and 1957 (Suchanek et al. 1993, 1998). The contamination of “El Valle” is mainly due to the activity of an alkali chlorine plant. The plant, built on an island within the Neuquén River, poured its wastewaters into a series of drainage pools from 1951 to 1979 (Arribére et al. 2003). After 1979, until its closure in 1995, water was stored in settling and drying pools (Arribére et al. 2003). The estimated annual discharge value of the plant is approximately 500 kg/ Hg/year (CRBAS 2012). This difference is reflected by the Hg concentrations detected in the sediment samples (18.3 mg/kg in Clear Lake; 1.3 mg/kg in the nearest sampling point to the area of higher contamination “El Valle”) (Suchanek et al. 1998; Arribére et al. 2003). In the case of “Bariloche” we can assume that this is a less polluted area than “El Valle” with mean concentrations and range of Hg lower than those detected in “El Valle” (mean Hg concentration “Bariloche” 0.6 mg/kg, range 0.49–0.6 mg/kg; mean Hg concentration “El Valle” 1.2 mg/kg, range 0.75–5.1 mg/kg) (Arriberé et al. 2003; Guevara et al. 2002). The levels found in “Bariloche” are similar to those found in areas considered to have received low levels of pollution and are similar to the study of turkey vulture in California, USA, by Wiemeyer et al. (1986), with mean concentrations of 0.11 mg/ kg (n = 5, female breeding), 0.12 mg/kg (n = 5, male non-breeding), and 0.098 mg/kg (n = 5, female non-breeding). Due to the scarcity of research on these species, no further studies have been found to contrast our results in an area of low contamination.

Risk assessment

The scarce number of studies relating Hg concentrations in feathers and their corresponding effects (e.g., toxic effects levels) makes further interpretation difficult. According to a study on black-headed gull chicks (*Chroicocephalus ridibundus*), concentrations of Hg from 5 to 40 mg/kg in feathers were associated with reproductive disorders (Lewis and Furness 1991). Studies with several bird species indicate that 40 mg/kg of Hg in feathers is associated with fertility problems, reproductive disorders, low hatching rate, and survival of chicks (Finley and Stendell 1978; Solonen and Lodenius 1984; Eisler 1985). Eisler (1985) indicates that Hg concentrations of 9–11 mg/kg in feathers cause reproductive and behavioral deficits in domestic mallards (*Anas platyrhynchos*). However, Bowerman et al. (1994) detected Hg concentration between 13 and 20 mg/kg in bald eagles (Haliaeetus leucocephalus) without associated signs of reproductive problems or decreasing population size. Scheuhammer (1991) hypothesizes that Hg concentrations above 20 mg/kg (dry weight) in feathers of growing piscivorous birds can result from diets containing Hg concentrations greater than 1 mg/kg (dry weight) and that these concentrations should be considered as indicative of a wetland habitat that may pose a significant threat to the reproductive success of piscivorous wildlife breeding there. In any case, Scheuhammer (1991) considered Hg concentrations of 1– 5 mg/kg (dry weight) in feathers of raptor birds as “normal”. Other authors consider Hg concentrations greater than 5 mg/kg in feathers as “dangerous to birds” (Burger and Gochfeld 1997; Palma et al. 2005; Eisler 2006; Albuja et al. 2012). No concentrations higher than 5 mg/kg were detected in this study. If we take the concentration of 5 mg/kg in this study as a reference, only one sample of turkey vulture from the El Valle area is close to this concentration (4.2 mg/kg). On the other hand, it should be pointed out that any of these studies quantify the possible sub-lethal effects at these concentrations, which might be relevant for long-living species, such as the scavenger birds in our study. In addition, as mentioned above, feathers trap Hg during their growth phase and are molted every year. Therefore, the results obtained from the analysis of the feathers must take this aspect into account.

Conclusions

This study brings together the concentrations of Hg in feathers of 69 samples of family Cathartidae and 21 *Caracara plancus*, and this is the first study of this magnitude of birds from Patagonia. The results of this study regarding the contamination of Hg coincide with the results of different studies in the same areas, suggesting higher concentrations of Hg contamination in “El Valle” than in “Bariloche”. The three species are common throughout the American continent (North, Central and South), have a high position in the trophic chain and the few studies where they have been used to determine Hg contamination in different environments of the American continent have some consistency of results. Therefore, we can consider valid the hypothesis that black vulture, turkey vulture, and southern crested caracara are good candidates for future biomonitoring studies. However, more studies are needed to assess more relationships between the values obtained. Another relevant aspect is that the three species share habitats with two endangered scavenger birds, the Californian condor and the Andean condor. Therefore, the results obtained with the species of this study might be relevant to evaluate risk to these endangered condors. This study is an important step in the collection of data on North Patagonia, the phenomena of Hg contamination in terrestrial ecosystems and the New World scavenger species, all of which have been little studied. Our results can be used as a comparison with future studies.

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CHAPTER III

Temporal changes in metal concentrations in Andean condor feathers: a potential influence of volcanic activity

Photo: Federico Grosso fotografía

Resumen

Las actividades geotérmicas (ej. erupciones volcánicas) representan una de las fuentes naturales más importantes de emisiones de metales (metales pesados y metaloides). Pueden ser uno de los principales riesgos para los ecosistemas en regiones como el norte de la Patagonia Argentina, una zona escasamente poblada, cerca de una extensa red de volcanes activos en la Cordillera de los Andes. La erupción de 2011 del Complejo Volcánico Puyehue-Cordón Caulle (Volcán Puyehue) ha sido el mayor evento volcánico de las últimas décadas. Los efectos de la exposición a las cenizas en la vida silvestre y los seres humanos han sido escasamente estudiados, y sólo un estudio de biomonitoreo ha utilizado especies de alta posición en la cadena trófica. La exposición a los metales de las especies de la Patagonia ha sido poco estudiada. Los principales objetivos de nuestro estudio fueron evaluar la detección de metales y valorar una posible relación entre los niveles de metales en la población de cóndor andino (*Vultur gryphus*) y la actividad volcánica de la zona. Investigamos los efectos de la erupción del Volcán Puyehue en 2011, utilizando muestras de plumas primarias de muda del cóndor andino, recogidas en nueve dormideros en los alrededores de Bariloche, Argentina (distancia máxima 85 km). Los datos disponibles sugieren que la muda de las plumas primarias del cóndor andino tiene una duración de 6 años. Se realizaron muestreos antes (2007, 2009) y después (2017) de la erupción volcánica (2011). Las plumas muestreadas en 2017 deberían haberse desarrollado en 2011-2012, reflejando la situación ambiental del período inmediatamente posterior a la erupción del Volcán Puyehue. Por primera vez, hemos examinado los metales en 48 plumas primarias fundidas de cóndor andino, mostrando los niveles de 9 metales y metaloides (Si, Cr, Cu, Zn, As, Se, Cd, Pb, Hg). Si, Zn, As, y Cd mostraron niveles más altos en las plumas muestreadas después de la erupción. Los niveles de Cr y Pb (aunque aparentemente no están relacionados con la erupción volcánica) en algunas muestras son compatibles con los posibles efectos adversos en los organismos vivos. Los resultados del examen representan una importante base de datos (la primera para esta especie) que puede utilizarse en futuros estudios con fines comparativos.

Abstract

Geothermal activities (e.g., volcanic eruptions) represent one of the most important natural sources of metal emissions (heavy metals and metalloids). They can be one of the main risks for the ecosystems in regions like North of Argentiniean Patagonia, a sparsely populated area, close to an extensive network of active volcanoes on the Andes Range. The 2011 eruption of the Puyehue-Cordon Caulle volcanic complex (PCCVC) has been the largest volcanic event of the last decades. The effects of exposure to ashes on wildlife and humans have been sparsely studied, and only one biomonitoring study has used higher trophic species. The exposure to metals of the species in Patagonia has been poorly studied. The main objectives of our study were to assess metal screening and to evaluate a possible relation between the levels of metals in the Andean condor (*Vultur gryphus*) population and the volcanic activity of the area. We investigated the effects of the eruption of the PCCVC in 2011, using samples of molt primary feathers of the Andean condor, collected in nine roosts around Bariloche, Argentina (maximum distance 85 km). Data available suggest the molt of the primary feathers of the Andean condor has a duration of 6 years. We carried out sampling before (2007, 2009) and after (2017) the volcanic eruption (2011). The feathers sampled in 2017 should have been developed in 2011–2012, reflecting the environmental situation of the period immediately following the eruption of the PCCVC. For the first time, we have screened metals in 48 molted primary feathers of Andean condor, showing the levels of 9 metals and metalloids (Si, Cr, Cu, Zn, As, Se, Cd, Pb, Hg). Si, Zn, As, and Cd showed higher levels in the feathers sampled after the eruption. The levels of Cr and Pb (although apparently not related to the volcanic eruption) in some samples are compatible with potential adverse effects in living organisms. The screening results represent an important database (the first for this species) that can be used in in future studies for comparative purposes.

Introduction

The north of Argentinian Patagonia is a sparsely populated territory, in many cases with difficult access. These factors have long protected these areas from anthropogenic pollution. The presence of large National Parks, such as the Nahuel Huapi (> 700,000 ha), has also contributed to the protection of this territory (Arribére et al. 2008, 2010; Rizzo et al. 2010). In spite of this, in the last 60 years, the development of human settlements, such as the city of San Carlos de Bariloche, has generated an increase in pollution by metals (heavy metals and metalloids) due to anthropogenic causes, such as tourism, fishing, untreated urban effluents, hydrocarbon extraction, and the activity of industrial areas (Arribére et al. 2010; Bubach et al. 2015; Rizzo et al. 2010). However, historically and nowadays, a main cause of pollution has been natural sources of metal emissions such as volcanic eruptions, a known source of this type of contamination. (Lee 1996), emitting also substantial quantities of toxic volatile trace metals into the atmosphere and oceans (Rubin 1997). The relatively high volatility of metals and many of their compounds suggests that a high temperature dispersal process is involved, and thus the volcanism appears to be a potentially significant factor (Mroz and Zoller 1975). Of particular environmental concern is the capacity of some metals (e.g., mercury, cadmium, lead) to bioconcentrate according to particular toxicokinetic processes and also their toxicity.

In the last 10 years, there have been several volcanic eruptions on the Chilean side of the Andes (Calbuco volcano in 2015; Puyehue-Cordon Caulle volcanic complex (PCCVC) in 2011; Chaitén volcano in 2008), which have directly affected northern Argentine Patagonia, the foraging areas of the Andean condor (*Vultur gryphus*) population of our study (Daga et al. 2014; Pistolesi et al. 2015). The eruption of PCCVC in 2011, the nearest to the area and lasting 8 months (Alarcón et al. 2015), had the biggest impact in NW Patagonia. More than 950 million tons of ash were expelled during the first 3 months of eruptive activity, most of which fell on the Argentine Patagonian steppe (Gaitán et al. 2011). Studies have been conducted after 2011 eruptions using plant species or zooplankton as bioindicators (Balseiro et al. 2014; Bubach et al. 2012; Conti et al. 2016; Juncos et al. 2014). Flueck and Smith-Flueck (2013a, 2013b) carried out some studies to evaluate fluoride effects on wild ungulates (Cervus elaphus) after PCCVC eruption, finding pathological development which could affects several ecological aspects of the deer communities and other parts of the ecosystem such as scavenger and plant communities (Flueck 2013, 2014;

Flueck and Smith-Flueck 2013a, 2013b). Although Wilson et al. (2013) evidenced the need to study and monitor the health impact risks in order to detect possible effects on human health, only one study has been carried out in this area to test whether volcanic ashes could promote inflammatory reactions in the epithelial cells of the human conjunctiva (Tesone et al. 2018). On the other hand, only one study has been carried out with higher trophic species (Andean condor, Plaza et al. 2019), evaluating blood metal levels and detecting the increase of respiratory pathologies related to the ashes present in the air.

Because of the high trophic level (i.e., apex predators and scavengers) in the terrestrial food chain and their wide distribution, birds of prey are exposed to contamination. For this reason, they are considered good biomonitors (García-Fernández et al. 1997, 2020; García-Fernández 2014). Bird species exposed to metal contamination can suffer alterations of various types (neurological, reproductive, growth) that can affect their survival (Abbasi et al. 2015). The use of terrestrial raptor feathers as a biomonitoring unit for studies on metal contamination has proven to be an effective alternative (non-invasive and non-lethal) instead of using internal tissues for the same purpose (Espín et al. 2014a, 2014b; Martínez-López et al. 2004, 2005). During the growth phase, due to the bloodstream circulation and rapid turnover rate, the feathers act as a depot tissue of trace elements, representing a detoxification pathway. When the growth of the feather is over, it becomes a stable and long-lasting trace element storage unit (Burger 1993). The Andean condor is a species distributed throughout the South American Andes, has a long-life span, low reproductive rate, and depends on high adult survival to maintain a stable population (Lambertucci 2007). It is the largest scavenger bird in the world and, therefore, could be used as a biomonitor for environmental pollution studies.

The objective of this study was to evaluate, for the first time, the levels of several metals in feathers of the Andean Condor population living in the north-western of Argentinian Patagonia. We also assessed the potential influence of the eruption of Puyehue-Cordon Caulle Volcanic Complex (PCCVC) in 2011 as an important cause of elevated exposure to some metals. For that, nine essential and nonessential metals, copper (Cu), zinc (Zn), chrome (Cr), silica (Si), cadmium (Cd), lead (Pb), total mercury (Hg), arsenic (As), and selenium (Se) were measured in forty-eight primary feathers (P1-P10) of Andean condor sampled during the period 2007–2017.

Material and methods

Study area, study species and sampling collection

Northern Patagonia Argentina is a sparsely populated area, where cattle ranching and tourism represent the main economic activities. Our study area was located in the surroundings of the Nahuel Huapi National Park, north-western Patagonia, Argentina. The area is characterized by the presence of two natural environments, the Andean woodlands and the Patagonian steppe, between which there is a transition environment called ecotone (Paruelo et al. 1998). The area has intense volcanic activity associated to the Andes Mountains both in the Argentinian and mainly in the Chilean side of the Andes. The Puyehue-Cordon Caulle Volcanic (PCCVC) eruption deposited a lot of tephra (ashes) on about 36 million ha of Argentina in June of 2011. Due to the weather conditions (mainly western winds), the largest deposits of volcanic products are found in the territory of the Nahuel Huapi National Park (Argentina) and in the Patagonian Steppe around it (Perez Catán et al. 2016). The eruptions of the Chaitén Volcano in 2008 and Calbuco Volcano in 2015 were not considered because of the molting of the Andean condor sampled did not cover them (explained in the "Results and discussion" section). The Andean condor, the largest scavenger bird of the world, inhabits the whole South America continent throughout the Andes (Del Hoyo et al. 1994). Their diet consists of medium to large vertebrate carcasses (Lambertucci et al. 2009a). Worldwide the species is classified near threatened (NT), due to the negative effects of several human disturbances (Alarcón and Lambertucci 2018; IUCN 2017; Lambertucci et al. 2009b, 2011). The population in the north of its distribution is critically endangered, in Chile the species is classified as Vulnerable (Inventario Nacional de Especies de Chile 2019) and in Argentina as threatened (MAyDS y AA 2017). Locally, the situation is different; the Andean condor population in our study area is estimated at least in 300 specimens (Lambertucci 2010).

The Andean condor belongs to the Cathartidae family known as "New World Vultures", a group of scarcely studied birds. We do not have any data on the molt dynamics of the Andean condor. The only information comes from the California condor (*Gymnogyps californianus*) and proposed that they can perform a highly seasonal, highly variable in sequence and highly asymmetric molt of the primary feathers every 2 years (Snyder et al. 1987). In recent years several studies (Zhang 2015; Hackett et al. 2008) have found Cathartidae family is related to the Accipitridae family, which includes "Old World Vultures". Therefore, we indirectly quantify the duration of the condor molt, comparing it with the big "Old World Vultures", estimating that in the Andean condor, the molt of the primary feathers should be every 5–7 years, as in the vultures of the genus *Gyps* (Zuberogoitia et al. 2013). The accumulation of metals in the structure of the feathers is verified only during the time of growth (for California condor, 3–6 months) (Snyder et al. 1987; Finkelstein et al. 2010). Once growth is complete, the feathers become inert structures, unable to accumulate more metals (García-Fernández et al. 2013). For this reason, the use of molted feathers in 2017 allows us to evaluate the levels of metals present in the environment approximately during the 2011 volcano eruption.

Forty-eight molted primary feathers of the Andean condor were sampled in nine roosts of the species located in the Patagonian steppe, in the surroundings of Nahuel Huapi National Park, in a radius of 85 km from the city of Bariloche (direction NE, E, SE) (Fig. 1). Sampling was carried out in different years (2007/2009/2017) during the austral spring (October–December).

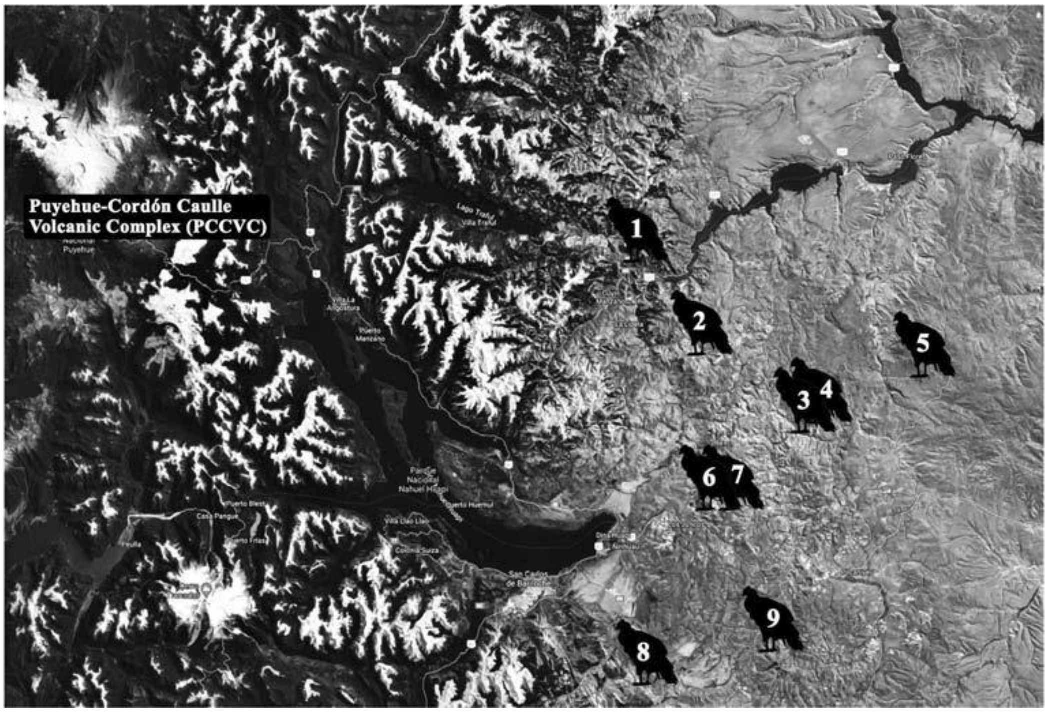


Fig. 1 Map of the study area in North-Western Patagonia, Argentina. Birds indicate the location of the condor's communal roost where we collected the feathers

Analytical procedure

Before the analysis of the feathers, they were subjected to a sequential series of baths with tap water, distilled water, and Milli-Q® water (ISO 3696) in order to eliminate possible contaminants deposited on the feathers. The feathers were dried at room temperature for 12 h (Espín et al. 2012). Once cleaned, the feathers were chopped to obtain a uniform sample of shaft and vane and stored in a sterile container. For the analysis of Si, Cr, Cu, Zn, As, Se, Cd, and Pb, the samples were placed in LDPE (low-density propylene) flasks with the addition of an acid mixture (nitric/perchloric/sulfuric, 8:4:1) for the organic matter disintegration (1 ml of acid mixture/ 100 mg of each feather). We transferred 1 ml of each predigested extract to a quartz tube, in order to dry the sample completely with a progressive heat treatment. When the tubes were cooled, we added purified water, transferring all of it to the measuring vessel, completing the final volume of 10 ml with 1% nitric acid.

After digestion, the detection and quantification were performed using inductively coupled plasma optic emission spectrometry (Agilent Technologies ICP-MS. Model 7900). The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The Ultra High Matrix Introduction (UHMI) system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences. The limits of detection were 0.065 ppm (Si), 0.073 ppb (Cr), 0.292 ppb (Cu), 0.871 ppb (Zn), 0.023 ppb (As), 0.816 ppb (Se), 0.061 ppb (Cd), and 0.046 ppb (Pb). For the analysis of total Hg, we used 0.05 g d.w. of each feather (vane and shaft), in a nickel boats and analyzed, following USEPA Method 7473 (sediments, soils, and sludges) (more details in Di Marzio et al. 2018a). We used a Milestone DMA-8 Direct Hg Analyzer by atomic absorption spectrophotometry, with a detection limit of 0.0001 ppm.

Statistical analysis

We first reported basic statistics (mean, median, SD, R). As the metal concentration data were not normally distributed, we used the nonparametric Mann–Whitney test to evaluate differences between years. We applied Spearman's nonparametric correlation test to evaluate the relationships between each metal. The significance level was set at $\alpha = 0.05$. Statistical analysis of the data was performed using SPSS v.25 (IBM SPSS Statistic) software.

In addition, we evaluated the Hg detoxification process through the calculation of the Hg:Se molar ratio described by Méndez-Fernández et al. (2014). This ratio was calculated as

$$\underline{\text{Hg:Se} = (\text{Hg } (\mu\text{g g-1 ww})/\text{Se } (\mu\text{g g-1 ww})) \times (78.96 \text{ (g mol-1)}/200.59 \text{ (g mol-1)})}$$

where 200.59 g mol-1 and 78.96 g mol-1 are the atomic mass of Hg and Se, respectively.

Results and discussion

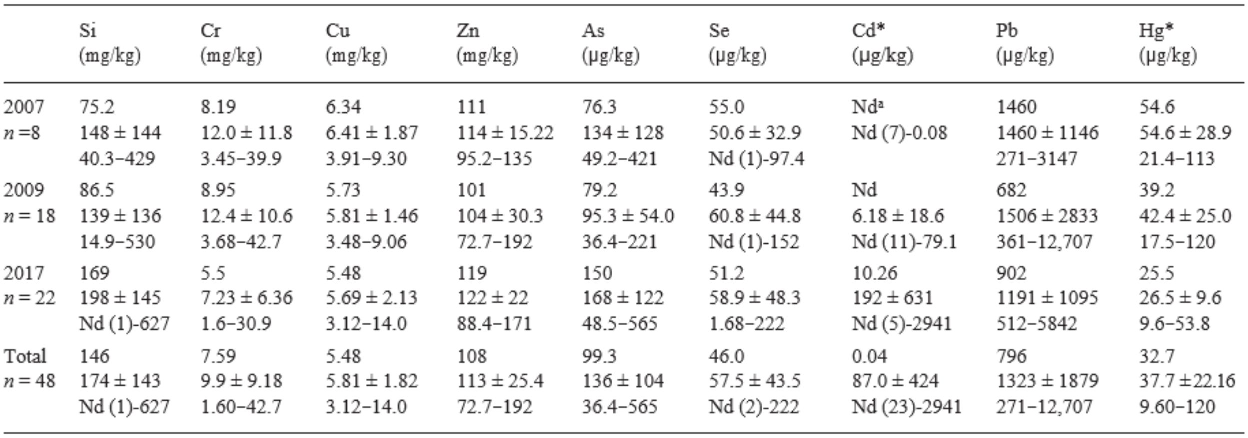
Metals levels in feathers of Andean condor

An overall metal assessment was conducted for first time in Andean condor feathers. Until now, information available was restricted only to lead concentrations (Lambertucci et al. 2011). We detected all 9 metals studied in primary flight feather from the Andean condor (Table 1). Detection rates of concentrations were 96–100% for all metals, except for Cd with detection rates of 46% (Table 1). The higher concentrations detected were for Si, followed by Zn > Pb > Cr > Cu > As>Se > Hg > Cd. Positive correlations were found between Si–As, Si–Zn, and Cu–Se, suggesting plausible similarities in the emissions sources and metabolic pathways of these elements (Table 2). The negative correlations observed between Cd–Hg and Cd–Cr have also been found in other studies associated with metallothionein binding (Elliott et al. 1992; Stewart et al. 1996).

Temporal evaluation of metals

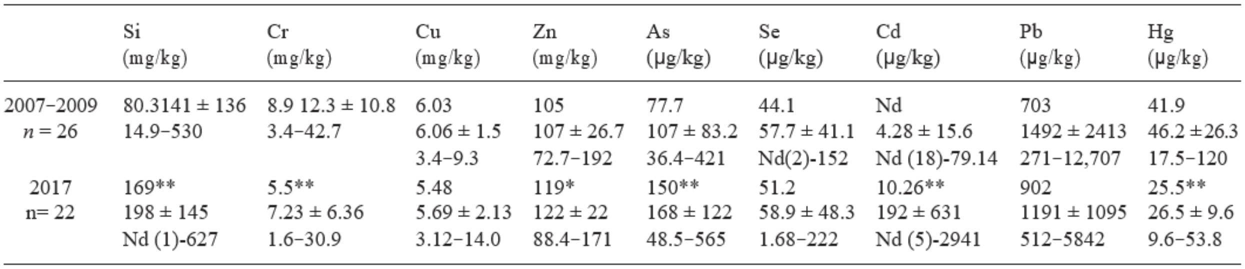
Regarding temporal trends, we found significant differences among sampling year only in the case of Cd (H = 17.132, p = 0.001) and Hg (H = 12.820, p = 0.005). No significant differences were found between 2007 and 2009, but there were significant differences between those years and the year 2017, the most important difference being between years 2009 and 2017 for Cr (U = 102, p = 0.008), Zn (U = 108, p = 0.014), As (U = 110, p = 0.016), Cd (U = 92, p = 0.003), and Hg (U =110, p = 0.016). Taking into account this data and our aim, we pooled the years in two groups (2007–2009 and 2017) in order to allow comparison considering the potential effect of the Puyehue–Cordón Caulle (PCCVC) eruption, to compare pre- and post-eruption data (Table 3).

The PCCVC 2011 volcanic eruption affected a large part of the steppe (Bertrand et al. 2014), and the ashes were deposited in the foraging areas of the condors. Condors can travel up to 350 km in a day (Lambertucci et al. 2014), but the average daily distance travel to feeding areas is 32 km in the study area, which can reach 176 km (Lambertucci et al. 2018). These data show high movements of these animals; however, during the volcanic eruption, they remained in the area both flying and feeding (Alarcón et al. 2015).



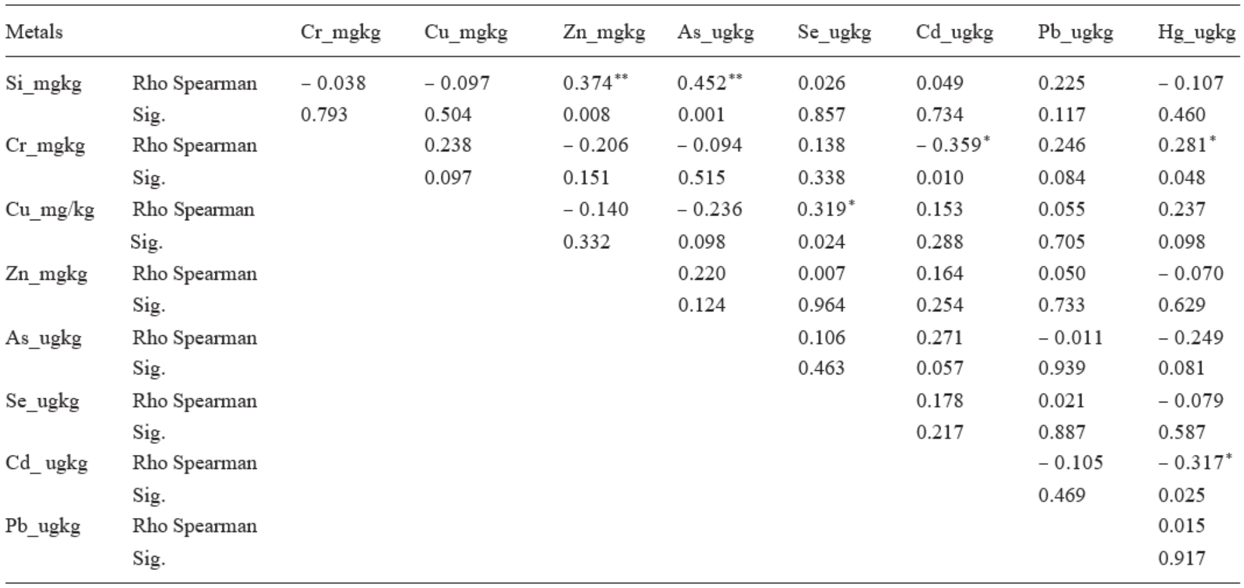
	Si (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (µg/kg)	Se (µg/kg)	Cd* (µg/kg)	Pb (µg/kg)	Hg* (µg/kg)
2007 *n* =8	75.2 148 ± 144 40.3–429	8.19 12.0 ± 11.8 3.45–39.9	6.34 6.41 ± 1.87 3.91–9.30	111 114 ± 15.22 95.2–135	76.3 134 ± 128 49.2–421	55.0 50.6 ± 32.9 Nd (1)-97.4	Nd[a] Nd (7)-0.08	1460 1460 ± 1146 271–3147	54.6 54.6 ± 28.9 21.4–113
2009 *n* = 18	86.5 139 ± 136 14.9–530	8.95 12.4 ± 10.6 3.68–42.7	5.73 5.81 ± 1.46 3.48–9.06	101 104 ± 30.3 72.7–192	79.2 95.3 ± 54.0 36.4–221	43.9 60.8 ± 44.8 Nd (1)-152	Nd 6.18 ± 18.6 Nd (11)-79.1	682 1506 ± 2833 361–12,707	39.2 42.4 ± 25.0 17.5–120
2017 *n* = 22	169 198 ± 145 Nd (1)-627	5.5 7.23 ± 6.36 1.6–30.9	5.48 5.69 ± 2.13 3.12–14.0	119 122 ± 22 88.4–171	150 168 ± 122 48.5–565	51.2 58.9 ± 48.3 1.68–222	10.26 192 ± 631 Nd (5)-2941	902 1191 ± 1095 512–5842	25.5 26.5 ± 9.6 9.6–53.8
Total *n* = 48	146 174 ± 143 Nd (1)-627	7.59 9.9 ± 9.18 1.60–42.7	5.48 5.81 ± 1.82 3.12–14.0	108 113 ± 25.4 72.7–192	99.3 136 ± 104 36.4–565	46.0 57.5 ± 43.5 Nd (2)-222	0.04 87.0 ± 424 Nd (23)-2941	796 1323 ± 1879 271–12,707	32.7 37.7 ±22.16 9.60–120

Table 1. Concentrations of metals in feather from Andean condor (*V. gryphus*) sampling in Nahuel Huapi National Park (2007–2017). Data are presented as median, mean ± standard deviation, minimum-maximum. n number of samples ([a] Only was detected in one sample; *Nd* non detected; *p < 0.01)



	Si (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (µg/kg)	Se (µg/kg)	Cd (µg/kg)	Pb (µg/kg)	Hg (µg/kg)
2007–2009 *n* = 26	80.3141 ± 136 14.9–530	8.9 12.3 ± 10.8 3.4–42.7	6.03 6.06 ± 1.5 3.4–9.3	105 107 ± 26.7 72.7–192	77.7 107 ± 83.2 36.4–421	44.1 57.7 ± 41.1 Nd(2)-152	Nd 4.28 ± 15.6 Nd (18)-79.14	703 1492 ± 2413 271–12,707	41.9 46.2 ±26.3 17.5–120
2017 n= 22	169** 198 ± 145 Nd (1)-627	5.5** 7.23 ± 6.36 1.6–30.9	5.48 5.69 ± 2.13 3.12–14.0	119* 122 ± 22 88.4–171	150** 168 ± 122 48.5–565	51.2 58.9 ± 48.3 1.68–222	10.26** 192 ± 631 Nd (5)-2941	902 1191 ± 1095 512–5842	25.5** 26.5 ± 9.6 9.6–53.8

Table 2. Concentrations of metals in feather from Andean condor (*V. gryphus*) sampling in Nahuel Huapi National Park before (2007– 2009) and after (2017) PCCVC (Puyehue-Cordon Caulle volcanic complex) eruption (2011). Data are presented as median, mean ± standard deviation, minimum-maximum. n number of samples (Nd no detected; *p < 0.05; **p < 0.01)



Metals		Cr_mgkg	Cu_mgkg	Zn_mgkg	As_ugkg	Se_ugkg	Cd_ugkg	Pb_ugkg	Hg_ugkg
Si_mgkg	Rho Spearman	− 0.038	− 0.097	0.374**	0.452**	0.026	0.049	0.225	− 0.107
	Sig.	0.793	0.504	0.008	0.001	0.857	0.734	0.117	0.460
Cr_mgkg	Rho Spearman		0.238	− 0.206	− 0.094	0.138	− 0.359*	0.246	0.281*
	Sig.		0.097	0.151	0.515	0.338	0.010	0.084	0.048
Cu_mg/kg	Rho Spearman			− 0.140	− 0.236	0.319*	0.153	0.055	0.237
	Sig.			0.332	0.098	0.024	0.288	0.705	0.098
Zn_mgkg	Rho Spearman				0.220	0.007	0.164	0.050	− 0.070
	Sig.				0.124	0.964	0.254	0.733	0.629
As_ugkg	Rho Spearman					0.106	0.271	− 0.011	− 0.249
	Sig.					0.463	0.057	0.939	0.081
Se_ugkg	Rho Spearman						0.178	0.021	− 0.079
	Sig.						0.217	0.887	0.587
Cd_ ugkg	Rho Spearman							− 0.105	− 0.317*
	Sig.							0.469	0.025
Pb_ugkg	Rho Spearman								0.015
	Sig.								0.917

Table 3. Correlations among metals concentrations in feather of Andean condor (*V. gryphus*) from Patagonia Argentina (*p < 0.05, **p < 0.01)

There are several studies on potentially toxic elements (As, Cu, Zn) introduced into ecosystems by volcanic ash (Cronin et al. 2014; Duggen et al. 2007; Ruggieri et al. 2012; Stewart et al. 2006). Volcanic material can affect aquatic ecosystems immediately (falling into water bodies), or decades later (falling inland) due to the effect of rain and snowmelt that carry toxic substances into water bodies (Bisson et al. 2005). The increase in total suspended solids generates changes in the aquatic habitats, with important and prolonged effects on the riparian macroinvertebrate communities. (Lallement et al. 2016; Miserendino et al. 2012). We identified significant differences for metal concentrations except in Cu, Se, and Pb levels where the concentrations were similar pre- and post-eruption. The concentrations of Si, Zn, As, and Cd were significantly higher in samples post eruption (Fig. 2), while the levels of Hg and Cr were lower after the eruption (Table 2). Volcanic eruptions represent an important source of Hg both in Latin America (Ribeiro Guevara et al. 2010) and globally. For example, in the Mediterranean region, Etna volcano is the main source of Hg deposition (Martin et al. 2008, 2012). However, there is uncertainty in our region. Higueras et al. (2014) show that in the PCCVC, it emits Hg in gaseous form, and its dilution in air does not make it dangerous for the condor. Perez Catán et al. (2016) detected Hg in the water column of the lakes of Nahuel Huapi National Park, without being able to clarify whether this was due to recently fallen ash or if it was a release of Hg from the bottom sediments due to the ash income. The presence of Hg in water column thus increased its bioavailability to the biota and could favor the bioaccumulation in aquatic organisms (Lacerda and

Malm 2008). Balseiro et al. (2014) indicate that pre-eruption conditions returned in a short-time period (1–3 years) for Hg. In our study area, the Andean condor diet is currently almost exclusively based on terrestrial wildlife (Lambertucci et al. 2018). Studies have shown that concentrations of Hg and Se are related, because Se can bind to this metal and have a detoxifying effect (Ohlendorf and Heinz 2011; Lourdes et al. 1991). The Hg:Se molar ratio was determined (([Hg]/200.59)/([Se]/78.96) in our samples, with a mean ratio 0.89. Given that the mean Hg:Se molar ratio was lower than the 1:1 M ratio, the selenium levels seems thus to be sufficient for binding to all body mercury in most individuals sampled. Only in 5 samples this ratio was higher than 1, indicating that the bioaccumulated selenium concentration is not quite sufficient for binding mercury body burdens in those cases. The mean concentration of Se in our samples was 60 µg/kg, although several studies consider normal values between 1000 and 4000 µg/kg of Se mainly in aquatic species (Burger 1993; Eisler 2000; Ohlendorf 1993). As mentioned above, the levels of Se are directly related to those of Hg. Due to the biomagnification processes that methyl-mercury (CH3Hg) is subject to in the aquatic environment, the levels of Se considered normal in these studies could be influenced by Hg levels.

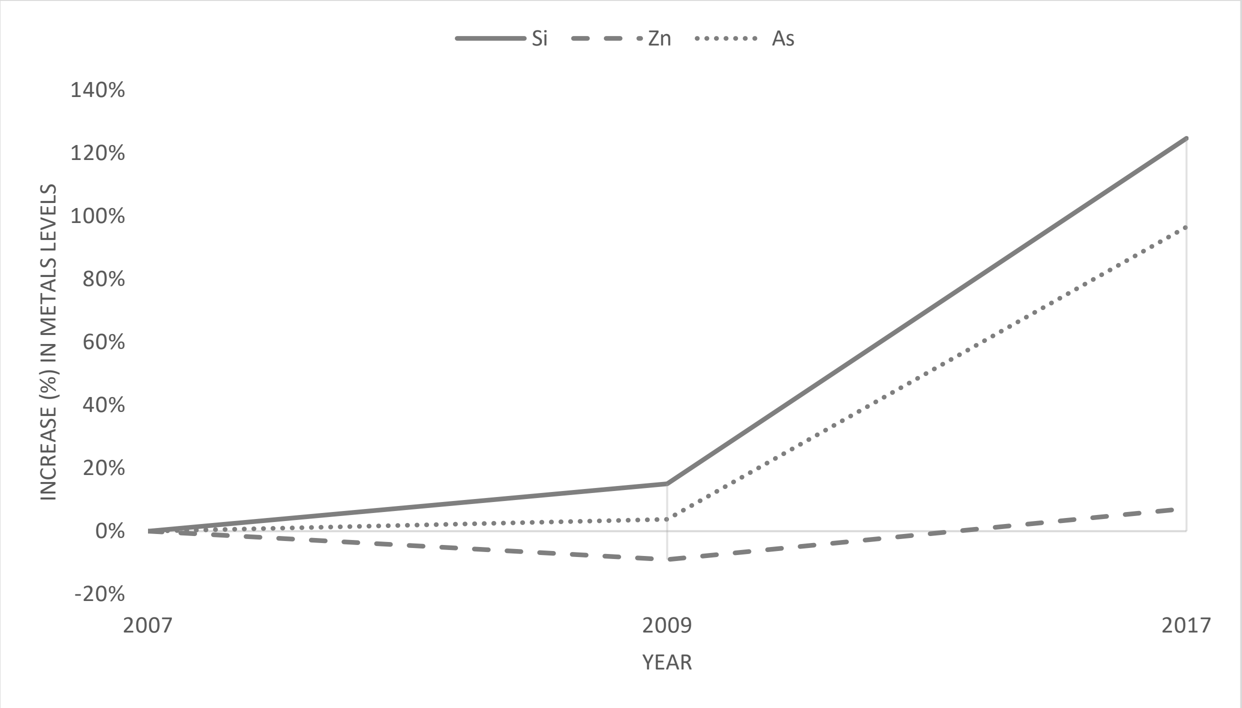


Fig. 2. Variations (percentage) in metals levels of the metals that significantly differs in concentrations between samples pre- and post-eruption. Base year = 2007 (y = %; x = year), Si = continuous line; Zn = dash line; As = dot line). Cd is not displayed because it was detected only in samples from 2017

The Cr concentrations in feather were also significantly lower post-eruption than in pre-eruption samples. As the final Cr concentrations during eruptive processes are related to the type of volcano and eruption, its analysis is very important for the identification of the emission source (Daga et al. 2008). Variations in Cr concentrations have been observed in different volcanic events (Nakada et al. 2005). Daga et al. (2008) conclude that Cr can be used as a source identification of recent events in Nahuel Huapi National Park. However, the retention of Cr by the soil particles during the infiltration process, the oxidation state, tri or hexavalent, and the solubility could affect the bioavailability of this element and its incorporation in the food chain (Han et al. 2004). On the other hand, the concentrations of Si, Zn, As, and Cd were significantly higher in post-eruption sampled feathers. Ruggieri et al. (2012) showed that the incorporation of certain elements (e.g., As, Fe, Ca, and Zn) represents the main environmental threat from volcanic ash. The PCCVC, a unique case in the Southern Volcanic Zone of the Andes Mountains among this type of volcanic structures, provides a magmatic composition where silica-rich rocks predominate over basaltic rocks (Daga et al. 2008; Lara et al. 2004). This data would explain the high levels of Si detected in our study. Contrary to our results, Bubach et al. (2012) did not find any bioindication of the emission of volatile forms of Zn by the Puyehue–Cordón Caulle volcanic complex. Zn is one of the elemental compositions of the primary components from tephra (Daga et al. 2008). Zinc's bioavailability is determined by complex interactions with the environment and is strongly dependent on the characteristics of that environment. The bioaccumulation of Zn in both aquatic and terrestrial plants (Singh and Kumar 2017), invertebrates (Heikens et al. 2001) and especially vertebrates (Çetin and Yur 2016) could be responsible for the increase of Zn concentration in the samples collected post-eruption. It is important to note the diet of Andean condor depends heavily (98.5%) on herbivores (Lambertucci et al. 2009a).

Due to the environmental and human health impact, there is special interest in As and Cd. Samples of deposited particles after eruptive activity and seismicity in 2000 of Volcano Copahue (located 314 km from PCCVC) showed an enrichment of several elements, As and Cd among others (Gómez et al. 2002). Moreover, the relationship between As concentrations in lichens and the distance to volcano is considered as an indication of the ash contribution, in addition to geological source, and is also associated with permanent geothermal emissions from PCCVC (Bubach et al. 2012).

To sum up, our results seem to indicate that increases in several metal concentrations in feathers of Andean condor post-eruption, including those more toxics to animal and human health, may reflect the incorporation of these elements in the environment and the trophic chain. Thus, this cathartid species can be used as a bioindicator (i.e., "the canary in the coal mine") to monitoring changes in the area where they live (see Plaza et al. 2020 for lead). In the whole area of study, it is difficult to think of other possible emission sources for the metals studied, except for Pb. Studies conducted in the area reveal that Pb contamination is due to hunting ammunition (Lambertucci et al. 2011; Plaza and Lambertucci 2019). The concentration values of Pb did not change in our study, unlike the other metals. However, the physical inspection of 43 condor specimens from the same study area a few months after the eruption of 2011 showed signs of pharyngitis in all specimens, probably caused by the inhalation of Si (Plaza et al. 2019).

Metal pattern distribution and risk assessment

Argentina, like most Latin America countries, has few biomonitoring studies of metal pollution in birds that inhabit terrestrial ecosystems (Di Marzio et al. 2019). Not all metals evaluated in our study have been previously investigated in bird feathers, limiting a comparison of results. In addition, threshold concentrations for lethal and sublethal effects in birds vary depending on several parameters (Burger 1993). According to the summary by Ansara-Ross et al. (2013), the reference values for feathers are < l mg/kg (dry weight, d.w.) for Cd (Burger 1993), <4 mg/kg (d.w.) for Pb (Scheuhammer 1987) and < 5 mg/kg for Se (d.w.) (Arnold et al. 1973). However, Hg concentration of 1–5 mg/kg (d.w.) in feathers of birds of prey are normal (Scheuhammer 1991).

We can distinguish essential metals such as Si, Zn, Cr, Cu, and Se and non-essential such as Pb, As, Hg, and Cd. Although Zn is an essential metal for body formation, organisms with high levels of Zn may encounter problems in maintaining homeostasis of tissue concentration of this particular element (Gasaway and Buss 1972). The feather concentrations of Zn found in this study are in the range of other studies on several other bird species (Ansara-Ross et al. 2013; Bustnes et al. 2013; Cahill et al. 1998; Grúz et al. 2018; Harmata and Restani 2013; Kavun 2004). Copper has an important role in several vital processes (Harms and Buresh 1987; Pesti and Bakalli 1996; Underwood and Suttle 1999), but chronic exposition to high levels could cause alterations on these processes, which have been shown in experimental studies (Chiou et al. 1999; Jackson et al. 1979;

Jackson and Stevenson 1981). Our results with a detected maximum value of 14 mg/kg and a mean concentration of 5.81 mg/kg (Table 1) are in concordance with other Cu mean levels found in feathers of Accipitridae and Cathartidae species (Haskins et al. 2013; Kavun 2004; Nighat et al. 2013; Wiemeyer et al. 1986).

One of the metals whose levels were significantly higher in samples post-eruption is Si. This metal has been scarcely studied in feathers. In any case, our concentrations are higher than those found in tail feathers of tawny owls (*Strix aluco*) by Bustnes et al. (2013) in Central Norway with a mean of 4.67 μg/g and similar to those found in cormorants by Skoric et al. (2012) (mean 119.96 mg/kg). This result is consistent with the fact that Si is the main component (ca. 70%) of the volcanic eruption we studied here (Caneiro et al. 2011). We have already commented above that in PCCVC silica-rich rocks predominate over basaltic rocks (Daga et al. 2008; Lara et al. 2004). On the other hand, Cr enters the body of birds by nutrition. Chromium levels in this study were higher than detected levels in raptor species from other regions of the planet (Ansara-Ross et al. 2013; Dauwe et al. 2003; Denneman and Douben 1993; Grúz et al. 2018; Harmata and Restani 2013; Van den Brink et al. 2003), with a mean value in our study from six and seven times higher than those detected by previous studies. Moreover, different studies indicated that levels of 2.80 mg/kg Cr detected in bird feathers may be related to adverse effects on the reproductive success of birds (Burger 1993; Burger and Gochfeld 2000; Kertész and Fáncsi 2003). Therefore, the high levels of Cr found in the feathers of Andean condor merit special attention since they may produce adverse effects.

For birds and other wild animals, small amounts of Se are necessary to maintain a good state of health. In bird feathers, values of Se between 1000 and 4000 μg/kg are considered normal; although frequently, the values are lower than 2000 μg/kg (Ohlendorf and Heinz 2011). In our study, Se levels (57.5 ± 43.5 μg/kg, range nd-222 μg/kg) were lower than those found in feathers of species of the same Andean condor family such as the turkey vulture (*Cathartes aura*) (940 ± 400 μg/kg) (Cahill et al. 1998) or from other raptors such as laggar falcon *(Falco biarmicus jugger)* (950– 5200 μg/kg), bald eagles (*Haliaeetus leucocephalus*) (800– 3200 μg/kg) (Bowerman et al. 1994; Movalli 2000), African grass owl (*Tyto capensis*) (22–3880 μg/kg), and barn owl (*Tyto alba*) (4–1120 μg/kg) (Ansara-Ross et al. 2013). In addition, there is evidence that in aquatic ecosystems Se reduces bioavailability and trophic transfer of Hg (Jones et al. 2013; Kehrig et al. 2013), reducing the toxic effects of Hg in some animals, including birds (Lourdes et al. 1991). Mercury is probably the nonessential metal of most concern for its toxic effects (Walker et al. 2001). The highest Hg concentration in our study was 120 μg/kg, which is not of concern since Hg concentrations of 1000–5000 μg/kg (d.w.) in raptor's feathers are considered normal (Scheuhammer 1991). Hg concentrations greater than 4100–5000 μg/kg in feathers have been related to adverse effects in birds (Burger and Gochfeld 1997; Palma et al. 2005). Studies conducted in the same area on turkey vultures and black vultures (*Coragyps atratus;* same family as the Andean condor) seem to indicate that the surroundings of Bariloche are an area of low Hg contamination (Di Marzio et al. 2018a). These species have been shown to be good subrogate species as they seem to reflect the same spatial-temporal variations in the Hg levels of the Andean condor (Di Marzio et al. 2018b). In any case, our data does not exceed this threshold level.

Arsenic concentration is 1500–2000 μg/kg in the earth's crust and can also enter the material cycle by volcano (Bundschuh and Maity 2015). In this sense, its levels are significantly higher in samples post eruption (Table 2), as we mentioned in previous section. The highest As concentration in our study was 570 μg/kg (Table 1), similar to those detected by Grúz et al. (2018) in several species from the Hortobágyi Madárpark (Bird Hospital Foundation) in Hungary. However, it was not close to As concentrations responsible for adverse effects (see Nighat et al. 2013; 11,070 μg/kg in Accipitridae and 19,610 μg/kg in Falconidae, which could be toxic). On the other hand, Cd showed low concentrations in feathers of Andean condor (Table 1). They were lower than 3000 μg/kg of cadmium, a value at which severe physiological, nutritional and behavioral disorders occurs in birds (Burger and Gochfeld 2000).

Finally, regarding Pb, we found a mean concentration of 1.3 mg/kg of lead in all Andean condor feathers. This mean value is similar to the one detected in Andean condors sampled in 2007 in the same area (Lambertucci et al. 2011). As mentioned, lead bullets for sport hunting have been identified as the most probable source of lead for scavengers in this area (Lambertucci et al. 2011; Plaza and Lambertucci 2019). Besides, three individuals showed lead concentrations higher than 4 mg/kg, the threshold suggested, levels above which reproduction could be affected (Burger and Gochfeld 2000).

Conclusions

We have reported the first reference values of several metals and metalloids for Andean condor feathers. These values, in addition to giving us information on the current state of health of the Andean condor population of northern Patagonia, Argentina, may in the future allow us to evaluate the evolution of the situation in the area and represent an important database against which to compare the results of future studies on the species. The results obtained in forty-eight Andean condor primary feathers from Patagonia collected in the period 2007–2017, pre and post Puyehue-Cordon Caulle volcanic eruption suggest a possible incorporation of metals in wildlife and the environment after the eruption. The high Si values seem to confirm the volcanic origin of the metals detected. Si, Zn, As, and Cd showed increased levels after the eruption, and concentrations of Zn, As, Se, and Cd (in addition to Cu and Hg) detected were lower than those causing adverse effects. The concentrations of Cr and Pb detected in some individuals showed levels compatible with the disorders of living organisms, but they were not related to the volcanic eruption. This study shows that the Andean condor reflects variations of the environment, suggesting that its use as a sentinel species at least for some metals could be useful inside its South American distribution, depending on the kind of metal investigated. However, to test this and interpret our results in a more comprehensive way, there is a need for deeper understanding of condor biology (e.g., molt pattern, physiology) to further support conservation efforts and to benefit the survival of this species and its habitat.

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CHAPTER IV

Testing Cathartidae of Northern Patagonia as a potencial biomonitor species for Hg ecotoxicological studies and surrogate species for Andean Condor *Vultur gryphus*

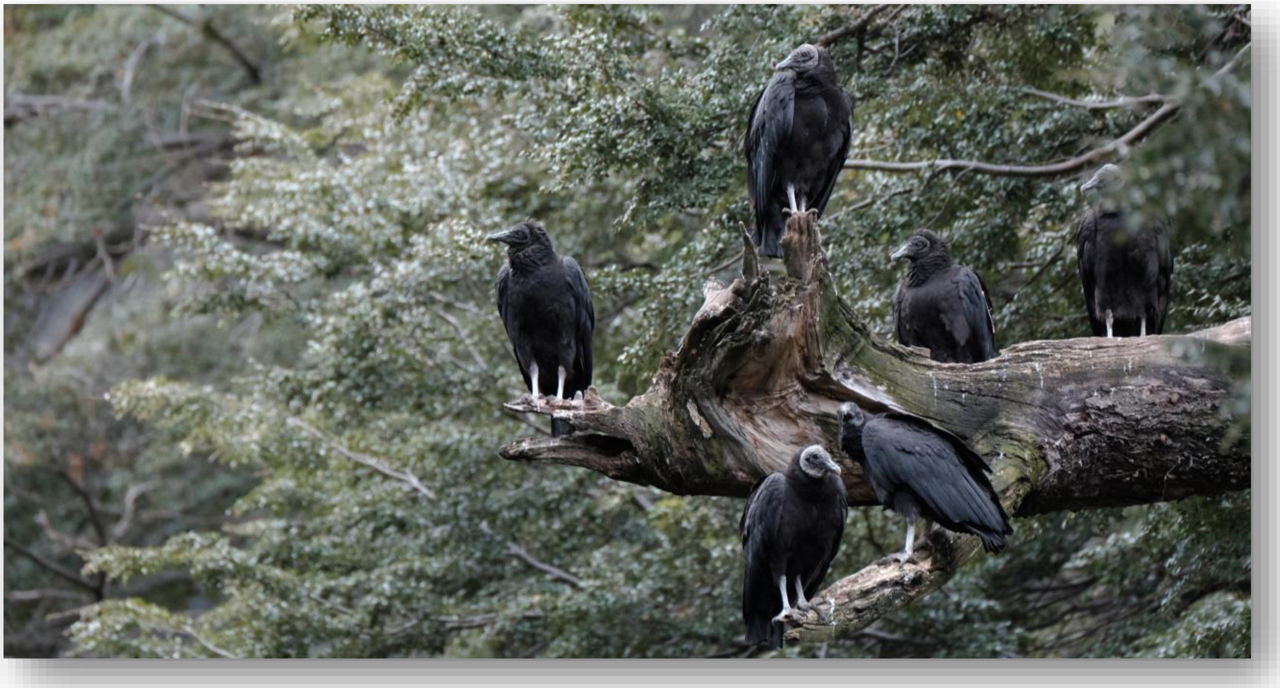

Photo: Marco D'Antonio fotografo

Resumen

Los estudios ecotoxicológicos son de gran interés para evaluar el estado de salud de la fauna y la flora silvestres y de las poblaciones humanas. Debido a su alta posición en la cadena trófica, las aves rapaces (y las aves carroñeras) son candidatos perfectos para el papel de biomonitor. Teniendo en cuenta que muchas aves rapaces están protegidas y algunas están amenazadas, es importante dar prioridad al uso de técnicas de muestreo no invasivas. Además, para el estudio de las especies amenazadas, podría evaluarse el uso de especies sustitutivas (con una función indicadora). Este enfoque se utiliza principalmente en los experimentos de laboratorio debido a la dificultad de comprender e interpretar las interacciones de todas las variables que intervienen en el estudio de las especies silvestres. En nuestro estudio pretendemos verificar si el jote negro *Coragyps atratus* y el jote colorado *Cathartes aura* podrían ser buenas especies de sustitución para inferir la contaminación por metales en el cóndor andino *Vultur gryphus*. Para ello comparamos los niveles de Hg detectados en las plumas de estas especies simpáticas del norte de la Patagonia (Argentina). Los resultados sugieren que el jote negro y el jote colorado no deben ser utilizados para la comparación directa con el condor andino. Si en futuros estudios se pudiera demostrar la existencia de un vínculo entre los niveles de Hg detectados en cóndor andin y jote negro, este ultimo podría ser una especie sustituta del cóndor andino. Al mismo tiempo, los resultados sugieren que el jote colorado puede reflejar los niveles de Hg tanto del medio ambiente terrestre como del acuático y por esta razón podría ser un buen biomonitor para nuestra área de estudio.

Abstract

Ecotoxicological studies are of great interest in order to assess the health status of wildlife and human populations. Due to their high position in the trophic chain, raptors (and scavenger birds) are perfect candidates for the role of biomonitor. Considering that many birds of prey are protected, and some are threatened, it is important to give priority to the use of non-invasive sampling techniques. In addition, for the study of threatened species, the use of surrogate species (with an indicator function) could be evaluated. This approachis mainly used in laboratory experiments due to the difficulty of understanding and interpreting the interactions of all the variables involved in the study of wild species. In our study we aim to verify if the Black Vulture *Coragyps atratus*and the Turkey Vulture *Cathartes aura* could be good surrogate species to infer metal contamination on the Andean Condor *Vultur gryphus*. For this we compared the Hg levels detected in feathers of these sympatric species from Northern Patagonia (Argentina). The results suggest that Black and Turkey Vultures should not be used for direct comparison with Andean Condors. If future studies could demonstrate the existence of a link between the Hg levels detected in Andean Condors and Black Vultures, Black Vulture might well be a surrogate species for the Andean Condor. At the same time the results suggest that the Turkey Vulture can reflect Hg levels from both, terrestrial and aquatic environment and for this reason it could be a good biomonitor for our area of study.

Introduction

Ecotoxicological biomonitoring studies help to assess geographical areas of the planet, identify possible risk sources for both wildlife and humans, and allow governments and/or environmental organizations to act in time to protect ecosystems (Beeby 2001). Many of the ecotoxicological studies conducted up to date, despite assessing contamination levels using animal samples (e.g. mollusk, fish), cannot be considered biomonitoring studies because the species employed does not satisfy the requirements to be considered biomonitors. (Burger, 2006; Di Marzio *et al.,* 2019). The main characteristics that a species needs to be considered a good biomonitor are: 1) high trophic level, 2) widely distributed and numerically abundant in the territory to be monitored, 3) sedentary (or with well-studied movement patterns), 4) its sampling must be simple and with an affordable cost (Hollamby *et al*., 2006; Holt and Miller, 2011). Based on this profile, raptors (including scavenger birds) can be considered good biomonitors (Espín *et al*., 2020; García-Fernández, 2014; García-Fernandez *et al*., 2008, 2020; Martínez-López *et al*., 2002).

To assess the risks that certain endangered species might be subjected to, research could be carried out with surrogate species. This approach is used in laboratory tests (Walker, 2014), mainly on fish species (Beyers, 1995; Besser *et al*., 2005). Although in field studies with raptors it began using at the beginning of the 1980s (Wiemeyer *et al*., 1986), until now, this approach hasn't been often used (but see, Naidoo, 2008; West *et al*., 2017; Herring *et al*., 2018; Plaza *et al*., 2020). The term "surrogate species" in these articles refers to a species that is indicative of environmental pollution of another species (Favreau et al., 2006) and has been used mainly to investigate surrogate species to evaluate metal exposition in the California Condor *Gymnogyps californianus*. In these papers, the Turkey Vulture *Cathartes aura*, species that belongs to

Cathartidae family as the California Condor, and the Common Raven *Corvus corax* whose diet is similar to the California Condor, have been used as surrogated species to infer mercury (Hg), lead (Pb) and others metals. The main limitations for these types of studies are represented by the differences (physiological, anatomical or ethological) among species (Carpenter *et al*., 2003; Plaza *et al*., 2020). The results of the studies mentioned above seem to confirm the complexity of interpreting the results obtained in studies with surrogate species.

The constant loss of biodiversity in recent years (WWF, 2018) imposes a careful use of endangered species for research. In the last decades, in South America the usage of endangered species for ecotoxicological studies have led to the sacrifice of captured specimens (Di Marzio *et al*. 2019). The use of non-invasive sampling techniques could reduce the negative impact that scientific research can produce on threatened wildlife. The use of raptor's molted feathers as a biomonitoring unit is an important technique in environmental biomonitoring studies, especially metal contamination studies (e.g. Hg). Feathers are easy to collect and store and metal levels detected in them are related to blood metal levels (Ansara-Ross *et al*. 2013; Espín *et al*., 2016; García-Fernández *et al*. 2013). A problem that can occur, using molted feathers, is that there is little information on molting strategies, as in the case of the Cathartidae. Recent studies suggest a closeness between the family Cathartidae and the family Accipitridae (Hackett *et al*., 2008; Zhang, 2015). Due to the lack of studies on the molt strategy of the Andean Condor *Vultur gryphus*, this data allows us to consider that this species could have a molt similar to the great Eurasian vultures (ca. 6 years) (Zuberogoitia *et al*., 2013). Chandler *et al*. (2010) consider Turkey Vulture to have annual molt, similar to what has been recorded in other Accipitridae with a similar size. Therefore, we can suppose Black Vulture *Coragyps atratus* also has the same molt strategy.

We sampled roosts of Black Vulture, Turkey Vulture (in 2011, 2013, 2017) and Andean Condor (in 2017), collecting primary molted feathers. The sampling was done in the in northwest Patagonia, Argentina. Our decision was motivated by several reasons: the presence of a large population of Andean Condors in this area, availability of scientific knowledge about the local populations of Andean Condors, Black Vultures and Turkey Vultures (Lambertucci *et al*., 2010; Barbar *et al*., 2015; Graña Grilli *et al*., 2017; Ballejo *et al*., 2018) and also certain investigations on Hg contamination in these terrestrial species, somewhat unusual in Latin America (Di Marzio *et al*., 2018). Furthermore, we know that the main sources of metal contamination detected until now are related to volcanic eruptions (Di Marzio *et al*., 2020), recent increase in the human population (Arribére *et al*., 2010; Rizzo *et al*., 2011; Bubach *et al*., 2015) and lead from sport hunting (Plaza and Lambertucci, 2018).

Benefiting from all this available information, which has helped us to interpret the results, our aim was test for the first time the use of the Black Vulture and the Turkey Vulture as possible surrogate species for the Andean Condor in ecotoxicological studies of Hg contamination. The Black Vulture and the Turkey Vulture share territory with both the Andean Condor (in South America) and the California Condor (in North America), and the results of this study could provide useful information for the conservation of both Condor species.

Materials and methods

Study area

Our sampling area was a territory of approximately 50 km2 west of the city of Bariloche, in the north of Argentinean Patagonia, near the Nahuel Huapi National Park (Figure 1). It is a rural area, sparsely populated (Rizzo *et al*..2011) where the main economic activities are extensive cattle raising, trout farming (located in several points of the Limay River, into our sampling area) and nature tourism. The represented ecotones are the sub-Antarctic forest and the steppe (Bustos and Rocchi 2008) (Figure 1).

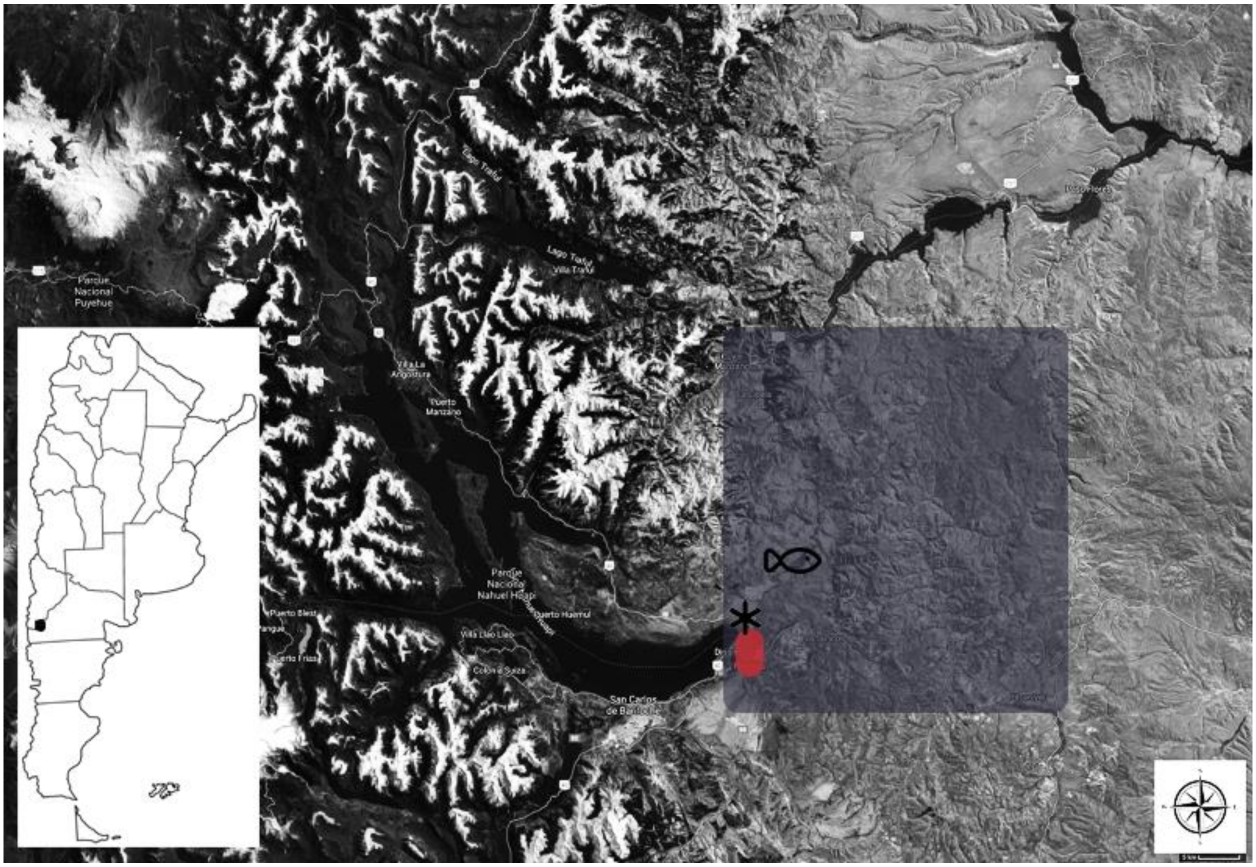

Fig. 1. Map of Argentina with the study area (black square) In the map of the surrounding of Bariloche, evidence of the sampling area (gray square). In red the location of the turkey vulture samples with the highest Hg values (>5mg/Kg) near a fish processing plant (*) and the fish farm area (sign of a fish)

Study species

We sampled communal roosts of Andean Condors, Black Vultures and Turkey Vultures. All three species belong to Cathartidae family, also known as "New World Vultures", not completely studied bird family (Di Marzio *et al.*, 2020). The Andean Condor (11-15 kg, body weight) is a sedentary species, ranging in the Andes (Del Hoyo et al., 1994; Ferguson-Lees and Christie, 2001) and classified by the IUCN Red List as "Near Threatened" at global level, although regionally its threat level can be higher. The north of Argentinian Patagonia population, not without threats, is one of the most abundant in Latin America (Lambertucci, 2010). Black Vulture (2 kg, body weight) is a sedentary species ranging all of South America and the southern part of the USA (Del Hoyo et al., 1994; Ferguson-Lees and Christie, 2001); its predilection for human settlements has allowed for its expansion in recent years (Barbar *et al*., 2015). Turkey Tulture (1.4 kg, body weight) is a migratory species (Graña Grilli *et al*., 2017) ranging from southern Canada to Tierra del Fuego (Del Hoyo et al., 1994; Ferguson-Lees and Christie, 2001). Chandler *et al*. (2010) consider Turkey Vulture to have annual molt, similar to what has been recorded in other Accipitridae with a similar size. Therefore, we can suppose Black Vulture also has the same molt strategy.

Sampling methods and Hg analysis

We analyze a total of 151 molted primary feathers (P1-P10) of the 3 species (22 from Andean Condor, 65 from Black Vulture and 64 from Turkey Vulture), collected in roosts during the southern springs of the years 2011, 2013, 2017. To reduce the possibility of pseudo-replication, we sampled the same type of feathers, from the same wing, in each roost. The feathers were measured, weighed, and cleaned using the methodology described by Espín *et al*. (2012). The feathers were then cut and a homogeneous sample (shaft and vane) of 0.05 g of each was analyzed by atomic absorption spectrophotometry using the Milestone DMA-80 Direct Hg Analyzer to detect Hg levels. The method was tested, using certified reference material (CRM; TORT-2, lobster hepatopancreas, National Research Council Canada), for precision and accuracy as described in Espín et al. (2014). Recovery percentage of total Hg from 5 replicates of CRM was 104.2 ± 11.8% (mean ± standard deviation). The coefficient of variation for the repeatability was 11.4%.

Statistical analysis

At first, we compared levels of mercury detected in the Andean Condor samples from 2017 (feathers grown in 2011-2012) with those detected in Black Vulture and Turkey Vulture (pooled samples from 2011 and 2013). Due to strong asymmetry in data, we used Mann-Whitney U test for rank distribution comparison (Sokal and Rohlf, 1995) between every species-pair. We used Benjamini & Yakutieli approach (Benjamini and Yekutieli, 2001) to account for false discovery rate in multiple testing during pairwise comparisons.

Further on, we evaluated ability of one species to be a surrogate for other in description of change of the mercury contamination levels. To analyze differences in temporal change, we used linear regression with interaction, to account for interspecies differences within sampling years. In general form fullest model is written as:

Relative_Mercury~Year+Species+Year:Species,

where “Year” is sampling year, “Species” is sampled species. Terms separated with “+” indicate main effects (overall difference for specific year or species), while “Year*Species” explaining year specific differences between species. We used year as a categorical variable. As mercury levels were highly asymmetrical, we first ln-transformed values, to reduce heteroscedasticity (Sokal and Rohlf, 1995; Zuur *et al.*, 2007). Then, as we care about change in levels specific for species, we scaled and centered species-specific ln-transformed values pooled over samples from 2011, 2013 and 2017, creating dependent variable “Relative_Mercury”. This procedure reduces possible artefacts due to value differences between species and allows investigation of change itself. We evaluated all the possible variable combinations and calculated AICc value. We chose model with least AICc as the best descriptor of information (Burnham and

Anderson, 2002). This approach resulted in model with main effect of species excluded, as species-specific differences were accounted for during scaling procedure.

We used software R for data analysis (R Core Team, 2019). We considered *p*-values less than 0.05 as statistically significant.

Results

Contamination levels

We have analyzed 151 feathers, detecting Hg in all the analyzed samples. Among the Turkey Vulture samples we found 4 samples in 2013 and 3 in 2017 with the highest levels (Table 1). As mentioned above, we estimate in 6 years the duration of the Andean Condor's molt, and annual the duration of the Black Vulture and Turkey Vulture molt. For this reason, we made a comparison of results between the 2017 Andean Condor samples and the 2011-2013 samples of the other two species. We found statistically significant difference in Hg levels between Andean Condor and the other two species with both p-values <0.001, but no statistically significant differences between Black Vulture and Turkey Vulture (p=0.87). (Figure 2).

Year	*Vultur gryphus*	*Coragyps atratus*	*Cathartes aura*
2011		0.17 0.22 ± 0.16 (n: 20)	0.13 0.13 ± 0,06 (n: 14)
2013		0.18 0.44 ± 0.62 (n: 9)	1,57 2,44 ± 2,4 (n: 13)
2017	0.025 0.026 ± 0.0096 (n:22)	0.15 0.42 ± 0.58 (n: 36)	0,32 1,31 ± 1,78 (n: 37)
Total	0.025 0.026 ± 0.0096 (n:22)	0.17 0.36 ± 0.5 (n: 65)	0,23 1,28 ± 1,87 (n: 64)

Table 1. Level of Hg (mg/Kg) in primary feathers of Andean condor, black vulture and turkey vulture (median, mean and SD, n= number of samples) according to year and species

Temporal change

Evaluation of temporal variation in Black and Turkey Vultures, indicate no overall temporal change from 2011 for data of both species together (Table 2, effects of year). Though, differences exist comparing species by year. Distributions of relative (to species specific average) mercury levels are similar between species in 2013 and 2017, but statistically significantly different in 2011 (Table 2, interaction terms). As analysis is carried out on species-specific relative contamination levels, this suggests overall stable detected mercury levels in Black Vulture (Table 2, Figure 3). Whereas, contamination levels in Turkey Vulture have significantly increased between 2011 and 2013 and have remained relatively high in 2017 (Table 2, Figure 3).

Variable	**Estimate**	**95% CI min**	**95% CI max**	**t-value**	**p-value**
Intercept	-0.0222	-0.4447	0.4004	-0.1039	0.9175
Year 2011	Reference				
Year 2013	0.1147	-0.6437	0.8732	0.2995	0.7651
Year 2017	0.0113	-0.5156	0.5383	0.0426	0.9661
Year 2011:Species	-0.7933	-1.4517	-0.1348	-2.3847	0.0186
Year 2013:Species	0.5427	-0.2767	1.3620	1.3110	0.1923
Year 2017:Species	0.0962	-0.3462	0.5385	0.4304	0.6677
F(5,123)=3.2727; p=0.0082; adjusted-R^2=0.0816					

Table 2. Linear regression results of scaled ln-tranfromed mercury level change over time and between species (black vulture as a reference) in time.

Discussion

The differences found between the Hg levels in the Andean Condor and the other two species (Figure 2) do not allow a direct comparison between the three species. Although eliminating the family variable, as all three species are Cathartidae, there are more intra- and inter-specific factors that create difficulty, as suggested by Carpenter *et al.* (2003). Even though all three species are strictly scavengers, the composition of the diet could be one of the factors to consider to explain these differences. There are differences in the composition of the diets in our area of study (Ballejo *et al*. 2018). The diet of Andean Condor is based mainly in remains of sheep, European Hare *Lepus europaeus*, European Deer *Cervus elaphus* and Wild Boar *Sus scrofa*. The diet of the Black Vulture is similar, although for this species the arthropods represent an important source of food (Ballejo *et al*., 2018).

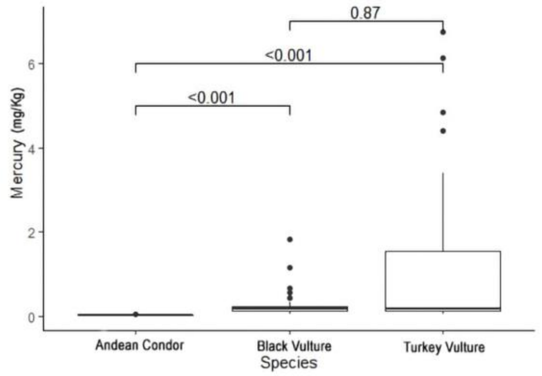

Fig. 2 Comparison of Hg levels (mg/Kg) of Andean Condor, Black Vulture and Turkey Vulture samples

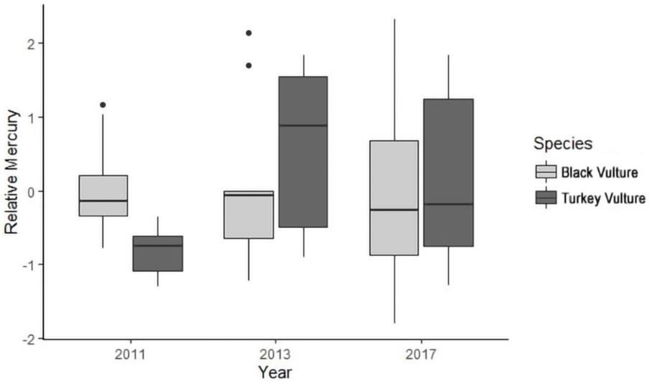

Fig. 3 Temporal variation of species-specific relative mercury contamination levels in Black Vulture and Turkey Vulture

Several studies show the importance of arthropods in the transfer of methylmercury from the aquatic to the terrestrial environment (Xie *et al*., 2008; Speir *et al*., 2014). In the aquatic environment, Hg bioaccumulation and biomethylation of inorganic Hg into organomercury (more toxic) are common (Eisler, 1985). This could, at least in part, explain the higher Hg values recorded in Black Vultures compared to the values in Andean Condors. Turkey Vulture has a more varied diet, including also remains of reptiles, fish and birds (Ballejo *et al*., 2018). The inclusion of fish in the diet of subordinate Turkey Vultures has also been documented by Blázquez *et al*. (2016) in Baja California, Mexico. For the same reasons explained above regarding the diet of the Black Vulture, Turkey Vultures feeding on fish could maybe have higher levels of Hg.

Turkey Vulture migration behavior could be another factor influencing Hg exposure. Chandler *et al*. (2010) describe the molt of the Turkey Vulture's primary feathers as serial. The molt of P1-P4 is completed before the reproductive period, followed by a suspension during the reproductive period, a resumption before the winter migration and a suspension again during the winter. The study of Graña Grilli *et al*. (2017) provides us the migratory routes of the Turkey Vulture of Bariloche. Considering the migration chronology, we can assume that the migration is subsequent to the development of the primary feathers, which would therefore reflect the contamination of our study area. Being hierarchical animals, the sex and age of the animals could be other factors responsible for the differences recorded, as proposed for other metals (Plaza *et al*., 2020). Access to food is strongly regulated by the hierarchy in Andean Condor, with adult males being the earliest eaters and young females being the last to eat (Donázar *et al.,* 1999). In Black Vulture and to a lesser extent in Turkey Vulture, hierarchy is determined by age, with adults getting priority access to food (Wallace and Temple, 1987). In addition, as demonstrated by Blázquez *et al*. (2016) subordinate animals may resort to alternative food sources, such as fish. As mentioned above, ecotoxicological studies with surrogate scavenger bird species are very scarce. One of them was carried out in Africa (Naidoo, 2008), using two species of the genus *Gyps* (classified as Least Concern) to test the toxicity of meloxicam (to use it as a substitute for diclofenac) and carrying out in vitro tests with chicks as a surrogate species for other more endangered vultures of the genus Gyps. West *et al*. (2017) studied the Turkey Vulture and Common Raven as surrogate species for the California Condor, but did not compare the results for these two species with the results on the California Condor. The study by Plaza *et al*. (2020), which was conducted in our same study area, compared the Pb levels detected in blood samples from Black Vulture and Andean Condor, suggesting caution on the use of Black Vultures as surrogate for Andean Condor perhaps because of differences in their access to carrion or because of a different susceptibility to this toxic metal. Wiemeyer *et al*. (1986) and Herring *et al*. (2018) conducted two studies comparing Hg levels (among other metals) in a Turkey Vulture and California Condor samples (Common Raven samples were also analyzed in both studies) discussing on the results obtained in the species without looking for correlations. Herring at al. (2018) refers to the need to investigate the spatial and temporal drivers of environmental contaminant-based threats in order to design reintroduction programs. In this perspective, in our study we have verified that the temporal trend of Hg values in Black Vulture and Turkey Vulture does not present significant differences between the species (Figure 2). In addition, we have found some analogies in the Hg values of our study with that of Herring *et al*. (2018) and in the Pb values between the study of Herring *et al*. (2018) and Plaza *et al*. (2020); although the studies refer to different samples (blood or feathers) of different species from different areas. Evaluating the results from the point of view of the values recorded in the "main" species (Andean Condor or California Condor), the Hg levels in the surrogate species are always overestimated, and the Pb levels underestimated.

Observing the Hg levels in the Turkey Vulture samples we can see, as mentioned above, that the levels rise in between 2011 and 2013, remaining high also in 2017 (Table 2, Figure 3). Various authors coincide that Hg levels in feathers higher than 4.1-5 mg/Kg are related to adverse effects in birds (Burger and Gochfeld, 1997; Palma *et al*., 2005). In our case seven Turkey Vulture specimens exceed these levels (Table 1). Di Marzio *et al*. (2018) considered the surroundings of Bariloche a low exposure area to Hg; in feathers sampled in this area in 2011 they detected median Hg levels of 0.12 mg/Kg in Southern Crested Caracara (*Caracara plancus*), 0.17 mg/Kg in Black Vulture and 0.13 mg/Kg in Turkey Vultures. In the case of Black Vulture, also the median values of 2013 and 2017 are similar (0.18 mg/Kg; 0.15 mg/Kg). It could be assumed that these values represent baseline area levels for high trophic level species; considering that also Bubach *et al.* (2012) consider 0.12-0.16 mg/kg basal levels for the same area in studies with lichens. As evidenced by Burger (1993), feathers represent the excretion pathway of 70-90% of MeHg of burden body. Several studies have shown that bird feathers contain levels of 90% MeHg in both aquatic and terrestrial species (Rimmer *et al*., 2005; Renedo *et al*., 2017). For these reasons, the high levels of Hg detected in Turkey Vulture feathers could refer to MeHg levels and their origin could be the aquatic ecosystems of the area. As seen above, Black Vultures do not show big variations in Hg levels, although they share the territory with Turkey Vultures. One of the main differences between the two species, as already mentioned, is the inclusion of fish in the diet of the Turkey Vulture. In addition, we have detected high Hg levels in Turkey Vulture feathers at another point in the Limay River (unpublished material).

Conclusions

Our results show, for the first time, that Turkey Vulture samples better reflect Hg contamination in our study area, possibly reflecting also the presence of Hg in the aquatic ecosystem. These data lead us to suppose that the Turkey Vulture would represent the most suitable biomonitoring species for ecotoxicological studies on Hg contamination in our study area, considering that there are no other raptors in the area that can feed on fish (e.g. Osprey *Pandion haliaetus*). Also for the first time, the levels of Hg detected in species with a high trophic chain in the area of Bariloche are compatible with toxic effects.With the information at our disposal, and in the absence of further studies, these results seem to indicate a worrying increase in total Hg (probably MeHg) levels. If confirmed, these data would indicate a potential risk even for the human population in the area.

Regarding the use of surrogates species, we can consider our results as interlocutory. In our test, based on the results, and thanks to all the existing data on the area and the species, we could say that for ecotoxicological studies on Hg contamination, the Black Vulture would represent a better option as a surrogate species of the Andean Condor than the Turkey Vulture.

Turkey Vulture could be considered as a suiteble surrogate species of the Andean Condor only for those habitats where the Andean Condor integrates its diet with fish. In order to apply the methodology of surrogates to other areas of distribution of the Andean Condor, considering the scarcity of scientific research in many parts of Latin America, more research is needed, with new approaches. The temporal trends and analogies of the relationships between Pb and Hg values detected in several studies with surrogate species, which at present are no more than simple speculations, could perhaps be these approaches. If links between these values can be verified, ecotoxicological studies with surrogate species could represent the future for biomonitoring of threatened species as Andean Condor.

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CHAPTER V

Evaluation of spacial and temporal changes in metal levels in feathers of five scavenger bird species from northern Patagonia (Argentina)

Photo: Alessandro Di Marzio

Resumen

La contaminacion por metales es un problema global, causada por fuentes naturales (ej. volcanes) y antropicas (actividades industriales, mineria et.). Las aves (especialmente rapaces), se consideran buenos biomonitores, asi como sus plumas son buena unidad biomonitora para estudios ecotoxicologicos sobre la contaminacion por metales. Nuestro estudio se centra en el estudio de los niveles de cinco metales (Hg, Pb, Zn, Cu, Cd) en el Norte de la Patagonia Argentina, empleando aves carroñeras como biomonitoras. Analizando los niveles, las tendencias temporales y las variaciones entre especies hemos podido ampliar las conocencias sobre fuentes de contaminacion y posibles amenazas, como en los casos del Complejo volcanico Puyehue-Cordon Caulle y de la represa hidroelectrica de "El Chocon". Por primera vez hemos detectado niveles de Hg que superan el umbral de seguridad de 5 mg/kg para Hg en plumas. Nuestros resultados parecen confirmar la utilidad de estudiar las tendencias temporales de los niveles de contaminacion, facilitando la intrepretacion de los resultados. Hemos ademas realizado por primera vez screening de los cinco metales en todas las especies estudiadas, creando un importante banco de dato para futuras investigaciones.

Abstract

Metal contamination is a global problem, caused by natural (e.g. volcanoes) and anthropic sources (industrial activity, mining etc.). Birds (especially birds of prey), are considered good biomonitors, as well as their feathers are good biomonitoring units for ecotoxicological studies on metal contamination. Our study is focused on the study of the levels of five metals (Hg, Pb, Zn, Cu, Cd) in Northern Patagonia Argentina, using carrion birds as biomonitors. By analysing the levels, temporal trends and variations between species, we have been able to increase our knowledge of contamination sources and possible threats, as in the cases of the Puyehue-Cordon Caulle Volcanic Complex and the "El Chocon" hydroelectric dam. For the first time we have detected Hg levels that exceed the safety threshold of 5 mg/kg for Hg in plumes. Our results seem to confirm the usefulness of studying the temporal trends of the contamination levels, facilitating the interpretation of the results. We have also carried out for the first time screening of the five metals in all the studied species, creating an important data bank for future investigations.

Introduction

Metal contamination is a global problem, particulary relevant in developing countries (Järup, 2003). Metals naturally form part of the earth's crust; and several are considered ubiquitous substances (Lepp, 2012). Some metals are considered micronutrients, necessary for the development of physiological functions of higher organisms (Alloway, 2013). Despite this, chronic (or high level) exposure can cause toxic effects in organisms (Flora et al., 2008). These effects vary according to the organism and the metal, but many of them are due to oxidative stress phenomena (Espín et al., 2014a). Metal emissions into the environment can have natural (geothermal activity, fires etc) or anthropogenic origins. Humans have known and used metals for thousands of years (Eisenreich et al. 1986, López-Costa et al., 2020). Almost all human productive activities generate metal emissions. Moreover, some activities can increase pollution. It is the case of hydroelectric dams, which reduce the water flow and increase metal deposition (Kummu and Varis, 2007; Wang et al., 2012). Due the impact of metal contamination on natural ecosystems and human health, continuous monitoring would be necessary to detect potential threats at an early stage, although in reality large areas of the planet have scarcely been studied (Di Marzio et al., 2019). These ecotoxicological studies require that biomonitoring species of high position in the trophic chain are used, in order to become also sentinel species for human health (Espín et al., 2016). Birds of prey are considered an optimal option as a biomonitoring species (García-Fernández et al., 2008, 2014; Martínez-López et al., 2004, 2005); many of them are protected and for that reason the use of nonlethal samples is strongly recommended (Ansara-Ross et al., 2013; Di Marzio et al., 2019; Movalli, 2000). Several studies demonstrate that feathers are suitable for this purpose (García-Fernández et al., 2013; Martínez-López et al., 2005). Feathers are easy to collect, store and analyse, showing the levels of contamination during the growing phase (García-

Fernández et al., 2013). The present study was carried out in Northern Patagonia, Argentina, analyzing the levels of five metals (Hg, Pb, Cd, Zn, Cu) in primary feathers of Andean condor (*Vultur gryphs*); Black buire (*Coragyps atratus*); Turkey vulture (*Cathartes aura*); Southern crested caracara (*Caracara plancus*) and Chimango caracara (*Milvago chimango*).

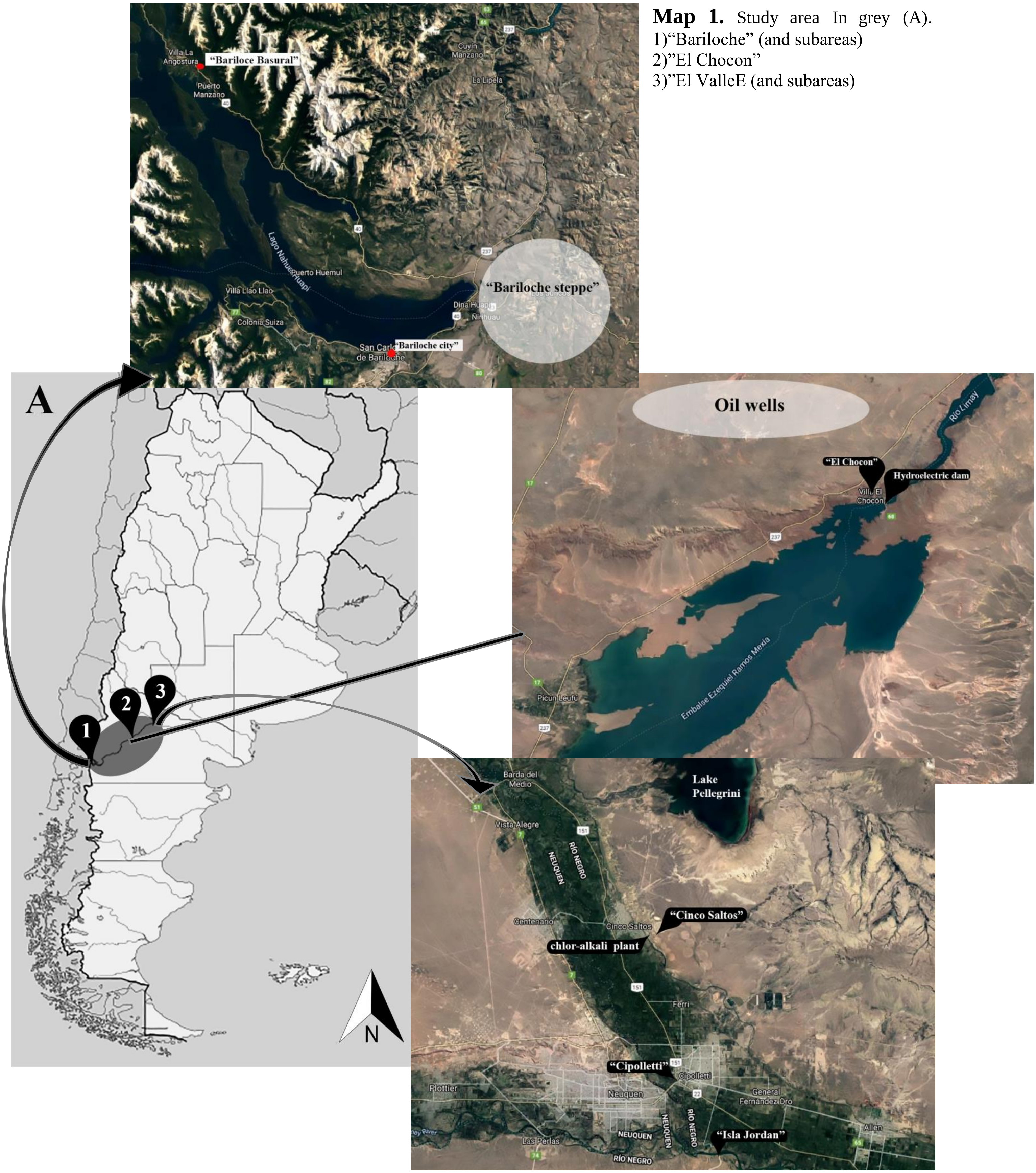
The aims of the study are the analysis of the levels of the metals mentioned above (particularly Hg), evaluating the main threats present in the territory and the collection of the levels of metals in species such as Chimango caracara, never before used in these types of studies, with an initial interpretation of the results.

Materials and methods

Study area

Our area of study includes the territory between the cities of Bariloche, (Rio Negro province) and the city of Neuquén (Neuquén province), in the north of the Argentine Patagonia, defining three sampling stations: "Bariloche", "El Chocón" and "El Valle" (Map 1). "Bariloche" includes the surroundings of the towns of Bariloche, Dina Huapi and Villa La Angostura, located on the border between the provinces of Neuquén and Río Negro, a rural area with low human density (Rizzo et al. 2011). The average elevation of the area is 893 meters above sea level (m.a.s.l.); the climate is humid continental (Köppen climate classification), with an average annual temperature of 8.1 °C and a total rainfall of 782.6 mm. The ecotones represented are the sub-Antarctic forest and the steppe (Bustos and Rocchi 2008).The area includes the Nahuel Huapi National Park with its main lake Nahuel Huapi and several other lakes. The main economic activities in the area are nature tourism, extensive cattle raising and trout farming (located at various points on the Limay River, the emissary of lake Nahuel Huapi). The area is interested by

Map 1. Study area In grey (A).
1)“Bariloche” (and subareas)
2)”El Chocon”
3)”El ValleE (and subareas)



an intense volcanic activity associated with the extensive network of active volcanoes present in the Andes Mountains both on the Argentine side and mainly on the Chilean side.

Following the hydrographic basin of the Limay River (which will take us until the last study area) we find "El Chocón". This study area includes the surroundings of Villa El Chocón, located downstream from Bariloche, 80 km from Neuquén. The village emerged around the construction of the hydroelectric dam on the Limay River, which is one of the main economic activities of the area.The ecotone represented is the steppe, an arid and temperate region with an average annual rainfall of between 80 and 300 mm. The average annual temperature is 15 °C, with a minimum of -13 °C in winter and a maximum of 43 °C in summer (El Chocón weather station). The main economic activities, in addition to those related to the hydroelectric dam, are extensive cattle raising and oil extraction.The third study area "El Valle" includes the confluence of the Neuquén and Limay rivers, between the towns of Cinco Saltos and the city of General Fernández Oro; an area of 100,000 ha (more than 40,000 ha are used for agriculture) (Pozo 2013). The average elevation of the area is 270 m above sea level; the climate is dry and cold desert (Köppen climate classification), with an average annual temperature of 14.5 °C and an annual rainfall of 186.9 mm (Bustos and Rocchi 2008). The ecotone represented is the steppe. It is a urbanized area (Neuquén with its 232,000 inhabitants is the most populated city in this part of Patagonia), where the main economic activities are represented by agriculture, industry and oil extraction (personal observations).

Species

The species studied in this work were: Andean condor (*Vultur gryphus*), Turkey vulture (*Cathartes aura*) and Black vulture (*Coragys atratus*) (Family *Cathartidae*), plus Southern crested caracara (*Caracara plancus*) and Chimango caracara (*Milvago chimango*) (Family *Falconidae*).

Cathartidae, also known as the "*New World Vultures*", are a family of birds that is still little studied. Andean condor, the largest scavenger bird of the world (11-15 kg, body weight), inhabits the whole South America continent throughout the Andes (Del Hoyo et al. 1994). Their diet consists of medium to large vertebrate carcasses (Lambertucci et al. 2009a). Worldwide the species is classified near threatened (NT), due to the negative effects of several human disturbances (Alarcón and Lambertucci 2018; IUCN 2017; Lambertucci et al. 2009b, 2011). Black vulture (2 kg, b.w.) is a sedentary scavenger bird species ranging all of South America and the southern part of the USA (Del Hoyo et al. 1994); its predilection for human settlements has allowed for its expansion in recent years (Barbar et al., 2015). The diet of Black vulture consists of carcasses of mammals and insects (Del Hoyo et al., 1994; Ballejo et al., 2018). Turkey vulture (1.4 kg, b.w.) is a migratory scavenger bird species ranging from southern Canada to Tierra del Fuego (Del Hoyo et al., 1994); the species has a more varied diet, including also remains of reptiles, fish and birds (Ballejo et al., 2018). The Southern crested caracara (2-3 kg, b.w.) is an opportunistic scavenger, ranging in South America (except Amazonia, Colombia, Peru, and high Andes), several Caribbean islands, Mexico, and is rarely encountered in the southern USA. (Del Hoyo et al. 1994). The diet of Southern crested caracara is primarily carcasses, juveniles, injured, slow moving birds, little rodents, reptiles, amphibians, fish, and arthropods (Del Hoyo et al., 1994). The Chimango caracara (0,3-0,4 kg, body weight) is an opportunistic scavenger, ranging in South America, southern of the Amazon forest (excluding the high Andean peaks) (Del Hoyo et al, 1994). The diet of the species includes insects, small vertebrates, carrion and fish (Biondi et al., 2005).

Sampling methods and metals analysis

We sampled and analyzed molted primary feathers (P1-P10) of the five species described, collected in roosts, during the southern springs (October-December) between 2009 and 2017 (see table 1). Before proceeding with the analyses, the feathers were submitted for washing, according to the methodology described by Espin et al. (2012), in order to clean the feathers and eliminate any possible sources of external contamination from our samples. After drying at room temperature for 12 hours, the feathers were measured, weighed and cut into pieces to obtain homogeneous samples of shaft and vane for analysis.

For Hg analyses 0.05 g of each was analyzed by atomic absorption spectrophotometry using the Milestone DMA-80 Direct Hg Analyzer to detect Hg levels. The method was tested, using certified reference material (CRM; TORT-2, lobster hepatopancreas, National Research Council Canada), for precision and accuracy as described in Espín et al. (2014b). Recovery percentage of total Hg from 5 replicates of CRM was 104.2 ± 11.8% (mean ± standard deviation). The coefficient of variation for the repeatability was 11.4%.

For Pb, Cu, Zn and Cd analysis in Black vulture, Turkey vulture, Southern crested caracara and Chimango caracara feather samples were digested to be analysed by anodic stripping voltammetry (ASV). Complete digestion was ensured by using high temperature digestion with a mixture of acids following the method described by Garcia-Fernandez et al. (1995). All the reagents used were Suprapur quality from Merck (Darmstadt, Germany). The quartz tubes used for the wet digestion were previously washed with 2% nitric acid for 48 h and then rinsed twice with tetradistilled water and dried in an oven at 100°C. Whole feathers were alternately washed in acetone and Triton X-100 diluted 1:400 (Hughes et al. 1997) to remove loosely adherent external contamination. They were then cut and dried at 80°C for at least 12 h. Then the samples were placed in LDPE (low-density propylene) flasks with an acid mixture (nitric/perchloric/sulfuric, 8:4:1) until the organic matter had completely disintegrated. One ml of acid mixture was used per each 100 mg of feather. Finally, 1 ml of the extract was submitted to a progressive thermal treatment and, once dried, was left to cool. Tetradistilled purified water was added and transferred to the measuring vessel, adjusting the final volume to 10 ml. The Andean condor samples, after the digestion process described above, were analyzed with using inductively coupled plasma optic emission spectrometry (Agilent Technologies ICP-MS. Model 7900). The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The Ultra High Matrix Introduction (UHMI) system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences. The limits of detection were 0.292 ppb (Cu), 0.871 ppb (Zn), 0.061 ppb (Cd), and 0.046 ppb (Pb). Some of the results were used in the previous chapters.

Statistics

All statistical analyses were performed with the IBM SPSS v. 24 statistical package. Metal concentrations in feathers are reported as mean ± standard deviation (SD), median and range (min-max). Generalized Linear Models (GLMs) were carried out to evaluate the effect of zone (Bariloche, El Chocon and El Valle) and year (2011, 2013 and 2017) on metal concentrations for each species. The concentration of each metal was selected as response variable, while year and zone were selected as explanatory factors in the models. The interaction between year and zone was also included in the models when possible. A second set of GLMs was performed only for Black vulture and Turkey vulture to evaluate the effect of the species, zone, year and their interactions on Hg concentrations. A backward stepwise procedure was followed to select the final models, excluding the explanatory variables when they had no significant effects. Concentration of metals were log10-transformed to make them better conform normal distribution. ANOVA followed by Tukey’s tests for multiple comparison were performed to test significant differences in metal concentrations between species (Black vulture vs. Turkey vulture vs. Andean condor), years and zones. The level of significance was set at $p \leq 0.05$ in all analyses.

Results

Feather metal concentrations in the five avian species by zone and year and the GLMs are presented in Table 1.

1. *Hg, Cd, Pb, Zn, Cu levels in Bariloche, El Chocon and Neuquen in Black vulture, Turkey vulture and Andean condor*

The best model explaining Hg concentrations in feathers in both Black vulture and Turkey vulture included year, zone and year x zone as explanatory factors, showing that Hg concentrations differed between years depending on the zone (Fig. 1). In general, Hg concentrations in feathers were higher in El Chocón than in Bariloche and El Valle for both species (Figure 1). Regarding the temporal trends, for Black vulture Hg levels were similar between years in Bariloche, while concentrations where higher in 2011 compared to 2017 in El Valle zone (Figure 1). The opposite trend was found in Turkey vulture, Hg levels being lower in 2011 compared to 2013 and/or 2017 in Bariloche and Chocon, while similar concentrations were found between years in El Valle. For other metals, no differences were found between zones in Black vulture, while lower Cu and Zn concentrations were observed in feathers of Turkey vulture from El Chocón compared to Bariloche and El Valle (Table 1).

When comparing metal concentrations among *Cathartidae* species, GLMs showed significant effects of year ($p<0.01$), zone ($p<0.01$), year x zone ($p<0.01$), year x species ($p<0.01$) and zone x species ($p=0.042$) on Hg concentrations in feathers. These results reflect that these species differed in feather Hg levels depending on the zone and the year. In this regard, Hg concentrations were lower in Black vulture compared to Turkey vulture in Bariloche, but similar concentrations were found in El Chocón and El Valle (Figure 2). On the other hand, Hg levels were lower in Black vulture in 2013 and 2017, but similar concentrations were observed for both species in 2011 (Figure 2). Similarly, Cd concentrations in feathers were lower in Black vulture compared to Turkey vulture in Bariloche, while levels of Pb, Cu and Zn were higher in Black vultures inhabiting this area (Figure 2). Zn concentrations were also higher in Black vulture compared to Turkey vulture in El Valle area (Figure 2).

Finally, Pb, Cu and Zn concentrations found in Andean condor in Bariloche (2017) were higher than those found in Black vulture and Turkey vulture in Bariloche in 2011 ($p<0.01$ in all cases; Table 1).

2. *Hg and Pb, levels in Bariloche and Neuquen in Chimango caracara and Southern crested caracara*

For Chimango caracara and Southern crested caracara, the best model explaining Hg concentrations included year and zone as explanatory variables (Table 1). Hg levels were higher in 2011 than 2017 for Chimango caracara in Bariloche, while the opposite trend was found in Southern crested caracara, with lower Hg concentrations in 2011 compared to 2017 in both Bariloche and El Valle (Figure 1). Regarding Pb concentrations in Chimango caracara, Pb levels were lower in 2011 compared to 2017 (Table 1).

3. *Comparison of Hg levels in Black vulture, Southern crested caracara and Chimango caracara from "Basural" sub area ("Bariloche")*

We decided to check the contamination levels at the "Basural" subarea of "Bariloche" (Map 1,2) sampling area. Black vulture and Chimango caracara, Hg concentrations did not differ between Bariloche (steppe for Black vulture, city center for Chimango caracara) and Basural in 2017 (mean ± SD in Black vulture: 323 ± 441 and 217 ± 315 ng/g, respectively, $p=0.99$; in

Chimango caracara: 400 ± 401 and 263 ± 205 ng/g, respectively, p=0.45). However, in Southern crested caracara, Hg levels were higher in "Basural" (1002 ± 792 ng/g) compared to Bariloche (steppe) (435 ± 526 ng/g; p<0.001) in 2017. In the subarea of Basural (Bariloche) in 2017, Southern crested caracara showed higher Hg concentrations (mean ± SD: 1002 ± 792 ng/g) than Chimango caracara and Black vulture (263 ± 205 and 217 ± 315 ng/g, respectively; p<0.001 in both cases).

Table 1. Feather metal concentrations (mean ± SD, median and range; ng/g) of four avian species from Patagonia (Argentina) in 2011, 2013 and 2017 and generalized linear models.

		Hg (ng/g)				**Cd (ng/g)**			**Pb (ng/g)**			**Cu (ng/g)**			**Zn (ng/g)**		
Species	**Zone**	**Year**	**N**	**Model (*p*)^a**	**Mean ± SD**	**N**	**Model (*p*)^b**	**Mean ± SD**	**N**	**Model (*p*)^b**	**Mean ± SD**	**N**	**Model (*p*)^b**	**Mean ± SD**	**N**	**Model (*p*)^b**	**Mean ± SD**
					Median (min-max)			**Median (min-max)**			**Median (min-max)**			**Median (min-max)**			**Median (min-max)**
Black vulture (Coragyps atratus)	Bariloche	2011	20	Year (<0.01) + Zone (<0.01) + Year x Zone (<0.01)	224.54 ± 156.61	20	None	13.83 ± 19.36	20	None	526.24 ± 538.27	20	None	3566.0 ± 3075.2	20	None	63401 ± 9910.2
					168.12 (85.76-654.27)			6.87 (0.06-76.68)			381.65 (179.99-2691.3)			2797.7 (2037.8-16375)			62629 (47496-94990)
		2013	9		437.22 ± 621.75												
					179.4 (54-1831.3)												
		2017	66		325.70 ± 486.69												
					161.65 (29.2-2193.3)												
	Chocon	2017	15		2198.5 ± 2582.8												
					1304.3 (202.9-8961.1)												
	El Valle	2011	5		1018.5 ± 891.90	5		7.81 ± 5.71	5		338.06 ± 73.23	5		2662.7 ± 445.01	5		69195 ± 7224.3
					864.81 (231.88-2441.9)			4.29 (4.17-17.31)			309.17 (271.17-459.8)			2791.2 (1909.1-3037.6)			70721 (58083-77754)
		2017	30		261.13 ± 387.81												
					111.75 (67.1-1707.3)												
Turkey vulture (*Cathartes aura*)	Bariloche	2011	14	Year (<0.01) + Zone (<0.01) + Year x Zone (<0.01)	133.82 ± 56.45	14	None	19.08 ± 6.38	14	None	288.35 ± 108.57	14	Zone (0.03)	2297.2 ± 247.60	14	Zone (<0.01)	55171 ± 9208.4
					133.57 (58.93-246.63)			17.23 (10.17-32.20)			233.25 (138.77-460.69)			2291.0 (1924.4-2909.6)			54714 (44236-73566)
		2013	13		2438.9 ± 2405.2												
					1569.3 (106-6743.5)												
		2017	37		1307.9 ± 1778.9												
					317.5 (59.7-6716.3)												
	Chocon	2011	15		405.54 ± 325.78	15		13.31 ± 4.57	15		385.52 ± 288.98	15		2083.4 ± 755.47	15		43301 ± 8325.0
					334.08 (39.34-1269.0)			13.23 (5.56-21.56)			305.08 (138.33-1322.9)			1938.2 (948.82-3993.1)			42054 (23179-56037)
		2017	27		2254.3 ± 2027.9												
					1990.7 (111.6-7322.9)												
	El Valle	2011	15		657.38 ± 1125.9	15		14.07 ± 14.08	15		1160.4 ± 2596.6	15		2484.9 ± 421.29	15		54623 ± 17856
					140.17 (51.1-4195.3)			12.81 (0.06-61.26)			203.12 (99.38-9468.0)			2514.8 (1847.9-3034.8)			52740 (35055-113332)
		2017	21		518.33 ± 885.67												
					140 (70.8-3564.8)												

Chimango caracara (*Milvago chimango*)	Bariloche	2011	3	Year (0.011) + Zone (0.015)*	955.16 ± 276.04 803.2 (788.5-1273.8)				3	Year (0.014)**	361.44 ± 343.24 179.22 (147.75-757.37)						
		2017	20		324.88 ± 307.52 239.4 (32.6-1161.9)				19		1226.3 ± 985.84 1013.7 (37.15-3707.8)						
	El Valle	2011	22		424.6 ± 487.97 177.4 (60.5-1865.7)				22		436.88 ± 303.73 334.65 (155.24-1466.3)						
Southern crested caracara (*Caracara plancus*)	Bariloche	2011	10	Year (<0.01) + Zone (<0.01)	134.92 ± 91.748 123.7 (31.2-360.8)												
		2017	57		756.74 ± 737.90 358.7 (73.9-3098)												
	El Valle	2011	11		541.22 ± 736.74 341.6 (87.9-2610.8)												
		2017	2		1707.2 ± 697.13 1707.2 (1214.3-2200.2)												
Andean condor *(Vultur gryphus)*	Bariloche	2017	-	NA	-	22	NA	192.75 ± 631.57 10.26 (<LOD-2941)	22	NA	1191.1 ± 1095.3 902.2 (512.3-5842.2)	22	NA	5698.7 ± 2139.0 5481.1 (3121.2-14081)	22	NA	122152 ± 22139

Model: indicates the most influential factor (explanatory variable) in the response variable "Metal concentration".

None = concentration of metal is not significantly influenced by any variable.

[a]Year, Zone and Year x Zone were included as factors in these models (*The interaction Year x Zone was not included in this model).

[b]Zone was included as factor in these models (**Year and Zone was included as factors in this model).

NA = Not applicable.

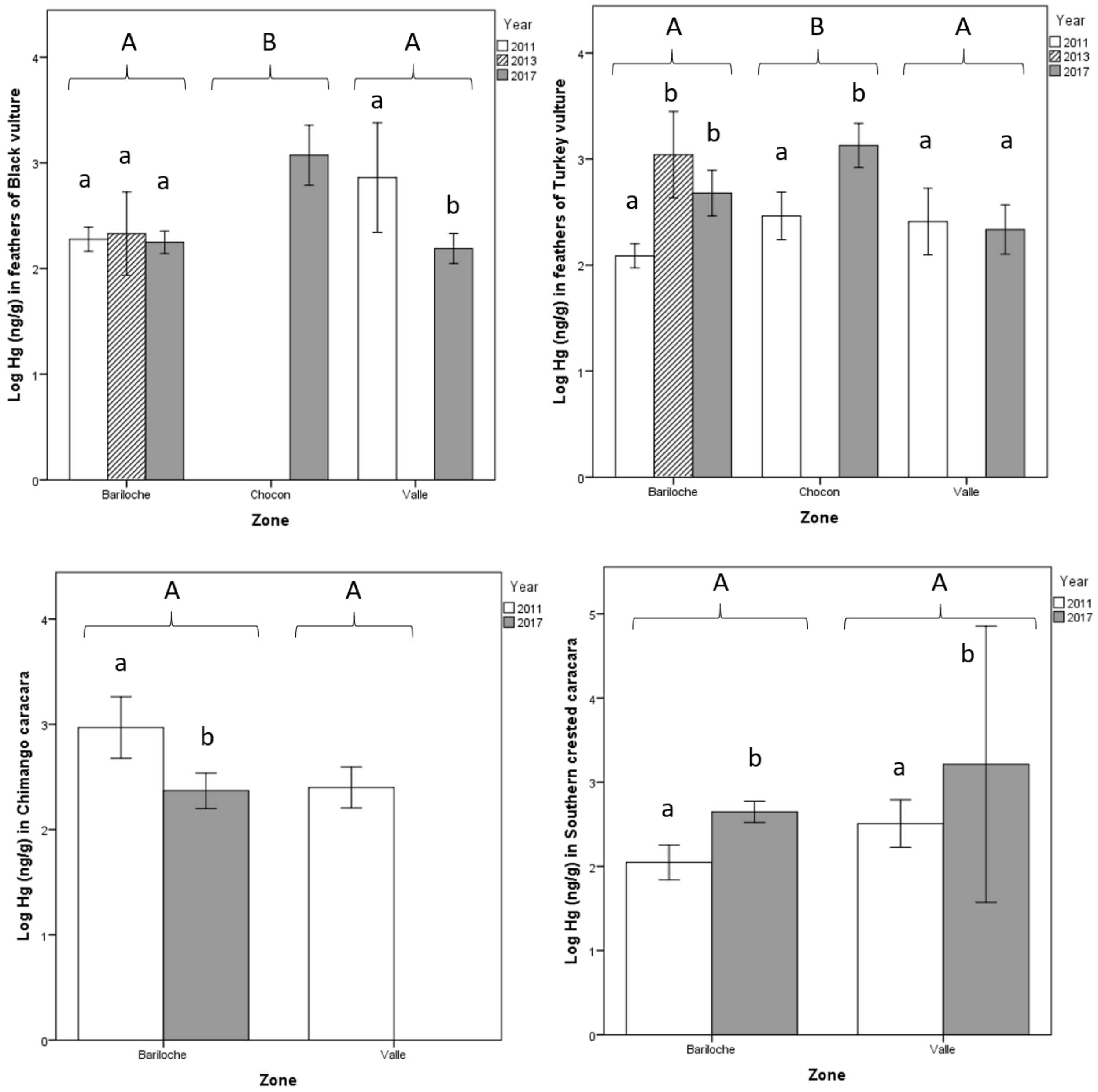


Figure 1. Log Hg concentrations (±95% CI) in feathers of four avian species from Patagonia (Argentina) by zone (Bariloche, Chocon and El Valle) and sampling year (2011, 2013 and 2017). Different letters above bars indicate significant differences between zones (capital letters) or between years within each zone (lowercase letters).

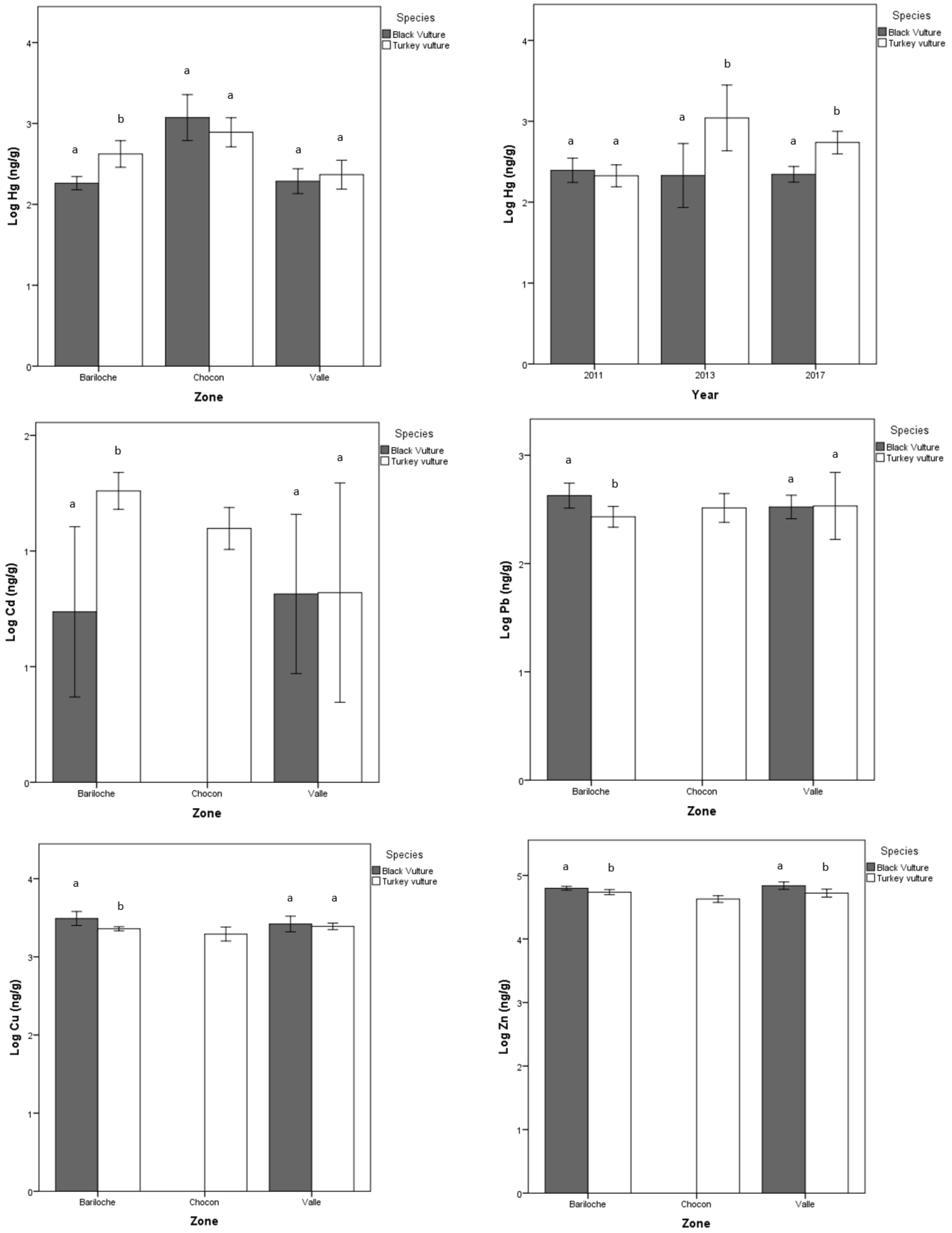


Figure 2. Log metal concentrations (±95% CI) in feathers of Black vulture and Turkey vulture from Patagonia (Argentina) by zone (Bariloche, Chocon and El Valle) and Log Hg concentrations by year (2011, 2013 and 2017). Different letters above bars indicate significant differences between species.

Discussion

Due to the various aspects discussed in point 1 of the results, we divided the discussion into two sections, to make it easier to read.

***1a.** Hg levels Bariloche, El Chocon and Neuquen in Black vulture and Turkey vulture*

The results of our study show that for both species the highest levels of Hg have been measured in "El Chocon" (Fig.1). In addition, the temporal trend of Hg levels in Turkey vulture seems to indicate seems to indicate that between 2011 and 2013 some punctual source has generated an increase in Hg, while in Black vulture the levels in the Bariloche area do not seem to suffer variations between 2011 and 2017 (Fig.2). On the other hand, Hg levels of Turkey vulture (2011, 2017) and Black vulture (2017) in the Neuquen area remain stable (Fig. 1). The 2011 Hg levels of Black vulture come from a very small pool of samples (n=5) with the presence of a sample with outlier values (2.44 mg/kg) (Table 1). As commented also by Di Marzio et al. (2018) this situation makes the interpretation of these datas difficult. For all these reasons, we can consider the existence, within our study area, of two zones that probably owe the detected Hg levels to different causes: Bariloche-El Chocón and Neuquén. The cause of this division is probably due to the presence of the El Chocon hydroelectric dam (Map 1,3). Hg contamination related to the presence of hydroelectric dams is a frequent problem in several South American countries and particularly studied in Brazil, due to the extensive network of hydroelectric dams present in the country (Di Marzio et al., 2019). Hydroelectric dams reduce water flow and thus the capacity of water to purify itself, accelerating the consequent decline of water quality, with increasing concentrations of metals and other contaminants (Kummu and Varis, 2007; Wang et al., 2012). The Hg levels detected in “Bariloche-El Chocon” area could be due to the June 2011-January 2012 eruption of the Puyehue-Cordón Caulle volcanic complex (PCCVC). As we have seen before, the area of Bariloche was particularly affected (Peréz Catán et al. 2016). Despite this, only a few ecotoxicological studies have been carried out on the possible impact of volcanic eruption on high trophic level species (Di Marzio et al., 2020; Plaza et al., 2020) and none of them focused exclusively on Hg. This is probably because very low levels of Hg have been detected in the ash of the PCCVC (Shkinev et al., 2016). Bubach et al. (2012), using lichens as bioindicators, seem to show that the Hg levels detected were not linked to this eruption. Di Marzio et al. (2020) also evidence that in Andean condor feathers from the Bariloche area (in samples related to the eruption) the Hg levels detected do not seem to indicate that the eruption has generated Hg emissions. The same conclusions could be reached analyzing the Hg levels detected in our study in Black vulture feathers in the Bariloche area. Bubach et al. (2015) report that numerous previous studies have linked Hg contamination to PCCVC activity. The PCCVC complex also has a fumarole system that emits Hg in gaseous form (Higueras et al., 2014). This kind of emission in normal conditions reaches Bariloche at basal levels although in 2007 high levels were recorded up to 50 km away from the fumarole. Normally, gaseous mercury is diluted in air and is not dangerous (Higueras et al., 2014), but these conditions may have changed during the 2011 eruption. The mobilization of mercury is greatly influenced by climatic factors such as temperature, rainfall, etc. (Nriagu and Becker, 2003; Pirrone et al., 2003) and it is possible that emissions from fumaroles may have precipitated in an aquatic environment. This would explain why studying lichens and birds of terrestrial food habits in Bariloche (Andean condor and Black vulture) have not detected elevated levels of mercury, while in samples of Turkey vulture, which as we have seen above integrates its diet with fish, these emissions are reflected. Arcagni et al. (2017) stated that the Hg present in the trophic chain of Lake Nahuel Huapi undergoes biomagnification processes in those autochthonous benthic species, but not in the introduced trout, result similar to those of the research by Rizzo et al. (2014); but it is necessary to consider that several aspects of Hg biomagnification are still not completely clear (Lavoie et al., 2013). Also, Balseiro et al. (2014) reported during a 2013 sampling thatthe aquatic ecosystem of the lakes around Bariloche were regenerating and that also in other occasions this process of regeneration after an eruption had a duration of 1-3 years. Although for the moment we cannot assert with absolute certainty if the emissions come from the eruption, the above explanation allows us to say that it is a plausible hypothesis.

Another result for the Bariloche area is that, as hypothesized by Di Marzio et al. (2018), the median levels of Hg detected in Black vulture (0.17 mg/Kg/2011, 0.18 mg/kg/2013, 0.16 mg/kg/2017) and the median levels detected in Turkey vulture in 2011 (0.13 mg/kg) (Table 1) can be considered the baseline levels for the Bariloche area. Also Bubach et al. (2012) identified as baseline levels for Bariloche, similar levels detected in lichens.

As mentioned above, the El Chocon area presents the highest Hg levels (8.96 mg/kg in Black vulture, 2017). Although "El Chocon" is an area less studied than Bariloche, denying us important information for the interpretation of data (e.g. diet composition), interpreting the studies of Ballejo et al. (2018) and using important data from direct observations (unpublished) we can try to explain the results of this area. We have already talked about the amplifying effect of the contamination exerted by the hydroelectric dam and we have seen that the eruption of the PCCVC could explain the detected Hg levels. As reported by Jackson et al. (2011), Hg contamination in rivers can be detected more than 100 km downstream from the point of emission; the distance between "Bariloche" and "El Chocón" is approximately 250 km. As reported by Jackson et al. (2011), Hg contamination in rivers can be detected more than 100 km downstream from the point of emission; the distance between "Bariloche" and "El Chocón" is approximately 250 km; the distance would be less if we calculate the range of the volcanic material. Another possible source of Hg could be the oil wells present in this area in the direction of "Neuquen". This area is part of the largest oil and gas deposits in Argentina, which began to be exploited around 1918 (Besil, 2020; Stratta, 2013). Despite the proximity of several oil wells (50-60 km), we would be at the limit of the home range of both species. Furthermore, the time trend in Turkey vulture does not seem to suggest that oil wells are the cause of the variations; eventual contamination by extractive activity would probably be responsible for the baseline levels. We can also hypothesize that unlike Bariloche, in "El Chocon" the Black vulture include fishes in its diet. Sazima (2007) reports that the species has an enormous plasticity in its diet and that in situations of scarcity of other resources, it can also feed on fish. In "Bariloche" Black vultures usually feed in the "*Mallín*" (a type of fertile meadow and wetland found in southern Chile and Argentina rich in wildlife and domestic animals) (personal observations). "El Chocon" is found in the steppe, a semi-desert environment where fauna is more scarce. This could justify the ipotesis of a more "ichthyophagous" behaviour by the Black vulture and consequently the high levels of Hg. As mentioned above, the "Neuquen" area seems not to have been influenced by the eruption of the PCCVC. The distance and the presence of the physical barrier of the El Chocón dam could be the reason.

Finally we analyze the area "El Valle". As we can see in Fig. 2, the levels of both species in "El Valle" are similar, although in Turkey vulture we have recorded high levels on occasion (4.2, 2011; 3.5, 2017). A possible source of Hg contamination in the area could be the old chlor-alkali plant at “Cinco Saltos” (Neuquen) (Di Marzio et al., 2018). Another possible source would be, as in the case of "El Chocón" the oil wells present in the area (personal observations). The lack of more information makes it difficult to interpret the data. We can however make a couple of considerations. The levels of Hg, quite stable over time and between species, could be indicating the basal levels of the area. In this case the median levels (Black vulture 0.11 mg/kg, 2017; Turkey vulture 0.14 in 2011 and 2017) are similar to the baseline levels of the Bariloche area. On the other hand, the samples with the highest Hg levels come from the subarea "Isla Jordan" downstream from the chlor-alkali plant, while the sampling carried out in the Black vulture colony of the subarea "Cinco Saltos" (Map 1d) (infront of the old chlor-alkali plant) has not shown high values. This could indicate that the contamination of the chlor-alkali plant (closed in 1998) (Arriberé et al., 2003) has less impact, although interviews with local inhabitants during the sampling have provided us with information about the behaviour of this colony. Interviewees reported that Black vultures move daily from their roost to Lake Pellegrini, which seem to be their foraging area. Whereas the lake upstream of the chlor-alkali plant, it wouldn't influence the Hg levels.

1b. *Cd, Pb, Zn, Cu levels in Bariloche, El Chocon and Neuquen in Black vulture, Turkey vulture and Andean condor*

For Pb, Cd, Zn and Cu analisis, we have samples only from Bariloche and El Valle from the year 2011 (Fig. 2). Interpreting these results is complex, due to the lack of information. The data is more relevant as a database. Still, we can make some considerations comparing the results of Black vulture (2011) and Turkey vulture (2011) with the results obtained in the Andean condor (2017) samples from Bariloche. For the Cd, comparisons are difficult. Black vulture and Turkey vulture samples are from 2011 (feathers grown in 2010), while the Andean condor samples are from 2017 (feathers grown in 2011-2012). As we have seen above, the 2011 Black vulture and Turkey vulture feathers seems to reflect the situation in Bariloche before the eruption of the PCCVC. Di Marzio et al. (2020) have shown that the 2017 Andean condor samples can be considered post-eruption samples. For this reason we focus on the interpretation of those data less related to the eruption (Pb and Cu), excluding the comparison of Cd levels and giving a new interpretation to Zn levels. Pb, Cu and Zn concentrations found in Andean condor in Bariloche

(2017) were higher than those found in Black vulture and Turkey vulture in Bariloche in 2011 ($p<0.01$ in all cases). Several studies have demonstrated that the primary source of Pb contamination in our study area comes from hunting ammunition (Lambertucci et al., 2011; Plaza and Lambertucci, 2019). Due to inter-specific hierarchies, Andean condors are the first to feed, followed by Black vultures and finally Turkey vultures (Donázar et al., 1999; Wallace and Temple, 1987). This implies that Andean condors are more exposed to lead bullets contamination, and that Pb levels in this species are higher than in other Cathartidae, as evidenced also for Plaza and Lambertucci, 2019. For Zn and Cu the interpretation of the results is more complex. These are essential elements in many physiological processes. The levels detected are beyond the safety limits for both. One explanation could be that these levels are normal for these species. On the other hand, Di Marzio et al. (2020) have shown that after the volcanic eruption of the PCCVC, the levels of Zn have risen; considering the geothermal activity the possible source of emission. Another explanation for these levels could be hunting, as for Pb. Both lead and lead-free ammunitions contain Cu and Zn, which can be deposited in the organisms that are shot, without reaching toxic levels (García-Fernández & Navas Ruiz, 2020; Schlichting et al., 2017)

2a. *Hg levels in Bariloche and Neuquen Southern crested caracara*

Hg levels were lower in 2011 compared to 2017 in both Bariloche and El Valle (Fig.1). The Hg levels of the Southern crested caracara in the Bariloche area show a similar trend to the Turkey vulture with higher levels in 2017. The 2011 Hg levels are in line with the basal levels in the Bariloche area, while in 2017 we see an increase in the median levels similar to those of Turkey vultures. The opportunistic diet of the Southern crested caracara, which also includes fish (Del Hoyo, 1994), could be the reason for this similarity.

2b. *Hg and Pb levels in Bariloche and Neuquen in Chimango caracara*

The discussion about the Chimango caracara data deserves prior clarification. The species is one of the most widespread bird of prey in all of South America (Del Hoyo et al., 1994). Despite this, there is little interest in this species, which has been little studied (Figueroa Rojas et al., 2015). In addition, the feathers, small in size, can be easily blown away by the wind, making sampling difficult (personal observations). Without previous data and with a limited volume of samples, our interpretations are relative. We consider, however, that since there are no previous studies, these data have enormous importance for future research. The Chimango caracara has a very small home range (approx. 15 km) (personal comunication). Hg levels were higher in 2011 than 2017 for Chimango caracara in Bariloche. We have investigated whether there were differences between the different sampling points in each study area. For Hg the point with the lowest average concentration of Hg was “Cipolletti” (El Valle) with 0.16 ± 0.31 mg/kg, while the highest average concentration was recorded in “Isla Jordan” (El Valle) with 1.41 ± 0.31 mg/kg. The distance between the two locations is 6 km, but “Cipolletti” is located on the banks of the Neuquen River, a few km before the confluence with the Limay River to form the Negro River, and “Isla Jordan” is located on the banks of the Negro River. The data is interesting, considering that “Cipolletti” is halfway between chlor-alkali plant and “Isla Jordan”, suggesting that there are other sources of Hg contamination, because if the Hg came only from the plant, it should be greater in “Cipolletti” and not in “Isla Jordan” as we have detected. This situation is similar to the one we have seen in the case of the Black vulture, and opens up the possibility that the Hg contamination in the "Jordan Island" sub-zone may have a different origin to the effluents of chlor-alkali plant.

No significant differences in Pb concentrations were observed among the tow studies areas (six sampling points) (F=2,198, p=0,075). Thus, considering “El Valle” as a whole and the area of

"Bariloche" plus the subareas "Basural"(Map 1b), significant differences in Pb concentrations were observed (F=3,266, p=0,031). In order to know which groups are significantly different, Tukey's test was carried out, and significant differences were observed between "El Valle" and "Bariloche" (p=0.020). With respect to "Basural", no significant differences were found with other groups, which could be due to the high deviation found. The highest value of Pb concentration was in "Basural" (3.70 mg/kg); . as for the minimum, also recorded at "Basural" (0.03 mg/kg). In this case it must be considered that the sampling in Bariloche was carried out within the city, a few meters away from a sheet metal workshop; which could represent the probable source of Pb.

3. *Comparison of Hg levels in Black vulture, Southern crested caracara and Chimango caracara from "Basural"* (dump) *sub area ("Bariloche")*

We also compared the Hg levels in the sub-zone "Basurero" (2017) in the Black vulture, Southern crested caracara and Chimango caracara, the three species present in the area (Fig.3).

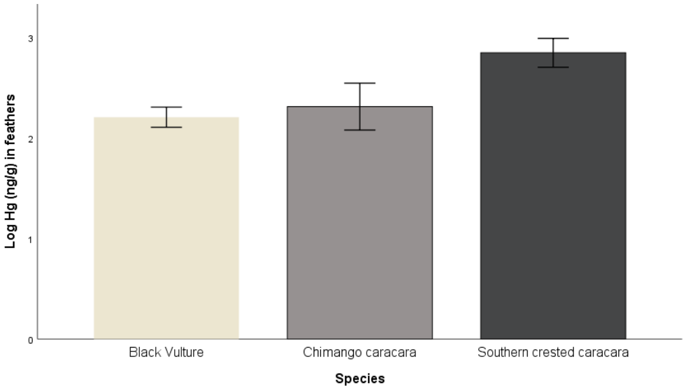


Fig 3. Log Hg concentrations (±95% CI) in feathers of three avian species in "Basural" 2017.

Southern crested caracara showed higher Hg concentrations (mean ± SD: 1002 ± 792 ng/g) than Chimango caracara and Black vulture (263 ± 205 and 217 ± 315 ng/g, respectively; p<0.001 in both cases). In Black vulture and Chimango caracara, Hg concentrations did not differ between Bariloche (city for Chimango caracara, steppe for Black vulture) and "El Basural" in 2017 (mean ± SD in Black vulture: 323 ± 441 and 217 ± 315 ng/g, respectively, p=0.99; in Chimango caracara:

400 ± 401 and 263 ± 205 ng/g, respectively, p=0.45). However, in Southern crested caracara, Hg levels were higher in “Basural” (1002 ± 792 ng/g) compared to Bariloche (steppe) (435 ± 526 ng/g; p<0.001) in 2017. Black vulture specimens marked in the subareas of "Basural" and "Bariloche "(steppe) have shown a sedentary behaviour, being observed almost exclusively in their marking area (personal communication) We can assume that the feeding of these animals is linked to the rubbish present in the dump. In addition to the variables considered above (species, diet), one factor which may influence this result is age. For Black vultures, adults rather than juveniles were observed during sampling. Similar observations were made with the Chimango caracara. For the southern crested caracara, most of the population consisted of immatures, using the dump as a school for hunting (mainly rats and insects) or to find easy food in the rubbish (personal observations).

Risk assessment

As previously mentioned, the use of feathers for the study of metal contamination is a widespread technique (García-Fernández et al., 2013); therefore we can count on good bibliographic references that indicate threshold level in feathers for some of the metals studied. For Zn and Cu we used results of research with Chatartidae for comparison of results (Table 2). The values referred below are indicative, because several parameters can influence the concentrations able to generate lethal and sublethal effects on birds (Burger 1993).

For the Cd, none of the samples exceeds the value of 2 mg/kg, estimated with the threshold level by Burger and Gochfeld (2000). Also the Pb levels detected in all samples are below the threshold level of 4 mg/kg (Scheuhammer, 1987). The Cu and Zn levels detected in our study are similar to those reported in the table. These levels are considered harmless in the corresponding studies.

Metal	***Threshold level (mg/kg; dry weight)***	***Reference***
Hg	<5	Burger and Gochfeld, 1997; Palma et al., 2005; Eisler, 2006; Albuja et al., 2012
Pb	< 4	Scheuhammer, 1987
Cd	< 2	Burger and Gochfeld, 2000
*Zn **	179 (*Cathartes aura*) 32-150 (*Cathartes aura*) 76-160 (*Gymnogyps californianus*)	Cahill et al., 1998; Haskins et al., 2013; Wiemeyer et al., 1986
*Cu**	2.6-7.8 (*Cathartes aura*) 1.7-7.6 (*Gymnogyps californianus*)	Haskins et al., 2013; Wiemeyer et al., 1986

Tab. 2 Threshold levels of Hg, Pb, Cd in bird feathers *Zn and Cu Levels detected in other Cathartiadae without adverse effects

Regarding the Cd, none of the samples exceeds the value of 2 mg/kg, estimated with the threshold level by Burger and Gochfeld (2000). Also the Pb levels detected in all samples are below the threshold level of 4 mg/kg (Scheuhammer, 1987). The Cu and Zn levels detected in our study are similar to those reported in the table. These levels are considered harmless in the corresponding studies. However, Hg levels generate some concern and deserve a more in-depth description. The average levels detected were under the threshold level of 5 mg/Kg, confirming that our study area can be considered a low Hg contamination area. Despite this we detected, for the first time in the areas "Bariloche" and "El Chocón", Hg levels between 4.4-8.9 mg/Kg in 14 samples of Turkey vulture and Black vulture. These levels, compatible with negative effects, could also harm human health.

Conclusions

We carried out an extensive multi-year study in Northern Patagonia, Argentina, detecting for the first time in this area, high levels of Hg compatible with adverse effects on wildlife and humans. These results would seem to show for the first time that during the 2011 eruption of the PCCVC there were Hg emissions. We also show the possible magnifying effect of the Hg contamination caused by the "El Chocon" hydroelectric dam. This type of environmental problem, investigated in other regions of Argentina, had not been studied before in our area of study. The study area between Bariloche and El Chocon, however, can be considered a low level of Hg exposure, although with episodic and potentially dangerous contamination. For these reasons, these data should be considered in the planning of new studies (especially in the eventuality of future eruptions), considering that in 2011 the possible contamination by Hg was under investigated. The Neuquen zone presents more stable levels over time and the possible sources of Hg emissions should be different from other areas. More research is needed in this area as well.

We also present the results of the first screening of various metals (Pb, Cd, Zn and Cu) in Black vulture and Turkey vulture feathers from Bariloche and El Valle, representing mainly an important database for future studies, given the lack of research on some of the metals analysed. Finally, we compared the data from both species in the Bariloche area with the data obtained by analysing the feathers of Andean condors (2017). The levels of none of the four metals appear to represent a health threat. These data, especially those relating to Hg levels seem to confirm that the Cathartidae are good choices as biomonitors in ecotoxicological studies. The Hg and Pb levels obtained from samples of Chimango caracara, the first for this species, although difficult to interpret represent another important database for future research. The Hg and Pb levels obtained from samples of Chimango caracara, the first for this species, although difficult to interpret represent another important database for future research. The Chimango caracara could represent an important tool for ecotoxicological studies in urban areas

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Discussion general

En el presente apartado se abordan los principales resultados de la tesis de forma global.

Vacío de datos

La falta de información científica sobre muchos aspectos de nuestro estudio ha sido un factor limitante importante que supuso varios retos para nosotros. En el *capítulo I* hemos podido constatar que la problemática no es circunscrita a la sola Argentina, que incluso representa uno de los países que ha producido el mayor esfuerzo estudiando la contaminación ambiental por metales pesados (Di Marzio et al., 2019). Se trata claramente de números relativos, que destacan parcialmente en el contexto Latinoamericano, pero que posicionan Argentina lejos de los promedios mundiales (Di Marzio et al., 2019). La situación económica de muchos países puede ser una de las razones que explique la escasez de estudios, aunque hemos visto por ejemplo que Cuba se posiciona por encima de México en nuestra clasifica. Las investigaciones, además de ser pocas, son mal distribuidas, con amplias áreas de este inmenso continente totalmente ignoradas. Los estudios se centran, aunque el trend parece estar cambiando, sobre especie con escaso potencial como especie biomonitoras (ej. moluscos) y se centran casi exclusivamente en ambientes acuáticos. Si por un lado se puede entender este interés, considerando que metales como el Hg y Pb (dos de los metales más estudiados en Latinoamérica) resultan más tóxicos en ambientes acuáticos (Eisler, 1987, 1988), por otra parte, hay que considerar que se trata de temáticas ampliamente estudiadas y que existen evidencias que estos metales pueden fácilmente pasar de los ambientes acuáticos a los terrestres, causando problemas (Speir et al., 2014; Xie et al., 2008). De los ecosistemas terrestres argentinos los únicos que han sido investigados se encuentran en nuestra área de estudio y son el resultado de nuestra labor en colaboración con el Laboratorio Ecotono de la Universidad del Comahue de Bariloche. Esta es la razón principal porque muchos de los resultados reportados en la presente tesis representan “primeros resultado”, como reportado en todos los capítulos. A todo lo comentado anteriormente hay que añadir la falta de información sobre nuestras especies de estudio. La familia Cathartidae presenta aspectos importantes de su biología que solo ahora se están empezando a estudiar. Hemos visto en el *capítulo II, III, IV y V* algunos de ellos que queremos aquí comentar de manera unitaria. Desde nuestro punto de vista, las principales limitaciones han surgido a raíz de la falta de información sobre el patrón y la fenología de la muda del cóndor Andino. De esta especie, tan emblemática desde el punto de vista ambiental y cultural para Latinoamérica, no existen estudios sobre este argumento. Y la poca información publicada (Snyder et al., 1987) se refiere al cóndor de California y se trata de material antiguo, incompleto y con conclusiones posiblemente erróneas. La situación es ligeramente mejor para el jote colorado (Chandler et al., 2010) y por comparación de resultados, para el jote negro también. Para el cóndor Andino ha sido difícil comparar con especies relacionadas, porque hasta la fecha la familia Cathartidae todavía no tiene una Orden cierta (Di Marzio et a., 2018). Estudios recientes parecen indicar su pertenencia a la orden Accipitriformes (la misma de los buitres euroasiáticos) (Zuberogoitia et al., 2013) y basándonos en estos estudios hemos considerado la duración de la muda del cóndor como la de los buitres del género *Gyps*. En los *capítulos IV y V*, gracias a esta información, hemos realizado por primera vez una comparación de los niveles de Hg detectados en plumas de cóndor andino, jote negro y jote colorado crecidas en el mismo lapso de tiempo. De los pocos trabajos que han investigados especies subrogadas del cóndor (en este caso cóndor de California) solo Wiemeyer et al. (1986) han empleados plumas como unidad biomonitora, sin tener en consideración este parámetro. Para carancho y chimango, presentes en los *capítulos II y V*, la situación ha sido mejor, disponiendo de más informaciones sobre la biología de las especies, aunque desde el punto de vista ecotoxicológico las dos especies han resultados muy poco estudiadas.

Contaminación por Hg en el norte de la Patagonia Argentina

El estudio de la contaminación por Hg en nuestra área de estudio ha sido el fil rouge de esta tesis, acompañado en los *capítulos III y V* por el análisis de otros metales. Nuestra área de estudio es un territorio no muy densamente poblado, con Bariloche y Neuquén que representan los dos únicos centros de mediano tamaño (observaciones personales). La zona “El Chocón”, pese a ser la menos poblada, ha resultado el área con los niveles de Hg más altos, con los niveles mayores detectados en 2013. Esto se debe con mucha posibilidad al efecto combinado de dos factores. Por un lado, la erupción del Complejo Volcánico Puyehue- Cordón Caulle (Volcán Puyehue) del año 2011 y por el otro la represa hidroeléctrica sobre el rio Limay, proprio a la altura del Villa El Chocón.

Como hemos visto en el *capítulo III y V* nuestra área de estudio, principalmente la zona de Bariloche, fue particularmente afectada en 2011 por la erupción del Volcán Puyehue, situado en el lado chileno de los Andes. La dirección del viento (desde oeste) fue responsable de empujar las cenizas hacia Bariloche y sus alrededores, depositándolas sobre una superficie de 36 millones de hectáreas (Pérez Catán et al., 2016). En el *capítulo III* vimos que las plumas mudadas de cóndor Andino muestreadas en 2017 (crecida en 2011-2012), habían almacenados metales emitidos por el volcán. Justificamos esta afirmación con la presencia de altos niveles de Si, que como evidenciado por varios estudios (Caneiro et al., 2011; Daga et al., 2008; Lara et al., 2004) representa el componente principal de las cenizas del volcán Puyehue. En nuestro trabajo los niveles de Hg detectados en las plumas de cóndor fueron bajos, y no parecían tener variaciones relacionadas con la erupción. Justificamos este dato con los resultados de Bubach et al. (2012), que evaluando los niveles de Hg en líquenes (utilizados en otras ocasiones con la misma finalidad) afirma que las cenizas del volcán Puyehue no contienen Hg. Pese a esto, los resultados del *capítulo V* parecen contradecir las afirmaciones de Bubach et al. (2012). En las muestras de jote colorado de la zona de Bariloche y de El Chocón (aquí junto con las muestras de jote negro) detectamos altos niveles de Hg en varios ejemplares en el año 2013, con valores > 6 mg/Kg (p.s.). En plumas, niveles de Hg >4 mg/Kg (p.s.) se consideran peligrosos (Burger and Gochfeld, 1997; Palma et al., 2005; Scheuhammer 1991). Es posible que el Hg durante la erupción haya salido en forma de gas, desde las fumarolas del volcán (Higueras et al., 2014). Por la temporalead (niveles de Hg más alto en 2013 respecto a 2011 y 2017) y por la falta de otras fuentes de emisión Hg conocidas, podemos pensar que el Hg proceda de la erupción. Hay que considerar también que la movilización del mercurio está muy influenciada por factores climáticos como temperatura, precipitaciones, etc. (Nriagu y Becker, 2003; Pirrone et al., 2003). Factores climáticos podrían haber condicionado la disponibilidad del Hg emitido, facilitado su deposición en el medio acuático. Esto justificaría porque no se han registrados altos niveles en las muestras de cóndor Andino y de jote negro (especies con hábitos alimentares prevalentemente terrestres en el área de Bariloche) y en los líquenes del estudio de Bubach et al. (2012). El jote colorado, por otro lado, como evidenciado por Ballejo et al. (2018) incluye pescado en su dieta en nuestra área de estudio.

Como hemos comentado, el otro factor que puede justificar los altos niveles Hg de “El Chocón” es la presencia de la represa hidroeléctrica. La presencia de barreras físicas de este tipo, que ralentizan el flujo de agua, aumenta la deposición de contaminantes y reducen la capacidad de los ríos de depurarse (Kummu and Varis, 2007; Wang et al., 2012). En el *capítulo I* hemos evidenciado la preocupación respecto al impacto de estas estructuras, reflejadas en un discreto número de estudios, principalmente en Brasil, país que cuenta con una extensa red de represas hidroeléctricas (Di Marzio et al., 2019). Nuestra observación debería ser la primera que evidencie en el norte de la Patagonia Argentina este tipo de riesgo potencial para la salud humana y la calidad del ecosistema.

Biomonitores, especies subrogadas y trend temporales

El capitulo II es debajo de muchos puntos de vista la piedra angular de esta tesis. Pese a que los resultados no sean concluyentes, nos ha permitido tener los primeros datos sobre los niveles de contaminación en el norte de la Patagonia Argentina. En él hipotizamos que los niveles de Hg detectados en jote negro, jote colorado y carancho pudieran representar los niveles basales para el área de Bariloche. En el *capitulo V* hemos podido comprobarlo, contrastando nuestras conclusiones con las que han obtenido Bubach et al. (2012) en líquenes, además hemos visto como estos niveles se mantienen constantes en el jote negro, pese a la contaminación generada posiblemente por el volcán Puyehue. Estos datos sugerirían el posible uso del jote negro como biomonitor para contaminación por Hg, principalmente en ecosistemas terrestres. En los *capítulos IV y V* evidenciamos también la posible capacidad del jote colorado de reflejar la contaminación por Hg en ecosistemas acuático, evidenciando como esta característica puede ser utilizada para estudios ecotoxicologicos en ambientes parecidos a nuestra área de estudio donde se registra la falta de aves rapaces acuáticas. También resulta curiosa la posición del carancho, cuyos valores reflejan los niveles basales de 2011 y niveles de contaminación intermedios a jote negro y jote colorado en 2017. En el *capítulo III* pudimos comprobar que el estudio de las plumas de cóndor Andino permite obtener informaciones sobre la contaminación generada por una erupción volcánica. Como comentado anteriormente, gracias a los datos del *capitulo V* pudimos comprobar que también las plumas de jote negro y jote colorado sirven, especialmente para casos como el Hg, como acabamos de comentar. Todos estos datos parecen demostrar que los Catarthidae puede ser buenas especies biomonitoras, a pacto de contextualizar su uso. Los datos preliminares que hemos obtenidos en muestras de chimango en el *capítulo V* son de difícil interpretación, principalmente por el reducido numero de muestras y por la falta de estudios previos sobre la especie. Una consideración importante sobre esta especie es que se trata se trata de la rapaz más común en Suramérica, con un *home range* extremadamente reducido (10-15 km, comunicaciones personales) respecto a jote negro (30-40 km) y de jote colorado (60-70 km) (Coleman y Fraser 1989; De Vault et al. 2004; Holland et al. 2017).

Hemos abordado también otra temática, el uso de especie subrogadas con finalidad de indicadores (Favreau et al., 2006), un argumento controvertido en estudios ecotoxicológicos. La técnica, conocida y utilizada en ensayos de laboratorio (principalmente con peces) (Besser et al., 2005; Beyers, 1995; Walker, 2014), no es tan usual en estudio de campo con aves necrófagas. Empleada por primera vez con subrogadas del cóndor de California (Wiemeyer et al., 1986), ha sido utilizada poco y con resultados no siempre fáciles de interpretar. Carpenter et al. (2003) avisaban que la comparación de resultados entre especies diferentes resulta complicado debido a las múltiples variables (a veces difíciles de estudiar) que interfieren. Los estudios realizados con cóndor de California (Herring at al., 2018; West et al., 2017; Wiemeyer et al., 1986) siempre ha utilizado como especies subrogadas el jote colorado y el cuervo (*Corvus corax*) sin realizar comparaciones de resultados. Herringer et al (2018) sugería focalizar las investigaciones en el estudio de tendencias temporales. En nuestro *capitulo IV y V* hemos realizado estos tipos de comparaciones, por primera vez en plumas de Cathartidae solos. Hemos evidenciado la presencia de tendencias temporales parecidos entre jote negro y jote colorado. Además, hemos observados algunas tendencias que se repiten en varios estudios y en nuestros *capítulos IV y V* (sobre contaminación por Hg y Pb), en tejidos diferentes (sangre y plumas) entre las dos especies de cóndor (Andino y Californiano) y los dos jotes (negro y colorado). (Herring et al., 2018; Plaza et al., 2020). Los niveles de Hg resultan siempre mas elevados en jote negro y jote colorado (en el caso del jote colorado más) respecto a los niveles detectados en cóndor (Herring et al., 2018). Al revés, como hemos visto también el nuestro *capitulo V,* los niveles de Pb son siempre mas elevado en las dos especies de cóndor respecto a los niveles de los dos jotes (Herring et al., 2018; Plaza et al., 2020). Desafortunadamente, por falta de estudios, nuestra interpretación de estos datos (de considerar anecdóticos en este momento) es puramente descriptiva.

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General conclusions

The specific conclusions of this thesis are presented in each of the chapters that make up this study. The following are the general conclusions of the doctoral thesis, providing some recommendations for future studies.

- Most of the results presented in this work represent first results on the different studied topics. Considering the scarcity of studies at Latin American level, its importance is to provide reference data for future research.
- We recommend the development of a work protocol common to all Latin American countries, so that the sharing of information between different work groups is more effective.
- The species studied seem to represent good options as biomonitors, contextualizing their use to specific needs. It is necessary to study more the species of our study, which given its wide distribution could allow the realization of comparable studies from different countries
- The temporal trends and the tendencies that seem to exist between certain species studied should be investigated more in depth, to verify their reliability and thus consent to a productive use of the surrogate species of the Andean and California condors.
- The Hg levels detected in the Bariloche area in the samples of black jote (2011-2017), red jote and carancho (2011) seem to represent the basal levels for the area. The real impact of Hg levels on public health and natural ecosystems in our area of study should be carefully studied. The results of our work have shown a worrying increase in levels, exceeding safety limits in some cases.

- Preliminary data obtained by studying chimango samples seem to indicate that the species could represent a good biomonitor for ecotoxicological studies in very precise hurban areas.

Extended abstract

Title: Assessment of environmental pollutant exposure in Argentinean Patagonian scavenger birds through the use of non-invasive samples.

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Introduction

Metals and metalloids are ubiquitarian substances, natural constituents of the earth's crust (Järup, 2003; Singh et al.2011). The natural emission sources for these substances mainly are geothermal activity and forest fires (Nriagu, 1990; Pirrone et al., 2010). Although natural emissions occasionally can represent a great treat (e.g. Hg emissions during the eruption of the Krakatoa volcano (1883) and Mt. St. Helens (1980) (UNEP, 2013)), the problem of environmental pollution by metals is generally related to human activities. Industry, mining, fuel production and urban pollution are the main sources of metal emissions (Da Rocha et al., 2015; Pereira et al., 2007; Soto-Jiménez and Flegal, 2009), but even agriculture (pollution by As contained in insecticide) can contribute (Peryea, 1998). Although some metals may be necessary for the development of normal cellular functions, exposure to high and/or prolonged doses may cause neurotoxic, genotoxic and carcinogenic alterations (Flora et al., 2008; Leonard et al., 2004), through mechanisms such as oxidative stress (Espín et al., 2014a, b, 2016a; Flora et al., 2008; García-Fernández et al., 2002).

Metals are very persistent in the environment and can accumulate in ecosystems and the species that inhabit them (García-Fernández et al., 2005). The negative effects induced by metal exposures are well known, however the problem remains and even in the developing areas of the world chronic exposure to metals is increasing (Järup, 2003; Suresh Kumar et al., 2015). One of concern areas is Latin America, where national legislation concerning environmental pollution has developed (or is developing) very slowly. Excluding Brazil, where environmental protection laws began to be implemented in the early 80s, the rest of the countries have taken decades to begin legislating on this matter (Lagos and Peters, 2010). In many cases, these laws are also adapted to the economic interests of large companies, such as the mining sector, in detriment of communitarian and environmental interests (Tafur, 2011). All that because mining is a strategic sector in Latin America, especially along the Andes and the Amazon River Basin. The 44.6% of the world's copper is extracted in Latin America. Four of the five countries with the highest mining activity of various minerals are in Latin America: Peru (1st silver, 2nd zinc, 3rd copper and tin, 4th molybdenum and lead, 5th gold), Chile (1st copper, lithium and iodine), Brazil (1st niobium, 2nd iron, 3rd bauxite, 5th tin) and Bolivia (3rd antimony and 4th tin) (Lagos and Peters, 2010). As mentioned above, mining is not the only source of pollution in Latin America. Agriculture, industry, fishing/hunting, mining and, in large megalopolises, urban pollution (Da Rocha et al., 2015; Pereira et al., 2007; Soto-Jiménez and Flegal, 2009) are important sources of contamination. And the US anthropogenic activities represent another source of pollution in the border areas (Soto-Jiménez and Flegal, 2009). These activities have been investigated and the results suggest that they are threatening wildlife (Ferreyra et al., 2014; Ronco et al., 2007; UNEP et al., 2010).

However, the information available about these other sources of pollution is incomplete and less structured than the information obtained from the annual reports of the mining sector (Burger, 2006; Utmazian and Wenzel, 2006).

Ecotoxicological biomonitoring studies help to assess environmental contamination, identifying possible sources of risk to wildlife and humans (Beeby 2001). The selection of an appropriate biomonitoring species is very important. The main characteristics that a species needs to be considered a good biomonitors are: 1) high trophic level, 2) widely distributed in the territory and numerically abundant in the territory to be monitored, 3) sedentary (or with well-studied movement patterns), 4) its sampling is simple and affordable cost (Hollamby et al., 2006; Holt and Miller, 2011). It is important to clarify that bioindicator and biomonitor species, in ecotoxicological studies, are two different things although many times the two words are used as synonyms. The bioindicators provide qualitative information about the contamination, while the biomonitors will provide quantitative data about the quality of the contamination (Weinstein and Davison, 2003). Birds, in particular birds of prey, can be considered good biomonitors (García-Fernández, 2014; García-Fernandez et al., 2008, 2020; Espín et al., 2020). The constant loss of biodiversity in recent years (WWF, 2018) imposes on the scientific community a careful use of endangered species for research. The use of non-invasive sampling techniques could reduce the negative impact that scientific research can sometimes have on threatened wildlife. The use of raptor's molted feathers as a biomonitoring unit is an important technique in environmental biomonitoring studies, especially metal contamination studies (e.g. Hg). During the growth phase, due to the bloodstream circulation and rapid turnover rate, the feathers act as a depot tissue of trace elements, representing a detoxification pathway (Martínez-López et al., 2004, 2005). When the growth of the feather is over, it becomes a stable and long-lasting trace element storage unit (Burger 1993). The feathers are easy to collect and store and the metal levels detected in feathers are related to hematic metal levels (Martínez-López et al., 2004, 2005; Ansara-Ross et al. 2013; García-Fernández et al. 2013; Espín et al., 2016).

Objectives

The general objective of this thesis is to study metal contamination in northern Patagonia, Argentina, evaluating natural and human emission sources and evidencing possible threats to wildlife and humans.

Objective 1. (Chapter I) **To compile the knowledge on the use of wildlife species as biomonitor/bioindicator of environmental contamination in Latin America.**

To achieve this objective an extensive bibliographic review of environmental pollution studies by metals in Latin America between 1990 and 2017 was carried out. This review was focused on the use of animal species as biomonitors/bioindicators. Special attention was paid to highlight the most studied areas, as well as the main metals, ecosystems and type of samples used.

Objective 2. (Chapter II**) To assess the influence of anthropogenic activities on mercury exposure in scavengers' birds from Patagonia (Argentina).**

To achieve this objective was to analyse, for the first time, the environmental contamination by Hg in terrestrial ecosystems in Northern Patagonia Argentina, using molted feathers of 3 species of scavenger birds as a biomonitoring tool.

Objective 3. (Chapter III) **To evaluate temporal trends and the volcanic activity on metal exposure in Andean condor.**

To achieve this objective was investigated the metal emissions during the eruption of Puyehue-Cordon Caulle volcanic complex (PCCVC) (2011) using molted primary feathers of Andean condor (*Vultur gryphus*) from the surroundings of Bariloche (Argentina). In addition, was performed the first screening of the levels 9 metals and metalloids (Si, Cr, Cu, Zn, As, Se, Cd, Pb, Hg) in Andean Condor.

Objective 4. (Chapter IV) **To evaluate the potential usefulness of Cathartidae of Northern Patagonia as a surrogate species for Andean condor (*Vultur gryphus*) and as a biomonitor regarding Hg exposure.**

To achieve this objective the Hg levels detected in Black vulture (*Coragyps atratus*) and the Turkey vulture (*Cathartes aura*) feathers with the Hg levels detected in Andean condor (*Vultur gryphus*) feathers were compared, to test the possible use of Black vulture and Turkey vulture as a surrogate species of Andean condor. At the same time, was found that the Turkey vulture could be a good biomonitor for studies on Hg contamination in our study area.

Objective 5. (Chapter V). **To evaluate temporal and spatial trends of Hg, Cd, Pb, Cu and Zn levels in the Limay River basin in anthropized and natural environments.**

To achieve this objective, the levels of Hg, Cd, Pb, Cu, Zn in Black in vulture and Turkey vulture samples, gathered in three sampling areas during several years, were compared for the first time. As well, for the first time a comparison of Cd, Pb, Cu, Zn levels in the Bariloche area between Andean condor, Black vulture and Turkey vulture was carried out. Also, Hg contamination in Southern crested caracara in two sampling areas and Pb and Hg contamination in Chimango caracara in two sampling areas was evaluated. In the case of Chimango caracara was possible to evaluate the contamination in subareas of sampling due to its reduced home range. Finally, was compared the Hg levels detected at the Villa la Angostura Dumpsite between Black vulture, Southern crested caracara and Chimango caracara.

Material and methods

Study area and species of study

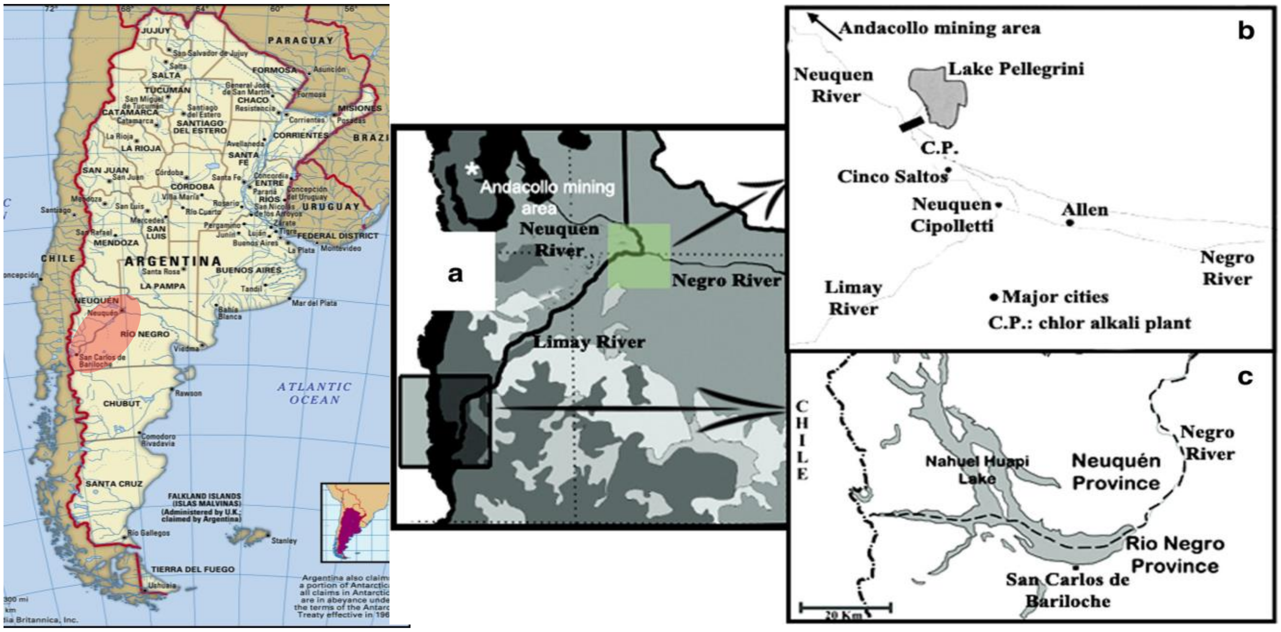


Fig. 1 Geographical representation of the study area (red circle in the Map of Argentina). A Sampling area. b Sampling area "El Valle." c Sampling area "Bariloche"

Northern Patagonia Argentina is a sparsely populated area, where cattle ranching, sporting hunting and tourism represent the main economic activities. Our area of study was the upper and middle basin of the Limay River (Rio Negro after the city of Neuquén) and its surroundings from Bariloche to Isla Jordan (Neuquén). Within this area, which is 380 km long and 80 km wide, we chose 3 sampling zones: Bariloche, El Chocón and El Valle. The first area "Bariloche" includes the surrounding of Bariloche city, Dina Huapi and Villa La Angostura, between the provinces of Neuquén and Rio Negro, a rural area with low human population density (Rizzo et al. 2011). The average elevation of the area is 893 amsl; the climate is humid continental (climatic classification of Köppen), with an average annual temperature of 8.1 °C and total rainfall of 782.6mm. The ecotone represented are the sub-Antarctic forest and the steppe (Bustos and Rocchi 2008). The area includes the Nahuel Huapi National Park with its main lake Nahuel Huapi and several other lakes. The main economic activity of the area are extensive cattle raising, trout farming (located in several points of the Limay River, into our sampling area) and nature tourism. The area has intense volcanic activity associated to the Andes Mountains both in the Argentinian and mainly in the Chilean side of the Andes. The second study area "El Chocón" include the surrounding of Villa El Chocón, located downstream from Bariloche, 80 km away from Neuquén. This is a temperate arid region with a mean annual precipitation between 80 and 300 mm, the ecotone represented is the steppe. The mean annual temperature is 15 °C, with a minimum temperature of –13 °C in winter and a maximum of 43 °C in summer (El Chocón meteorological station). The main economic activities are extensive livestock farming, oil extraction and hydroelectric power production. The third study area "El Valle" includes the confluence of the Neuquén river and Limay river, between the localities of 5 Saltos and General Fernandez Oro city an area of 100,000 ha (over 40,000 ha are used for agriculture) (Pozo 2013; Romero Gámez 2013). The average elevation of the area is 270 m above mean sea level (amsl); the climate is dry and cold desert (climatic classification of Köppen), with an average annual temperature of 14.5 °C and annual rainfall of 186.9 mm (Bustos and Rocchi 2008). The ecotone represented is the steppe. It is a highly urbanized area, where the main economic activities are represented by agriculture (fruit and vegetable production), industry and oil extraction.

The study species have been: Andean condor (*Vultur gryphus*), Turkey vulture (*Cathartes aura*) and Black vulture (*Coragys atratus*), of the Family Cathartidae, plus Southern crested caracara (*Caracara plancus*) and Chimango caracara (*Milvago chimango*) of the family Falconidae. The Cathartidae, also known as the "New World Vultures", are a family of birds that is still little studied. Andean condor, the largest scavenger bird of the world (11-15 kg, body weight), inhabits the whole South America continent throughout the Andes (Del Hoyo et al. 1994). Their diet consists of medium to large vertebrate carcasses (Lambertucci et al. 2009a). Worldwide the species is classified near threatened (NT), due to the negative effects of several human disturbances (Alarcón and Lambertucci 2018; IUCN 2017; Lambertucci et al. 2009b, 2011). Black vulture (2 kg, b.w.) is a sedentary scavenger bird species ranging all of South America and the southern part of the USA (Del Hoyo et al. 1994); its predilection for human settlements has allowed for its expansion in recent years (Barbar et al., 2015). The diet of Black vulture consists of carcasses of mammals and insects (Del Hoyo et al., 1994; Ballejo et al., 2018). Turkey vulture (1.4 kg, b.w.) is a migratory scavenger bird species ranging from southern Canada to Tierra del Fuego (Del Hoyo et al., 1994); the species has a more varied diet, including also remains of reptiles, fish and birds (Ballejo et al., 2018). The Southern crested caracara (2-3 kg, b.w.) is an opportunistic scavenger (Ferguson-Lees and Christie 2001), ranging in South America (except Amazonia, Colombia, Peru, and high Andes), several Caribbean islands, Mexico, and is rarely encountered in the southern USA. (Del Hoyo et al. 1994). The diet of Southern crested caracara is primarily carcasses, juveniles, injured, slow moving birds, little rodents, reptiles, amphibians, fish, and arthropods (Ferguson-Lees and Christie 2001). The Chimango caracara (0,3-0,4 kg, body weight) is an opportunistic scavenger, ranging in South America, southern of the Amazon forest (excluding the high Andean peaks) (Del Hoyo et al, 1994). The diet of the species includes insects, small vertebrates, carrion and fish (Biondi and Favero, 2005).

Samples

Fresh-molted primary flight feathers (P1–P10) collected from the roosting areas were used in this research. The sampling was conducted during the austral spring of 2007, 2009, 2011, 2013 and 2017 (October to December); this time of year, covers the courtship, mating, and breeding periods of the species studied in the sampling region. Due to the type of sample and the sampling methodology, age or sex of the individuals are unknown. To reduce the possibility of pseudo replication, only one feather per sampling point (or more than one if it was the same primary feather from the same wing) was collected. Each sample was kept in an individual paper bag, labelled (study area, day, and species), and stored at room temperature in a dry place until analysis. A total of 441 primary feathers were collected.

Table 1. Total of the samples used for all the chapters of the thesis

	2007	*2009*	*2011*	*2013*	*2017*
Vultur gryphus	8	18	-	-	22
Coragyps atratus	-	-	25	9	111
Cathartes aura	-	-	44	13	85
Caracara plancus	-	-	21	-	39
Milvago chimango	-	-	25		21

Before the analysis of the feathers, they were subjected to a sequential series of baths with tap water, distilled water, and Milli-Q® water (ISO 3696) in order to eliminate possible contaminants deposited on the feathers. The feathers were dried at room temperature for 12 h (Espín et al. 2012). Once cleaned, the feathers were chopped to obtain a uniform sample of shaft and vane and stored in a sterile container. In all samples we measure the Hg levels. For the analysis of total Hg, we used 0.05 g d.w. of each feather (vane and shaft), in a nickel boats and analysed, by a Milestone DMA-8 Direct Hg Analyzer by atomic absorption spectrophotometry, with a detection limit of 0.0001 ppm, following USEPA Method 7473 (sediments, soils, and sludges). The applicability of this method to the analysis of biotic samples has been previously demonstrated (Haynes et al. 2006). The calibration curve was calculated with 11 points (in duplicate) from 0 to 1004 ng of Hg. The precision and accuracy of the method were tested using certified reference material (CRM) (n = 11; Hg standard for AAS, Fluka, 1000 mg/L Hg in 12% nitric acid). Recovery of total Hg from seven replicates of CRM diluted to 1 ppm was 98.14 ± 3.52% (mean ± standard deviation). The coefficient of variation for repeatability was 3.58%.

For the analysis of Si, Cr, Cu, Zn, As, Se, Cd, and Pb planned in the objective 3, the feather samples were placed in LDPE (low-density propylene) flasks with the addition of an acid mixture (nitric/perchloric/sulfuric, 8:4:1) for the organic matter disintegration (1 ml of acid mixture/100 mg of each feather). We transferred 1 ml of each predigested extract to a quartz tube, in order to dry the sample completely with a progressive heat treatment. When the tubes were cooled, we added purified water, transferring all of it to the measuring vessel, completing the final volume of 10 ml with 1% nitric acid. After digestion, the detection and quantification were performed using inductively coupled plasma optic emission spectrometry (Agilent Technologies ICP-MS. Model 7900). The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The Ultra High Matrix Introduction (UHMI) system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences. The limits of detection were 0.065 ppm (Si), 0.073 ppb (Cr), 0.292 ppb (Cu), 0.871 ppb (Zn), 0.023 ppb (As), 0.816 ppb (Se), 0.061 ppb (Cd), and 0.046 ppb (Pb).

For the analysis of Pb, Cd, Zn, Cu planned in the chapter V feather samples were digested to be analysed by anodic stripping voltammetry (ASV). Complete digestion was ensured by using high temperature digestion with a mixture of acids following the method described by Garcia-Fernandez et al. (1995). All the reagents used were Suprapur quality from Merck (Darmstadt, Germany). The quartz tubes used for the wet digestion were previously washed with 2% nitric acid for 48 h and then rinsed twice with tetradistilled water and dried in an oven at 100°C. Whole feathers were alternately washed in acetone and Triton X-100 diluted 1:400 (Hughes et al. 1997) to remove loosely adherent external contamination. They were then cut and dried at 80°C for at least 12 h. Then the samples were placed in LDPE (low-density propylene) flasks with an acid mixture (nitric/perchloric/sulfuric, 8:4:1) until the organic matter had completely disintegrated. One ml of acid mixture was used per each 100 mg of feather. Finally, 1 ml of the extract was submitted to a progressive thermal treatment and, once dried, was left to cool. Tetradistilled purified water was added and transferred to the measuring vessel, adjusting the final volume to 10 ml.

For the objective 1 we performed a systematic and extensive review using specific key words (Contamination, Heavy metals, Caribbean, Central America, Latin America, South America, plus each country separately), in Google Scholar, PubMed & Scopus. In this way we looked for identifying studies on heavy metals contamination in Latin-America. We included articles from 1990 to 2017, this chronological range was decided because of the difficulty to find online material published before 1990. In addition, we reviewed and included those papers cited in the references of the articles found that were relevant. A total of 5450 papers were found and from them 690 were pertinent and thus included in the database. The review was done in English and Spanish. The study area was delimited in the north by the border between Mexico and the United States, in the south by the Beagle Channel, in the west by the Pacific Ocean and in the east by the Atlantic Ocean, including the Galapagos Islands to the west and the Falkland Islands (Malvinas Islands) to the east. The other criteria to include a paper were: 1) the species used as biomonitor must be an animal, and 2) the metals investigated were Hg, Cd, Cr, Cu, Pb, Ni, Zn, Fe, Mn, As, and Se. The following data were collected for each study: 1) the year of publication, 2) the country where the study was carried out, 3) the species used, 4) the type of environment where the study was carried out (terrestrial/aquatic), 5) the types of samples used, 6) the sampling techniques used (live/dead/sacrificed animals). Only studies analysing metals in samples from the field were selected, excluding toxicity assessments performed in the laboratory. In addition, we searched in Scopus to find the total number of studies conducted by national institutions (using the "affiliation country" filter) of each country in the area of Environmental Sciences (in English and Spanish) between 1990 and 2017. A second search was also carried out keeping the same parameters. The filter "country of affiliation" was replaced by "keywords, title or summary". In this way we obtained the number of studies in the area of Environmental Sciences carried out in each country by foreign institutions. In order to quantify each country's interest in environmental issues, we compared the number of studies carried out by national institutions with the number of studies carried out by foreign institutions.

Statistical analysis

In the *Chapter II* all analyses were carried out using the SPSS v.15.0 statistical package. Reported Hg concentrations represent median, mean ± standard deviation, and range. We used the Mann-Whitney test for the comparison between species. We used generalized linear models (GLM, normal distribution) to analyse the concentrations of Hg in each sample, using Hg concentration in each sample as the response variable. The explanatory variables considered were the study area and species. Four models were compared: (a) the null model, (b) the model with the variable “study area” (c) the model with the variable “species” (d) the model with two variables (study area + species), and finally, (e) the model with the interaction of both variables (study area × species). The level of significance for these tests was set at $\alpha = 0.05$. Furthermore, the quality of each model relative to each of the other models.

In the *Chapter III* all statistical analysis of the data was performed using SPSS v.25 (IBM SPSS Statistic) software. We first reported basic statistics (mean, median, SD, R). As the metal concentration data were not normally distributed, we used the nonparametric Mann–Whitney test to evaluate differences between years. We applied Spearman’s nonparametric correlation test to evaluate the relationships between each metal. The significance level was set at $\alpha = 0.05$. In addition, we evaluated the Hg detoxification process through the calculation of the Hg:Se molar ratio described by Méndez-Fernández et al. (2014). This ratio was calculated as Hg:Se = (Hg (μg g-1 ww)/Se (μg g-1 ww)) × (78.96 (g mol-1)/200.59 (g mol-1)), where 200.59 g mol-1 and 78.96 g mol-1 are the atomic mass of Hg and Se, respectively.

In the *Chapter IV* we used software R for data analysis (R Core Team, 2019). We considered p-values less than 0.05 as statistically significant. At first, we compared levels of mercury detected in the Andean Condor samples from 2017 (feathers grown in 2011-2012) with those detected in Black vulture and Turkey vulture (pooled samples from 2011 and 2013). Due to strong asymmetry in data, we used Mann-Whitney U test for rank distribution comparison (Sokal and Rohlf, 1995) between every species-pair. We used Benjamini&Yakutieli approach (Benjamini and Yekutieli, 2001) to account for false discovery rate in multiple testing during pairwise comparisons. Further on, we evaluated ability of one species to be a surrogate for other in description of change of the mercury contamination levels. To analyse differences in temporal change, we used linear regression with interaction, to account for interspecies differences within sampling years. In general form fullest model is written as:

Relative_Mercury~Year+Species+Year:Species,

where “Year” is sampling year, “Species” is sampled species. Terms separated with “+” indicate main effects (overall difference for specific year or species), while “Year*Species” explaining year specific differences between species. We used year as a categorical variable. As mercury levels were highly asymmetrical, we first ln-transformed values, to reduce heteroscedasticity (Sokal and Rohlf, 1995; Zuur et al., 2007). Then, as we care about change in levels specific for species, we scaled and centered species-specific ln-transformed values pooled over samples from 2011, 2013 and 2017, creating dependent variable “Relative_Mercury”. This procedure reduces possible artefacts due to value differences between species and allows investigation of change itself. We evaluated all the possible variable combinations and calculated AICc value. We chose model with least AICc as the best descriptor of information (Burnham and Anderson, 2002). This approach resulted in model with main effect of species excluded, as species-specific differences were accounted for during scaling procedure.

In the *Chapter V* all statistical analyses were performed with the IBM SPSS v. 24 statistical package. Metal concentrations in feathers are reported as mean ± standard deviation (SD), median and range (min-max). Generalized Linear Models (GLMs) were carried out to evaluate the effect of zone (Bariloche, Chocon and El Valle) and year (2011, 2013 and 2017) on metal concentrations for each species. The concentration of each metal was selected as response variable, while year and zone were selected as explanatory factors in the models. The interaction between year and zone was also included in the models when possible. A second set of GLMs was performed only for Black vulture and Turkey vulture to evaluate the effect of the species, zone, year and their interactions on Hg concentrations. A backward stepwise procedure was followed to select the final models, excluding the explanatory variables when they had no significant effects. Concentration of metals were log10-transformed to make them better conform normal distribution. ANOVA followed by Tukey's tests for multiple comparison were performed to test significant differences in metal concentrations between species (Black vulture vs. Turkey vulture vs. Andean condor), years and zones. The level of significance was set at $p \leq 0.05$ in all analyses.

Results and discussion

Chapter 1. From Mexico to the Beagle Channel: A review of metal and metalloid pollution studies on wildlife species in Latin America.

Information about emissions of metals and metalloids (Hg; Cd; Cr; Cu; Pb; Ni; Zn; Fe; Mn; As; Se) is scarce and fragmented in Latin America, limiting research which could benefit from these data. To know the state of the research, we reviewed the studies of environmental pollution by metals and metalloids carried out on animal species in Latin America.

Temporary distribution

We found 690 scientific papers on metal and metalloid pollution corresponding to our search criteria. The general trend of scientific research on pollution by metals and metalloids in Latin America shows an increase in the last 28 years. Until the year 2008 the number of papers never exceed 30 articles per year, while since 2009 (excluding 2017) this value is always exceeded. These data are in line with the trend of scientific research in Latin America evidenced by Santa and Herrero Solana (2010) and with the global trend evidenced by Burger (2006).

Geographical distribution and ecosystems

The papers we found were distributed in 27 countries; Brazil, Mexico and Argentina were the first countries in terms of number of publications, equivalent to 74% of the total. Checking whether the research was carried out by local or foreign groups, we found that of the first nine countries (those with more than 10 studies) Mexico, Bolivia and Peru are the only countries where scientific research is carried out mainly by foreign research groups. To try to give an interpretation we have evaluated the average GDP (Gross Domestic Product) (1990–2017) and the average percentage of GDP devoted to scientific research (1996–2017) in each country, using World Bank data (www.worldbank.org). The first country by percentage of GDP devoted to scientific research were Brazil (6409 USD/capita; 1.07% for research) followed by Argentina (8309 USD/capita; 0.48% for research), Cuba (3970 USD/per capita; 0.48% for research), Peru (3382 USD/per capita; 0.44% for research), Chile (8344 USD/capita; 0.36% for research), Bolivia (1550 USD/per capita; 0.28% for research), Colombia (3926 USD/per capita; 0.20% for research), Mexico (7247 USD/per capita; 0.08% for research). It is very important to remind that the levels of GDP and the percentage invested in the research represent only one of the multiple factors that influence the scientific output of each country.

With 96% of the studies (n=662) concentrated in aquatic ecosystems (marine and freshwater), Latin America is well above the global trend of approximately 40% (n=214) (Burger, 2006). This situation generates a gap of information that makes difficult to interpret data from new research on terrestrial ecosystems, slowing down scientific progress in this field (Di Marzio et al., 2018; Martínez-López et al., 2015). The studies show a wide and unequal distribution, with large unstudied areas and focal points where research efforts have been concentrated. In the Caribbean area, we found only 1 study investigating terrestrial ecosystems, from a total of 35 studies carried out in the area. Central America and Mexico follow a similar pattern with only 9 studies on terrestrial habitat from a total of 155 studies. The largest number of studies (n > 80) are concentrated in the Gulf of California (Mexico), an area of high ecological and economic importance, according to the Mexican government (Soto-Jiménez and Flegal, 2009). The Gulf of California is one of the most economically developed areas of the country, with a strong presence of mining, industry and agriculture; activities that generate pollution by metals (Sánchez-Rodríguez et al., 2001; Soto-Jiménez and Flegal, 2009). The presence of Pb in this area is also partly caused by the industrial's emissions from the U.S.A. (Soto-Jiménez and Flegal, 2009). In South America we found the same trend as in Central America and the Caribbean, with 18 studies on terrestrial ecosystems out of a total of 518 studies. We found four areas where most studies are concentrated. The first is the Amazon river basin (n=80). In this vast area of Brazil, the studies are distributed along the river, from the border with Peru to the mouth of the Atlantic Ocean. The Madeira river basin in its stretch within the State of Rondônia, the Tapajos river basin in the State of Pará and the Río Negro basin (including the confluence in Manaus) are the most studied areas of the entire Amazon basin. In these three river basins mercury (Hg) has been used in artisanal gold mining since the XVI (Malm, 1998). The problem of mining pollution is amplified by the presence of a vast network of hydroelectric dams and their reservoirs, that increases concentrations of metals and other pollutants (Kummu and Varis, 2007; Wang et al., 2012). The problems of the impact of hydroelectric dams as well as contamination by mining are also studied in French Guiana, Chile, Venezuela, Argentina, Peru (Alcalde and Gil, 2000; Cid et al., 2009; Copaja et al., 2016; Diringer et al., 2015; Durrieu et al., 2005; Rondon and Pérez, 1999). The second area covers the coastal strip from Rio De Janeiro to Curitiba (n=83), a highly populated area. Urban pollution and industrial activities represent the main problems of this area (Avelar et al., 2000; Pereira et al., 2007; Quiterio et al., 2004). Behind this strip we detected another zone where pollution studies are concentrated, the area between the states of Mina Gerais and Mato Grosso do Sul (n=24). The contamination in this area is generated by mining, industry and agriculture (Da Rocha et al., 2015; Jordão et al., 1996; Veado et al., 2006). La Plata River estuary represents another point where a high number of studies (n > 40) of metallic contamination are concentrated. The Paraná-La Plata river system is subjected to metal pollution from the intensive agricultural activities upstream (Cr, Cd, Cu, Zn, Ni, Pb) (Carnelo et al., 1997; Salazar et al., 2012) and urban and industrial activities in the delta area, where almost half of the population of Argentina is concentrated (Ronco et al., 2007). Another area investigated, although in a lower level (n=21) is the area of Bariloche, in the Province of Rio Negro. This area contains numerous lakes and natural parks; thus, this is an area of low environmental pollution (Di Marzio et al., 2018). However, one of the main sources of metal contamination there could be volcanic eruptions (e.g. As, Cu, Zn, Cr, Si) (Bubach et al., 2015; Conti et al., 2016; Di Marzio et al., 2020; Ruggieri et al., 2012), or lead from hunting (Lambertucci et al., 2011; Wiemeyer et al., 2017).

According to our Scopus search, studies included in our data base represent only 0.9% of studies of environmental sciences in Latin America, suggesting that the study of environmental pollution by metals and metalloids using animal samples is increasing but it is not still a priority in Latin America.

Metals and metalloids

We found studies related to 11 metals and metalloids (Hg, Cd, Cr, Cu, Pb, Ni, Zn, Fe, Mn, As, Se); the most studied metals in the three geographical areas of Latin America were: Caribbean: Hg > Pb > Cu > Cd > Zn; Central America: Cd > Pb > Hg > Cu > Zn; South America: Hg > Cd > Cu > Zn > Pb. In South America, the most studied metal was Hg (n=317). The interest for Hg in South America (n=317), is caused by the use of this substance in the extraction of gold in artisanal mining (Malm, 1998). Studies on pollution by other metals (Cd, Cu, Zn, Pb) could be motivated by the presence of other important sources of emissions such as industry, intensive agriculture, among others (Plaza et al., 2018; Ronco et al., 2007). In Central America, the first two metals studied were Cd and Pb. In both cases, this is due to the predominance of studies from Mexico, where there is contamination by Cd in the Gulf of California. Part of that contamination may be originated in the sedimentary and volcanic rocks of Monterrey formation, rich in Cd and used to produce fertilizer (Mendez and Paez- Osuna, 1998). Pb pollution is related with the mining areas of the Sierra Madre (Espinosa and Armienta, 2007; 2009; Soto- Jiménez and Flegal, 2009) and harbours drainage (Gutiérrez-Galindo et al., 2010). Hg seems to be an emerging problem, as the increased number of studies in the last 9 years may reflect. In the case of the Caribbean, wastewater inputs, mining (bauxite), agriculture, industry and hydrocarbon extraction are indicated as possible causes of pollution (Fernandez et al., 2007). The predominance of Hg as the most researched pollutant could be explained due to the concern that the elements with the high level of biomagnification in the aquatic environment produce.

Biomonitoring species and samples

We have classified the species used in the studies according to their taxonomical class, except for invertebrates that are grouped together in the subphylum “invertebrates”, and the classes Actinopterygii and Chondrichthyes that are grouped under the generic definition of “fish”. The most studied groups in the three geographical areas have been the same, with some minor differences (Caribbean: Invertebrates=Fish; Central America: Invertebrates > Fish; South America: Fish > Invertebrates). The fish group presents an important variability in the percentage of studies using Chondrichthyes, depending on the area (Caribbean 3%; Central America 37%; South America 8%). This value is influenced by the tradition of shark fishing in Mexico (de Borhegyi, 1961; Sosa-Nishizaki et al., 2008). The common trend of the last evaluated period (2008–2017) shows an increase of the studies on Mammalia and a decrease of studies using invertebrates in all areas. Several studies consider species that occupy high positions in a trophic pyramid to be good biomonitors (Burger, 2006; Gómez-Ramírez et al., 2014). Many papers reviewed, although they use animal species to determine pollution levels, do not consider (nor evaluate) whether these species could be good biomonitors. Only a small percentage of studies (12%) have used live animals as biomonitors, although in recent years there has been much emphasis on the responsible use of species for scientific research (Ansara-Ross et al., 2013). In terms of sample type, soft tissues were the most common choice in the studies (88%). Soft tissues mainly provide indices of recent exposure and therefore provide a more reliable source of information (Ansara-Ross et al., 2013). Searching for how many investigations carried out in the last 28 years in Latin America have used endangered species as biomonitors, we found 49 species of various classes (Mammalia, Actinopterygii, Aves, Reptilia, Chondrichthyes) classified as Near Threatened (NT, n=16), Vulnerable (VU, n=25), Endangered (EN, n=7) and Critically Endangered (EN, n=1) according to the IUCN (International Union for Conservation of Nature) Red List categories (Table 1). No-lethal studies showed percentages for species (n=49) of 37% (Mammalia=10%; Actinopterygii=4%; Reptilia=8%; Aves=14%) and in each species group, the specimens (n=3350) percentages of 20% (Mammalia=3%; Actinopterygii=0.2%; Reptilia= 10%; Aves=7%). The studies that resorted to the sacrifice of biomonitors showed percentages for species/specimens of 39%/29% (Mammalia=2%/0.3%; Chondrichthyes=12%/21.6%; Reptilia= 8%/4%; Birds=16%/3%); of the sacrificed species 4 were NT, 12 VU and 2 EN. It is very striking that specimens of species classified EN have been sacrificed. These are 10 specimens of *Pelecanoides garnotii*, captured for the American Museum of Natural History collection (Ochoa-Acuna et al., 2002) and 570 specimens of *Mustelus schmitti*, captured at 7 sampling stations in the Bahia Blanca estuary (Argentina) during 1985–1986 (Marcovecchio et al., 1991). A high number of samples come from animals fished intentionally or accidentally (and eggs from the reptiles' nests). In this case the percentages of species/ specimens have been 53%/51% (Mammalia=6%/10.8%; Actinopterygii= 4%/0.2%; Chondrichthyes=26%/13%; Reptilia=8%/24%; Aves=8%/3%). In the case of Reptilia (all species are turtles), the turtles that have been sacrificed come from the Amazon basin, while the marine species were not sacrificed. In the case of mammals, the 306 specimens were from La Plata dolphin (*Pontoporia blainvillei*) accidentally fished. Also, in this case is striking the high number of species and specimens of Chondrichthyes captured (n=426). All specimens of sharks and rays investigated have died, either by sacrifice or because they were caught accidentally or intentionally.

***Chapter II.* Mercury in the feathers of bird scavengers from two areas of Patagonia (Argentina) under the influence of different anthropogenic activities: a preliminary study.**

Primary flight feathers (P1–P10) from the roosting areas were used in this study. The sampling was conducted during the austral spring of 2011 (October to December); in tow sampling area ("Bariloche" and "El Valle"). A total of 90 primary feathers were collected: 44 in "Bariloche" from Black vultures (n = 20), Turkey vultures (n = 14), and Southern crested caracaras (n = 10) and 46 in "El Valle" from Black vultures (n = 5), Turkey vultures (n = 30), and Southern crested caracaras (n = 11).

Table 2 Hg levels for the three species of the study in the two sampling areas. Median, mean ± SD (minimum-maximum) (n = number of samples)

	Bariloche	**El Valle**
Black vultures	0.17; 0.22 ± 0.16; (0.09–0.65) (n = 20)	0.86; 1.02 ± 0.89; (0.23-2.44) (n=5)
Turkey vultures	0.13; 0.13±0.06; (0.06-0.25) (n=14)	0.29; 0.53 ± 0.82; (0.04-4.2) (n=30)
Southern crested caracaras	0.12; 0.13 ± 0.09; (0.03-0.36) (n=10)	0.34; 0.54 ± 0.74; (0.09-2.61) (n=11)

Hg concentrations were detected in all feather samples. In "Bariloche" area, Hg concentrations were similar between species; but in "El Valle", the highest mean and median concentrations (1.02 mg/kg and 0.86 mg/kg, respectively) were found in the Black vulture. The two highest concentrations of Hg feather concentrations were found in a Turkey vulture (4.20 mg/kg) and in a Southern crested caracara (2.61 mg/ kg). However, no significant differences among species were found. The application of GLM to study the effect of the sampling area and species on concentrations of Hg in feathers shows that the model including only the variable "study area" was significant (D2 = 72.89, p = 0.005) and with the best Akaike index (AIC = 1225.12). The value was significantly higher in "El Valle" than "Bariloche" for the three species.

We can consider "El Valle" contaminated by Hg as a result of the activity of a chlor-alkali plant (Arribére et al. 2003); our results are consistent with the data from several Hg and heavy metal contamination studies, carried out using samples of sludge and biota from rivers and lakes from the same area (Guevara et al. 2002; Arribére et al. 2003; Rizzo et al. 2011). We compared our results with other studies in feathers of different bird species, including scavengers, from different areas around the world. Cahill et al. (1998) found an average of 1.26 mg/kg Hg (n = 36) in turkey vulture feathers, higher than in the same species from "El Valle" (0.53 mg/kg Hg, n = 30), found in our study. In this case, the difference could be due to the different degree of contamination of the two areas. Clear Lake (California) has a history of contamination due to the activities of now abandoned Sulphur Bank Hg Mine that poured 100 tons of Hg into the lake between 1872 and 1957 (Suchanek et al. 1993, 1998). The contamination of "El Valle" is apparently due to the activity of an alkali chlorine plant. The plant, built on an island within the Neuquén River, poured its wastewaters into a series of drainage pools from 1951 to 1979 (Arribére et al. 2003). After 1979, until its closure in 1995, water was stored in settling and drying pools (Arribére et al. 2003). The estimated annual discharge value of the plant is approximately 500 kg/ Hg/year (CRBAS 2012). This difference is reflected by the Hg concentrations detected in the sediment samples (18.3 mg/kg in Clear Lake; 1.3 mg/kg in the nearest sampling point to the area of higher contamination "El Valle") (Suchanek et al. 1998; Arribére et al. 2003). In the case of "Bariloche," we can assume that this is a less polluted area than "El Valle," with mean concentrations and range of Hg lower than those detected in "El Valle" (mean Hg concentration "Bariloche" 0.6 mg/kg, range 0.49–0.6 mg/kg; mean Hg concentration "El Valle" 1.2 mg/kg, range 0.75– 4.2 mg/kg) (Arriberé et al. 2003; Guevara et al. 2002). The levels found in "Bariloche" are similar to those found in areas considered to have received low levels of pollution and are similar to the study of turkey vulture in California, USA, by Wiemeyer et al. (1986), with mean concentrations of 0.11 mg/ kg (n = 5, female breeding), 0.12 mg/kg (n = 5, male nonbreeding), and 0.098 mg/kg (n = 5, female non-breeding). Due to the scarcity of research on these species, no further studies have been found to contrast our results in an area of low contamination.

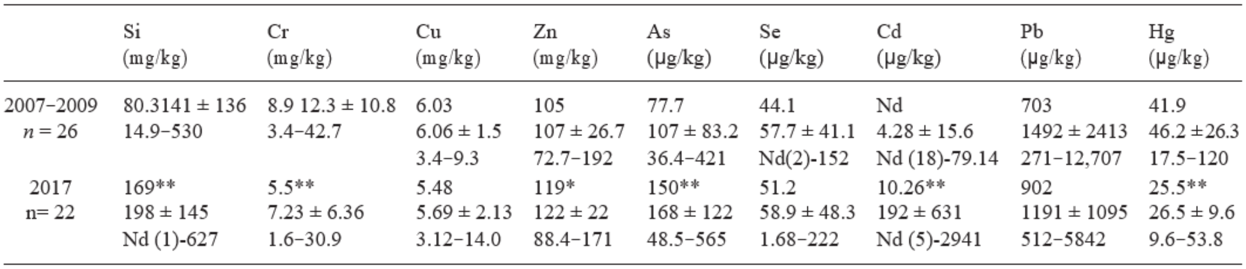
Risk assessment

Scheuhammer (1991) considered Hg concentrations of 1– 5 mg/kg (d. w.) in feathers of raptor birds as "normal". Other authors consider Hg concentrations greater than 5 mg/ kg in feathers as "dangerous to birds" (Burger and Gochfeld 1997; Palma et al. 2005; Eisler 2006; Albuja et al. 2012). No concentrations higher than 5 mg/kg were detected in this study. Only one sample of Turkey vulture from the "El Valle" area is close to this concentration (4.2 mg/kg). It's important to emphasize that any of these studies quantify the possible sub-lethal effects at these concentrations, which might be relevant for long-living species, such as the scavenger birds in our study.

Chapter III. **Temporal changes in metal concentrations in Andean condor feathers: a potential influence of volcanic activity.**

Forty-eight molted primary feathers (P1- P10) of the Andean condor were sampled in nine roosts of the species located in the Patagonian steppe (Bariloche; Argentina) in different years, during the austral spring (October–December). Due to the lack of studies on the phenology of the molt of the Andean Condor, we can estimate that in the molt of the primary feathers should be every 5–7 years, as in the vultures of the genus Gyps (Zuberogoitia et al. 2013). For this reason, the use of molted feathers in 2017 allows us to evaluate the levels of metals present in the environment approximately during the 2011 volcano eruption of Puyehue-Cordon Caulle volcanic complex (PCCVC) (Chile).

Table 3 Levels of metals in feather from Andean condor sampling before and after PCCVC eruption (2011). Data are presented as median, mean ± standard deviation, minimum-maximum. *n* number of samples



	Si (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (μg/kg)	Se (μg/kg)	Cd (μg/kg)	Pb (μg/kg)	Hg (μg/kg)
2007–2009	80.3141 ± 136	8.9 12.3 ± 10.8	6.03	105	77.7	44.1	Nd	703	41.9
n = 26	14.9–530	3.4–42.7	6.06 ± 1.5	107 ± 26.7	107 ± 83.2	57.7 ± 41.1	4.28 ± 15.6	1492 ± 2413	46.2 ±26.3
			3.4–9.3	72.7–192	36.4–421	Nd(2)-152	Nd (18)-79.14	271–12,707	17.5–120
2017	169**	5.5**	5.48	119*	150**	51.2	10.26**	902	25.5**
n= 22	198 ± 145	7.23 ± 6.36	5.69 ± 2.13	122 ± 22	168 ± 122	58.9 ± 48.3	192 ± 631	1191 ± 1095	26.5 ± 9.6
	Nd (1)-627	1.6–30.9	3.12–14.0	88.4–171	48.5–565	1.68–222	Nd (5)-2941	512–5842	9.6–53.8

Metals levels in feathers of Andean condor

We detected all 9 metals studied (Si, Cr, Cu, Zn, As, Se, Cd, Pb, Hg) in primary flight feather from the Andean condor. Detection rates of concentrations were 96–100% for all metals, except for Cd with detection rates of 46%. The higher concentrations detected were for Si, followed by Zn > Pb > Cr > Cu > As > Se > Hg > Cd. Positive correlations were found between Si–As, Si–Zn, and Cu–Se, suggesting plausible similarities in the emissions sources and metabolic pathways of these elements. The negative correlations observed between Cd–Hg and Cd–Cr have also been found in other studies associated with metallothionein binding (Elliott et al. 1992; Stewart et al. 1996).

Temporal evaluation of metals

We found significant differences among sampling year only in the case of Cd (H = 17.132, p = 0.001) and Hg (H = 12.820, p = 0.005). No significant differences were found between 2007 and 2009, but there were significant differences between those years (as a pool) and the year 2017, the most important difference being between years 2009 and 2017 for Cr (U = 102, p = 0.008), Zn (U = 108, p = 0.014), As (U = 110, p = 0.016), Cd (U = 92, p = 0.003), and Hg (U = 110, p = 0.016).

Although it occurred in Chile, the 2011 PCCVC volcanic eruption affected a large part of the steppe near Bariloche (Bertrand et al. 2014), and the ashes were deposited in the condor foraging areas. The condors, during the volcanic eruption, remained in the area both flying and feeding (Alarcón et al. 2015). Volcanic material can affect aquatic ecosystems immediately (by falling into water bodies) or decades later (by falling inland) through the effect of rain and snowmelt that carry toxic substances into water bodies (Bisson et al. 2005), with significant and prolonged effects on riparian macroinvertebrate communities (Lallement et al. 2016; Miserendino et al. 2012). Si, Zn, As, and Cd were significantly higher in samples posteruption, while the levels of Hg and Cr were lower after the eruption. Volcanic eruptions represent an important source of Hg both in Latin America (Ribeiro Guevara et al. 2010) and globally. However, there is uncertainty in our region. Higueras et al. (2014) show that in the PCCVC, it emits Hg in gaseous form, and its dilution in air does not make it dangerous for the condor.

Studies have shown that concentrations of Hg and Se are related, because Se can bind to this metal and have a detoxifying effect (Ohlendorf and Heinz 2011; Lourdes et al. 1991). The Hg:Se molar ratio was determined:

$$\underline{([Hg]/200.59) / ([Se]/78.96)}$$

in our samples, with a mean ratio 0.89. Given that the mean Hg:Se molar ratio was lower than the 1:1 Mratio, the Se levels seems thus to be sufficient for binding to all body Hg in most individuals sampled, except for 5 samples were this ratio was higher than 1. As mentioned above, the levels of Se are directly related to those of Hg. Due to the biomagnification processes that methyl-mercury (CH3Hg) is subject to in the aquatic environment, the levels of Se considered normal in these studies could be influenced by Hg levels.

The Cr concentrations in feather were also significantly lower posteruption than in preeruption samples. Cr concentrations during eruptive processes are related to the type of volcano and eruption, its analysis is very important for the identification of the emission source and Cr can be used as a source identification of recent events in Nahuel Huapi National Park (Daga et al. 2008). However, the retention of Cr by the soil particles during the infiltration process, the oxidation state, tri- or hexavalent, and the solubility could affect the bioavailability of this element and its incorporation in the food chain (Han et al. 2004).

The concentrations of Si, Zn, As, and Cd were significantly higher in posteruption sampled feathers. The PCCVC, a unique case in the Southern Volcanic Zone of the Andes Mountains among this type of volcanic structures, provides a magmatic composition where silica-rich rocks predominate over basaltic rocks (Daga et al. 2008; Lara et al. 2004). This data would explain the high levels of Si detected in our study. Zn is one of the elemental compositions of the primary components from tephra (Daga et al. 2008). Zinc's bioavailability is determined by complex interactions with the environment and is strongly dependent on the characteristics of that environment. The bioaccumulation of Zn in all ecosystems (Singh and Kumar 2017; Heikens et al. 2001; Çetin and Yur 2016) could be responsible for the increase of Zn concentration in the samples collected posteruption. It is important to note the diet of Andean condor depends heavily (98.5%) on herbivores (Lambertucci et al. 2009a). Due to the environmental and human health impact, there is special interest in As and Cd. Samples of deposited particles after eruptive activity and seismicity in 2000 of Volcano Copahue (located 314 km from PCCVC) showed an enrichment of several elements, As and Cd among others (Gómez et al. 2002). Moreover, the relationship between As concentrations in lichens and the distance to volcano is considered as an indication of the ash contribution, in addition to geological source, and is also associated with permanent geothermal emissions from PCCVC (Bubach et al. 2012).

To sum up, our results seem to indicate that increases in several metal concentrations in feathers of Andean condor posteruption, including those more toxics to animal and human health, may reflect the incorporation of these elements in the environment and the trophic chain. Thus, this cathartid species can be used as a bioindicator (i.e., "the canary in the coal mine") to monitoring changes in the area where they live (see Plaza et al. 2020 for lead). In the whole area of study, it is difficult to think of other possible emission sources for the metals studied, except for Pb. Studies conducted in the area reveal that Pb contamination is due to hunting ammunition (Lambertucci et al. 2011; Plaza and Lambertucci 2019). The concentration values of Pb did not change in our study, unlike the other metals.

Metal pattern distribution and risk assessment

Argentina, like most Latin America countries, has few biomonitoring studies of metal pollution in birds that inhabit terrestrial ecosystems (Di Marzio et al. 2019). Not all metals evaluated in our study have been previously investigated in bird feathers, limiting a comparison of results. According to the summary by Ansara-Ross et al. (2013), the reference values for feathers are < l mg/kg (dry weight, d.w.) for Cd (Burger 1993), < 4 mg/kg (d.w.) for Pb (Scheuhammer 1987) and < 5 mg/kg for Se (d.w.) (Arnold et al. 1973). However, Hg concentration of 1–5 mg/kg (d.w.) in feathers of birds of prey are normal (Scheuhammer 1991). Although Zn is an essential metal for body formation, organisms with high levels of Zn may encounter problems in maintaining homeostasis of tissue concentration of this particular element (Gasaway and Buss 1972). The feather concentrations of Zn found in this study are in the range of other studies on several other bird species (Ansara- Ross et al. 2013; Cahill et al. 1998). Copper has an important role in several vital processes (Underwood and Suttle 1999), but chronic exposition to high levels could cause alterations on these processes, which have been shown in experimental studies (Chiou et al. 1999). Our results with a detected maximum value of 14 mg/kg and a mean concentration of 5.81 mg/kg are in concordance with other Cu mean levels found in feathers of Accipitridae and Cathartidae species (Haskins et al. 2013; Kavun 2004; Nighat et al. 2013; Wiemeyer et al. 1986). One of the metals whose levels were significantly higher in samples posteruption is Si. This metal has been scarcely studied in feathers. In any case, our concentrations are higher than those found in tail feathers of tawny owls (Strix aluco) by Bustnes et al. (2013) in Central Norway with a mean of 4.67 µg/g and similar to those found in cormorants by Skoric et al. (2012) (mean 119.96 mg/kg). This result is consistent with the fact that Si is the main component (ca. 70%) of the volcanic eruption we studied here (Caneiro et al. 2011). Chromium levels in this study were higher than detected levels in raptor species from other regions of the planet (Ansara-Ross et al. 2013; Dauwe et al. 2003; Harmata and Restani 2013), with a mean value in our study from six and seven times higher than those detected by previous studies. Moreover, different studies indicated that levels of 2.80 mg/kg Cr detected in bird feathers may be related to adverse effects on the reproductive success of birds (Burger 1993; Burger and Gochfeld 2000; Kertész and Fáncsi 2003). Therefore, the high levels of Cr found in the feathers of Andean condor merit special attention since they may produce adverse effects. In bird feathers, values of Se between 1000 and 4000 µg/kg are considered normal; although frequently, the values are lower than 2000 µg/kg (Ohlendorf and Heinz 2011). In our study, Se levels (57.5 ± 43.5 µg/kg, range nd-222 µg/kg) were lower than those found in feathers of species of the same Andean condor family such as the turkey vulture (*Cathartes aura*) (940 ± 400 µg/kg) (Cahill et al. 1998) or from other raptors such as laggar falcon (*Falco biarmicus jugger*) (950– 5200 µg/kg), bald eagles (*Haliaeetus leucocephalus*) (800– 3200 µg/kg) (Bowerman et al. 1994; Movalli 2000), African grass owl (*Tyto capensis*) (22–3880 µg/kg), and barn owl (*Tyto alba*) (4–1120 µg/kg) (Ansara-Ross et al. 2013). Mercury is probably the nonessential metal of most concern for its toxic effects (Walker et al. 2001). The highest Hg concentration in our study was 120 µg/kg, which is not of concern since Hg concentrations of 1000– 5000 µg/kg (d.w.) in raptor's feathers are considered normal (Scheuhammer 1991). Hg concentrations greater than 4100–5000 µg/kg in feathers have been related to adverse effects in birds (Burger and Gochfeld 1997; Palma et al. 2005). Studies conducted in the same area on turkey vultures and black vultures seem to indicate that the surroundings of Bariloche are an area of low Hg contamination (Di Marzio et al. 2018). In any case, our data does not exceed this threshold level. Arsenic concentration is 1500–2000 µg/kg in the earth's crust and can also enter the material cycle by volcano (Bundschuh and Maity 2015). In this sense, its levels are significantly higher in samples post eruption, as we mentioned in previous section. The highest As concentration in our study was 570 µg/kg, similar to those detected by Grúz et al. (2018) in several species from the Hortobágyi Madárpark (Bird Hospital Foundation) in Hungary. However, it was not close to As concentrations responsible for adverse effects (see Nighat et al. 2013; 11,070 µg/kg in Accipitridae and 19,610 µg/kg in Falconidae, which could be toxic). Cd showed low concentrations in feathers of Andean condor. They were lower than 3000 µg/kg of cadmium, a value at which severe physiological, nutritional and behavioral disorders occurs in birds (Burger and Gochfeld 2000). Finally, regarding Pb, we found a mean concentration of 1.3 mg/kg of lead in all Andean condor feathers. This mean value is similar to the one detected in Andean condors sampled in 2007 in the same area (Lambertucci et al. 2011). As mentioned, lead bullets for sport hunting have been identified as the most probable source of lead for scavengers in this area (Lambertucci et al. 2011; Plaza and Lambertucci 2019). Besides, three individuals showed lead concentrations higher than 4 mg/kg, the threshold suggested, levels above which reproduction could be affected (Burger and Gochfeld 2000).

***Chapter IV.* Testing Cathartidae of Northern Patagonia as a potencial biomonitor species for Hg ecotoxicological studies and surrogate species for Andean condor (*Vultur gryphus)*.**

We sampled roosts of Black vulture, Turkey vulture (in 2011, 2013, 2017) and Andean condor (in 2017), collecting primary molted feathers. The sampling was done in the in northwest Patagonia, Argentina. Our decision was motivated by several reasons: the presence of a large population of Andean condors in this area, availability of scientific knowledge about the local populations of Andean condors, Black vultures and Turkey vultures (Lambertucci et al., 2010; Barbar et al., 2015; Graña Grilli et al., 2017; Ballejo et al., 2018) and also certain investigations on Hg contamination in these terrestrial species, somewhat unusual in Latin America (Di Marzio et al., 2018). Furthermore, we know that the main sources of metal contamination detected until now are related to volcanic eruptions (Di Marzio et al., 2020), recent increase in the human population (Arribére et al., 2010; Rizzo et al., 2011; Bubach et al., 2015) and lead from sport hunting (Plaza and Lambertucci, 2018).

Table 4. Level of Hg (mg/Kg) in primary feathers of Andean condor, black vulture and turkey vulture (median, mean and SD, n= number of samples) according to year and species

Year	***Andean condor***	***Black vulture***	***Turkey vulture***
2011		0.17; 0.22 ± 0.16 (n: 20)	0.13; 0.13 ± 0,06 (n: 14)
2013		0.18; 0.44 ± 0.62 (n: 9)	1,57; 2,44 ± 2,4 (n: 13)
2017	0.025; 0.026 ± 0.0096 (n:22)	0.15; 0.42 ± 0.58 (n: 36)	0,32; 1,31 ± 1,78 (n: 37)
Total	0.025; 0.026 ± 0.0096 (n:22)	0.17; 0.36 ± 0.5 (n: 65)	0,23; 1,28 ± 1,87(n: 64)

We have analyzed 151 feathers, detecting Hg in all the analyzed samples. Among the Turkey vulture samples we found 4 samples in 2013 and 3 in 2017 with the highest levels. As mentioned above, we estimate in 6 years the duration of the Andean condor's molt, and annual the duration of the Black vulture and Turkey vulture molt. For this reason, we made a comparison of results between the 2017 Andean condor samples and the 2011-2013 samples of the other two species. We found statistically significant difference in Hg levels between Andean Condor and the other two species with both p-values <0.001, but no statistically significant differences between Black vulture and Turkey vulture ($p=0.87$).

Evaluation of temporal variation in Black and Turkey vultures, indicate no overall temporal change from 2011 for data of both species together. Though, differences exist comparing species by year. Distributions of relative mercury levels are similar between species in 2013 and 2017, but statistically significantly different in 2011. As analysis is carried out on species-specific relative contamination levels, this suggest overall stable detected mercury levels in Black vulture. Whereas, contamination levels in Turkey vulture have significantly increased between 2011 and 2013 and have remained relatively high in 2017.

The differences found between the Hg levels in the Andean Condor and the other two species do not allow a direct comparison between the three species. Although eliminating the family variable, as all three species are Cathartidae, there are more intra- and inter-specific factors that create difficulty, as suggested by Carpenter et al. (2003). There are differences in the composition of the diets in our area of study (Ballejo *et al*. 2018). The diet of Andean Condor is based mainly in remains of sheep, European Hare *Lepus europaeus*, European Deer *Cervus elaphus* and Wild Boar *Sus scrofa*. The diet of the Black vulture is similar, although for this species the arthropods represent an important source of food (Ballejo *et al*., 2018). Several studies show the importance of arthropods in the transfer of methylmercury from the aquatic to the terrestrial environment (Xie *et al*., 2008; Speir *et al*., 2014). In the aquatic environment, Hg bioaccumulation and biomethylation of inorganic Hg into organomercury (more toxic) are common (Eisler, 1985). This could, at least in part, explain the higher Hg values recorded in Black vultures compared to the values in Andean Condors. Turkey vulture has a more varied diet, including also remains of reptiles, fish and birds (Ballejo *et al*., 2018). The inclusion of fish in the diet of subordinate Turkey vultures has also been documented by Blázquez *et al*. (2016) in Baja California, Mexico. For the same reasons explained above regarding the diet of the Black vulture, Turkey vultures feeding on fish could maybe have higher levels of Hg. Being hierarchical animals, the sex and age of the animals could be other factors responsible for the differences recorded, as proposed for other metals (Plaza *et al*., 2020). Access to food is strongly regulated by the hierarchy in Andean condor, with adult males being the earliest eaters and young females being the last to eat (Donázar *et al.*, 1999). In Black vulture and to a lesser extent in Turkey vulture, hierarchy is determined by age, with adults getting priority access to food (Wallace and Temple, 1987). In addition, as demonstrated by Blázquez *et al*. (2016) subordinate animals may resort to alternative food sources, such as fish.

We have found some analogies in the Hg values of our study with that of Herring *et al*. (2018) and in the Pb values between the study of Herring *et al*. (2018) and Plaza *et al*. (2020); although the studies refer to different samples (blood or feathers) of different species from different areas. Evaluating the results from the point of view of the values recorded in the "main" species (Andean Condor or California Condor), the Hg levels in the surrogate species are always overestimated, and the Pb levels underestimated.

Observing the Hg levels in the Turkey vulture samples we can see, as mentioned above, that the levels rise in between 2011 and 2013, remaining high also in 2017. Various authors coincide that Hg levels in feathers higher than 4.1-5 mg/Kg are related to adverse effects in birds (Burger and Gochfeld, 1997; Palma *et al*., 2005). In our case seven Turkey vulture specimens exceed these levels. Di Marzio *et al*. (2018) considered the surroundings of Bariloche a low exposure area to Hg; in feathers sampled in this area in 2011 they detected median Hg levels of 0.12 mg/Kg in Southern crested caracara, 0.17 mg/Kg in Black vulture and 0.13 mg/Kg in Turkey vultures. In the case of Black vulture, also the median values of 2013 and 2017 are similar (0.18 mg/Kg; 0.15 mg/Kg). It could be assumed that these values represent baseline area levels for high trophic level species; considering that also Bubach *et al.* (2012) consider 0.12-0.16 mg/kg basal levels for the same area in studies with lichens. As evidenced by Burger (1993), feathers represent the excretion pathway of 70-90% of MeHg of burden body. Several studies have shown that bird feathers contain levels of 90% MeHg in both aquatic and terrestrial species (Rimmer *et al*., 2005; Renedo *et al*., 2017). For these reasons, the high levels of Hg detected in Turkey vulture feathers could refer to MeHg levels and their origin could be the aquatic ecosystems of the area. As seen above, Black vultures do not show big variations in Hg levels, although they share the territory with Turkey vultures. One of the main differences between the two species, as already mentioned, is the inclusion of fish in the diet of the Turkey vulture. In addition, we have detected high Hg levels in Turkey vulture feathers at another point in the Limay River.

Chapter V. Metal levels in feathers of five scavenger bird species from northern Patagonia (Argentina)

For this study we used samples of Andean condor, Black vulture, Turkey vulture, Southern crested caracara and Chimango caracara. Some of the results were used in the previous chapters.

We have analyzed the levels of Hg, Pb, Cd, Zn and Cu using the five species mentioned above. Sampling has been done in 2011, 2013 and 2017 in the sampling areas of Bariloche, El Chocon and Neuquén.

Table 5. Total samples used divided by species and year

	Andean condor	***Black vulture***	***Turkey vulture***	***Southern crested caracara***	***Chimango caracara***
2011		25	44	21	25
2013		9	13		
2017	22	111	85	39	21

Hg, Cd, Pb, Zn, Cu levels in Bariloche, El Chocon and Neuquen in Black vulture, Turkey vulture and Andean condor

1) We compared, for the first time, the Hg levels in Black vulture and Turkey vulture feathers collected in the years 2011, 2013, 2017 in three sampling areas (Bariloche, El Chocon and Nequen).

2) Also, for the first time we compared Cd, Pb, Zn and Cu levels in feathers of Black vulture and Turkey vulture from Bariloche and El Valle (2011).

3) Finally, from the area of Bariloche, a comparison of Pb, Cd, Zn and Cu levels between Andean condor (2017), Black vulture and Turkey vulture (2011) was done.

1) The best model explaining Hg concentrations in feathers in both Black vulture and Turkey vulture included year, zone and year x zone as explanatory factors, showing that Hg concentrations differed between years depending on the zone. In general, Hg concentrations in feathers were higher in El Chocon than in Bariloche and El Valle for both species. Regarding the temporal trends, for Black vulture Hg levels were similar between years in Bariloche, while concentrations where higher in 2011 compared to 2017 in El Valle zone. The values of Bariloche (0.17 mg/Kg/2011, 0.18 mg/kg/2013, 0.16 mg/kg/2017) can be considered the baseline levels in this area, as confirmed also by Bubach et al. (2012) in their study with lichens. With respect to the levels in the El Valle area, one possible explanation could be the constant reduction of pollution generated by the alkali chlorine plant present in this area, closed in the early 2000's (Di Marzio et al., 2018). The opposite trend was found in Turkey vulture, Hg levels being lower in 2011 compared to 2013 and/or 2017 in Bariloche and El Chocon, while similar concentrations were found between years in El Valle. Probably it depends on the presence of El Chocon hydroelectric dam. Hg contamination related to the presence of hydroelectric dams is a frequent problem in several South American countries and particularly studied in Brazil (Di Marzio et al., 2019). Hydroelectric dams reduce the water flow and hence the self-purification capacity of the water,

accelerating the consequent decline in water quality, with increasing concentrations of metals and other pollutants (Kummu and Varis, 2007; Wang et al., 2012). When comparing metal concentrations among *Cathartidae* species, GLMs showed significant effects of year (p<0.01), zone (p<0.01), year x zone (p<0.01), year x species (p<0.01) and zone x species (p=0.042) on Hg concentrations in feathers. These results reflect that these species differed in feather Hg levels depending on the zone and the year. In this regard, Hg concentrations were lower in Black vulture compared to Turkey vulture in Bariloche, but similar concentrations were found in El Chocon and El Valle. Hg levels were lower in Black vulture in 2013 and 2017, but similar concentrations were observed for both species in 2011. A possible interpretation to these data could come from June 2011-January 2012 eruption of the Puyehue-Cordón Caulle volcanic complex (PCCVC). This eruption, despite being on Chilean territory, due to the reduced distance and favorable environmental conditions (western winds) particularly affected our study area (Perez Catán et al. 2016). Despite this, only a few ecotoxicological studies have been carried out on the possible impact of volcanic eruption on high trophic level species (Di Marzio et al., 2020; Plaza et al., 2020). Although Bubach et al. (2012) determined that the eruption had not caused Hg emissions, numerous studies seem to indicate that the PCCVC emits Hg during eruptive activity (Bubach et al., 2015; Ribeiro Guevara et al., 2010; Rizzo et al., 2011). Higueras et al. (2014) showed how the fumaroles of the PCCVC represent, in addition to the ashes, a source of gaseous Hg emission. The probable Hg emissions generated by the eruption could explain the low levels of Hg detected in both species in 2011 (pre-eruption feathers). Ballejo et al. (2017) evidence that fish are part of the diet of Turkey vulture, in the area of Bariloche. Considering that Hg levels in this area are lower in Black vulture, Andean condor (Di Marzio et al., 2020) and also in the study of Bubach et al. (2012) with lichens, we can assume that some factor (e.g. precipitation) has accelerated the accumulation of Hg in the aquatic environment to the detriment of deposits in the terrestrial environment. Due to the distance, the presence of the dam in El Chocon and the different trend we can assume that the sources of contamination in the area of El Valle are different from those in Bariloche, such as the alkali chlorine plant evidenced by Di Marzio et al. (2018). However, this area is less studied than the Bariloche area and further interpretation is difficult.

2) For other metals, we have samples only from Bariloche and El Valle from the year 2011. No differences were found between zones in Black vulture, while lower Cu and Zn concentrations were observed in feathers of Turkey vulture from Chocon compared to Bariloche and El Valle. Interpreting these results is more complex, due to the lack of information. Comparing metal concentrations among *Cathartidae* species, GLMs showed that Cd concentrations in feathers were lower in Black vulture compared to Turkey vulture in Bariloche, while levels of Pb, Cu and Zn were higher in Black vultures inhabiting this area. Zn concentrations were also higher in Black vulture compared to Turkey vulture in El Valle area.

3) Finally, Pb, Cu and Zn concentrations found in Andean condor in Bariloche (2017) were higher than those found in Black vulture and Turkey vulture in Bariloche in 2011 (p<0.01 in all cases). Regarding the Cd, as reported by Di Marzio et al. (2020), the source of emission should be volcanic eruptions, at least in the Bariloche area. None of the samples exceeds the value of 4000 ng/g, considered a normal value for Cd in bird feathers (Burger, 1993). Several studies have demonstrated that the primary source of Pb contamination in our study area comes from hunting

ammunition (Lambertucci et al., 2011; Plaza et al., 2019). Due to inter-specific hierarchies, condors are the first to feed, followed by Black vultures and finally Turkey vultures ((Donázar *et al.,* 1999; Wallace and Temple, 1987). This implies that condors are more exposed to contamination from lead bullets, and that Pb levels in this species are higher than in other *Cathartidae* (Plaza et al., 2019). For Zn and Cu the interpretation of the results is more complex. These are essential elements in many physiological processes. The levels detected are beyond the safety limits for both. One explanation could be that these levels are normal for these species. On the other hand, Di Marzio et al. (2020) have shown that after the volcanic eruption of the PCCVC, the levels of Zn have risen; considering the geothermal activity the possible source of emission. Another explanation for these levels could be hunting, as for Pb. Both lead and lead-free ammunitions contain Cu and Zn, which can be deposited in the organisms that are shot, without reaching toxic levels (García-Fernández & Navas, 2020; Schlichting et al., 2017)

Hg and Pb, levels in Bariloche and Neuquen in Chimango caracara and Southern crested caracara

1). We evaluate the Hg levels in Southern crested caracara and Chimango caracara from "Bariloche" and "·El Valle" (2011, 2017)

2). We also evaluated Pb levels in Chimango caracara from "Bariloche" and "El Valle" (2011, 2017)

1). For Chimango caracara and Southern crested caracara, the best model explaining Hg concentrations included year and zone as explanatory variables. Hg levels were higher in 2011 than 2017 for Chimango caracara in Bariloche, while the opposite trend was found in Southern crested caracara, with lower Hg concentrations in 2011 compared to 2017 in both Bariloche and El Valle.

2) Regarding Pb concentrations in Chimango caracara, Pb levels were lower in 2011 compared to 2017. The Hg levels of the Southern crested caracara in the Bariloche area show a similar trend to the Turkey vulture. In 2011 the levels are similar to what we have previously called the basal levels of the area. And in 2017 the levels are similar to those of the Turkey vulture. This could depend on the diet, as it is an opportunistic scavenger that includes fish in its diet (Blázquez et al., 2016).

The discussion about the Chimango caracara data deserves prior clarification. It is the most widespread bird of prey in all of South America (Ferguson-Lees and Christie 2001). Despite this, it is a species that has never been used in ecotoxicological studies. In addition, the feathers, which are small in size, can be easily blown away by the wind, making sampling difficult (personal observations). Without previous data and with a limited volume of samples, our interpretations are relative. We consider, however, that since there are no previous studies, these data have enormous importance for future research. The Chimango caracara has a very small home range (approx. 15 km). We have investigated whether there were differences between the different sampling points in each study area. For Hg the point with the lowest average concentration of Hg was Cipolletti (El Valle) (Map 1,3) with 0.16 ± 0.31 mg/kg, while the highest average concentration was recorded in Isla Jordan (El Valle) (Map 1,3) with 1.41 ± 0.31 mg/kg. The distance between the two locations is 6 km, but Cipolletti is located on the banks of the Neuquen River, a few km before the confluence with the Limay River to form the Negro River, and Jordan Island is located on the banks of the

Negro River. The data is interesting, considering that Cipolletti is halfway between the alkali chlorine plant and Jordan Island, suggesting that there are other sources of Hg contamination, because if the Hg came only from the plant, it should be greater in Cipolletti and not in Jordan Island as we have detected. No significant differences in Pb concentrations were observed among the six sampling points (F=2,198, p=0,075). Thus, considering El Valle as a whole and the sampling points of Bariloche and "Basural", significant differences in Pb concentrations were observed (F=3,266, p=0,031). In order to know which groups are significantly different, Tukey's test was carried out, and significant differences were observed between El Valle and Bariloche (p=0.020). With respect to the dump, no significant differences were found with other groups, which could be due to the high deviation found. The highest value of Pb concentration was 3.70 mg/kg and was obtained at "Basural". As for the minimum, it was also recorded at "Basural" with a value of 0.03 mg/kg. In this case it must be considered that the sampling in Bariloche was carried out within the city, a few meters away from a sheet metal workshop. It is plausible to think that the Pb contamination detected comes from this activity.

Comparison of Hg levels in Black vulture, Southern crested caracara and Chimango caracara from "Basural" sub area ("Bariloche")

1). We check Hg levels in the "Basural" subarea (2017) in Black vulture, Southern crested caracara and Chimango caracara, the three species residents on the area.

Southern crested caracara showed higher Hg concentrations (mean ± SD: 1002 ± 792 ng/g) than Chimango caracara and Black vulture (263 ± 205 and 217 ± 315 ng/g, respectively; p<0.001 in both cases). In Black vulture and Chimango caracara, Hg concentrations did not differ between Bariloche (city for Chimango caracara, steppe for Black vulture) and "Basural" in 2017 (mean ± SD in Black vulture: 323 ± 441 and 217 ± 315 ng/g, respectively, p=0.99; in Chimango caracara: 400 ± 401 and 263 ± 205 ng/g, respectively, p=0.45). However, in Southern crested caracara, Hg levels were higher in "Basural" (1002 ± 792 ng/g) compared to Bariloche (steppe) (435 ± 526 ng/g; p<0.001) in 2017. In addition to factors such as species and diet, one factor that may influence this result is age. In the case of Black vultures, adults rather than juveniles were observed during the sampling. The same was observed with the Chimango caracara. In the case of the Southern crested caracara, most of the population was made up of immatures, which use the dumpster as a

Table 1. Feather metal concentrations (mean ± SD, median and range; ng/g) of four avian species from Patagonia (Argentina) in 2011, 2013 and 2017 and generalized linear models.

			Hg (ng/g)			Cd (ng/g)			Pb (ng/g)			Cu (ng/g)			Zn (ng/g)		
Species	**Zone**	**Year**	**N**	**Model (p)^a**	**Mean ± SD** **Median (min-max)**	**N**	**Model (p)^b**	**Mean ± SD** **Median (min-max)**	**N**	**Model (p)^b**	**Mean ± SD** **Median (min-max)**	**N**	**Model (p)^b**	**Mean ± SD** **Median (min-max)**	**N**	**Model (p)^b**	**Mean ± SD** **Median (min-max)**
Black vulture (Coragyps atratus)	Bariloche	2011	20	Year (<0.01) + Zone (<0.01) + Year x Zone (<0.01)	224.54 ± 156.61 168.12 (85.76-654.27)	20	None	13.83 ± 19.36 6.87 (0.06-76.68)	20	None	526.24 ± 538.27 381.65 (179.99-2691.3)	20	None	3566.0 ± 3075.2 2797.7 (2037.8-16375)	20	None	63401 ± 9910.2 62629 (47496-94990)
		2013	9		437.22 ± 621.75 179.4 (54-1831.3)												
		2017	66		325.70 ± 486.69 161.65 (29.2-2193.3)												
	Chocon	2017	15		2198.5 ± 2582.8 1304.3 (202.9-8961.1)												
	El Valle	2011	5		1018.5 ± 891.90 864.81 (231.88-2441.9)	5		7.81 ± 5.71 4.29 (4.17-17.31)	5		338.06 ± 73.23 309.17 (271.17-459.8)	5		2662.7 ± 445.01 2791.2 (1909.1-3037.6)	5		69195 ± 7224.3 70721 (58083-77754)
		2017	30		261.13 ± 387.81 111.75 (67.1-1707.3)												
Turkey vulture (*Cathartes aura*)	Bariloche	2011	14	Year (<0.01) + Zone (<0.01) + Year x Zone (<0.01)	133.82 ± 56.45 133.57 (58.93-246.63)	14	None	19.08 ± 6.38 17.23 (10.17-32.20)	14	None	288.35 ± 108.57 233.25 (138.77-460.69)	14	Zone (0.03)	2297.2 ± 247.60 2291.0 (1924.4-2909.6)	14	Zone (<0.01)	55171 ± 9208.4 54714 (44236-73566)
		2013	13		2438.9 ± 2405.2 1569.3 (106-6743.5)												
		2017	37		1307.9 ± 1778.9 317.5 (59.7-6716.3)												
	Chocon	2011	15		405.54 ± 325.78 334.08 (39.34-1269.0)	15		13.31 ± 4.57 13.23 (5.56-21.56)	15		385.52 ± 288.98 305.08 (138.33-1322.9)	15		2083.4 ± 755.47 1938.2 (948.82-3993.1)	15		43301 ± 8325.0 42054 (23179-56037)
		2017	27		2254.3 ± 2027.9 1990.7 (111.6-7322.9)												
	El Valle	2011	15		657.38 ± 1125.9 140.17 (51.1-4195.3)	15		14.07 ± 14.08 12.81 (0.06-61.26)	15		1160.4 ± 2596.6 203.12 (99.38-9468.0)	15		2484.9 ± 421.29 2514.8 (1847.9-3034.8)	15		54623 ± 17856 52740 (35055-113332)
		2017	21		518.33 ± 885.67 140 (70.8-3564.8)												

Chimango caracara (*Milvago chimango*)	Bariloche	2011	3	Year (0.011) + Zone (0.015)*	955.16 ± 276.04				3	Year (0.014)**	361.44 ± 343.24					
					803.2 (788.5-1273.8)						179.22 (147.75-757.37)					
		2017	20		324.88 ± 307.52				19		1226.3 ± 985.84					
					239.4 (32.6-1161.9)						1013.7 (37.15-3707.8)					
	El Valle	2011	22		424.6 ± 487.97				22		436.88 ± 303.73					
					177.4 (60.5-1865.7)						334.65 (155.24-1466.3)					
Southern crested caracara (*Caracara plancus*)	Bariloche	2011	10	Year (<0.01) + Zone (<0.01)	134.92 ± 91.748											
					123.7 (31.2-360.8)											
		2017	57		756.74 ± 737.90											
					358.7 (73.9-3098)											
	El Valle	2011	11		541.22 ± 736.74											
					341.6 (87.9-2610.8)											
		2017	2		1707.2 ± 697.13											
					1707.2 (1214.3-2200.2)											
Andean condor *(Vultur gryphus)*	Bariloche	2017	-	NA	-	22	NA	192.75 ± 631.57 10.26 (<LOD-2941)	22	NA	1191.1 ± 1095.3 902.2 (512.3-5842.2)	22	NA	5698.7 ± 2139.0 5481.1 (3121.2-14081)	22	NA	122152 ± 22139

Model: indicates the most influential factor (explanatory variable) in the response variable "Metal concentration".

None = concentration of metal is not significantly influenced by any variable.

[a]Year, Zone and Year x Zone were included as factors in these models (*The interaction Year x Zone was not included in this model).

[b]Zone was included as factor in these models (**Year and Zone was included as factors in this model).

NA = Not applicable.

Conclusions

Chapter 1. From Mexico to the Beagle Channel: A review of metal and metalloid pollution studies on wildlife species in Latin America.

This review catalogs studies of environmental pollution by metals and metalloids in wildlife inform Latin America for the first time. We found high levels of metal and metalloid contamination in the three geographical areas from Latin America with industrial activity, intensive agriculture, urban contamination indicated as the main emission sources. In some cases, the contaminant levels detected in certain species have been among the highest globally. Several studies conducted on species of interest for human consumption show values that exceed safety limits; for instance, Barrera-García et al. (2012), detected 1.69 ± 0.18 μg/g−1 ww Hg level in shark sample from Mexico, while the limit established for human consumption by international agencies, such as US Food and Drug Administration, World Health Organization (WHO) and the Mexican Official Norm (NOM 242- SSA1) is 1.0 μg/g−1 ww. The study by Akagi and Naganuma (2000) detected levels of Hg ranged from 0.08 to 3.82 ppm in fish from the Tapajos River system, Brazil. In this case the levels of mercury exceeded the Brazilian permitted limit of 0.5 ppm. Other studies show that despite detecting levels of pollution below the limits, they can represent risks for health to pregnant women, children and those populations that base their diet almost exclusively on aquatic animals coming from contaminated areas. We provide here a useful database for researchers and governments to develop environmental research strategies, promoting the protection of biodiversity, including human beings. One of the gaps observed in Latin Americas research is the lack of a single working protocol that facilitates the comparison of data, unifying the results of the different studies. It would be advisable that the different countries create biomonitoring schemes of contaminants and adopt a common protocol, as it has been done in others regions of the world. The development of research with biomonitors rather than bioindicators, which currently represent most studies, should be a priority. Following the global trend, we suggest researchers should use non-lethal sampling techniques and use non-threatened species as biomonitoring, increasing studies on subrogate species. The use of sentinel species with a high position in the food chain would be advisable; in this way the results could be used to detect possible risks to human's health at an early stage. Considering the possible effects of chronic exposure at doses slightly below the safety limits established for food resources and the needs of the population most sensitive to contaminants, further research should be undertaken to define safety limits more in line with these conditions. To assess their actual pollution status, in addition to aquatic ecosystems, terrestrial ecosystems should also be the subject of further research.

Chapter II. Mercury in the feathers of bird scavengers from two areas of Patagonia (Argentina) under the influence of different anthropogenic activities: a preliminary study.

This study brings together the concentrations of Hg in feathers of 69 samples of family Cathartidae and 21 Caracara plancus, and this is the first study of this magnitude of birds from Patagonia. The results of this study regarding the contamination of Hg coincide with the results of different studies in the same areas, suggesting higher concentrations of Hg contamination in “El Valle” than in “Bariloche”. The three species are common throughout the American continent (North, Central, and South), have a high position in the trophic chain and the few studies where they have been used to determine Hg contamination in different environments of the American continent have some consistency of results. Therefore, we can consider valid the hypothesis that black vulture, turkey vulture, and southern crested caracara are good candidates for future biomonitoring studies. However, more studies are needed to assess more relationships between the values obtained. Another relevant aspect is that the three species share habitats with two endangered scavenger birds, the Californian condor and the Andean condor. Therefore, the results obtained with the species of this study might be relevant to evaluate risk to these endangered condors. This study is an important step in the collection of data on North Patagonia, the phenomena of Hg contamination in terrestrial ecosystems and the New World scavenger species, all of which have been little studied. Our results can be used as a comparison with future studies and geographic areas. This and other research on ethology and phenology, developed from the joint sampling of 2011, have provided us with new information, which has been used for new sampling that will try to shed light on some of the questions arisen from this preliminary study.

Chapter III. Temporal changes in metal concentrations in Andean condor feathers: a potential influence of volcanic activity.

We have reported the first reference values of several metals and metalloids for Andean condor feathers. These values, in addition to giving us information on the current state of health of the Andean condor population of northern Patagonia, Argentina, may in the future allow us to evaluate the evolution of the situation in the area and represent an important database against which to compare the results of future studies on the species. The results obtained in forty-eight Andean condor primary feathers from Patagonia collected in the period 2007–2017, pre and post Puyehue-Cordon Caulle volcanic eruption suggest a possible incorporation of metals in wildlife and the environment after the eruption. The high Si values seem to confirm the volcanic origin of the metals detected. Si, Zn, As, and Cd showed increased levels after the eruption, and concentrations of Zn, As, Se, and Cd (in addition to Cu and Hg) detected were lower than those causing adverse effects. The concentrations of Cr and Pb detected in some individuals showed levels compatible with the disorders of living organisms, but they were not related to the volcanic eruption. This study shows that the Andean condor reflects variations of the environment, suggesting that its use as a sentinel species at least for some metals could be useful inside its South American distribution, depending on the kind of metal investigated. However, to test this and interpret our results in a more comprehensive way, there is a need for deeper understanding of condor biology (e.g., molt pattern, physiology) to further support conservation efforts and to benefit the survival of this species and its habitat.

Chapter IV. Testing Cathartidae of Northern Patagonia as a potential biomonitor species for Hg ecotoxicological studies and surrogate species for Andean Condor (*Vultur gryphus)*.

Our results show, for the first time, that Turkey vulture samples better reflect Hg contamination in our study area, possibly reflecting also the presence of Hg in the aquatic ecosystem. These data lead us to suppose that the Turkey vulture would represent the most suitable biomonitoring species for ecotoxicological studies on Hg contamination in our study area, considering that there are no other raptors in the area that can feed on fish (e.g. Osprey *Pandion haliaetus*). Also, for the first time, the levels of Hg detected in species with a high trophic chain in the area of Bariloche are compatible with toxic effects. With the information at our disposal, and in the absence of further studies, these results seem to indicate a worrying increase in total Hg (probably MeHg) levels. If confirmed, these data would indicate a potential risk even for the human population in the area.

Regarding the use of surrogate species, we can consider our results as interlocutory. In our test, based on the results, and thanks to all the existing data on the area and the species, we could say that for ecotoxicological studies on Hg contamination, Black vulture would represent a better option as a surrogate species of Andean condor than Turkey vulture. Turkey vulture could be considered as a suitable surrogate species of Andean vondor only for those habitats where Andean condor integrates its diet with fish. Black vulture and Turkey vulture should not be considered as good options as surrogate species of Andean condor. In order to apply the methodology of surrogates to other areas of distribution of the Andean condor, considering the scarcity of scientific research in many parts of Latin America, more research is needed, with new approaches. The temporal trends and analogies of the relationships between Pb and Hg values detected in several studies with surrogate species, which at present are no more than simple speculations, could perhaps be these approaches. If links between these values can be verified, ecotoxicological studies with surrogate species could represent the future for biomonitoring of threatened species as Andean condor.

Chapter V. **Evaluation of spacial and temporal changes in metal levels in feathers of five scavenger bird species from northern Patagonia (Argentina)**

Our study shows, for the first time, data on contamination by Hg, Cd, Pb, Cu, Zn in the North of the Argentine Patagonia, employing carrion birds and evaluating the possible use of these species for future studies. The Hg levels detected in Turkey vulture and Black vulture samples in three sampling areas over several years have allowed us to evidence the potential risk to mercury exposure in El Chocon area. This exposure, possibly influenced by the presence of the hydroelectric dam, has probably been amplified by the 2011 burping of the PCCVC. The impact of the probable Hg emissions from the volcano, not fully demonstrated to date, should be further studied to assess the long-term effects on wildlife and humans living in the area. The importance of time trend comparisons has also become apparent once again. Furthermore, the data suggests that the use of Black vulture as biomonitor species offers information on baseline Hg levels, while Turkey vulture and perhaps Southern crested caracara would offer evidence of contamination from the aquatic ecosystem.

Pb exposure from hunting activities could also be associated with Cu and Zn exposure, although in this case the levels of these elements would not represent a health threat. Finally, the data obtained in Chimango caracara, although partial, represent an important first study of the species. The results seem to indicate that the species, if adequately researched, could represent an important tool for point studies of contamination, especially in relation to hurban environments. We hope that these results will encourage and support future research.

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