Universidad de Concepción
Facultad de Ciencias Ambientales
Programa de Doctorado en Ciencias Ambientales con mención en Sistemas Acuáticos Continentales

ESTUDIO DE ELEMENTOS TRAZA EN ECOSISTEMAS ACUÁTICOS DE LA PENÍNSULA ANTÁRTICA

Tesis para optar al grado de

Doctor en Ciencias Ambientales con mención en Sistemas Acuáticos Continentales

Winfred Eliezer Espejo Contreras

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規律は、最終的に知能を期限切れ

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RESUMEN

Los elementos traza (ETs) poseen propiedades fisicoquímicas que son fundamentales para la fabricación de muchos productos de uso diario, los cuales han sido claves para el desarrollo de las civilizaciones. Esta demanda sigue en aumento con el descubrimiento de nuevas propiedades de elementos antes desconocidos, los cuales son muy importantes para el desarrollo de nuevas tecnologías (energías renovables, electrónica, automóviles, aeronaves, biomedicina). Sin embargo, el uso extensivo de los ETs ha modificado las concentraciones naturales de algunos metales en la litósfera. Esta mayor actividad antropogénica se suma a los ciclos naturales de los ETs modificando su distribución, llegando en algunos casos a niveles tóxicos para los seres vivos. A pesar de su gran relevancia económica poco se sabe sobre los ciclos ecológicos de los metales no tradicionales. Estos ciclos son a escala global ya que el aumento de las concentraciones de metales a nivel local puede aumentar en la zona de emisión, y también pueden ser transportados a grandes distancias llegando incluso a zonas remotas como la Antártica. En esta zona polar, es vital la conservación de la biodiversidad, considerada como la más prístina del planeta. Dentro de la fauna antártica, los pingüinos son valiosos representantes debido a que constituyen la mayor biomasa de esta región. Siendo aves acuáticas, los pingüinos tienen la capacidad de vivir tanto en el mar como en tierra, lo que los convierte en el principal nexo entre ambos ecosistemas. Se ha observado un leve pero sostenido incremento de los niveles de ETs en la fauna antártica, lo cual se debería a una mayor actividad antrópica local y global. Los pingüinos pueden verse afectados por los ETs a ciertas concentraciones, pero éstos también actúan como biovectores de estos químicos, es decir pueden transportar ETs desde el mar donde los ingieren por la dieta y los depositan luego en la tierra por medio de las excretas y las plumas. Se observaron mayores concentraciones de algunos ETs en suelos impactados por la presencia de colonias de pingüinos con relación a los sitios de referencia. Sin embargo, hay que tener presente que no se observaron diferencias estadísticamente significativas en todos los casos, siendo estas especie-sitio específicas. Los ETs que se encuentran en los lugares donde estas aves anidan podrían constituir una potencial

fuente de exposición para organismos terrestres que viven allí, así como para los polluelos de pingüinos. Por otro lado, los ETs depositados en tierra podrían volver al mar mediante escorrentía e ingresar a las tramas tróficas acuáticas costeras. A este respecto, la transferencia trófica de Cd mostró evidencia de biomagnificación en invertebrados acuáticos (TMS 0,66 – 0,84) y Ta mostró evidencia de biomagnificacion en invertebrados y vertebrados acuáticos (TMS entre 0,05 - 0,18). Esto tiene importantes implicaciones, especialmente con respecto a Ta, dado que se sabe muy poco sobre la toxicidad potencial y dado que existe una mayor demanda de este elemento por sus aplicaciones tecnológicas. El biotransporte y la transferencia trófica son fundamentales en ciclo de los ETs, lo que amerita más estudios para comprender la distribución de estos elementos y cómo influyen las actividades antropogénicas locales y globales en los ciclos naturales de estos químicos.

Capítulo I: INTRODUCCIÓN

1.1 Antecedentes generales de los elementos traza en el medioambiente

Entre los elementos químicos, los metales se distinguen por poseer propiedades químicas y físicas muy distintivas, tales como una alta conductividad eléctrica y térmica, maleabilidad, ductilidad y capacidad de formar aleaciones (Gunn 2014). La abundancia de metales en la corteza terrestre varía enormemente (Figura 1), lo que influye en que exista una mayor o menor disponibilidad (Gunn 2014). Aproximadamente el 99,7% de la corteza terrestre está compuesta de un número relativamente pequeña de elementos, denominados elementos mayoritarios (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K y P), mientras que los elementos traza (Cd, Ta, Hg, As, Pb, Cu o Zn entre otros), también llamados metales traza o metales pesados, sólo representan el 0,3% restante (Fan 1996, Rudnick y Gao 2003). Los elementos traza, reciben su nombre debido a que las concentraciones ambientales son menores a los elementos mayoritarios, reportándose en microgramos hasta picogramos por gramo. A pesar de sus bajas concentraciones ambientales, el descubrimiento y uso de varios elementos traza por parte de la humanidad se remonta a varios miles de años: Au (6000 AC), Cu (4200 BC), Ag (4000 BC) o Pb (3500 BC), entre otros (Cobelo-García et al. 2015). Los elementos traza han jugado un papel fundamental en el desarrollo de las civilizaciones, resultando esencial para el desarrollo económico de los pueblos, especialmente después de la revolución industrial en el siglo XIX, para el funcionamiento de la sociedad y para mejorar la calidad de vida de las personas (Ashraf et al. 2015). Es la demanda por más y nuevos productos lo que genera la necesidad por obtener minerales, más que la demanda del mineral en sí. Sin minerales, los países desarrollados no disfrutarían del estilo de vida que poseen y al que todos aspiran. Sin el desarrollo continuo de la tecnología para la exploración, procesamiento y fabricación de minerales, la sociedad no podría contar con productos baratos y confiables, como aviones, automóviles, computadoras, teléfonos móviles y una gran cantidad de otros productos electrónicos personales portátiles, los cuales se siguen masificando (Gunn 2014). El avance de la tecnología ha sido posible gracias a las nuevas aplicaciones de ciertos elementos metálicos que antes no se conocían, como el Ta y otros (Filella et al. 2017). Los usos más notables están en el desarrollo y producción de semiconductores, superconductores, vidrios metálicos, aleaciones magnéticas y acero de alta resistencia y baja aleación, y más recientemente en nanotecnología (IARC 2006, 2010).

La distribución de elementos traza en la corteza terrestre se debe a fenómenos naturales, dependiendo de su composición geológica. Como es el caso de ciertas áreas volcánicas, el agua subterránea puede contener altas concentraciones de compuestos arsenicales (Juncos et al. 2016). No obstante, las actividades antropogénicas pueden aumentar las concentraciones de metales presentes de forma natural en diversos compartimentos ambientales, y llegar a producir contaminación ambiental. Las principales actividades humanas que contribuyen al aumento de elementos traza en el ambiente son la minería, las fundiciones, la quema de combustibles fósiles, la agricultura a través de los pesticidas, y el uso de metales en la industria (Capuano et al. 2005; Ouyang et al. 2006). Por su parte, el creciente uso de elementos traza poco conocidos en equipos tecnológicos junto con el aumento de desechos electrónicos, podría llevar a un aumento de estos elementos en el ambiente en el futuro (Cobelo-García et al. 2015). Estas actividades antropogénicas pueden causar emisiones puntuales de elementos traza al ambiente, pero en algunos casos las actividades humanas pueden provocar emisiones difusas que indirectamente pueden provocar cambios en el medio ambiente, movilizando elementos traza que de otra manera estarían depositados en formas estables (Dore et al. 2014). El transporte a gran distancia de contaminantes atmosféricos no sólo contribuye al aumento de la carga de metales en ecosistemas apartados, sino que también altera la movilidad de los metales (Gunn 2014). La distancia que pueden recorrer los elementos traza desde la fuente que los produce hasta su deposición final puede en ocasiones ser superior a 1000 km por transporte aéreo (Chung et al. 2014). Pudiendo llegar a ecosistemas apartados como es la Antártica.

1.2 Características generales de los ecosistemas acuáticos de la Antártica

La Antártica, es el único continente no habitado por seres humanos (salvo por la presencia de bases científicas, que incluyen a nuestro país). Debido a que el tratado Antártico ha decretado que esta es una región del mundo dedicada a la paz y la ciencia. La mayoría de la superficie de este continente está cubierta de hielo, solo en periodo estival aproximadamente el 2% de su superficie perteneciente a zonas costeras, permanece libre de hielo (Bargagli, 2008).

La biota Antártica presenta características particulares debido a las bajas temperaturas y marcada variación estacional de la radiación solar que presenta este continente. Las principales características son: i) Organismos con ciclos de vida largos y con clara tendencia hacia el gigantismo debido a las bajas temperaturas (De Broyer 1977); esto hace que las tasas metabólicas de estos organismos sean más lentas (Peck 2001, Chapman 2005), con un mínimo uso de energía (Clarke 1979, Chapman 2005), lo que hace tengan una mayor bioacumulación de elementos traza en comparación con especies de otras regiones del planeta; ii) en la Antártica, las especies acuáticas se encuentran más vinculadas entre si energéticamente (Van den Brink et al. 2011).

1.3 Pesencia de ETs en la Antártica.

La Antártica había sido considerada por muchos años como un continente prístino, libre de contaminación e intervención humana. Debido a la globalización y el incremento industrial del último siglo los niveles de contaminantes en el ambiente han aumentado, los cuales por transporte global a gran escala están llegando a zonas frías como es la Antártica. Lo anterior sumado a un aumento de las actividades antropogénicas locales en la Antártica, principalmente en las Islas Shetland del sur y la parte norte de la Península Antártica, debido al incremento de las actividades científicas, militares y turísticas. La contaminación por elementos trazas es de gran preocupación ya que pueden influir en la integridad funcional y estructural de los ecosistemas (Malik & Zeb, 2009). Estos elementos se pueden encontrar de forma natural en la Antártica, pero las actividades antropogénicas locales y globales pueden influir en los niveles naturales presentes en la Antártica. Actualmente, existe un creciente número de publicaciones que han reportado concentraciones de elementos traza en la Antártica (Carravieri et al, 2013,2014,2016; Celis et al, 2012, 2014, 2015; Espejo et al 2014, Jerez et al, 2011, 2013a, 2013b). Se ha observado un leve, pero sostenido incremento de los niveles de ETs en la fauna antártica, lo cual se debería a una mayor actividad antrópica local y global.

1.4 Los pingüinos como bioindicadores de niveles de ETs

Los pingüinos son, las especies de aves marinas características, particularmente convenientes para estudiar la distribución de elementos traza en los ecosistemas marinos, pues son de amplia distribución geográfica, relativamente fácil de identificar, ocupan posiciones altas en las cadenas alimentarias, y son especies sensibles a las perturbaciones ambientales (Burger et al. 2000, Smichowski et al. 2006, Boersma 2008). Las aves marinas pueden reflejar la bioacumulación de elementos trazas que adquieren por la dieta. Este grupo de especies son altamente utilizadas en programas de biomonitoreo de contaminación ambiental, permitiendo evaluar la distribución espacial y temporal de los contaminantes. Lo cual es de gran utilidad para programas de protección y regulación de contaminantes (Cid et al 2009).

Los pingüinos, a parte de las características propias de toda ave marina, son las especies más representivas en la Antártica, las cuales constituyen la mayor biomasa de la Antártica (Boersma, 2008). Las plumas son excelentes como matriz de monitoreo, ya que poseen grupos sulfhídricos los que tienen elevada afinidad con los metales (Metcheva et al., 2006), además la muda anual permite tener una tendencia temporal de los niveles de exposición (Metcheva et al., 2006). Por otra parte, las excretas son una ruta importante de eliminación de metales en pingüinos

(Ancora et al. 2002), por lo que sus heces son una fuente directa de contaminación ambiental y adecuada para el biomonitoreo (Metcheva et al., 2006). Algunos estudios previos han demostrado que los excrementos de pingüinos Gentoo (*Pygoscelis papua*), pingüinos de Chinstrap (*Pygoscelis antarctica*) y Pinguinos Adelia (*Pygoscelis adeliae*) son bio-monitores efectivos para la contaminación de metales en la Antártica, mostrando que los niveles de metales traza en excretas son un criterio importante para la salud ambiental, específicamente para condiciones polares (Celis et al.,2012, 2015; Espejo et al. 2014). El estudio de estas especies sirve para evaluar la variación espacial y temporal de las concentraciones de elementos traza en el ambiente antártico.

1.5 Biotransporte de ETs en la Antártica

El 95 % de las aves marinas forma colonias (Schreiber y Burger 2001) y éstas se encuentran entre los mayores asentamientos de aves en el mundo. Es allí cuando ocurre la incubación y la recreación de estas aves, donde se liberan grandes cantidades de contaminantes al suelo por medio del guano (excrementos). Es por ello que las aves marinas son biovectores de compuestos químicos desde el mar a la tierra (Liu et al. 2006, Evenset et al. 2007, Michelutti et al. 2009; Choy et al. 2010, Mallory et al. 2015). Este biotransporte trae consigo el aumento de elementos traza y otros elementos mayores (como nutrientes), los cuales pueden actuar como un subsidio crítico a los ecosistemas terrestres (Blais et al. 2005). La deposición de excrementos de aves marinas ha tenido un efecto significativo en la composición geoquímica de algunas áreas polares terrestres (Sun y Xie 2001; Xie y Sun 2008). Existen estudios que indican que los suelos del Ártico, próximos a los sitios de anidación se ven fuertemente afectados por los elementos traza de las heces de gaviota (Headley 1996, Michelutti et al. 2009). Por su parte, la biota terrestre que vive cerca de una colonia de aves marinas tiende a incorporar estas sustancias químicas, presentando concentraciones superiores a las observadas en organismos que viven en hábitats similares pero que están lejos de las colonias de aves (Ligeza y Smal 2003, Choy et al. 2010).

Los estudios sobre biotransporte de ETs en la Antártica son escasos. Huang et al. (2014) observaron que en los sitios de colonias de pingüinos emperadores en Bahía Amanda (Antártica Oriental) las concentraciones de P, Se, Hg y Zn fueron más altas que en los sitios de control, con la excepción del Cu y el Pb. Ellos argumentan que debido al alto nivel trófico de la especie y alta dinámica de transferencia de elementos, esta ave puede ser capaz de transportar una gran cantidad de nutrientes y ETs del mar a la tierra, idependientemente del tamaño de la población. Otro estudio (Qin et al., 2014) reveló un importante biotransporte de P debido a la presencia de pingüinos en distintos lugares de la Antártica, con aportes que variaron entre 12.349 a 167.036 kg anuales. Un estudio reciente demostró que el suministro continuo de heces de pingüinos determina un fuerte cambio en el dominio de los taxones de la comunidad bacteriana del suelo aledaño a los sitios de anidación (Santamans et al., 2017).

1.6 Trofodinámica de elementos traza en la Antártica

La trofodinámica, estudia la transferencia trófica de químicos (Cd, Ta y entre otros), que corresponde a la forma en que las concentraciones de un químico se mueven a lo largo de diferentes niveles tróficos, grupo de organismos con necesidades energéticas y nutricionales similares (Garvey y Whiles 2016). Las concentraciones de los compuestos químicos pueden aumentar a mayor nivel trófico (biomagnificación), disminuir (biodilución) o no presentar tendencia alguna (Luoma y Rainbow 2008). Los estudios de la transferencia trófica están limitados por la dificultad de discriminar los niveles tróficos de los organismos. El conocimiento acerca de los isótopos estables de nitrógeno puede ser muy útil para determinar el flujo de energía a través del tiempo, así como la constitución alimentaria en las comunidades ecológicas (Cabana y Rasmussen 1994; Croteau et al. 2005). El análisis de isotopos estables de nitrógeno utiliza las emisiones radiactivas (Hop et al. 2002, Squeo y Ehleringer 2004), facilitando el establecimiento de las relaciones tróficas en redes tróficas. Existen dos isótopos estables de nitrógeno (^{15}N y ^{14}N). Estos isótopos son utilizados para estimar el flujo de energía a través de las cadenas alimenticias marinas, calculando el enriquecimiento del isótopo más pesado por sobre el más liviano (Ruus et al. 2002). Esta diferencia entre isótopos más pesados y más livianos se manifiesta por un proceso denominado fraccionamiento isotópico, el cual está dado por transformaciones biológicas del organismo y las diferencias en el peso atómico del isótopo más pesado con respecto al más liviano (Fry 2008). El fraccionamiento isotópico es la ruptura mediante una reacción bioquímica de una molécula separando el isótopo liviano del resto de la molécula, debido a que los enlaces químicos son más fuertes en el primero. El resultado de estas diferencias genera señales que son incorporadas dentro de los predadores al asimilar la materia orgánica. Así, el tejido de los organismos superiores se enriquece con el isotopo más pesado, resultando en un $\delta^{15}N$ mas alto a medida que consume presas que conforman su dieta (Ruus et al. 2002). Es por ello que la relación $^{15}N/^{14}N$ ($\delta^{15}N$) se ha utilizado para evaluar el nivel trófico en la biota acuática. Por su parte, los isotopos de carbono ($^{13}C/^{12}C$; $\delta^{13}C$) entregan información acerca de las interacciones tróficas mediante el establecimiento de la contribución de las fuentes de C, es decir, si son organismos pelágicos o bentónicos (Figura 2).

Para tener mayor precisión en el nivel trófico, los valores brutos de $\delta^{15}N$ se ajustan restando el promedio de $\delta^{15}N$ de los consumidores primarios obteniendo así valores de $\delta^{15}N_{adj}$ (Cabana et al. 1996; Anderson y Cabana 2007). Del mismo modo, los valores brutos de $\delta^{13}C$ son ajustados con lípidos para garantizar que los organismos se vinculen energéticamente. De esta manera, los valores del consumidor $\delta^{15}N$ se convierten en niveles tróficos (TL) de acuerdo con la siguiente ecuación:

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \Delta^{15}N + \lambda \quad (1)$$

Donde, λ es el nivel trófico del organismo de referencia. TLconsumer es el nivel trófico de un consumidor dado, y $\delta^{15}N_{consumer}$ y $\delta^{15}N_{baseline}$ son valores brutos de $\delta^{15}N$ de un consumidor determinado y el organismo de referencia, respectivamente. Se utiliza un factor de discriminación trófica para $\delta^{15}N$ (Δ15N).

La transferencia trófica de algún metal se puede evaluar mediante regresiones lineales como sugiere Lavoie et al. (2013), tal como se indica a continuación:

$$\log_{10} [Ta] = b\ \delta^{15}N + a \quad (2)$$

$$\log_{10} [Ta] = b\ TL + a \quad (3)$$

Donde, b en la ecuación 2 se conoce como la pendiente de magnificación trófica (TMS, sigla en inlgés), y el antilogaritmo de la pendiente de la ecuación 3 como el factor de magnificación trófica (TMF).

La comparación entre las concentraciones químicas y los niveles tróficos mediante regresión lineal puede mejorar la comprensión de los fenómenos biológicos de los elementos traza en el ambiente y la posible exposición humana a través de la dieta, tema que ha recibido especial atención durante las últimas décadas (Kelly et al. 2007; Luoma y Rainbow 2008).

La comparación entre las concentraciones de Hg y contaminantes orgánicos persistentes (COPs) con los niveles tróficos está bien documentada, mostrando una clara tendencia hacia la biomagnificación (Lavoie et al. 2013; Walters et al. 2016). Estudios recientes con otros elementos traza como Cd, Cu y Zn han mostrado que las concentraciones aumentan a niveles tróficos más altos, dependiendo del sitio y factores específicos de la especie, más que de las características del elemento (Ikemoto 2008; Zeng et al. 2013, Majer et al. 2014). Se ha evidenciado que hay biomagnificación de Cu y Cd en el estuario del río Pearl en China (Cheung y Wang 2008; Zeng et al. 2013), mientras que en el delta del río amarillo (China) se han reportado incrementos de los niveles de Cu y Cd en la trama trófica del río (Cui et al. 2011). También se ha reportado biomagnificación de Cd en mamíferos del Ártico (Dehn et al. 2006), así como en la Bahía de San Francisco (Croteau et al. 2005). Se desconoce aún lo que sucede en otras localidades y con metales menos estudiados. Hay razones teóricas para sospechar que Se, Be, Co y tal vez otros elementos traza podrían experimentar cierto grado de biomagnificación en algunas circunstancias, aunque sólo Se ha sido evaluado en investigaciones a nivel de campo (Stewart et al. 2004). Con respecto a Cu y Zn, estos elementos traza son esenciales, por lo tanto, posiblemente hay un enriquecimiento de estos elementos en lugar de biomagnificación. Sólo existen pruebas que demuestran biomagnificación de nanopartículas (como Au, Ti) en las redes tróficas a nivel de laboratorio (Ferry et al. 2009; Kubo-Irie et al. 2016).

En lo concreto, hay una carencia de estudios que permitan conocer las concentraciones de elementos emergentes (como es el caso de Ta) en matrices bióticas, así como la movilidad a lo largo de diferentes niveles tróficos, especialmente en un escenario donde el Ta ha adquirido una gran demanda debido al desarrollo de tecnologías emergentes (Cobelo-García et al. 2015 Filella et al. 2017). Esto es importante porque si Ta se biomagnifica en los ecosistemas acuáticos podría constituir una vía de exposición en aquellas especies que dominan los niveles superiores de las cadenas alimentarias (Kales y Goldman 2002).

En cuanto a estudios sobre trofodinámica en la Antártica, Bargagli et al. (1998) reportaron por primera vez niveles de Hg en diferentes especies que habitan en la Bahía de Terra Nova, observando un progresivo aumento de sus concentraciones a medida que aumentaba el nivel trófico. Estudios en la Bahía Admiralty han revelado ciertos procesos de biomagnificación de Hg (Santos et al. 2006) y Cd (Majer et al. 2014). Sin embargo, existe un sólo estudio en especies bentónicas que contrastó las concentraciones de As, Cd, Cu, Ni, Pb y Zn con isótopos estables de nitrógeno ($\delta^{15}N$) donde se indica un incremento de las concentraciones de Cd a mayores niveles tróficos (Majer et al. 2014).

1.7 Elementos traza como objeto de estudio

De todos los elementos traza existente los elementos objeto de estudio de la presente investigación fueron elementos traza más conocidos (Cd, Cu, Mn, Ni, Pb, Zn), poco conocidos (Co, Mo, Sr, V) y un elemento que cual se desconoce su comportamiento ambiental (Ta). Los elementos fueron considerados relevantes en esta investigación por su importancia en ecosistemas acuáticos, por ser reportados o preverse incremento de estos elementos por acción antrópica y por su capacidad de provocar efectos toxicológicos. De los cuales a continuación se presenta una breve reseña.

El Cadmio (Cd), es un elemento no esencial sin función biológica conocida, y está clasificado como uno de los elementos traza más peligrosos (Ravera 1984). Este metal se encuentra ampliamente distribuido en la corteza terrestre debido a procesos naturales (erosión y volcanes) y actividades antrópicas (metalurgia, galvanoplastia, pinturas, combustión de carbón y petróleo) (Kakkar y Jaffery 2005). Existe evidencia que indica que el Cd posee la capacidad de bioacumularse y tener un grado de toxicidad en los organismos acuáticos (Bargagli et al. 1996).

El cobre (Cu) es un elemento esencial que participa en actividades biológicas, y que está muy distribuido en la corteza terrestre, constituyendo diferentes minerales. Sus principales usos son en equipos electrónicos y maquinarias industriales, ya que presenta alta resistencia a la corrosión. Recientemente se ha descubierto que posee propiedades antibacterianas, razón por la cual se utiliza en hospitales y productos textiles. El exceso de Cu en los organismos puede producir alteraciones hepáticas, renales y cerebrales (Repetto, 1995).

EL zinc (Zn) es un elemento esencial formando parte de más de 200 enzimas y distintas proteínas. Es esencial en el desarrollo del sistema nervioso y sistema óseo. Este elemento se encuentra en diferentes minerales y es abundante en diversos alimentos. Su principal uso es en la fabricación de acero galvanizado, tuberías, y barcos donde se utiliza como recubrimiento para protegerlo de la corrosión. Además, es utilizado en pilas y baterías eléctricas. La exposición sub-crónica de Zn genera acumulación en corazón, hígado, riñones y huesos. El Zn es considerado teratogénico (Soria et al., 1995).

El plomo (Pb) es un elemento no esencial que tiende a bioacumularse en organismos acuáticos (Moreno-Gaw, 2003). La principal fuente antropogénica de Pb corresponde al masivo uso como aditivo en combustibles de gasolina, razón por la cual se se emitieron grandes cantidades de Pb a la atmosfera; desde su prohibición han disminuido sus emisiones en algunos países desarrollados, aunque no ha sido una tendencia general (Yu, 2001). Actualmente diversos productos industriales siguen utilizando Pb, tales como baterias de automóviles, materiales de construcción, municiones, entre otros. Además, diversos procesos industriales son potenciales fuentes de emisión de Pb (fundiciones, refinerías, incineradoras, etc.). La exposición crónica de Pb provoca efectos neurotóxicos, alteraciones hepáticas, anemia, entre otras alteraciones (Soria et al., 1995).

El niquel (Ni) es un micronutriente para plantas, invertebrados, aves y mamíferos (Eisler, 1998). Este elemento se encuentra ampliamente distribuido en los medios bióticos como abióticos y se puede acumular en organismos terrestres y acuáticos (ATSDR, 2005). Las fuentes naturales son por actividad volcánica y erosión de suelos y rocas, mientras que las principales fuentes antropogénicas son las industrias químicas, electrónicas, termoeléctricas y cerámicas. Los productos que más lo utilizan son el acero inoxidable, baterías, pinturas, prótesis y cintas magnéticas. Otras fuentes de emisiones antrópicas son la quema de combustibles fósiles e incineración de residuos urbanos. (ATSDR, 2005; Soria et al., 1995; WHO, 1991).

El manganeso (Mn) es un elemento esencial para las proteínas, el metabolismo energético, la mineralización de huesos y la protección frente a radicales libres (ATSDR, 2008). El Mn es un compuesto de más de 100 minerales, siendo la erosión de los suelos y rocas la principal fuente de emisión natural (ATSDR, 2008). El Mn es empleado en la fabricación de baterias, pilas, fuegos artificiales, porcelanas y como aditivo en plaguicidas y fertilizantes (Soria et al., 1995). La exposición crónica a Mn causa alteraciones neuronales, pulmonares, reproductivas e inmunes (ATSDR 2008).

El vanadio (V) se encuentra en cerca de 65 minerales diferentes, siendo importante en la estabilización de productos de acero; además es utilizado en cerámicas e imanes supercoductores. Las fuentes naturales de V son principalmete las emisiones volcánicas. Las fuentes antropogénicas son la refinería del petróleo, termoeléctricas y carbón. La ingesta de este elemento puede producir trastornos digestivos, disminución de glóbulos rojos, aumento de la presión sanguínea, efectos neurológicos y alteración del desarrollo (ATSDR 2012).

El estroncio (Sr) proviene principalmete de la erosión de rocas y suelos. Sus principales usos son en pirotecnia, imanes de ferrita, fábrica de ceramicas, productos de vidrio, pigmentos para pinturas, lámparas fluorescentes y actualmente en la fabricación de relojes atómicos más modernos y precisos. El organismo absorbe Sr como si fuera Ca, pero sólo el ^{90}Sr (su forma radiactiva) puede ocasionar varias enfermedades y desórdenes en los huesos, tales como el cáncer óseo primario (ATSDR 2004a).

El cobalto (Co) se encuentra en las rocas, el suelo, el agua, plantas y animales. El Co se usa para producir aleaciones en la manufactura de motores de aviones, imanes, herramientas para triturar y cortar, y articulaciones artificiales para la rodilla y la cadera. También para colorear vidrio, cerámicas y pinturas y como secador de esmaltes y pinturas para porcelana. El Co tiene efectos tanto beneficiosos como perjudiciales para la salud de seres humanos, resultando beneficioso ya que forma parte de la vitamina B12. La exposición a niveles altos de Co puede producir alteraciones pulmonares, cardíacas, dérmicas y hepáticas (ATSDR 2004b).

El molibdeno (Mo) es un metal esencial desde el punto de vista biológico y se utiliza en la industria sobre todo en aceros aleados, como catalizador del petróleo, pigmentos de pinturas y plásticos. También en aparatos electrónicos como un metal conductor. Los altos niveles de Mo pueden interferir con la absorción de Cu, produciendo deficiencia de este último elemento. La ingesta crónica de más de 10 mg/día de Mo puede causar diarrea, retraso en el crecimiento, infertilidad, y bajo peso al nacer. También puede afectar a los pulmones, los riñones y al hígado (Opresko 1993).

El Tántalo (Ta), es un elemento químico de transición número 73 en la Tabla Periódica, azul grisáceo, duro y altamente resistente a la corrosión (O'Neil 2001). Este elemento conserva sus propiedades mecánicas hasta 1000°C, siendo químicamente estable en el aire a 300°C (Pokross 1990). Estas propiedades lo hacen muy atractivo para tecnologías avanzadas como las energías renovables, la eficiencia energética electrónica, la industria automotriz, aeroespacial y biomedicina (Henderson 2013; Gunn 2014). Se estima que los nuevos usos del Ta permitirán un aumento en su demanda global (Gunn 2014). La producción mundial de Ta ha aumentado en las últimas dos décadas, aunque todavía su extracción sigue siendo baja (alrededor de 1000 toneladas métricas por año) (United Nations, 2002). A pesar de su relevancia económica, se sabe poco sobre su distribución en matrices biológicas y abióticas (Filella et al. 2017).

A modo de resumen, en la Figura 1 se presenta el modelo conceptual sobre el cual se sustenta la presente investigación.

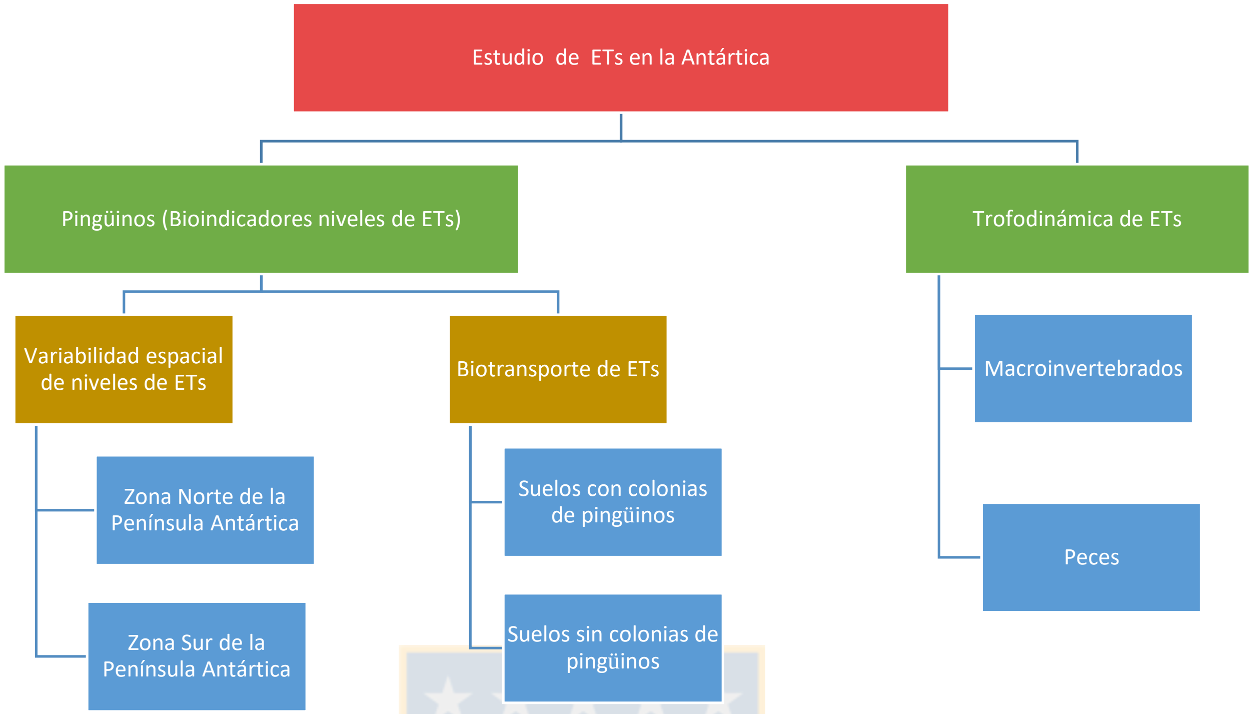

Figura 1. Mapa conceptual en el cual se basó la estructuración de la presente investigación.

Hipótesis

A causa del aumento de la población y de la industrialización, el riesgo a una mayor exposición de contaminantes ha crecido, y es por ello que también ha aumentado la preocupación de la comunidad científica, de los gobiernos y la sociedad por la salud humana y la protección del medioambiente (Nordberg et al. 2014). Sin embargo, todavía existen brechas considerables en el conocimiento que impiden comprender a cabalidad los ciclos ecológicos de los metales. La fauna acuática ha sido señalada como un indicador biológico potencial para estudiar contaminación (Burger y Gochfeld 2004). Si se considera que las especies antárticas tienen ciclos de vida largos, con clara tendencia hacia el gigantismo (De Broyer 1977), y tasas metabólicas son lentas (Peck 2001, Chapman 2005), existiría una mayor bioacumulacion de elementos traza en comparación con especies de otras regiones del planeta. Lo anterior, sumado al hecho de que las especies antárticas se encuentran más vinculadas entre si energéticamente (Van den Brink et al. 2011), significaría que los procesos de transferencia trófica podrían verse incrementados. A pesar de que la contaminación directa por elementos traza en la Antártica es más leve que en otras zonas geográficas, el biotransporte podría jugar un papel importante en el enriquecimiento de los suelos en esas regiones polares. En consecuencia, la hipótesis planteada se sustenta en las siguientes afirmaciones:

1) Existe un enriquecimiento significativo de ETs en los suelos debido al biotransporte que realizan los pingüinos vía excretas.

2) Las TMS de ETs (Cd y Ta) son más fuertes en los ecosistemas acuáticos Antárticos que las de otras regiones más templadas.

Objetivo general

Evaluar el biotransporte de ETs y determinar la transferencia trófica de Cd y Ta en distintos ecosistemas marinos.

Objetivos especificos

Determinar si existe diferencias significativas entre las concentraciones de ETs y parámetros fisicoquímicos en zonas de anidamiento de pingüinos en comparación con zonas sin intervención.

1. Determinar los niveles tróficos de diferentes especies acuáticas que habitan en la Antártica mediante el análisis de isotopos estables de nitrógeno ($\delta^{15}N$)

2. Determinar las concentraciones de Cd y Ta en diferentes especies acuáticas.

3. Correlacionar los niveles tróficos con las concentraciones de Cd y Ta.

4. Comparar las pendientes de biomagnificación de ecosistemas acuáticos Antárticos con ecosistemas acuáticos de la Patagonia Chilena y Costa Norte de Chile.

Estructura organizativa de la presente tesis

Esta tesis está estructurada en introducción, desarrollo (sobre la base de cinco capítulos), resumen final y conclusiones generales.

El **primer capítulo** corresponde a una **introducción** que incluye conceptos generales sobre los elementos traza, biotransporte y transferencia trófica. Esta sección incluye también las hipótesis y los objetivos de la tesis y un apartado explicativo sobre la organización del trabajo.

El **segundo capítulo** corresponde a una revisión bibliográfica sobre una visión global de los niveles de exposición y los efectos biológicos de los oligoelementos en los pingüinos. Este Capítulo ha sido publicado en la revista *Reviews of Environmental Contamination and Toxicology* [Espejo W., Celis J.E., González-Acuña D., Banegas A., Barra R., Chiang G. (2017) A Global Overview of Exposure Levels and Biological Effects of Trace Elements in Penguins. In: Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews). Springer, New York, NY]. IF4.7 (2017).

El **tercer capítulo** incluye los resultados obtenidos de los análisis de elementos traza en excretas plumas y suelos desde diferentes colonias de pingüinos papua (*Pygoscelis papua*) que habitan en la Península Antártica. Este capítulo se encuentra publicado en la revista *Water, Air, & Soil Pollution* [Celis, J. E., Barra, R., Espejo, W., González-Acuña, D., & Jara, S. (2015). Trace element concentrations in biotic matrices of Gentoo penguins (*Pygoscelis Papua*) and coastal soils from different locations of the Antarctic Peninsula. Water, Air, & Soil Pollution, 226: 2266, doi:10.1007/s11270-014-2266-5. IF1.7 (2017)

El **cuarto capítulo** corresponde a los resultados de los análisis de elementos traza y nutrientes en muestras de suelo superficial en sitios de anidación en dos colonias de pingüinos de Humboldt (*Spheniscus humboldti*) ubicadas en el noroeste de Chile y tres colonias de pingüinos Adelia (*Pygoscelis adeliae*) en el área de la Península Antártica. Las muestras control se tomaron fuera de las colonias dentro de los sitios adyacentes a las áreas de anidación, observándose que no se vieron afectadas por los excrementos de las aves. Este capítulo se encuentra publicado en la revista *Water, Air, & Soil Pollution* [Espejo, W., Celis, J. E., Sandoval, M., González-Acuña, D., Barra, R., & Capulín, J. (2017). The Impact of Penguins on the Content of Trace Elements and Nutrients in Coastal Soils of North Western Chile and the Antarctic Peninsula Area. Water, Air, & Soil Pollution, 228: 116, doi:10.1007/s11270-017-3303-y. IF1.7 (2017)

El **quinto capitulo** corresponde a los resultados de los análisis de las concentraciones de Cd e isótopos estables de nitrógeno en 32 especies recolectadas en el verano austral de 2014, desde dos lugares de la Patagonia Occidental y dos lugares de la Península Antártica. El contenido de este capítulo se enviará para su publicación a la revista científica *Marine Pollution Bulletin*.

El **sexto capítulo** corresponde a los resultados de los análisis de las concentraciones de Ta e isótopos de nitrógeno, tomando como base 4 tramas tróficas: i) un lugar de la costa noroeste de Chile, ii) dos localidades de la Patagonia oeste de Chile, y iii) un lugar del área de la Península Antártica. Este capítulo se encuentra publicado en la revista *Enviromental Science & Tecnology Letters.* 2018 DOI: 10.1021/acs.estlett.8b00051. IF 5.3 (2017).

Esta tesis concluye con la **Discusión general** y las **Conclusiones finales** extraídas de los resultados obtenidos.

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Capitulo II. A global overview on exposure levels and biological effects of trace elements in penguins

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List of Acronyms

Al Aluminum

As Arsenic

Ca Calcium

Cd Cadmium

Co Cobalt

Cr Chromium

Cu Copper

Fe Iron

Hg Mercury

Mn Manganese

Ni Nickel

Pb Lead

Se Selenium

V Vanadium

Zn Zinc

Highlights

• Most of the studies on metals in penguins have been carried out in Antarctic and subantarctic islands. However, there is a lack of data from lower latitudes where other important penguin species inhabit.

• The levels of metals are reported mainly in feathers and excreta. Further research in other biological matrices such as internal organs and blood is required.

• Further research in the issue of biological effects caused by metals is needed.

• Little is known about the interaction between the metals which could activate certain mechanisms of detoxification of the body of the penguins.

1 Introduction

Trace metal toxicity is one of the major stressors leading to hazardous effects on biota (Zhang and Ma 2011). In aquatic environments, trace elements contamination is a great concern due to the implications these chemicals may have on both wildlife and human health (Lavoie et al. 2013; Prashanth et al. 2016). These elements enter the water through natural erosion, geochemical cycles, industrial processes and agricultural practices (Burger and Gochfeld 2000a). In birds, some metals can produce severe adverse effects such as difficulty in flying, walking and standing, paralysis and an increase in mortality (Newman 2015). In order to monitor the occurrence of environmental pollutants in marine ecosystems, the use of aquatic birds has greatly increased, because they can accumulate trace elements in diverse tissues, such as eggs, feathers or liver, thus be used to indirectly evaluate in a proper way the toxicological status of the marine ecosystem under study (Savinov et al. 2003). Moreover, seabird diet and feeding ecology can differ in response to climate change, thus affecting exposure to metals over time (Braune et al. 2014). Evidence indicates that the concentrations of certain pollutants in seabirds have a lower variation coefficient than that observed in fishes or marine mammals, so that the analysis of a relatively low number of samples of birds is similar to that obtained by analyzing a significantly higher number in other groups of animals (Pérez-López et al. 2005). Birds tend to be more sensitive to environmental contaminants than other vertebrates (Zhang and Ma 2011), thus ecotoxicological studies on seabirds have proliferated in recent years (Casini et al. 2001; Barbieri et al. 2010; Barbosa et al. 2013; Celis et al. 2014; Kehrig et al. 2015).

The study of trace elements in penguins is valuable, because they are animals that exclusively inhabit the Southern Hemisphere and represent about 90% of the bird biomass of the Southern Ocean (Williams 1990). Penguins are present in different systems in the Antarctic, subantarctic islands of the Pacific, Atlantic and

Indian oceans, as well as on the coasts of Australia, South Africa, South America and the Galapagos (García and Boersma 2013). Penguins are useful indicators of the degree of contamination by trace elements in the environment, because they are highly specialized animals that swim and dive in search for food, are widely distributed, and are organisms usually found at the top of the trophic web (De Moreno et al. 1997; Boersma 2008; Fig. 1). Additionally, penguins are extremely interesting as bioindicators because of their intense molting process (Carravieri et al. 2014), and because they can be finicky eaters with a restricted diet (Lescroël et al. 2004; Jerez et al. 2011).

The different penguin species (order Sphenisciformes, family Spheniscidae) can be classified in the genera *Aptenodytes*, *Eudyptes*, *Eudyptula*, *Megadyptes*, *Pygoscelis* and *Spheniscus*. These species have in common the fact of presenting serious risks of survival in the future, because about two thirds of penguin species are on the Red List of Threatened Species of the International Union for Conservation of Nature (UICN 2016). Contamination, climate change, fishing, alterations of ecosystems, diseases and even tourism are their major threats (García and Boersma 2013). The lack of knowledge about the effects of trace elements in seabirds is a main threat to their population sustainability (Sanchez-Hernandez 2000).

In the toxicology of trace elements ingested by penguins, the study of the biological effects of such elements is of great relevance, because it may contribute to the knowledge of possible consequences in nature (Nordberg and Nordberg 2016). Moreover, the evidence revealed that some trace elements such as As, Cd, Hg, Mn, Pb, and Zn can affect the endocrine system of animals and humans, producing alterations in physiological functions (Iavicoli et al. 2009). Given the wide distribution of penguins, data concerning trace element concentrations in different biotic matrices of penguins are summarized here to be used as a first background database for contamination detection in marine ecosystems.

2 Materials and methods

In order to identify the interaction of penguin species and trace elements, a systematic study of the existing literature on the concentrations of trace elements in different biotic matrices studied so far was conducted. These biological matrices correspond to guano, feathers, eggs, blood, stomach contents and internal organs. By using databases such as Direct, Springer, Scopus and Web of Science, different keywords were used. Among them, “trace element”, “heavy metal”, “trace metals”, “mercury”, “aluminum", "arsenic", "cadmium", "lead", "zinc", "copper", “pollution”, “persistent pollutants”, “monitoring”, “biomonitoring”, “penguin”, “seabirds”, “eggs”, “guano”, “droppings, “feathers”, “tissues”, “organs” and “Antarctic” can be mentioned. Furthermore, the list of references of each publication was reviewed to identify additional documents on the issue not previously found. Selection criteria strictly corresponded to trace element concentrations on the basis of dry weight (dw) based on studies performed *in situ*.

Subsequently this information was summarized in tables. Mean levels of exposure to trace elements in penguins were compared with other marine, aquatic or terrestrial birds from different parts of the world. In addition, maps of the distribution of penguins are included along with records of trace elements, based on the identification of the different colonies of penguins worldwide (Boersma 2008). Information about the investigations related to the exposure and effect of trace elements in penguins was also considered.

Mean metal concentrations were correlated between the livers and stomach contents, as well as renal metal concentrations. A bubble chart was built upon mean concentrations of each trace element reported in gentoo penguins (*Pygoscelis papua*) at South Shetland Islands to see similarities and differences according to biological matrices. First, concentration values were normalized by log (x+1) to remove any weighting from dominant peaks and then analyzed with a Bray-Curtis similarity matrix (Clarke et al. 2006). Finally, the resultant similarity matrix was analyzed in a two-dimensional multidimensional scaling (MDS) plot.

Values of the trophic transference coefficient (TTC), defined as the ratio between the concentration of a certain element in the body of an animal (internal organs) and the concentration of the element in the stomach contents (Suedel et al. 1994), were calculated for penguins taking into consideration the mean concentration of each element.

3 Exposure to trace elements and its effects on penguins

Trace element concentrations measured in different biotic matrices of different species of penguins are presented in Tables 1 to 9. The most commonly reported trace elements in penguins are Al, As, Cd, Cu, Hg, Mn, Pb and Zn. Gentoo penguin is the species that display the highest concentration of most trace elements studied with around 33%. Others trace elements such as Co, Cr, Fe, Ni, and Se have been poorly studied (Szopińska et al. 2016). Levels of Fe (23.37-164.26 µg/g) have been reported in feathers of pygoscelid penguins from Antarctica (Metcheva et al. 2006; Jerez et al. 2011). In the same matrix and region, levels of Se (1.8-2.0 µg/g) have been linked with a major exposure to Cd and Hg (Jerez et al. 2011), since Se is known to have a detoxifying effect of these metals (Smichowski et al. 2006). Cobalt levels have been reported in feathers of chinstrap and gentoo penguins from Antarctica (0.17-0.25 µg/g, Metcheva et al. 2006). Nickel has been reported in chinstrap penguins from Antarctica in feces (3.2-3.7 µg/g), liver (0.07 µg/g) and muscle (<0.03 µg/g) by Metcheva et al. (2006), and in feathers of pygoscelid penguins (0.24-1.18 µg/g, Jerez et al. 2011). In the organs of most avian wildlife species from unpolluted ecosystems, Ni concentrations may vary greatly (0.1-2.0 µg/g, Outridge and Scheuhammer 1993). Chromium levels (1.15-8.08 µg/g) were reported by Jerez et al. (2011) in feathers of pygoscelid penguins from Antarctica. Metals such as Mo, V or Y have not been reported in penguins.

Feathers constitute the most common biological matrix used *in situ* for determining trace elements in penguins (Table 1). Metals are delivered mainly by the blood supply, which is linked to the feeding habits of the bird (Metcheva et al.

2006). Some evidence shows that the concentration of Hg in feathers reflects levels in the blood during formation (Dauwe et al. 2005). Trace element burdens in feathers express past exposure and accumulation during the inter-moult period, thus they are more representative of long-term rather than acute exposure, at least for Hg (Furness et al. 1986). Reports in penguin feathers comprise ten species, most of which are from Antarctica and subantarctic islands (Table 1). Similarly, there is plenty of information of trace elements in penguin guano, particularly from Antarctica (Table 8), but there are few data on other species that live in lower latitudes, except for a study of Humboldt penguins (*Spheniscus humboldti*) from the coast of Chile (Celis et al. 2014).

There is very little information on trace elements in penguin eggshells (Table 2), bones (Table 3) and kidneys (Table 4). Concentrations of trace elements in blood, brain, testicles, embryo, spleen and heart of penguins have been poorly investigated (Table 9). Studies of trace elements in the liver of penguins (Table 5) correspond mostly to species that inhabit the Antarctica and subantarctic islands. Data of trace elements in muscles of penguins are scarce and they are exclusively focused on species that inhabit Antarctica (Table 6). Studies on metals in stomach contents of penguins are scarce and all of them have been carried out in Antarctica (Table 7).

Trace elements are chemicals that occur in natural and perturbed environments in small amounts (Prashanth et al. 2016). Their inadequate intake can damage the function of cells, causing physiological disorders and disease (Soria et al. 1995). These chemicals can be classified according to their biological significance, as non-essential and essential trace elements. Non-essential elements, such as Pb, Be, Cd, Hg, As, Sb and Ti, have no known function in the animal body, and their presence may produce toxicity. Essential elements (Cr, Co, Cr, Cu, Fe, Mo, Se, Zn and Mn) are required in small amounts because they perform vital functions for the maintenance of animal life, growth and reproduction (Nordberg and Nordberg 2016). Some trace elements such as Ni, Sn, V and Al cannot be yet classified as essential, as their role is not clear in animals, including humans (Prashanth et al. 2016). In general, the information available on concentrations of trace elements is fragmented in time and space, so it is not possible to build trends. Therefore, implementations of monitoring programs that incorporate these variables are required.

3.1 Non-essential trace elements

3.1.1 Aluminum

The maximum concentrations of Al have been found in stomach contents of gentoo penguins from King George Island, Antarctica (2,595 µg/g, Table 7) and in feathers of adult chinstrap penguins from Deception Island, Antarctica (203.1 µg/g, Table 1). On the contrary, the lowest concentration of Al (0.55 µg/g) has been reported in livers of Adélie penguins from Avian Island, Antarctica (Table 5). The high Al levels found in penguins from King George and Deception Islands could be linked to the abundance of this metal in bioavailable forms in the sediments of these areas (dos Santos et al. 2005; Deheyn et al. 2005).

Concentrations of Al in penguin feathers (0.71-203.13 µg/g) are highest in adult chinstrap penguins at Deception Island, whereas the lowest concentrations are in juvenile Adélie penguins at Avian Island (Table 1). This range is lower than the concentrations of Al (96-866 µg/g) in feathers of birds from Europe and North America (Rattner et al. 2008; Lucia et al. 2010). Highest concentrations of Al (866 µg/g) have been measured in feathers of osprey eagles (*Pandion haliaetus*) (Rattner et al. 2008).

Only one study reports the concentration of Al in penguin eggshells (28.96 µg/g, Table 2), which is higher than that found by Custer et al. (2007) in seagulls of North America (3.3 µg/g).

Concentrations of Al in penguin bones (4.16-69.95 µg/g) are the highest in gentoo penguins from King George Island, while the lowest concentrations are in chinstrap penguins from the same location (Table 3). Studies on levels of Al in bones of seabirds are scarce, and the few data in penguins are all from species from genera

Pygoscelis and *Aptenodytes*, being higher than those of birds from the Northern Hemisphere (1.37-6.9 µg/g, Dauwe et al. 2005).

In kidneys, Al concentrations (0.69-14.12 µg/g, Table 4) are highest in Adélie penguins from Avian Island and are lowest in chinstrap penguins from King George Island. In comparison, Al levels in kidneys of aquatic birds from the Southwest coast of France (6.1-8.9 µg/g, Lucia et al. 2010) are within the range reported in the penguin kidneys from Antarctica.

Concentrations of Al in the liver (0.55-15.52 µg/g, Table 5) are highest in chinstrap penguins from Deception Island (Table 5), whereas the lowest concentrations of Al correspond to Adélie penguins from Avian Island. There is little information about the levels of Al in livers of seabirds, although is possible to see that the concentrations of Al in penguins are within the range reported in the aquatic and terrestrial birds from Europe (0.18-37.3 µg/g) (Scheuhammer 1987; Dauwe et al. 2005).

In muscles, Al concentrations (1.07-114.9 µg/g, Table 6) are highest in chinstrap penguins from Deception Island, whereas the lowest concentrations are in the same species from King George Island. Despite the lack of data on seabirds from other regions, levels of Al in penguin muscles are higher than those found in a study carried out from Europe in muscles of the Great tit (0.08-1.46 µg/g, Dauwe et al. 2005).

Concentrations of Al in penguin stomachs (46.80-2,594.6 µg/g, Table 7) are highest in gentoo penguins from King George Island, whereas the lowest concentrations are in Adélie penguins from Avian Island. This range is higher than the concentrations of Al (0.22-23.5 µg/g) in stomach contents of wild birds from Europe (Dauwe et al. 2005).

There is only one measurement of Al in excreta (316 µg/g, Table 8), which is from gentoo penguins at Livingston Island, showing a deficit of information for this element. No Al was found in guano of other seabirds. Birds are most likely exposed to Al through their diets, and most Al is excreted via the feces and only a fraction is retained (Sparling and Lowe 1996).

In blood, the only existing study corresponds to the little penguin, and Al concentrations (3.19-4.22 µg/g, Table 9) present less variability and are within the range reported in the seagulls of the Northern Hemisphere (1.34-4.11 µg/g, Kim et al. 2013).

The main toxic effects of Al that have been reported in animals are produced in the central nervous system, though long lasting exposures can also affect the skeletal system, decreasing its rate of formation and increasing the risk of fractures. The functioning of the renal, endocrine, reproductive and cardiac systems is also affected by a chronic exposure to this metal (Sjögren et al. 2007). In birds, Al is poorly absorbed and its potential for toxicity is low, thus Al levels in soft tissue do not necessarily reflect toxicity to the individual (Scheuhammer 1987). Nevertheless, there is some evidence indicating that Al found in the bone marrow tissue of humeri of wild pied flycatchers (*Ficedula hypoleuca*) can produce small clutches, defective eggshell formation, and intrauterine bleeding, similar to the symptoms of Al intoxication in mammals (Nyholm 1981). Al interferes with the deposition of Ca, resulting in weak bones and eggs, besides affecting the reproductive capacity (Nayak 2002). No studies have been performed in penguins to see any possible effects produced by this metal.

3.1.2 Arsenic

The maximum As concentrations in tissues and organs are in the liver of Adélie penguins (*Pygoscelis adeliae*) from King George Island (1.2 µg/g, Table 5) and kidneys of the same species and location (1.07 µg/g, Table 4). In contrast, the lowest concentration of As (0.01 µg/g) has been reported in feathers of adult chinstrap penguins (*Pygoscelis antarctica*) from Livingston Island, Antarctica (Table 1). Arsenic tends to accumulate in almost all organs, mainly in the liver where biomethylation of As takes place producing some kind of acids, such as monomethylarsonic and dimemethylarsonic (Khan et al. 2014).

Concentrations of As in feathers (0.01-0.88 µg/g, Table 1) are highest in adult gentoo penguins that inhabit Livingston Island, and are lowest in adult chinstrap penguins from Livingston Island. Arsenic concentrations in feathers of black-legged kittiwakes (*Rissa tridactyla*) and black oystercatchers (*Haematopus bachmani*) from Alaska (0.17-0.34 µg/g, Burger et al. 2008) and in feathers of black-tailed gull chicks (*Larus crassirostris*) from Korea (0.15-0.44 µg/g, Kim et al. 2013) are within the range found in the penguins. There is no information on the levels of As in penguin eggshells (Table 2).

In bones, concentrations of As are highest in gentoo penguins that inhabit Byers Peninsula (Table 3), while the lowest concentrations are in chinstrap penguins from Deception Island. Concentrations of As in penguin bones (0.04-0.19 µg/g) are within the range reported in the aquatic and terrestrial birds of the Northern Hemisphere (< 0.0001-1.60 µg/g, Lebedeva 1997).

In kidneys, As concentrations (0.38-1.07 µg/g, Table 4) are highest in Adélie penguins from King George Island and lowest in the same species from Avian island. Arsenic levels in penguin kidneys are within the range found in the kidneys of passerine birds from the Northern Hemisphere (0.071-1.81 µg/g, Sánchez-Virosta et al. 2015).

In the liver, the content of As (0.30-1.20 µg/g) is highest in adult Adélie penguins from King George Island, and the lowest in juvenile same species, location and sampling date (Table 5). The highest levels of As in adult penguins is due to this element probably tends to be accumulated into the animal body, which is directly related to the age of the individuals (Khan et al. 2014). The concentrations of As in penguin livers are lower than those found in seabirds of the Northern Hemisphere (0.22-5.62 µg/g) (Lucia et al. 2010; Ribeiro et al. 2009; Skoric et al. 2012).

In muscles, As concentrations (0.18-1.04 µg/g, Table 6) are highest in chinstrap penguins from Deception Island, and lowest in Adélie penguins from King George Island. The concentrations of As in penguin muscles are higher than those reported in wild birds from the Northern Hemisphere (0.01-0.35 µg/g, Gasparik et al. 2010).

In the stomach, As concentrations are highest in Adélie penguins from Avian Island, and are lowest in the same species from King George Island (Table 7). Arsenic concentrations in penguin stomach content (0.47-3.22 µg/g) are higher than those levels found in wild birds from Europe (0.006-0.76 µg/g, Dauwe et al. 2005).

In excreta, As concentrations are highest in Humboldt penguins from Cachagua Island (Chile), and lowest in gentoo penguins from Base O'Higgins, Antarctic Peninsula (Table 8). In general, levels of As in penguin guano (0.15-7.86 µg/g) are lower than those levels found in guano of wild birds from the Northern Hemisphere (0.42-16.03 µg/g, Dauwe et al. 2000; Kler et al. 2014).

In blood, the highest As concentration is in the little penguin (*Eudyptula minor*) from Australia (Table 9). Levels of As in penguin blood (0.67-3.72 µg/g) are higher than those levels observed in black-tailed gull chicks of the Northern Hemisphere (0.26-0.48 µg/g, Kim et al. 2013).

Generally, in birds As is initially accumulated in liver and kidneys and subsequently it is redistributed to feathers and claws (Sánchez-Virosta et al. 2015). Against exposure to As, organisms have biotransformation mechanisms to decrease its toxicity, in which inactive As metabolites are formed (monomethylarsenic and dimethylarsenic), which are more easily removed by the kidneys (Soria et al. 1995; ATSDR 2007). In ducklings, clinico-pathological effects caused by sodium arsenate at 30-300 µg/g can produce liver congestion, necrosis and fibrosis, severe degeneration of brain, and increase mortality (Khan et al. 2014).

In general, the levels of As reported in feathers, blood and organs of penguins are below 3 µg/g, the limit considered normal in living organisms (Jerez et al. 2013a), except the concentrations of As in blood of little penguins that inhabit St Kilda, on the coast of Australia (3.72 µg/g, Table 6). All the studies performed in penguins reveal that the concentrations of As are below 50 µg/g as to produce toxic effects and that can lead to endocrine disorders (Neff 1997).

3.1.3 Cadmium

This metal is known to bioaccumulate in marine biota from both natural and anthropogenic sources (Espejo et al. 2014). The maximum concentrations of Cd (351.8 µg/g) have been found in kidneys of Adélie penguins from Avian Island, Antarctic Peninsula (Table 4). On the contrary, the lowest concentration of Cd (< 0.001 µg/g) has been reported in muscles of Adélie penguins from Potter Cove, Antarctica (Table 6). Generally, birds accumulate Cd in their bodies through the food chain, and Cd is first accumulated in the liver and then transported to several organs (Lee 1996). Cadmium concentrations in penguins tended to be higher in kidneys than in the liver, as also noted in different species of Anseriformes (Jin et al. 2012).

In feathers, the maximum Cd concentrations have been found in adult gentoo penguins from Livingston Island, and the minimum in juvenile Adélie penguins from King George Island (0.01-0.50 µg/g, Table 1). In general, Cd concentrations in penguin feathers are lower than those found in seabirds of the Northern Hemisphere (0.04-1.28 µg/g) (Kim et al. 1998; Agusa et al. 2005; Mansouri et al. 2012). In eggshells, there is no information on the levels of Cd in penguins (Table 2).

In bones, Cd concentrations (<0.001-0.17 µg/g, Table 3) are maximum in Adélie penguins from Avian Island, and minimum in chinstrap penguins from King George Island. The concentrations of Cd in penguin bones are lower than those reported in bones of seabirds of the Northern Hemisphere (0.03-0.33 µg/g, Kim et al. 1998).

In kidneys, Cd concentrations (0.2-351.8 µg/g, Table 4) are highest in Adélie penguins from Avian Island, and are lowest in gentoo and Adélie penguins, both from King George Island. Cadmium levels in kidneys of gulls (0.90-44.4 µg/g) of south-western Poland and the Artic (Orłowski et al. 2007; Malinga et al. 2010) are within the range reported in penguin kidneys. A study found that Cd levels in kidneys of scoters (*Melanitta perspicillata*) from the Queen Charlotte Islands in Canada were as high as 390.2 µg/g, a concentration potentially associated with renal damage (Barjaktarovic et al. 2002).

In the liver, Cd concentrations (0.06-27.7 μg/g, Table 5) are highest in Emperor penguins from the Weddell Sea, and are lowest in Adélie penguins from King George Island. Cadmium concentrations in penguin livers reveal that 9 of 19 reports (47.4%) exceeded the threshold levels of toxicity for wild birds (3μg/g, Scheuhammer 1987). The Cd levels in penguin livers are comparable with those levels (0.05-15.1 μg/g) found in seabirds of the Northern Hemisphere (Elliot et al. 1992; Kim and Koo 2007; Pérez-López et al. 2005).

In muscles, Cd levels of seabirds (0.26-0.52 μg/g) from the Northern Hemisphere (Orłowski et al. 2007; Malinga et al. 2010) are within the range reported in penguins (<0.001-2.63 μg/g, Table 6). The highest Cd levels are in Adélie penguins from Avian Island, and are lowest in the same species from Potter Cove.

In the stomach, Cd concentrations (0.09-2.9 μg/g, Table 7) are highest in Adélie penguins from Edmonson Point, and are lowest in gentoo penguins from King George Island. The levels of Cd in penguin stomach contents are far below those levels of Cd detected in the stomach contents of seabirds from industrialized areas of Korea (96-217 μg/g) (Kim and Oh 2014b; Kim and Oh 2014c).

In excreta, Cd levels are linked to high dietary Cd intake (Ancora et al. 2002). Cd concentrations (0.16-47.7 μg/g, Table 8) are highest in Humboldt penguins from Pan de Azúcar Island (Chile), and are lowest in Adélie penguins from the Antarctic Peninsula. Levels of Cd in penguin excreta are higher than those observed in wild bird species (0.12-1.88 μg/g) of the Northern Hemisphere (Kaur and Dhanju 2013; Kler et al. 2014).

In birds, the accumulation of Cd can have adverse effects on health, such as renal and testicular damage, disorder in the balance of Ca and the skeletal integrity, reduced feed intake and growth rate, decreased egg laying, thinning eggshells or alteration in the behavior of the bird, among other effects (Burger 2008; Furness 1996; Larison et al. 2000; Rodrigue et al. 2007). However, seabirds seem to be less vulnerable to the exposure to high levels of Cd than other wild organisms and birds of terrestrial environments (Burger 2008; Furness 1996). Highest Cd concentrations in tissues of marine birds were in kidney tissue of oceanic birds (Elliot et al. 1992; Pérez-López et al. 2005; Orłowski et al. 2007; Kim and Koo 2007; Malinga et al. 2010). In *Pygoscelis* penguins from the South Shetland Islands, a ratio kidney/liver for Cd concentrations of about 4 means a higher Cd affinity for renal tissue (Jerez et al. 2013b), thus indicating a chronic or sub-chronic exposure to Cd due to maternal transfer of this metal during egg development, as occurs in other seabirds (Agusa et al. 2005). A high exposure to Cd causes significant accumulation of this metal in the soft tissues, because a small proportion is excreted, and release of Cd from kidney is very slow (Eisler 1985). Thus, under conditions of chronic dietary exposure, kidney concentrations of Cd may express long-term accumulation (Scheuhammer 1987).

Toxic effects of Cd appear in humans and other mammals when kidney Cd levels reach about 100 µg/g ww (Scheuhammer 1987) or about 400 µg/g dw (assuming a moisture content of 75% in the sample). Seabirds accumulate a large amount of metals such as Hg in their liver because they usually occupy the highest trophic positions in the marine food web and have a long life span (Thompson 1990). However, birds are relatively resistant to some metals, like Cu (Eisler 1998). The process of the metal detoxification in liver of seabirds is well described by Ikemoto et al. (2004). In penguins, some metals interact with others to activate certain phase I detoxification mechanisms in the organism. A study carried out by Kehrig et al. (2015) evidenced a correlation between Se and metallothioneins in liver samples of Magellanic penguins (*Spheniscus magellanicus*), indicating that Se would be involved in detoxification of Cd, Pb and Hg. Another study showed a positive correlation between Se and Cd in tissues of chinstrap, gentoo and Adélie penguins, which would be related to the detoxifying function played by Se against the toxicity of Cd (Jerez et al. 2011). In this sense, Jerez et al. (2013a) stated that high levels of Se (30.6 µg/g) and Zn (126.05 µg/g) can protect chinstrap penguins of Deception Island at least partially against toxic effects of high levels of Cd (27.54 µg/g). However, the accumulation of Cd and Se, and likely other heavy metals, can cause teratogenic effects in a wide range of birds and animal species (Hoffman 2002; Gilani and Alibhai 1990; Ohlendorf et al. 1988; Franson et al. 2007), and even micromelia in penguins (Raidal et al. 2006). High Se levels of over 10 µg/g in liver of aquatic birds can produce hepatic toxicity (Lemley 1993). A study found that 47% of the samples of livers of penguins from Antarctic Peninsula had Se levels above the mentioned toxicity threshold (Jerez et al. 2013a). However, when evaluating Se toxicity and oxidative stress, nutritional factors should be taken into consideration (Franson et al. 2007).

Studies carried out in colonies of some penguins from Antarctica have shown that kidney samples collected at Weddell sea and Avian Island present high concentrations of Cd (270.2 and 351.8 µg/g, Table 4), implying that those seabirds probably presented a chronic exposure to this metal, with levels above the toxicity threshold established for birds (Furness 1996).

3.1.4 Mercury

The maximum concentrations of Hg (8.16 µg/g) have been found in feathers of adult gentoo penguins from Crozet Islands (Table 1). In contrast, the lowest concentration of Hg (0.02 µg/g) has been reported in bones of Adélie penguins from East Antarctica (Table 3). As with most seabirds, penguin feathers constitute an important way of detoxification of Hg (Yin et al. 2008).

In feathers, Hg concentrations (0.0014-8.16 µg/g, Table 1) are highest in adult gentoo penguins from Crozet Islands. The lowest Hg levels have been reported in juvenile Magellanic penguins from the coasts of Argentina (Frias et al. 2012, Table 1). Mercury concentrations in penguin feathers are lower than those found in different species of seagulls and terns from Northern Hemisphere (0.31-20.2 µg/g) (Goutner et al. 2000; Zamani-Ahmadmahmoodi et al. 2014) and in feathers of birds from various locations of the Chilean coast (0.11-13 µg/g, Ochoa-Acuña et al. 2002). Of the thirty-two reports in penguin feathers, only two studies are in the range of Hg levels (5 to 40 µg/g) linked to reduced hatch of egg laid in various bird species (Eisler 1987). Concentrations of Hg of 9-20 µg/g in feathers can decrease reproductive success in some piscivorous birds (Fimreite 1974; Scheuhammer 1987; Beyer et al. 1997; Evers et al. 2008). The range of Hg concentrations reported in penguin feathers are below those known to cause adverse health and reproductive effects in birds.

In eggshells, Hg concentrations (0.005-0.26 µg/g, Table 2) are highest in Adélie penguins from Terra Nova Bay (Bargagli et al. 1998) and are lowest in the same species in Almiranty Bay. Mercury levels in penguin eggshells are lower than those reported in marine, aquatic and terrestrial birds of other latitudes (0.05-36.37 µg/g) (Yin et al. 2008; Daso et al. 2015).

In bones, Hg concentrations (0.02-0.07 µg/g, Table 3) are highest in gentoo penguins from Fildes Peninsula, and are lowest in Adélie penguins that inhabit the surroundings of the Zhongshan Station (Yin et al. 2008). In general, data of Hg in bones of birds are not abundant, because this metal is not precisely stored in this biotic matrix, making comparisons difficult. In any case, levels of Hg in penguin bones are 50% lower than those detected in bones of seagulls from the coasts of Japan (Agusa et al. 2005) and lower than those in great cormorants (*Phalacrocorax carbo*) from Europe (1.4-1.72 µg/g, Skoric et al. 2012).

In kidneys, Hg concentrations (0.15- 2.47 µg/g, Table 4) are highest in Magellanic penguins from the coast of Southern Brazil (Kehrig et al. 2015). The lowest levels are reported in Adélie penguins that inhabit King George Island (Smichowski et al. 2006). Mercury concentrations in penguin kidneys are lower than those detected in kidneys of seabirds from the Northern Hemisphere (0.3-5 µg/g) (Arcos et al. 2002; Zamani-Ahmadmahmoodi et al. 2014).

In livers, Hg concentrations (0.16-5.7 µg/g, Table 5) are highest in Magellanic penguins from the coasts of Southern Brazil (Kehrig et al. 2015). The lowest concentrations of Hg have been reported in Adélie penguins from Terra Nova Bay (Bargagli 2008). Mercury concentrations in penguin livers are below those reported in seabirds of the Northern Hemisphere (4.9-306 µg/g, Kim et al. 1996). In birds, sublethal effects of Hg on growth, development, reproduction, blood and tissue chemistry, metabolism, behaviour, histopathology and bioaccumulation have been found between 4 and 40 mg/kg (dietary intake) (Eisler 1987). The concentrations of

Hg in liver of Magellanic penguins from Rio Grande du Sul, Brazil (5.7 µ/g, Table 5) are higher than the threshold of toxicity for Hg (Kehrig et al. 2015).

In muscles, Hg is reported by a single study in Adélie penguins (0.6 µg/g, Table 6) from Terra Nova Bay (Bargagli et al. 1998). Levels of Hg in penguin muscles are lower than those reported in terns and gulls from Asia (0.9-2.5 µg/g, Zamani-Ahmadmahmoodi et al. 2014).

In stomachs, Hg is only reported in Adélie penguins (0.08 µg/g) from Terra Nova Bay (Bargagli et al. 1998) and from Edmonson Point (0.10 µg/g, Ancora et al. 2002) (Table 7). It is difficult to find reports of Hg levels in bird stomachs. Levels of Hg detected in penguin stomach are lower than those measured in intestines of cormorants from Europe (1.29-2.49 µg/g, Skoric et al. 2012).

In excreta, Hg concentrations (0.10-6.60 µg/g, Table 8) are highest in gentoo penguins from Base O'Higgins, and are lowest in Adélie penguins from Yalour Island, both locations of the Antarctic Peninsula. Levels of Hg in penguin excreta are higher than those in other marine birds worldwide (0.10-0.75 µg/g, Yin et al. 2008).

Mercury concentrations in penguin blood (0.84-2.75 µg/g) and in penguin brains (0.43 µg/g) are been measured in little penguins from Australia and Adélie penguins from Terra Nova Bay (Antarctica), respectively (Table 9). Those levels are higher than those found in the blood of black-tailed gull chicks and Great tits from the Northern Hemisphere (0.03-0.26 µg/g) (Dauwe et al. 2000; Kim et al. 2013). Mercury concentrations of over 3 µg/g in blood can affect endocrine systems of Arctic birds with negative consequences for reproduction (Tartu et al. 2013). In loons (*Gavia immer*), Evers et al. (2008) reported adverse effect threshold for adult birds at 3 µg/g (w.w) in blood and reproductive failure when adult blood Hg levels reach 12 µg/g /(w.w). Tartu et al. (2016) found that Hg levels (1.0-1.5 g/g) in blood of adult kittiwakes can disrupt prolactine secretion (a pituitary hormone involved in parental care) which could lead to reduced chick survival.

Chronic exposure to metals may imply a threat to penguins. Some evidence shows that the survival and breeding success decreased with increasing Hg levels in blood of Artic seabird (2.28±0.42 μg/g, Goutte et al. 2015). Mercury in its organic form (methylmercury, ethylmercury) is more lipophilic, which favors its accumulation mainly in the liver, kidneys, brain and feathers. Inorganic Hg is mostly accumulated in kidneys, due to its affinity to metallothioneins presented by renal cells (Byrns and Penning 2011). In seabirds, habitat type and functional feeding group may influence organic Hg bioaccumulation rates at higher trophic levels (Chen et al. 2008). The direct effects of elevated organic Hg on marine biota can include changes in brain neurochemical receptor density (Scheuhammer et al. 2008). In pinnipeds, adverse effects may manifest as immunosuppression (Lalancette et al. 2003). There are few studies on the effects of metals in feathers and blood of birds, but evidence exits indicating that concentrations of Hg of 5 μg/g in feathers of birds can cause reproductive impairment (Burger and Gochfeld 1997), including smaller egg size, lower hatching rate, decreased chick survival and even impaired territorial fidelity in waterfowls (Rothschild and Duffy 2005). The few studies reveal that the concentrations of Hg in biotic matrices of penguins from Antarctica are below the above mentioned threshold of toxicity for Hg. In general, Hg levels are lower in most of biological matrices of penguins than birds from the Northern Hemisphere.

3.1.5 Lead

Excepting excreta, the maximum concentrations of Pb (1.90 μg/g) have been found in feathers of adult gentoo penguins from Livingston Island (Table 1) and in bones of Adélie penguins from East Antarctica (1.60 μg/g, Table 3). In contrast, the lowest concentration of Pb (< 0.001 μg/g) has been reported in muscles of gentoo penguins from King George Island (Table 6). Lead is not metabolically regulated (Gochfeld et al. 1996), and unlike Cd, tends to be accumulated in bird feathers (Jerez et al. 2011).

In feathers, Pb concentrations (0.0008-1.9 μg/g, Table 1) are highest in adult gentoo penguins from Livingston Island. On the other hand, Pb levels are lowest in juvenile Adélie penguins from King George Island. The highest concentration of Pb in penguin feathers is directly related to major human activity (Jerez et al. 2011, Jerez et al. 2013a). Levels of Pb in penguin feathers are lower than those concentrations found in feathers (0.34-7.15 μg/g) of different seabirds of the Northern Hemisphere (Kim et al. 1998; Burger et al. 2008; Ribeiro et al. 2009; Skoric et al. 2012; Kim et al. 2014b). Lead concentrations of 4 μg/g (dw) in feathers are known to be a threshold level for toxicity (Burger and Gochfeld 2000b).

In eggshells, the highest Pb concentrations (0.75 μg/g, Table 2) have been found in gentoo penguins from Fildes Peninsula (Yin et al. 2008). Levels of Pb (0.68-0.75 μg/g) in eggshells of penguins are lower than those reported in seabirds (1.25-3.10 μg/g) of the Northern Hemisphere (Yin et al. 2008; Kim and Oh 2014a).

In bones, Pb concentrations (<0.001-1.60 μg/g, Table 3) are highest in Adélie penguins from Zhongshan Station (Yin et al. 2008) and are lowest in *Pygoscelis* penguins from King George Island and Byers Peninsula (Barbosa et al. 2013; Jerez et al. 2013a). The concentrations of Pb in bones of penguins are lower than those reported in bones of marine, aquatic and terrestrial bones (0.04-42.32 μg/g) of the Northern Hemisphere (Lebedeva 1997; Kim et al. 1998; Orłowski et al. 2007; Yin et al. 2008). Lead is known to be toxic metal, and the skeleton is the main depot for these elements (Lebedeva 1997). Lead levels > 10 μg/g in bone of wild birds are considered to be toxic, and so may be interpreted as a result of relatively polluted habitats (Scheuhammer 1987). Bone Pb concentrations higher than 20 μg/g are considered as excessive exposure for waterfowls (Franson 1996). Levels in penguin bones are far below those threshold values, which suggest that the biological effect should be neglected.

In kidneys, Pb concentrations (<0.001-0.55 μg/g, Table 4) are highest in Magellanic penguins from the coast of Southern Brazil (Kehrig et al. 2015), and are lowest in gentoo penguins from King George Island (Jerez et al. 2013b).

Concentrations of Pb in penguin kidneys are lower than those of seabirds from the Northern Hemisphere (0.14-11.18 µg/g) (Kim et al. 1998; Orłowski et al. 2007). Lead concentrations > 68 µg/g in kidneys of snowy owls (*Nyctea scandiaca*) is linked to bird´s mortality (Franson 1996).

In the liver, Pb levels varies from <0.001 to 0.58 µg/g (Table 5) with the highest concentrations in Magellanic penguins from the coasts of Southern Brazil (Kehrig et al. 2015). The lowest levels are reported in gentoo penguins from King George Island (Jerez et al. 2013b). Concentrations of Pb in penguin livers are lower than values (0.50-3.71 µg/g) found in seabirds of Asia (Kim et al. 1998; Kim and Koo 2007; Kim and Oh 2014c). A study conducted in South Korea (Kim and Oh 2014c) found that high levels of Pb in liver (6.2 µg/g) could negatively affect both behavior and growth of chicks of the black-tailed gull. Concentrations of Pb in livers of penguins are far below this threshold value. Hepatic Pb concentrations of over 30 µg/g in waterfowls can produce Pb poisoning, which is characterized by impaction of the upper alimentary tract, submandibular edema, myocardial necrosis and biliary discoloration of the liver (Beyer et al. 1998).

In muscles, Pb concentrations (<0.001-0.60 µg/g, Table 6) are highest in gentoo penguins from Livingston Island (Metcheva et al. 2010), and are lowest in the same species of King George Island (Jerez et al. 2013b). The levels of Pb in penguin muscles are lower than those reported in seabirds (0.014-3.59 µg/g) of the Northern Hemisphere (Kim et al. 1998; Orłowski et al. 2007).

In the stomach, Pb concentrations (0.03- 0.71 µg/g, Table 7) are highest in gentoo penguins from King George Island, and are lowest in chinstrap penguins from King George Island. The levels of Pb in stomach contents of penguins are lower than those levels of Pb (0.059-105.0 µg/g) detected in stomach contents of seabirds from the Northern Hemisphere (Kim et al. 1998; Kim and Oh 2014b; Kim and Oh 2014c).

In excreta, Pb concentrations (0.08-12.79 µg/g, Table 8) are highest in Humboldt penguins from Cachagua Island (Celis et al. 2014), while the lowest levels were reported in gentoo penguins from Neko Harbor, Antarctic Peninsula (Celis et al.

2015b). In general, levels of Pb in penguin guano are lower than the concentrations of Pb (3.90-124.8 μg/g) in guano of aquatic and terrestrial birds from the Northern Hemisphere (Dauwe et al. 2000; Martinez-Haro et al. 2010; Kler et al. 2014).

In blood, Pb concentrations (0.04-0.07 μg/g, Table 9) have been measured only in little penguins from the coast of Australia. Those Pb levels are below the deleterious effect level of 4 μg/g (Finger et al. 2015), and are also lower than those reported in gulls from the Northern Hemisphere (0.06-0.18 μg/g, Kim et al. 2013). Some biological functions of birds can be altered when Pb levels in blood > 3 μg/g, and Pb levels > 6 μg/g can produce uremic poisoning (Franson 1996).

In birds, it has been observed that the exposure to Pb in young individuals of the herring gull (*Larus argenteus*) and the common tern (*Sterna hirundo*) affects the behavioral development, growth, locomotion, balance, search for food, thermoregulation and recognition between individuals (Burger and Gochfeld 2000a). Pb is transported through blood bonded to hemoglobin, reaching the liver, kidneys, bone marrow and central nervous system. Nevertheless, Pb can be stored in tissues rich in Ca such as hairs, feathers and bones, where it can remain for many years (O'Flaherty 1998). Lead in penguin bones is accumulated throughout the lifetime of the individual, and so its presence in bones may be considered an indicator of long-term exposure (Barbosa et al. 2013). A study for *Pygoscelis* penguins from Antarctica found that Cd, Ni, Pb, and Se levels in muscles are long-term dependent (Jerez et al. 2013a). High concentrations of Cu can increase the toxic effects caused by Pb (Eisler 1988).

Feces can be used to detect adverse toxicological effects in wildlife by means of porphyrins, which can be correlated with metals measured in the same sample (Mateo et al. 2016). A study showed a strong affinity between the levels of Pb with porphyrins in excreta of gentoo penguins (Celis et al. 2012), which may be associated to hepatic and renal damage (Casini et al. 2003). Available data indicate that concentrations of Pb in guano of penguins in the Antarctica have increased in the last 200 years as a result of greater local anthropogenic activity (Sun and Xie

2001). Studies that are able to show the possible biological effects of Pb on these populations of polar seabirds are needed.

Negative correlations between Pb-Cu and Pb-Fe have been found in livers of *Pygoscelis* penguins (Jerez et al. 2013a), indicating the capability of Pb (a metal directly linked to various anthropogenic activities) to use the transport mechanisms of the essential cations, preventing them from performing their metabolic function (Ballatori 2002). Penguin species from higher latitudes could be more vulnerable to the effects of trace elements due to their less effective immunological systems in such environments in comparison to other species of penguins that live in lower latitudes (Boersma 2008; Cooper et al. 2009).

There are few studies on the exposure to heavy metals in penguins and it is necessary to progress in the use of non-destructive biomarkers and non-invasive matrices (i.e. feathers or fecal material) or semi-invasive such as blood tissue. Porphyrins have proved to be useful biomarkers of exposure to contaminants (Casini et al. 2003), because they are capable of bonding to metals and they can be detected in different biological matrices (De Matteis and Lim 1994). Some trace metals can interfere with the biosynthesis of hemoglobin and cause alterations in the porphyrins, which are accumulated or excreted (Casini et al. 2001). Byproducts such as copro- uro- and protoporphyrins are not toxic in normal concentrations, but when there is an excess they can affect the liver and bone marrow (Lim 1991). A study showed a positive correlation between the levels of porphyrins and those of Hg and Pb in guano of gentoo penguins (Celis et al. 2012). Another study carried out in Humboldt penguins found that the levels of porphyrins were directly correlated with the concentrations of As, Pb and Cu, thus there exist a high probability that these penguins might develop hepatic and renal damage because of the exposure to these metals (Celis et al. 2014). The higher concentrations of metals in penguin excreta suggest a physiological mechanism of detoxification (Ancora et al. 2002), although this also imply that those trace elements are not absorbed at the intestinal level. It has been observed that when bird present renal damage caused by Cd, the levels of this metal in excreta are increased (Goyer 1997). Lead concentrations in all of the biotic matrices of penguins studied are lower than those Pb levels found in marine, aquatic and terrestrial birds of the Northern Hemisphere, which is highly industrialized and where human population is concentrated.

3.2 Essential trace elements

3.2.1 Copper

In general, there are not enough data available on the toxicity of copper to avian wildlife. Birds, when compared to lower forms, are relatively resistant to Cu (Eisler 1998). With the exception of excreta, the maximum concentrations of Cu have been found in the liver of gentoo penguins from King George Island (386.1 µg/g, Table 5). In contrast, the lowest concentration of Cu (0.06 µg/g) has been reported in bones of Adélie penguins from the same location (Table 3). There is evidence showing that Cu levels of 1,050 µg/g in the livers of eiders can cause liver necrosis and fibrosis (Norheim and Borch-Iohnsen 1990). In pygoscelid penguins, Cu levels over 24 µg/g in the liver (Szefer et al. 1993) could represent an additional stress to birds already facing stressful conditions, such as starvation (Debacker et al. 2000).

In feathers, Cu concentrations (6.87-21.5 µg/g, Table 1) are highest in adult gentoo penguins from Fildes Peninsula (Yin et al. 2008), whereas are lowest in juvenile of the same species from King George Island (Jerez et al. 2013b). Levels of Cu in penguin feathers are higher than those concentrations found in feathers of different seabirds (7.56-11.2 µg/g) of the Northern Hemisphere (Kim et al. 1998; Malinga et al. 2010).

In eggshells, Cu concentrations (1.24-10 µg/g, Table 2) are highest in chinstrap penguins from Fildes Peninsula, and are lowest in gentoo penguins from Livingston Island. Copper concentrations in penguin eggshells are comparable to those Cu levels reported in eggshells of birds from other latitudes (0.42-7.54 µg/g) (Dauwe et al. 2000; Yin et al. 2008; Kim and Oh 2014a).

In bones, Cu concentrations (0.06-57.81 µg/g, Table 3) are highest and lowest in colonies of Adélie penguins from King George Island. Concentrations of Cu in penguin bones are comparable to those Cu levels found in bones of marine, aquatic and terrestrial birds of the Northern Hemisphere (0.37-60 µg/g) (Lebedeva 1997; Kim et al. 1998; Orłowski et al. 2007; Yin et al. 2008).

In kidneys, Cu has been reported between 1.6 and 19.1 µg/g (Table 4), with the highest concentrations in Emperor penguins from Weddell Sea, whereas the lowest levels correspond to Adélie penguin from Potter Cove (King George Island). Levels of Cu in penguin kidneys are lower than those found in kidneys of Artic seabirds (12.2-27.8 µg/g) (Kim et al. 1998; Malinga et al. 2010).

In livers, Cu concentrations (10.91-386.1 µg/g, Table 5) are highest in colonies of gentoo penguins from King George Island, and are lowest in Adélie penguin from the same location. The levels of Cu in livers of Antarctic penguins are higher than those detected in other seabirds of Asia and Europe (0.26-92.5 µg/g) (Kim and Koo 2007; Pérez-López 2005; Ribeiro et al. 2009; Malinga et al. 2010). A study found that mute swans (*Cygnus olor*) from estuaries in Britain had more than 2,000 µg/g of Cu in their blackened livers (Bryan and Langston 1992).

In muscles, Cu concentrations (4.43-9.70 µg/g, Table 6) are highest in colonies of chinstrap penguins that inhabit the Antarctic Peninsula (Szefer et al. 1993), whereas the lowest are in Adélie penguin from King George Island (Jerez et al. 2013b). Levels of Cu in penguin muscles are within the range reported in the muscles of seabirds from Northern Hemisphere (3.59-18.3 µg/g) (Kim et al. 1998; Malinga et al. 2010; Orłowski et al. 2007).

In stomachs, Cu levels (4.85-66.42 µg/g, Table 7) presented the highest value in Adélie penguins from Avian Island, and the lowest levels in the same species from King George Island. The levels of Cu in penguin stomach contents are higher than those detected in seabirds of the Northern Hemisphere (4.89–14.0 µg/g) (Kim et al. 1998; Kim and Oh 2014b).

In excreta, Cu concentrations (37.6-585.8 µg/g) are highest in colonies of Adélie penguins from Kopaitic Island, and are lowest in chinstrap penguin from the Antarctic Peninsula. Levels of Cu in penguin guano are higher than those values (10-150.8 µg/g) found in excrement birds from other parts of the world (Dauwe et al. 2000; Yin et al. 2008; Kler et al. 2014). A study in excreta of Humboldt penguins found that the levels of porphyrins were directly correlated with the concentrations of As, Pb and Cu (Celis et al. 2014), and those birds might present some hepatic and renal disorder (Casini et al. 2003).

In blood, Cu concentrations (2.14-2.48 µg/g, Table 9) are only reported in little penguins from Australia. Copper concentrations in penguin blood are within the range reported in the seagulls, eiders and ducks of the Northern Hemisphere (0.64-2.56 µg/g) (Franson et al. 2003; Kim et al. 2013).

In general, marine birds retain a very small portion of Cu and other metals ingested (Bryan and Langston 1992). Although Cu is an essential metal, in excess it can produce a series of metabolic, pulmonary, hepatic and renal toxic effects (Soria et al. 1995). Copper can increase the toxic effects caused by Pb in birds, fishes and invertebrates (Eisler 1988). In birds, Cu is accumulated in the liver and bone marrow, being associated to metallothionein and thus preventing an excess of free ions of this element (Eisler 1998). However, this protective mechanism is limited and lesions can be produced in the liver (ATSDR 2004).

3.2.2 Manganese

Excepting excreta and stomach contents, the maximum concentrations of Mn have been found in bones (18.35 µg/g, Table 3) and the liver (15.83 µg/g, Table 5) of gentoo penguins from Byers Peninsula and Adélie penguins from King George Island, respectively. In contrast, the lowest concentration of Mn (<0.01 µg/g) has been reported in feathers of juvenile Adélie penguins from Avian Island, Antarctica (Table 1).

In feathers, Mn concentrations range <0.01 to 3.26 µg/g (Table 1), with the highest levels in chinstrap penguins at Deception Island and the lowest in Adélie penguins at Avian Island. This range in penguin feathers is lower than those found in seabirds from the Northern Hemisphere (0.003-19.29 µg/g) (Burger et al. 2008; Kim et al. 2013), and also is lower than Mn levels detected in feathers of adult seabirds from industrialized and populated areas, such as the Brazilian coasts (11.4 µg/g, Barbieri et al. 2010).

In eggshells, there is only one study reporting concentrations of Mn (0.82 µg/g, Table 2) in gentoo penguins from Livingston Island (Metcheva et al. 2011). Manganese concentration in penguin eggshells is within the range reported in the seabirds of the United States and Spain (0.29-4.63 µg/g) (Gochfeld 1997; Morera et al. 1997).

In bones, Mn concentrations (2.5-18.35 µg/g, Table 3) are highest in gentoo penguins from Byers Peninsula (Barbosa et al. 2013), and are lowest in the same species from Livingston Island (Metcheva et al. 2010). Concentrations of Mn in bones of penguins are within the range reported in the bones of marine, aquatic and terrestrial birds of the Northern Hemisphere (1.06-30.6 µg/g) (Lebedeva et al. 1997; Kim et al. 1998).

In kidneys, Mn concentrations (3.78-11.18 µg/g, Table 4) are highest in chicks of Adélie penguins from King George Island during the 2008-2009 austral summer season, and are lowest in adult Adélie penguins from the same location during austral summers of 2007 to 2010. Manganese concentrations were increased in penguin chicks when compared with adult specimens. Although Mn levels detected in individuals of the same species seem to show a temporal variability, the age of the birds seems to be relevant; birds regulate Mn primarily by excretion in the feces (Kler et al. 2014), and probably Mn intake from food in chicks exceeds excretion (Skoric et al. 2012). Concentrations of Mn in penguin kidneys are within the range found in the kidneys of Arctic seagulls (<0.01-20.1 µg/g; Malinga et al. 2010).

In the liver, Mn concentrations (6.8-15.83 µg/g, Table 5) are highest in Adélie penguins from King George Island and are lowest in the same species from the Antarctic Peninsula. Levels of Mn in penguin livers are comparable to values reported in seabirds from Asia and Artic (4.14-20.3 µg/g) (Kim et al. 1998; Malinga et al. 2010).

In muscles, Mn concentrations (0.51-2.55 µg/g, Table 6) are highest in chinstrap penguins from Deception Island and are lowest in Adélie penguins from Antarctic Peninsula. Concentrations of Mn in penguin muscles are slightly lower than the concentrations of Mn in muscle tissues of Arctic birds (1.84-2.56 µg/g) (Campbell et al. 2005; Burger et al. 2008).

In the stomach, Mn levels (2.20-82.43 µg/g, Table 7) are highest in gentoo penguins from King George Island and are lowest in Adélie penguins from Avian Island. The levels of Mn in penguin stomach contents are higher than those of Mn (0.98-15.9 µg/g) detected in stomach contents of seabirds from the Northern Hemisphere (Kim et al. 1998; Kim and Koo 2007).

In excreta, Mn levels (12.3-138.0 µg/g, Table 8) are highest in chinstrap penguins from the Antarctic Peninsula and are lowest in gentoo penguins from Livingston Island. Concentrations of Mn in penguin droppings are within the range (0.03-221.8 µg/g) found in the guano of different avian species from Asia (Lebedeva et al. 1997; Kaur and Dhanju 2013; Kler et al. 2014).

In animals, Mn is a neurotoxic metal that can affect several neural activities, and at concentrations of about 1,000 µg/g, it has negative effects on certain brain functions (Šaric and Lucchini 2007). Mn is distributed via blood linked to transferring and albumin, being accumulated in tissues rich in mitochondria, such as hepatic and renal tissue (Erikson and Aschner 2003; Soria et al. 1995). Effects produced by an acute exposure to Mn include irritation in the digestive tract, respiratory disorders, cardiac ailments, coma and even death (Soria et al. 1995). In turn, chronic intoxication with this metal generates neurological, reproductive, pulmonary and immune alterations (ATSDR 2008). The elimination of this metal is produced mainly through the gastrointestinal tract (Roth 2006).

No research has been done related to the effects of Mn on penguins. It is an issue because increases in the environmental Mn levels have been related to the current use of Mn as additive in combustibles (Burger and Gochfeld 2000b). There is recent evidence showing that Mn levels in hepatic tissues of Antarctic penguins (Jerez et al. 2013a) are slightly higher than those detected two decades ago (Honda et al. 1986; Szefer et al. 1993).

3.2.3 Zinc

With the exception of excreta, the maximum concentrations of Zn (330.3 µg/g) have been found in livers of chinstrap penguins from King George Island (Table 5) and in bones of the same species from Byers Peninsula, Antarctica (Table 3). In contrast, the lowest concentration of Zn (4.07 µg/g) has been reported in eggshells of gentoo penguins from Livingston Island, Antarctica (Table 2).

In feathers, the range of Zn concentrations (33.3-180.5 µg/g, Table 1) indicates that the highest concentrations are in adult gentoo penguins from Fildes Peninsula and the lowest are in juvenile same species from Doumer Island. Zn levels in penguin feathers are similar to those Zn levels found in feathers of different seabirds of the Northern Hemisphere (42.9-189.2 µg/g) (Kim et al. 1998; Ribeiro et al. 2009; Lucia et al. 2010; Kim et al. 2013).

In eggshells, Zn concentrations (4.07-20 µg/g, Table 2) are highest in gentoo penguins from Fildes Peninsula, and are lowest in gentoo penguins from Livingston Island. The levels of Zn in penguin eggshells are lower than those detected in water birds and seabirds of the United States and the Artic (9.04-58.1 µg/g) (Custer et al. 2007; Malinga et al. 2010).

In bones, the range of Zn (81-244.6 µg/g, Table 3) indicates the highest concentrations are in gentoo penguins from Byers Peninsula, whereas the lowest concentrations are in the same species from Livingston Island. The concentrations of Zn in penguin bones are similar to those Zn levels reported in bones of marine birds of the Northern Hemisphere (83.9-202 µg/g) (Kim et al. 1998; Yin et al. 2008; Skoric et al. 2012).

In kidneys, Zn concentrations (85.7-234.3 µg/g, Table 4) are highest in Adélie penguins from Avian Island. In contrast, the lowest Zn concentrations are in the same species from King George Island. Levels of Zn in penguin kidneys are higher than those Zn levels found in kidneys of marine birds from the North Pacific and Artic seabirds (30.2-183 µg/g) (Kim et al. 1998; Sagerup et al. 2009; Malinga et al. 2010).

In the liver, Zn concentrations (72-330.3 µg/g, Table 5) are highest in chinstrap penguins from King George Island and are lowest in gentoo penguins from Livingston Island. Concentrations of Zn in penguin livers are above those Zn levels found in seabirds of the Northern Hemisphere (14.92-541 µg/g) (Parslow et al. 1973; Kim and Koo 2007; Pérez-López et al. 2005). A study found that a high concentration of Zn (541 µg/g) in livers of northern gannets (*Morus bassanus*) could be the main cause of the bird´s mortality (Parslow et al. 1973).

In muscles, Zn concentrations (24-163.7 µg/g, Table 6) indicate the highest concentrations are in Adélie penguins from King George Island, while the lowest concentrations are in gentoo penguins from Livingston Island. Levels of Zn in seabirds (53.2-75.5 µg/g) of the Northern Hemisphere (Kim et al. 1998; Malinga et al. 2010) are within the range found in the penguin muscles.

In stomachs, Zn levels (19.8-71.16 µg/g, Table 7) are highest in Adélie penguins from King George Island, and are lowest in gentoo penguins from the same location. Concentrations of Zn in penguin stomach contents are within the range (6.64-102 µg/g) found in the seabirds of the Northern Hemisphere (Kim et al. 1998; Kim and Koo 2007).

In excreta, the range of Zn (0.83-487.1 µg/g, Table 8) shows the highest concentrations in Humboldt penguins from Pan de Azúcar Island, while the lowest levels are in the same species from Cachagua Island. Concentrations of Zn in penguin droppings are lower than those (100-721.8 μg/g) found in marine birds and different avian species of the Northern Hemisphere (Yin et al. 2008; Kaur and Dhanju 2013).

In blood, only a single study has measured Zn levels in little penguins (33.47-38.77 μg/g, Table 9). These levels are within the range detected in the long-tailed ducks (*Clangula hyemalis*) and nesting common eiders (*Somateria mollissima*) from Alaska (18.2-39 μg/g) (Franson et al. 2003).

Despite the fact that Zn is an essential metal, pancreas histological damage has been found in birds (Eisler 1993). In birds, Zn accumulated in liver bonded to metallothionein, though it can also be accumulated in muscles and bones (Wastney et al. 2000). In penguins, there is a significant positive correlation between renal Zn and Cd (Fig. 2), which evidences a possible effect of metallothionein synthesis caused by Cd accumulation, as observed in other investigations for seabirds (Honda et al. 1990; Malinga et al. 2010). Evidence shows that Zn poisoning in birds usually occurs when the concentration of this metal exceeds 2,100 μg/g in the liver or kidney (Eisler 1993). The concentrations of Zn in livers of penguins are below 200 μg/g (Table 5), considered as the threshold value of physiological importance in different species of seabirds (Honda el at. 1990), except that found in liver of chinstrap penguins (330.3 μg/g) and in livers of gentoo penguins (237.2 μg/g) from King George Island. These levels of Zn seem to be related to the great concentration of human activities present in King George Island (Tin et al. 2009).

4 Similarities and differences of trace elements

4.1 Distribution of trace elements

There is great similarity (82%) between concentrations of trace elements in guano and stomach contents of penguins (Fig. 3). Likewise, the levels of trace elements in kidneys and livers present great similarity (81%), which may be due to the fact that both organs have similar mechanisms of detoxification and biotransformation of elements (Sánchez-Virosta et al. 2015). Furthermore, there is also 85% of similarity between the concentrations of trace elements in feathers and muscles. Due to sampling constraints, it is not easy to establish relationships between the concentrations in feathers and the concentrations found in the internal tissues of penguins, even though some previous works have found some relationship between feathers and muscle tissues for some trace elements in birds (Del Hoyo et al. 1992). Metal levels in eggs and bones presented no correlation with the other biotic matrices. Some metals (as Pb, Cd) are not metabolically regulated and tend to be immobilized in bird bones (Lebedeva 1997) and eggshell (Kim and Oh 2014a). Both biological matrices are mainly composed of Ca, which is one of the most important plasma constituents in mammals and birds, and provides structural strength and support to bones and eggshell (De Matos 2008). Trace elements such as Pb and Cd might interact with the metabolic pathway of Ca (Scheuhammer 1987).

A high content of elements in penguin excreta imply a physiological mechanism of detoxification (Ancora et al. 2002), but also imply that elements are not necessarily absorbed at the intestinal level, which reinforces the fact that high concentrations of trace elements in feces are likely the result of low intestinal absorption rather than detoxification mechanisms, and much of the elements ingested by these seabirds are being excreted. It is observed that when some bird present renal damage caused by Cd, the levels of this metal in excreta is increased (Goyer 1997). In penguins, feathers play an important role in detoxification of Hg and Pb, because a large amount of these metals from their diets can be transferred into their plumages (Stewart et al. 1997; Ancora et al. 2002; Jerez et al. 2011). Other metals such as Cd, Cu and Zn are mainly eliminated via the feces (Ancora et al. 2002; Yin et al. 2008). In general, sequestration of metals (such as Hg or Pb) in bird´s feathers results in decreased internal bioavailability (Jerez et al. 2011; Calle et al 2015). Diet, exposure levels, physiological conditions and the toxic-kinetic mechanisms regulate the arrival of metals to feathers, as for the case of Hg (Becker et al. 2002). Redistribution to plumage occurs during feather growth when the feather is connected to blood vessels, and metals are incorporated in the keratin structure (Burger et al. 2011). When the feather matures, blood vessels shrivel and the feather is no longer supplied with blood, at which point the metal deposition to feather ceases (Burger 1993). Hg elimination is possible via deposit in eggs, excreta, uropygial gland, and feathers (Dauwe et al. 2000). In seabirds, Hg concentrations in feathers reflect the uptake and storage of this heavy metal during the period between molts rather than short-term uptake (Furness et al. 1986).

In general, trace element levels in penguins are scarce and fragmented; therefore no correlation analysis is possible now. Data of trace elements available in *Pygoscelis* penguins of the South Shetland Islands (Fig. 4), showed that concentrations of Cd in the liver and stomach contents are significantly correlated. It indicates that the levels of Cd in gentoo, chinstrap and Adélie penguins that live there are strongly influenced by diet, which has been also noted in populations of seagulls from the Northern Hemisphere (Kim and Oh 2014c). In birds, trace element levels in blood reflect recent dietary exposure and often correlate strongly with those in internal tissues (Monteiro and Furness 2001). A study evidenced that blood provides a more precise indicator of penguin body burden for Al, As, Cd, Cu, Fe, Hg, Pb, Se, and Zn than feathers (Finger et al. 2015).

At present, most Hg pollution resides in aquatic environments, where it is converted to methylmercury (Chen et al. 2008). Because of its high affinity with sulphydryl groups of proteins, this heavy metal is easily incorporated into the food chain, bioaccumulating in aquatic organisms, and bioamplyfying from one trophic level to the next (Fitzgerald et al. 2007). Some metals such as Zn and Cd among others might be biomagnified under certain environments such as Antarctica, a cold place where trophic chains are short and highly dependent on krill (Majer et al. 2014). In Table 10 the values of the trophic transference coefficient (TTC) are shown only for penguins of the genus *Pygoscelis*, because there is no information on the levels of metals in stomach contents for other species of penguins. TTC is defined as the ratio between the concentration of a certain metal in the body of an animal (internal organs) and the concentration of the metal in the stomach contents (Suedel et al. 1994). The value of TTC is usually < 1 for trace metals (Anan et al. 2001), except for those metals highly cumulative in the organism which are biomagnified in the trophic chain, such as Hg (Lavoie et al. 2013). In the case of penguins that live in Antarctica, Hg, Cd and Zn showed a high cumulative power in gentoo, chinstrap and Adélie penguins, with TTC values far above unity. Scientific evidence indicates that Zn, Se, Cu and Cd tend to bioaccumulate in aquatic trophic chains (Dehn et al. 2006; Mathews and Fisher 2008). This suggests the possibility of metal biomagnification under specific circumstances. It has been found that biomagnification of Hg is expressed more strongly in cold environments with simple trophic chains (Lavoie et al. 2013). This issue should be addressed in depth in further studies, considering the diversity of marine environments in which the different species of penguins feed.

4.2 Geographical differences

Most studies on levels of trace elements in penguins have been carried out in Antarctica and nearby islands (~85%). The most reported trace elements are Pb, Cd, Cu, and Zn. On the contrary, Al and Mn are the less reported elements. The lack of studies on trace elements in penguins from the coasts of Australia, South Africa, and Galapagos Islands is clearly noted (Table 11, Fig. 5). Most of the field studies on trace elements are concentrated in the Antarctic and sub-Antarctic areas (90%), specifically in the Antarctic Peninsula and South Shetland Islands (74%); the rest of the studies are concentrated in the coasts of South America (8%) and coasts of Australia (2%). Further studies are needed in order to overcome the huge gap of data between Antarctica and other territories of more temperate zones where there are colonies of other species of penguins. Differences in trace element concentrations in the same species at different sites are evidenced in gentoo penguins, because they have a large distribution and a very plastic diet depending on site. Gentoo penguins at Crozet Islands have higher feather Hg concentrations (Carravieri et al. 2016) than those reported at Antarctic locations (Bargagli et al.

1998). Gentoo penguins at subantarctic areas have higher feather Hg concentrations than those reported at Antarctic locations (Table 1). Gentoo penguins at higher latitudes feed largely on krill (Carlini et al. 2009), whereas they prey mainly on fish at lower latitudes (Lescroël et al. 2004). Krill is a pelagic low-trophic prey very abundant in Antarctica with lower Hg burden compared to fish (Bargagli et al. 1998; Bustamante et al. 2003).

Due to the lack of data, the comparison of the levels of trace elements between different species and populations of penguins must be performed with caution. In general, the concentrations of trace elements are fragmented from the spatio-temporal point of view, which prevents for now conducting an analysis of tendencies. Hence, the implementation of monitoring programs that incorporate these variables is required.

4.3 Interspecific differences

There are 18 species of penguins that inhabit the planet (García and Boersma 2013), but trace metals have been reported only in 11 species (Table 12), evidencing the information gap in species such as *Eudyptes pachyrhynchus, Eudyptes sclateri, Eudyptes robustus, Eudyptes schlegeli, Spheniscus demersus, Spheniscus mendiculus* and *Megadyptes antipodes.* The species with more elements reported are *P. papua*, *P. adeliae* and *P. antarctica*. On the other hand, the less studies species are *E. chrysocome* and *E. chrysolophus*. It is necessary to state that the distribution of trace elements by species from different studies, species and individuals presents serious limitations because of temporal variation, spatial variation, diet, individual specialization, physiological condition, and sex.

In Adélie, gentoo and chinstrap penguins, concentrations of the essential trace elements Cu, Mn and Zn in all biotic matrices exhibited less inter-species variation than the non-essential Al, As, Cd, Hg and Pb, expressed through the coefficient of variation. These results are in concordance with similar findings of other investigations in seabirds (Honda et al. 1990; Lock et al. 1992). Penguins, by virtue of its members exhibiting a wide range of trace element burdens, along with variation in diet, varying moult strategies and variation in their average life-spans may explain inter-species pattern of metal accumulation, storage and elimination (Thompson 1990; García and Boersma 2013). This is an issue that needs to be more investigated.

Diet is one of the most important factors that explain differences in trace element concentrations among the species. Penguins are useful bioindicators of Hg contamination in their food webs (Carravieri et al. 2016). Feather Hg concentrations in *Eudyptes* and *Pygoscelis* penguins are lower than *Aptenodytes* penguins, because they feed at lower trophic levels (Carravieri et al. 2013). One study showed that the concentrations of Zn, Al and Mn in feathers were significantly higher in gentoo than in chinstrap penguins, which could be explained by the different diet and feeding habits of these species (Metcheva et al. 2006). During the Antarctic summer, when the breeding season takes place, gentoo penguins feed inshore, eating mainly crustaceans (68 %) and fish (32 %), even though foraging areas may also be included in their diet (Croxall et al. 1997). Also chinstrap penguins feed almost exclusively on krill, but can feed beyond the continental shelf areas (Espejo et al. 2014). Concentration of trace elements can differ among colonies of the same species that live far from each other owing to diet and the presence of chemicals in waters (Jerez et al. 2011). Similarly, Yin et al. (2008) mention that the difference in Cu levels among seabirds might be related to different food resources for the species. The trophic level of the species which is given by diet can be determined by means of stable isotopes of nitrogen, a method infrequently used in studies of trace elements in penguins (Brasso et al. 2013; Brasso et al. 2014; Carravieri et al. 2016).

5 Summary and conclusions

In the environment, trace elements are persistent and come from both natural cycling in the biosphere, as well as from anthropogenic activities (Nordberg and Nordberg 2016). For this reason there is a concern about the possible negative effects these contaminants may have on animals and marine ecosystems (Szopińska et al. 2016). Birds are excellent indicators of the degree of pollution in the environment, because they rapidly express the biological impacts of the contaminants that can even be extrapolated to humans (Ochoa-Acuña et al. 2002; Cifuentes et al. 2003; Zhang and Ma 2011), a remarkable issue considering humans are the most sensitive species to the toxic effects of some trace elements (Byrns and Penning 2011). Human population will increase and also marine-derived protein consumption. Most penguins include fish in their diets, such as sprats, myctophid fish, anchovies, silversides, jack mackerel, and common hake, among others (García and Boersma 2013). Many fish are also consumed by humans, thus these birds might be used as a bioindicator for human health and as exposure assessment. Most penguins are on the upper side of the trophic chain and they depend on few species for food. Consequently, the effects on a particular species might loom a serious threat to penguins.

Investigations of trace elements in penguins report mostly the levels of Al, As, Cd, Cu, Hg, Mn, Pb and Zn. The most reported metal is Pb, whereas Al is the less reported. Other metals such as Co, Cr, Fe or Ni have been poorly studied (Jerez et al. 2013a; Szopińska et al. 2016). The oldest data dates back to the fifties and it was aimed at determining the Hg levels in feathers of King penguins (*Aptenodytes patagonicus)* from Crozet Islands, South East of Indian Ocean (Carravieri et al. 2016).

There are 18 species of penguins around the world and trace elements have been reported in 11 of them (*P. papua, P. antarctica, P. adeliae, Aptenodytes forsteri, A. patagonicus, Spheniscus magellanicus, S. humboldti, Eudyptes chrysolophus, E. chrysocome, E. minor* and *E. moseleyi*). Most studies of concentrations of trace elements in penguins have been focused on the genus *Pygoscelis*, mainly on gentoo penguins, followed by Adélie penguins, and finally chinstrap penguins. Other penguin species such as *E. pachyrhynchus, E. sclateri, E. robustus, E. schlegeli, S. demersus, S. mendiculus* and *Megadyptes antipodes* have not received any attention.

The most studied penguin biological matrices are feathers and then excreta, followed by the liver, kidneys, bones, muscles and stomach contents. On the other hand, studies carried out to measure trace elements in blood and internal organs such as heart, testicles, spleen or brains of penguins are scarce (Bargagli et al. 1998; Finger et al. 2015; Metcheva et al. 2010; Metcheva et al. 2011). The species which display the highest concentration of most trace elements is gentoo penguin (33%), followed by Adélie penguin (31%), chinstrap penguin (19%), Humboldt penguin (7%), Magellanic penguin (6%), and Emperor penguin (4%).

The maximum concentrations (µg/g, dw) of Al (≈2,595) have been found in stomach contents of gentoo penguins from King George Island, and Cd (351.8) in the liver of Adélie penguins from Antarctic Peninsula. The highest levels of As (7.9) and Pb (12.8) were found in excreta of Humboldt penguins from the Central Coast of Chile. Maximum concentrations of Hg (6.6) and Cu (585.8) have been reported in excreta of gentoo penguin and Adélie penguin, respectively, both from the Antarctic Peninsula, whereas the maximum Zn levels (487.1) was found in excreta of Humboldt penguins of Northern Chile. Finally, excepting excreta and stomach contents, maximum levels of Mn (18.35) are in bones of gentoo penguins from Byers Peninsula, South Shetland Islands. The large variation in trace element concentrations detected in different biotic matrices of penguins in Antarctica might be explained because in this continent many pristine places coexist with locations having major human presence, a situation which rarely occurs in others areas of the world. Additionally, several other factors can force variation in trace element concentrations in penguin tissues such as feeding ecology, physiological state, species, age class, molting patterns, among others.

In general, Hg, Pb and Cd concentrations in penguins are lower than those reported in other seabirds from the Northern Hemisphere, whereas the concentrations of Al and As are otherwise. The concentrations of Cu, Mn and Zn tend to be within the range reported in the marine birds of the Northern Hemisphere, suggesting that those elements are regulated in seabirds (Gibbs 1995). The highest levels of Cu and Cd correspond to penguins that live in Antarctica, which might be related to the high levels of these metals detected in the Antarctic krill (Nygard et al. 2001). On the other hand, it has been observed that in the Antarctic Peninsula there is a natural enrichment of Cd, As and Al in the trophic chains, due to the local volcanism (Deheyn et al. 2005). Nevertheless, comparisons could be influenced by the differences in the diet composition of each of the species (Jerez et al. 2011).

Studies on the effects of trace elements on penguins are scarce. For that reason, the comparison of data reported in penguins with those obtained from studies performed on birds at other locations and ecologically different to penguins was unavoidable. Hence, any comparison to toxic thresholds of trace elements in terrestrial birds should be taken with extreme caution, because seabirds appear to be more resistant to toxic effect of most pollutants than are mammals or terrestrial birds (Beyer et al. 1996). In general, the concentrations of trace elements in the different organs of penguins are below the toxicity thresholds with negative biological consequences for seabirds. Some colonies of Humboldt penguins located in areas with human presence on the coast of Chile might present some pathological problems due to As, Cu and Pb (Celis et al. 2014). Some negative effects in the liver and kidneys of gentoo penguins from the Antarctic Peninsula could be linked to local Pb contamination (Celis et al. 2012, Jerez et al. 2013a). Levels of Zn in livers of some colonies of gentoo and chinstrap penguins from King George Island (Jerez et al. 2013a) exceeded in 19% and 65% the threshold value of physiological importance for seabirds, respectively (Honda et al. 1990). It seems to be related to areas of greatest human activities in Antarctica, which are concentrated precisely on King George Island (Tin et al. 2009). Levels of Cd in livers of some colonies of gentoo, Adélie, chinstrap and Emperor penguins that inhabit the Antarctic Peninsula area, and Magellanic penguins from southern Brazil, which together represent almost 48% of the reported colonies might be associated with physiological and ecological problems (>3 µg/g, Scheuhammer 1987). Cadmium concentrations found in kidneys of Adélie, chinstrap and Emperor penguin from some locations of the Antarctic Peninsula (270.2-351.8 µg/g, Table 4), such as Avian Island, Deception Island, and Weddell Sea, indicate that these birds have suffered some degree of chronic exposure to this metal (Furness 1996). Further studies that correlate the levels of trace metals found in non-invasive samples with biological effects on penguins are required.

Most studies of concentrations of trace elements in penguins have been carried out on the Antarctica and subantarctic islands, thus evidencing a lack of data from other areas where penguins live also, such as Australia, South Africa, South America and Galapagos Islands. It is surprising to find studies mainly in Antarctica, since researchers require an adequate implementation and a firm determination to work under extreme climatic conditions. Perhaps the urge to travel to remote and poorly explored regions is more important than the simple desire of performing research in more populated places with more temperate climates where the species of threatened penguins could be more exposed to contaminants by being in areas with major human presence.

The trophic transfer coefficient, calculated from the levels of metals available in gentoo, chinstrap and Adélie penguins, suggests a possible biomagnification of Cd and Zn. Due to the fact that scientists have always believed that metals, except Hg, are not biomagnified, this issue needs to be studied more in different environments inhabited by penguins.

Most studies of penguins have focused on measuring the levels of exposure in different biotic matrices. The concentration of metals in tissues and organs of penguins may have a great toxicological importance. In humans, diseases related to deficiency of essential trace elements are well known (Nordberg and Nordberg 2016). Further studies with biomarkers are needed in order to evaluate the actual risks associated with the levels of these contaminants in polar environments with low ecological diversity, which can increase diseases with consequences for the health of penguin populations (Boersma 2008).

Little is known about the interaction of metals that might activate certain detoxification mechanisms of the organism of penguins. It is suspected that Se could play an important role in the detoxification processes of Hg. The study with species in captivity could be a good alternative to evaluate the physiological mechanisms of these species at a given concentration of metals, under a controlled environment (Falkowska et al. 2013).

In the short term, studies of trace elements in penguins should take into account the following aspects:

- Incorporation of other metals such as Co, Ni or Cr and their possible effects in the organism of the different species of penguins in order to perform more accurate risk assessments.
- Further toxico -kinetics studies on trace element levels in penguins, including other tissues and organs, are needed to better understand the overall toxicity in seabirds.
- Information on metals of the following species is crucial: *Eudyptes pachyrhynchus, Eudyptes moseleyi, Eudyptes sclateri, Eudyptes robustus, Eudyptes schlegeli, Spheniscus demersus, Spheniscus mendiculus* and *Megadyptes antipodes.*
- Correlation between the levels of metals in different biological matrices with their effects on different species and geographic locations is required.
- Interspecific variation of metals should be addressed more in depth, being the isotopes of nitrogen a good tool to understand differences among species.
- The implementation of monitoring programs that incorporate spatial-temporal data is required for conducting an analysis of tendencies.
- It is crucial to uniform monitoring protocols to help unify the data and make it more comparable.

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1 **Table 1** Mean trace metal levels (µg/g, dw) in feathers of different penguin species worldwide.

Species	N	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	3	-	-	-	-	0.82±0.13	-	-	-	Terra Nova Bay[1]	1989-90	Bargagli et al. (1998)
	1	-	-	-	-	1.40	-	61.5	-	Admiralty Bay[2]	2004	Santos et al. (2006)
	n/i	-	-	-	1.50	0.60	-	-	-	Zhongshan Station[9]	2001	Yin et al. (2008)
	1	3.56	0.04	0.12	<0.01	-	16.21	70.41	0.21	King George Is.[2]	2007-10	Jerez et al. (2013a)
	2	0.71±0.43	0.06±0.001	0.08±0.01	0.06±0.09	-	16.22±0.51	60.59±2.02	<0.01	Avian Is.[3]	2007-10	Jerez et al. (2013a)
	1**	52.44	0.08	0.01	<0.01	-	19.29	83.90	1.15	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	64.3±61.75	0.17±0.11	0.13±0.08	0.24±0.38	-	13.32±8.22	61.11±20.3	2.01±0.52	King George Is.[2]	2008-9	Jerez et al. (2013b)
	4	-	-	0.30	0.50	0.90	-	-	-	Edmonson Point[1]	1995	Ancora et al. (2002)
	25	43.36±69.03	-	-	0.64±1.09	-	12.68±7.09	50.84±17.38	1.30±1.16	King George Is.[2]	2005-7	Jerez et al. (2011)
	21	8.62±6.41	0.07±0.04	0.04±0.05	0.32±0.36	-	13.41±2.6	82.45±13.1	1.16±1.26	Yalour Is.[3]	2005-7	Jerez et al. (2011)

	22	5.08±3.03	0.07±0.03	0.04±0.02	0.14±0.21	-	13.16±3.04	77.69±15.17	0.34±0.49	Avian Is.[3]	2005-7	Jerez et al. (2011)
	10	-	-	-	-	0.66±0.2	-	-	-	Adélie land[10]	2007	Carravieri et al. (2016)
	12	-	-	-	-	0.43±0.13	-	-	-	Adélie land[10]	2006	Carravieri et al. (2016)
	10**	-	-	-	-	0.19±0.06	-	-	-	Adélie land[10]	2007	Carravieri et al. (2016)
P. antarctica	25	132.4±198.09	0.10	-	1.76±1.74	-	20.29±8.3	77.12±45.15	1.66±0.98	King George Is.[2]	2007	Jerez et al. (2011)
	10	26.07±9.97	0.01±0.0001	0.04±0.03	0.15±0.12	-	14.93±6.1	72.21±28.96	0.92±0.59	Livingston Is.[2]	2000	Jerez et al. (2011)
	25	203.1±194.65	0.10±0.1	0.08±0.04	0.32±0.22	-	16.39±3.44	82.40±15.64	3.26±2.68	Deception Is.[2]	2005-7	Jerez et al. (2011)
	20	14.26±9.72	0.05±0.03	0.10±0.05	0.14±0.09	-	19.23±3.65	97.27±21.35	0.29±0.39	Ronge Is.[3]	2005-7	Jerez et al. (2011)
	3	8.99±7.86	0.07±0.01	0.31±0.22	0.81±0.84	-	19.60±1.7	62.29±20.01	0.05±0.03	Deception Is.[2]	2005-7	Jerez et al. (2013a)
	2	7.99±10.02	0.31±0.21	0.01±0.01	0.02±0.03	-	15.29±0.34	94.75±2.37	0.21±0.30	King George Is.[2]	2005-7	Jerez et al. (2013a)
	5	142.0±206.33	0.48±0.3	0.02±0.03	0.06±0.04	-	18.57±2.78	94.99±5.29	2.25±3.17	Deception Is.[2]	2007-10	Jerez et al. (2013b)
	29	26±8	0.45±0.2	0.30±0.07	1.73±0.94	-	18.5±3	87.0±5	1.5±0.7	Livingston Is.[2]	2007-10	Metcheva et al. (2006)
	32	26±11	2.4*	0.2*	1.66±1.2	-	18±2	75±7	1.6±0.43	Livingston Is.[2]	2008-9	Metcheva et al. (2006)

P. papua	1	-	-	-	-	0.54	-	90.70	-	Admirantly Bay[2]	2002	Santos et al. (2006)
	14	40±10	0.88±0.32	0.21±0.06	1.7±1.3	-	17±4	106±8	1.5±0.73	Livingstone Is.[2]	2002	Metcheva et al. (2006)
	28	46±22	(<0.6-4.0)$^{\Delta}$	(0.15-0.43)$^{\Delta}$	1.57±1.13	-	16±2	89±7	2.6±0.99	Livingstone Is.[2]	2003	Metcheva et al. (2006)
	1^{+}	-	-	0.50±0.12	<1.90	-	15.9±0.12	73.0±0.54	1.65±0.15	Livingston Is.[2]	2002-6	Metcheva et al. (2010)
	14	37.0±6.9	(0.3-0.69)$^{\Delta}$	(0.05-0.41)$^{\Delta}$	1.52±0.5	-	17.0±3.1	92.0±4.6	1.70±0.2	Livingstone Is.[2]	2006-7	Metcheva et al. (2011)
	20	39.76±24.74	0.05±0.04	0.03±0.03	0.51±0.46	-	16.44±3.16	85.12±14.84	1.80±1.28	King George Is.[2]	2005-7	Jerez et al. (2011)
	14	40.17±42.37	0.02±0.02	0.02±0.01	0.17±0.23	-	13.88±2.89	75.41±15.85	1.17±1.05	Livingston Is.[2]	2005-7	Jerez et al. (2011)
	17	22.92±27.87	0.04±0.02	0.03±0.01	0.25±0.44	-	16.02±2.09	72.89±7.46	0.46±0.58	Ronge Is.[3]	2005-7	Jerez et al. (2011)
	8	17.19±11.9	0.07	0.03±0.04	0.31±0.09	-	13.42±5.09	61.71±18.01	0.68±0.66	Paradise Bay [3]	2005-7	Jerez et al. (2011)
	11	-	-	-	-	5.90±1.91^{b}	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
	12	-	-	-	-	5.23±1.12	-	-	-	Crozet Is.[7]	2006	Carravieri et al. (2016)
	12$^{@}$	-	-	-	-	1.88±0.46	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
	4**	15.72±19.24	0.09±0.07	0.02±0.01	0.07±0.13	-	16.02±5.40	119.72±21.81	0.27±0.35	King George Is.[2]	2007-10	Jerez et al. (2013a)

	2	6.71±5.15	0.10±0.05	0.02±0.004	0.33±0.31	-	19.26±0.94	69.49±6.32	0.06±0.09	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	68.55±76.39	0.12±0.05	0.06±0.04	0.87±0.86	-	6.87±1.54	80.59±10.85	0.95±0.69	King George Is.[2]	2008-9	Jerez et al. (2013b)
	10	-	-	0.05±0.07	0.06±0.04	-	13.74±1.81	36.89±6.26	0.75±0.28	Neko Harbor[3]	2014	Celis et al. (2015b)
	10	-	-	0.09±0.07	0.10±0.17	-	14.98±4.09	33.26±4.04	0.27±0.37	Doumer Is.[3]	2014	Celis et al. (2015b)
	10	-	-	0.14±0.09	0.60±0.34	-	19.65±2.25	64.19±10.67	1.23±0.46	Stranger Point[2]	2014	Celis et al. (2015b)
	10	-	-	0.21±0.28	0.63±0.27	-	20.89±4.3	64.07±10.73	1.19±0.68	Base O´Higgins[3]	2014	Celis et al. (2015b)
	55	-	-	-	-	0.97±0.67	-	-	-	South Georgia[9]	2009	Pedro et al. (2015)
	29	-	-	-	-	1.1±0.62	-	-	-	South Georgia[9]	2011	Pedro et al. (2015)
	12	-	-	-	-	5.85±3.00	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	4.96±2.44	-	-	-	Kerguelen Is.[7]	2006	Carravieri et al. (2013)
	12[@]	-	-	-	-	2.45±0.67	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	1.44±0.44	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
A. forsteri	3	-	-	-	-	0.98±0.21	-	-	-	Terra Nova Bay [1]	1989-90	Bargagli et al. (1998)

	17	-	-	-	-	1.77±0.37	-	-	-	Adélie Land[10]	2007	Carravieri et al. (2016)
	12**	-	-	-	-	0.61±0.11	-	-	-	Adélie Land[10]	2007	Carravieri et al. (2016)
A. patagonicus	31	-	-	-	-	1.98±0.73	-	-	-	Crozet Is.[7]	2000-1	Scheifler et al. (2005)
	10	-	-	-	-	2.66±0.86	-	-	-	Crozet Is.[7]	1966-74	Scheifler et al. (2005)
	12	-	-	-	-	2.22±0.59	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	2.17±0.52	-	-	-	Kerguelen Is.[7]	2006	Carravieri et al. (2013)
	12**	-	-	-	-	1.79±0.55	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12@	-	-	-	-	1.12±0.16	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	2.94±0.47	-	-	-	Crozet Is.[7]	2006	Carravieri et al. (2016)
	12	-	-	-	-	2.89±0.73	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
	12**	-	-	-	-	1.80±0.24	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
E. chrysolophus	12	-	-	-	-	2.24±0.29	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	2.08±0.35	-	-	-	Kerguelen Is.[7]	2006	Carravieri et al. (2013)

	12[@]	-	-	-	-	0.36±0.07	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	2.48±0.35	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
	12	-	-	-	-	2.09±0.31	-	-	-	Crozet Is.[7]	2006	Carravieri et al. (2016)
	12[**]	-	-	-	-	0.43±0.10	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
S. magellanicus	21	-	-	-	-	0.206±0.098	-	-	-	Punta Tombo [5]	2007	Frias et al. (2012)
	18[π]	-	-	-	-	0.123±102	-	-	-	Punta Tombo [5]	2007	Frias et al. (2012)
	37[**]	-	-	-	-	0.033±0.052	-	-	-	Punta Tombo [5]	2007	Frias et al. (2012)
	22	-	-	0.13±0.07	0.14±0.08	0.78±0.44	-	-	-	Rio Grande do Sul[6]	n/i	Kehrig et al. (2015)
E. chrysocome	12	-	-	-	-	1.96±0.41	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	1.92±0.35	-	-	-	Kerguelen Is.[7]	2006	Carravieri et al. (2013)
	12[**]	-	-	-	-	0.27±0.06	-	-	-	Kerguelen Is.[7]	2007	Carravieri et al. (2013)
	12	-	-	-	-	1.79±0.37	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
	12	-	-	-	-	1.62±0.35	-	-	-	Crozet Is.[7]	2006	Carravieri et al. (2016)

	12**	-	-	-	-	0.34±0.05	-	-	-	Crozet Is.[7]	2007	Carravieri et al. (2016)
E. minor	13	40.38±22.96	0.16±0.05	0.04±0.02	0.42±0.20	4.13±0.98	11.42±2.19	84.77±11.28	-	St. Kilda [4]	2012	Finger et al. (2015)
	12	16.78±16.11	0.18±0.1	0.04±0.03	0.08±0.03	2.70±0.37	10.77±1.5	80.58±8.06	-	Phillip Is.[4]	2012	Finger et al. (2015)
	10	6.25±2.79	0.09±0.03	0.06±0.02	0.10±0.05	1.50±0.82	10.54±2.07	76.80±7.15	-	Notch Is.[4]	2012	Finger et al. (2015)
E. moseleyi	12	-	-	-	-	2.10±0.36	-	-	-	Amsterdam Is.[11]	2006	Carravieri et al. (2016)
	12	-	-	-	-	1.82±0.30	-	-	-	Amsterdam Is.[11]	2007	Carravieri et al. (2016)
	15**	-	-	-	-	0.34±0.07	-	-	-	Amsterdam Is.[11]	2007	Carravieri et al. (2016)

*Sample collection, ** Juvenile, [@]Chicks, [π]Young adults, [+]Duplicates were performed from the sample, [b](max 8.16 μg/g), [Δ]Authors only report min and max values, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Several locations of the Antarctic Peninsula (West Antarctica), [4]Victoria, Australia, [5]Coast of Argentina, [6]Coast of Brazil, [7]South East of Indian Ocean, [8]Mirror Peninsula (East Antarctica), [9]Subantarctic area of the Atlantic Ocean, [10]East Antarctica, [11]Southern of the Indian Ocean. n/i = not informed.

Table 2 Mean trace metal levels (μg/g, dw) measured in eggshells of different penguin species.

Species	N	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	13	-	-	-	-	0.26±0.08	-	-	-	Terra Nova Bay[1]	1989-90	Bargagli et al. (1998)
	89	-	-	-	-	0.02±0.01	-	-	-	King George Is.[2]	2006-11	Brasso et al. (2014)
	1	-	-	-	-	0.005	-	8.30	-	Admiranty Bay[2]	2004	Santos et al. (2006)
P. antárctica	92	-	-	-	-	0.07±0.05	-	-	-	King George Is.[2]	2006-11	Brasso et al. (2014)
P. papua	n/i	-	-	-	-	0.75	-	-	-	Fildes Peninsula[2]	2002	Yin et al. (2008)
	12	28.96±4.3	<0.3	<0.05	0.68±0.3	-	1.24±0.4	4.07±0.6	0.82±0.08	Livingston Is.[2]	2006-7	Metcheva et al. (2011)
	90	-	-	-	-	0.02±0.01	-	-	-	King George Is.[2]	2006	Brasso et al. (2014)

*Sample collection, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), n/i = not informed.

1 **Table 3** Mean trace metal levels (µg/g, dw) in bones of different penguin species.

Species	n	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	n/i	-	-	-	1.60	-	-	-	-	Zhongshan Station[1]	2001	Yin et al. (2008)
	1	8.49	0.07	0.03	<0.001	-	0.06	138.38	7.44	King George Is.[2]	2007-10	Jerez et al. (2013a)
	1	5.61	0.12	0.17	0.10	-	0.17	106.15	7.56	Avian Is.[3]	2007-10	Jerez et al. (2013a)
	5	11.89±3.69	0.13±0.08	0.01±0.004	0.04±0.1	-	0.96±0.53	227.01±121.11	831±3.11	King George Is.[2]	2008-9	Jerez et al. (2013b)
P. antarctica	2	4.16±1.02	0.08±0.07	<0.001	<0.001	-	0.17±0.22	221.3±9.19	6.66±0.57	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4	7.30±6.09	0.04±0.01	0.07±0.03	0.21±0.12	-	0.19±0.1	138.77±20.63	8.40±1.25	Deception Is.[2]	2007-10	Jerez et al. (2013a)
	5	7.38±2.93	0.14±0.13	0.004±0.001	0.14±0.02	-	0.71±0.36	235.01±40.62	12.5±2.13	Deception Is.[2]	2008-9	Jerez et al. (2013b)
P. papua	1	69.95	0.13	0.001	0.19	-	0.79	184.1	11.01	King George Is.[2]	2008-9	Jerez et al. (2013b)
	13	32.28±14.96	0.19±0.04	0.008±0.003	<0.001	-	1.15±0.33	244.6±7.99	18.35±2.28	Byers Peninsula[2]	2009	Barbosa et al. (2013)
	8	18.63±4.44	0.12±0.01	0.005±0.001	0.02±0.01	-	0.74±0.09	223.82±21.91	9.81±1.21	Hannah Point [2]	2009	Barbosa et al. (2013)
	2	7.59±0.2	0.06±0.01	0.002±0.002	0.15±0.19	-	0.20±0.11	180.05±29.4	8.27±1.78	King George Is.[2]	2007-10	Jerez et al. (2013a)
	1**	-	-	0.10±0.02	0.30±0.14	-	0.90±0.028	81.0±0.6	2.50±0.23	Livingston Is.[2]	2002-6	Metcheva et al. (2010)

*Sample collection, **Duplicates were performed from the sample, [1]Mirror Peninsula (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Southern of the Antarctic Peninsula.

1 **Table 4** Mean trace metal levels (μg/g, dw) in kidneys of different penguin species.

Species	N	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	1	-	-	-	-	1.20	-	-	-	Terra Nova Bay[1]	1989-90	Bargagli et al. (1998)
	1	0.74	1.07	54.41	<0.01	-	10.74	163.71	3.78	King George Is.[2]	2007-10	Jerez et al. (2013a)
	1**	3.48	0.45	0.68	<0.01	-	12.66	119.90	7.79	King George Is.[2]	2007-10	Jerez et al. (2013a)
	2	14.12±3.86	0.38±0.12	351.8±0.08	0.21±0.17	-	14.78±3.04	234.3±62.24	5.77±1.36	Avian Is.[3]	2007-10	Jerez et al. (2013a)
	5	4.09±7.05	0.44±0.24	0.20±0.15	0.05±0.12	-	11.85±3.69	85.74±19.49	11.18±6.12	King George Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	0.547±0.033	0.339±0.012	0.144±0.007	0.146±0.004	1.6±0.12	-	9.4±0.2	Potter Cove[4]	2002-3	Smichowski et al. (2006)
	5	-	-	263.8±216.6	-	-	17.80±4.1	-	-	Weddell Sea[5]	1982-3	Steinhagen-Schneider (1986)
P. antarctica	2	0.69±0.38	0.52±0.6	0.49±0.32	<0.01	-	17.13±2.63	107.79±23.57	10.13±2.37	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4	0.75±0.76	0.58±0.12	263.93±139.77	0.18±0.01	-	15.33±4.67	149.8±49.23	5.35±0.75	Deception Is.[2]	2007-10	Jerez et al. (2013a)
	5	10.93±10.57	0.50±0.09	0.54±0.29	0.14±0.02	-	13.64±2.28	92.83±32.19	10.19±2.63	Deception Is.[2]	2008-9	Jerez et al. (2013b)
A. forsteri	4	-	-	270.2±126.8	-	-	19.10±3.0	-	-	Weddell Sea[5]	1982-3	Steinhagen-Schneider (1986)

P. papua	3	2.13±0.62	0.67±0.41	11.37±14.1	0.07±0.03	-	13.99±2.91	93.14±42.13	6.40±3.07	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4[**]	4.8±4.24	0.43±0.17	1.54±0.71	<0.01	-	19.99±6.83	152.14±18.51	7.33±3.38	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	6.91±3.95	0.40±0.23	0.20±0.05	<0.001	-	14.26±4.33	125.43±12.60	7.54±3.47	King George Is.[2]	2008-9	Jerez et al. (2013b)
	1***	-	-	41.20±0.67	0.10±0.5	-	8.10±0.45	232.0±2.67	4.90±0.44	Livingston Is.[2]	2002-6	Metcheva et al. (2010)
S. magellanicus	22	-	-	46.50±33.55	0.55±0.3	2.47±1.42	-	-	-	Rio Grande do Sul[6]	n/i	Kehrig et al. (2015)

*Sample collection, **Juvenile, *** Duplicates were performed from the sample, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Several locations of the Antarctic Peninsula, [4]King George Island (South Shetland Islands, West Antarctica), [5]Northeast of the Antarctic Peninsula, [6]Coast of Brazil.

Table 5 Mean trace metal levels (µg/g, dw) in liver of different penguin species.

Species	N	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	1	4.19	1.20	4.41	0.05	-	10.91	136.3	8.58	King George Is.[2]	2007-10	Jerez et al. (2013a)
	2	0.55±0.11	0.33±0.04	22.03±10.47	0.06±0.03	-	15.34±1.87	141.75±4.21	11.55±4.55	Avian Is.[3]	2007-10	Jerez et al. (2013a)
	1**	1.93	0.30	0.18	<0.01	-	22.89	182.58	15.83	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	6.81±11.91	0.60±0.4	0.06±0.05	0.04±0.07	-	92.06±74.53	133.88±71.42	12.01±5.8	King George Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	0.499±0.024	0.102±0.007	0.202±0.009	0.269±0.01	18.0±1	-	10.0±0.2	Potter Cove[4]	2002-3	Smichowski et al. (2006)
	3	-	-	7.20±0.12	0.30±0.05	-	11.90±0.5	140.0±4	6.80±0.2	AP[5]	1989	Szefer et al. (1993)
	5	-	-	7.50±2.4	-	-	19.90±5.8	-	-	Weddell Sea[6]	1982-3	Steinhagen-Schneider (1986)
P. antarctica	2	1.0±0.14	0.37±0.36	0.16±0.08	0.05±0.01	-	24.26±11.18	330.34±293.26	14.76±4.17	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4	2.02±1.47	0.67±0.15	27.54±14.47	0.15±0.06	-	14.95±0.67	126.05±25.18	9.30±2.06	Deception Is.[2]	2007-10	Jerez et al. (2013a)
	5	15.52±15.55	0.47±0.14	0.11±0.08	0.18±0.02	-	95.10±48.67	132.2±64.4	11.42±3.24	Deception Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	-	10.70±0.3	0.01	-	12.60±0.4	126.0±1	7.50±0.5	AP[5]	1989	Szefer et al. (1993)
A. forsteri	4	-	-	27.7±15.6	-	-	23.4±4	-	-	Weddell Sea[6]	1982-3	Steinhagen-Schneider (1986)
P. papua	3	2.19±0.52	1.01±0.9	1.05±1.43	0.10±0.07	-	102.57±155.93	112.56±72.69	7.71±6.34	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4**	1.62±1	0.79±0.63	0.40±0.18	<0.01	-	386.13±174.48	237.19±22.38	8.30±0.57	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	2.12±2.05	0.45±0.18	0.08±0.04	<0.001	-	142.4±63.85	152.91±45.53	10.51±3.74	King George Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	-	3.19±0.1	0.48±0.12	-	26.50±0.9	100.0±4	8.80±0.8	AP[5]	1989	Szefer et al. (1993)
	1***	-	-	2.32±0.27	0.50	-	24.70±0.19	72.0±0.53	7.10±0.17	Livingston Is.[2]	2002-6	Metcheva et al. (2010)
S. magellanicus	22	-	-	7.25±4.71	0.58±0.32	5.70±3.73	-	-	-	Rio Grande do Sul[7]	n/i	Kehrig et al. (2015)

*Sample collection, **Juvenile, ***Duplicates were performed from the sample, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Antarctic Peninsula, [4]King George Island (South Shetland Islands, West Antarctica), [5]Several locations of the Antarctic Peninsula, [6]Northeast of the Antarctic Peninsula, [7]Coast of Brazil.

Table 6 Mean trace metal levels (µg/g, dw) in muscles of different penguin species.

Species	N	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	1	-	-	-	-	0.6	-	-	-	Terra Nova Bay[1]	1989	Bargagli et al. (1998)
	1	3.27	0.37	1.09	<0.01	-	7.43	149.95	0.63	King George Is.[2]	2007-10	Jerez et al. (2013a)
	2	1.78±1.91	0.30±0.16	2.63±2.09	0.15±0.11	-	8.53±2.41	66.26±57.77	1.11±0.39	Avian Is.[3]	2007-10	Jerez et al. (2013a)
	1**	3.41	0.18	<0.01	<0.01	-	6.97	163.75	0.91	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	6.14±6.72	0.39±0.25	0.01±0.02	0.04±0.01	-	5.52±1.97	104.34±49.7	1.13±0.4	King George Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	0.815±0.007	<0.001	0.12±0.007	-	6.4±0.4	-	1.50±0.1	Potter Cove[4]	2002-3	Smichowski et al. (2006)
	9	-	-	0.46±0.42	0.04±0.01	-	7.90±0.7	46.7±10	0.51±0.16	AP[5]	1989	Szefer et al. (1993)
	5	-	-	0.32±0.02	-	-	8.20±2.4	-	-	Weddell Sea[6]	1982	Steinhagen-Schneider (1986)
P. antarctica	2	1.07±0.36	0.57±0.62	0.01±0.001	<0.01	-	8.07±1.28	139.91±40.94	0.87±0.28	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4	12.32±10.04	1.04±0.27	1.83±0.63	0.17±0.08	-	6.69±1.73	118.8±40.73	1.17±0.68	Deception Is.[2]	2007-10	Jerez et al. (2013a)
	5	114.88±125.59	0.59±0.3	0.01±0.01	0.20±0.06	-	6.82±1.2	105.08±55.41	2.55±1.53	Deception Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	-	0.57±0.02	0.01	-	9.70±0.3	37.0±2.9	0.76±0.31	AP[5]	1989	Szefer et al. (1993)
P. papua	3	1.39±0.95	0.63±0.53	0.11±0.18	0.18±0.05	-	7.97±1.15	103.07±60.55	0.85±0.39	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4**	2.01±1.96	0.36±0.21	0.01±0.01	<0.01	-	9.95±2.08	139.39±46.68	0.52±0.06	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	43.71±21.93	0.40±0.23	0.01±0.01	<0.001	-	4.43±1.46	106.6±37.42	1.46±0.43	King George Is.[2]	2008-9	Jerez et al. (2013b)
	3	-	-	0.02	0.01	-	8.20±0.5	35.7±3	0.46±0.03	AP[5]	1989	Szefer et al. (1993)
	1***	-	-	0.5±0.12	<0.60	-	5.60±0.31	24.0±0.24	1.4±0.13	Livingston Is.[2]	2002-6	Metcheva et al. (2010)
A. forsteri	4	-	-	0.35±0.09	-	-	5.50±2	-	-	Weddell Sea[6]	1982-3	Steinhagen-Schneider (1986)

*Sample collection, **Juvenile, ***Duplicates were performed from the sample, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Antarctic Peninsula, [4]King George Island (South Shetland Islands, West Antarctica), [5]Antarctic Peninsula (locations not specified), [6]Northeast of the Antarctic Peninsula, n/i = not informed.

Table 7 Mean trace metal levels (µg/g, dw) in stomach contents of different penguin species.

Species	N	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	5	-	-	-	-	0.08±0.01	-	-	-	Terra Nova Bay[1]	1989-90	Bargagli et al. (1998)
	1	349.72	0.47	0.45	0.07	-	4.85	26.57	6.64	King George Is.[2]	2007-10	Jerez et al. (2013a)
	2	46.80±54.31	3.22±0.06	1.10±0.8	0.28±0.19	-	66.42±34.43	38.99±14.05	2.20±0.11	Avian Is.[3]	2007-10	Jerez et al. (2013a)
	5	282.01±235.63	2.00±1.58	0.23±0.17	0.40±0.26	-	57.81±35.82	71.16±48.82	10.57±8.76	King George Is.[2]	2008-9	Jerez et al. (2013b)
	45	-	-	2.90	0.20	0.10	-	-	-	Edmonson Point[1]	1995	Ancora et al. (2002)
P. antarctica	2	641.07±255.18	1.44±1.83	0.17±0.06	0.03±0.04	-	51.07±49.14	49.39±8.3	9.33±4.97	King George Is.[2]	2007-10	Jerez et al. (2013a)
	1	193.52	1.77	0.71	0.12	-	54.86	46.67	5.99	Deception Is.[2]	2007-10	Jerez et al. (2013a)
	5	477.85±192.75	1.92±1.11	0.32±0.34	0.33±0.11	-	65.67±50.01	31.04±10.02	12.4±6.46	Deception Is.[2]	2008-9	Jerez et al. (2013b)
P. papua	2	2594.61±1306.72	2.0±0.09	0.09±0.11	0.71±0.42	-	30.51±35.73	19.84±4.63	82.43±27.49	King George Is.[2]	2007-10	Jerez et al. (2013a)
	4[**]	854.88±1000.14	0.28±0.07	0.12±0.07	0.05±0.02	-	7.33±1.13	41.09±16.40	16.27±15.69	King George Is.[2]	2007-10	Jerez et al. (2013a)
	5	2010.15±3231.82	2.04±2.92	0.24±0.15	0.17±0.14	-	58.69±28.48	31.46±12.52	36.89±66.39	King George Is.[2]	2008-9	Jerez et al. (2013b)

*Sample collection, [**]Juvenile, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Southern of the Antarctic Peninsula.

Table 8 Mean trace metal levels (μg/g, dw) in excreta of different penguin species.

Species	n	Al	As	Cd	Pb	Hg	Cu	Zn	Mn	Locations	Date*	References
P. adeliae	7	-	-	-	-	0.17±0.1	-	-	-	Terra Nova Bay[1]	1989-90	Bargagli et al. (1998)
	14	-	-	5.5	0.30	0.20	-	-	-	Edmonson Point[1]	1995	Ancora et al. (2002)
	n/i	-	-	-	0.5-3.7	0.15-0.25	-	-	-	Zhongshan Station[5]	2001	Yin et al. (2008)
	27	-	1.14±0.39	3.96±2.36	1.96±0.86	0.52±0.31	558.9±217.73	262.7±91.89	-	Arctowski[2]	2012-13	Celis et al. (2015a)
	18	-	0.95±0.41	2.77±0.92	1.53±0.75	0.40±0.18	585.8±196.22	215.8±42.47	-	Kopaitic Island[3]	2012-13	Celis et al. (2015a)
	10	-	0.72±0.47	1.78±0.39	0.59±0.5	0.10±0.08	402.9±54.76	215.7±91.18	-	Yalour Is.[3]	2012-13	Celis et al. (2015a)
	10	-	0.66±0.53	1.63±0.43	0.45±0.39	0.13±0.08	362.9±38	188.4±41.82	-	Avian Is.[3]	2012-13	Celis et al. (2015a)
P. antarctica	3	-	-	0.16±0.01	3.80±0.2	-	37.6±2	456.0±4.0	138.0±8	AP [6]	1989	Szefer et al. (1993)
	n/i	-	-	-	1-1.8	0.06-0.15	-	-	-	Barton Peninsula[2]	2000	Yin et al. (2008)
	4	-	0.43±0.24	3.30±0.18	1.06±0.6	-	168.9±40.8	295.7±59.01	-	Hydrurga Rocks[3]	2011-12	Espejo et al. (2014)
	10	-	0.40±0.15	1.89±0.35	1.07±1.5	-	229.9±39.23	246.8±53.37	-	Cape Shirreff[2]	2011-12	Espejo et al. (2014)
	9	-	0.70±0.26	3.13±0.59	1.31±0.78	-	259.99±79.51	227.8±63.9	-	Narebski Point[2]	2011-12	Espejo et al. (2014)

	9	-	0.55±0.31	1.88±0.65	1.27±0.35	-	286.7±85.75	210.0±115.58	-	Kopaitic Island[3]	2011-12	Espejo et al. (2014)
P. papua	n/i	-	-	-	0.11	0.15	-	-	-	Fildes Peninsula[2]	2002	Yin et al. (2008)
	10	316±47.5	5.13±1.79	1.03±0.36	<0.4	-	104.0±2.1	145.0±2.9	12.3±1.2	Livingston Is.[2]	2006-7	Metcheva et al. (2011)
	10	-	0.33±0.22	2.51±0.89	2.89±1.07	-	199.95±62.47	379.99±82.73	-	Base O'Higgins[3]	2011-12	Espejo et al. (2014)
	4	-	0.44±0.38	2.15±0.47	0.78±0.22	-	114.7±41.55	192.2±39.32	-	Yankee Harbour[2]	2011-12	Espejo et al. (2014)
	3	-	0.37±0.28	3.35±0.23	2.55±1.02	-	184.5±10.81	324.3±106.51	-	Mikkelsen Harbor[3]	2011-12	Espejo et al. (2014)
	4	-	0.43±0.13	2.16±0.35	0.87±0.53	-	130.05±20.02	195.38±32.84	-	Danco Is.[3]	2011-12	Espejo et al. (2014)
	10	-	0.36±0.29	2.14±0.91	2.74±1.3	-	222.51±85.48	201.2±63.39	-	Base G. Videla[3]	2011-12	Espejo et al. (2014)
	5	-	0.52±0.16	2.40±0.83	2.05±1.95	-	154.2±28.41	172.92±62.85	-	Yelcho Station[3]	2011-12	Espejo et al. (2014)
	4	-	0.33±0.17	1.98±0.11	2.51±0.84	-	148.8±32.3	246.95±30.37	-	Brown Station[3]	2011-12	Espejo et al. (2014)
	10	-	0.15±0.097	0.73±0.27	0.74±0.953	6.60±4.153	-	-	-	Base O'Higgins[3]	2011	Celis et al. (2012)
	10	-	0.38±0.176	1.72±0.832	0.34±0.388	1.15±0.828	-	-	-	Base G. Videla[3]	2011	Celis et al. (2012)
	11	-	-	1.58±1.11	0.08±0.08	-	146.0±76.17	142.97±35.51	22.43±8.57	Neko Harbor[1]	2014	Celis et al. (2015b)
	10	-	-	1.24±0.25	0.09±0.1	-	201.5±64.14	108.74±25.23	17.84±13.22	Doumer Is.[3]	2014	Celis et al. (2015b)

	10	-	-	1.97±0.86	1.46±0.49	-	222.51±85.48	201.2±63.39	36.62±16.97	Stranger Point[2]	2014	Celis et al. (2015b)
	10	-	-	2.92±0.81	1.68±0.58	-	266.83±42.77	317.92±46.6	44.75±10.67	Base O'Higgins[3]	2014	Celis et al. (2015b)
S. humboldti	20	-	1.84±2.65	47.70±38.71	1.80±0.3	0.77±0.83	147.79±146.42	487.11±395.15	-	Pan de Azúcar Is.[4]	2011-12	Celis et al. (2014)
	19	-	0.36±0.4	21.24±18.35	1.59±2.12	0.46±0.19	69.62±24.98	222.55±59.2	-	Chañaral Island[4]	2011-12	Celis et al. (2014)
	24	-	7.86±4.88	42.47±45.55	12.79±9.97	0.61±0.4	199.67±81.78	0.83±0.33	-	Cachagua Island[4]	2011-12	Celis et al. (2014)
A. forsteri	5	-	-	-	-	0.31±0.03	-	-	-	Terra Nova Bay[1]	1989-90	Bargagli et al. (1998)
A. patagonicus	n/i	-	-	-	0.6-1.1	0.25-0.35	-	-	-	Zhongshan Station[5]	2001	Yin et al. (2008)

*Sample collection, [1]Victoria Land (East Antarctica), [2]South Shetland Islands (West Antarctica), [3]Antarctic Peninsula (West Antarctica), [4]Coast of Chile, [5]Mirror Peninsula (East Antarctica), [6]Antarctic Peninsula (locations not specified), n/i = not informed.

Table 9 Mean trace metal levels (μg/g, dry weight) in blood, brain, testicles, embryo, spleen and heart of different penguin species.

Matrix	**n**	**Species**	**Al**	**As**	**Cd**	**Pb**	**Hg**	**Cu**	**Zn**	**Mn**	**Locations**	**Date***	**References**
Blood	10	*E. minor*	3.89±1.26	3.72±1.76	-	0.07±0.02	2.75±0.85	2.48±0.44	37.97±5.28	-	St. Kilda[1]	2012	Finger et al. (2015)
Blood	11	*E. minor*	3.19±0.84	1.07±1.22	-	0.04±0.01	0.86±0.23	2.14±0.42	33.47± 3.27	-	Phillip Is.[1]	2012	Finger et al. (2015)
Blood	10	*E. minor*	4.22±1.67	0.67±0.43	-	0.04±0.01	0.84±0.37	2.32±0.40	38.77±6.76	-	Notch Is.[1]	2012	Finger et al. (2015)
Brain	1	*P. adeliae*	-	-	-	-	0.43	-	-	-	Terra Nova Bay[2]	1989-90	Bargagli et al. (1998)
Testicles	1	*P. adeliae*	-	-	-	-	0.42	-	-	-	Terra Nova Bay[2]	1989-90	Bargagli et al. (1998)
Embryo	12	*P. papua*	14.56±2.4	<0.3	<0.05	<0.4	-	2.82±0.7	25.27±2.5	0.67±0.06	Livingston Is.[3]	2006-7	Metcheva et al. (2011)
Spleen	1**	*P. papua*	-	-	3.5±0.41	< 0.95	-	24.7±0.19	232.0±2.67	6.30±0.15	Livingston Is.[3]	2002-6	Metcheva et al. (2010)
Heart	1**	*P. papua*	-	-	0.1±0.02	0.20±0.09	-	11.3±0.09	91.5±0.67	1.0±0.25	Livingston Is.[3]	2002-6	Metcheva et al. (2010)

*Sample collection, **Duplicates were performed from the sample, [1]Victoria (Australia), [2]Victoria Land (East Antarctica), [3]South Shetland Islands (West Antarctica).

Table 10 Whole body trophic transfer coefficient (TTC) for *Pygoscelis* penguins.[1]

	Trace elements [2]							
Species	**Al**	**As**	**Cd**	**Pb**	**Hg**	**Cu**	**Zn**	**Mn**
P. papua	0.01	0.21	39.55	0.21	n/d	1.45	6.90	0.05
P. adeliae	0.02	0.24	27.12	0.67	5.29	0.38	3.07	1.03
P. antarctica	0.03	0.27	54.63	0.65	n/d	0.28	3.47	0.79

[1]TTC was calculated as the ratio of the concentration in the penguin's body (liver, kidneys, bones and muscles together) to the concentration in diet (stomach content). n/d = no data available for Hg levels in stomach content. [2]Data reported in different biotic matrices of pygoscelid penguins from Antarctic Peninsula area (Bargagli et al. 1998; Bargagli 2008; Barbosa et al. 2013; Jerez et al. 2013a; Jerez et al. 2013b; Smichowski et al. 2006; Steinhagen-Schneider 1986; Szefer et al. 1993).

Table 11 Locations and number of studies performed on trace elements in different species of penguins worldwide.

Region	Location	Ψ	Coordinates	Studies*	Metals reported	Reference
West Antarctica	Barton Peninsula	1	62°14'S, 58°46'W	1	Pb, Hg	Yin et al. 2008
	Potter Cove	2	62°14'16"S, 58°39'59"W	1	As, Cd, Pb, Hg, Cu, Mn	Smichowski et al. 2006
	Stranger Point	3	62°15'32.00"S, 58°36'54.00"W	1	Cd, Pb, Cu, Zn, Mn	Celis et al. 2015b
	Arctowski	4	62° 9'36"S, 58°28'25"W	1	As, Cd, Pb, Hg, Cu, Zn	Celis et al. 2015a
	King George Island	5	62°02′S, 58°21′W	4	Al, As, Cd, Pb, Hg, Cu, Zn, Mn	Brasso et al. 2014; Jerez et al. 2013a,b
	Admiralty Bay	6	62° 4'52"S, 58°23'41"W	1	Hg, Zn	Santos et al 2006
	Narebski point	7	62°12′S, 58°45′W	1	As, Cd, Pb, Hg, Cu, Zn	Espejo et al. 2014
	Fildes Peninsula	8	62°12′S, 58°58′W	1	Pb, Hg	Yin et al. 2008
	Yankee Harbour	9	62°31'60.00"S, 59°46'41.0"W	1	As, Cd, Pb, Cu, Zn	Espejo et al. 2014
	Livingston Island	10	62°37′S 60°27′W	4	Al, As, Cd, Pb, Cu, Zn, Mn	Metcheva et al. 2006, 2010, 2011; Jerez et al. 2011
	Cape Shirreff	11	62°28′S, 60°47′W	1	As, Cd, Pb, Cu, Zn	Espejo et al. 2014
	Byers Peninsula, Livingston Is.	12	62°38′S, 61°05′W	1	Al, As, Cd, Pb, Cu, Zn, Mn	Barbosa et al. 2013
	Hannah Point, Livingston Is.	13	62°39′16″S, 60°36′48″W	1	Al, As, Cd, Pb, Cu, Zn, Mn	Barbosa et al. 2013
	Deception Island	14	62°56'27"S, 60°35'39"W	3	Al, As, Cd, Pb, Cu, Zn, Mn	Jerez et al. 2011; Jerez et al. 2013a,b
	Base O´Higgins	15	63°19′15″S, 57°53′55″W	3	Al, As, Cd, Pb, Hg, Cu, Zn, Mn	Celis et al. 2012; Espejo et al. 2014, Celis et al. 2015b
	Kopaitic	16	63°18'59"S, 57°54'47"W	2	As, Cd, Pb, Hg, Cu, Zn	Celis et al. 2015a, Espejo et al. 2014
	Mikkelsen Harbor	17	63°53'22"S, 60°47'3"W	1	As, Cd, Pb, Hg, Cu, Zn	Espejo et al. 2014
	Hydrurga Rocks	18	64° 8'40"S, 61°40'22"W	1	As, Cd, Pb, Cu, Zn	Espejo et al. 2014
	Danco Island	19	64°43'53"S, 62°35'44"W	1	As, Cd, Pb, Cu, Zn	Espejo et al. 2014
	Ronge Island	20	64°43′S, 62°41′W	1	Al, As, Cd, Pb, Cu, Zn, Mn	Jerez et al. 2011
	Neko Harbor	21	64°50′S, 62°33′W	1	Cd, Pb, Cu, Zn, Mn	Celis et al. 2015b
	González Videla Base	22	64° 49′ 26″ S, 62° 51′ 26″ W	2	As, Cd, Pb, Hg, Cu, Zn	Celis et al. 2012; Espejo et al. 2014
	Brown Station	23	64°53'43.2"S, 62°52'13.7"W	1	As, Cd, Pb, Cu, Zn	Espejo et al. 2014
	Paradise Bay	24	64°53′S, 62°53′W	1	Al, As, Cd, Pb, Cu, Zn, Mn	Jerez et al. 2011

	Yalour Island	25	65°14'2"S, 64°13'26"W	2	Al, As, Cd, Pb, Hg, Cu, Zn, Mn	Jerez et al. 2011; Celis et al. 2015a
	Yelcho Station	26	64°52′33″S 63°35′02″W	1	As, Cd, Pb, Cu, Zn	Espejo et al. 2014
	Doumer Island	27	64°51′S, 63°35′W	1	Cd, Pb, Cu, Zn, Mn	Celis et al. 2015b
	Avian Island	28	67°46'12"S, 68°53'40"W	2	Al, As, Cd, Pb, Hg, Cu, Zn, Mn	Celis et al. 2015a; Jerez et al. 2013a
	Weddell Sea	29	77°S 49°W	1	Cd, Cu	Steinhagen-Schneider 1986
East Antarctica	Edmonson Point	30	74°20´S 165°8´E	1	Cd, Pb, Hg	Ancora et al. 2002
	Terra Nova Bay	31	74°47'30"S, 164°51'35"E	1	Hg	Bargagli et al. 1998
	Adélie Land	32	66°40´ S, 140°01´ E	1	Hg	Carravieri et al. 2016
	Zhongshan Station	33	69°22´ S,76°22´ E	1	Pb, Hg	Yin et al. 2008
West Coast of South America	Pan de Azúcar Is., Chile	34	26° 9'S, 70°41'29"W	1	As, Cd, Pb, Hg, Cu, Zn	Celis et al. 2014
	Chañaral Is., Chile	35	29° 1'33"S, 71°34'5"W	1	As, Cd, Pb, Hg, Cu, Zn	Celis et al. 2014
	Cachagua Is., Chile	36	32°35'6"S, 71°27'24"W	1	As, Cd, Pb, Hg, Cu, Zn	Celis et al. 2014
East Coast of South America	Rio Grande do Sul, Brazil	37	31°11'20"S, 50°51'47"W	1	Cd, Pb, Hg	Kehrig et al. 2015
	Punta Tombo, Argentina	38	44° 2'17"S, 65°12'2"W	1	Hg	Frias et al. 2012
Subantarctic areas	South Georgia Is.	39	54° 16′ 53″ S, 36° 30′ 28″ W	1	Hg	Pedro et al. 2015
	Crozet Islands	40	46°26′S, 51°45′E	2	Hg	Scheifler et al. 2005; Carravieri et al. 2016
	Kerguelen Islands	41	49°21′S, 70°18′E	1	Hg	Carravieri et al. 2013
	Amsterdam Island	42	37°50´ S, 77°31´ E	1	Hg	Carravieri et al. 2016
Coast of Australia	Victoria	43	38°48'53"S, 146° 5'36"E	1	Al, As, Cd, Pb, Hg, Cu, Zn	Finger et al. 2015

$^{\Psi}$Number of the position pointed in Fig. 5; *Number of studies performed.

Table 12 Mean concentrations (µg/g) of trace elements in different species of penguins around the world.[1]

Species	Al	As	Cd	Pb	Hg	Cu	Zn	Mn
A. forsteri	-	-	99.42±148.53 (n=12)	-	0.92±0.63 (n=17)	16.0±9.34 (n=12)	-	-
A. patagonicus	-	-	-	0.06 (n= n/d)	1.98±0.82 (n=156)	-	-	-
E. chrysocome	-	-	-	-	1.32±0.79 (n=72)	-	-	-
E. chrysolophus	-	-	-	-	1.61±0.95 (n=72)	-	-	-
E. minor	12.45±14.59 (n=66)	0.98±1.39 (n=66)	0.05±0.01 (n=35)	0.13±0.15 (n=66)	2.13±1.29 (n=66)	6.61±4.72 (n=66)	58.73±24.29 (n=66)	-
E. moseleyi	-	-	-	-	1.42±0.95 (n=39)	-	-	-
P. antárctica	86.55±160.45 (n=203)	0.60±0.60 (n=235)	10.62±48.13 (n=219)	0.57±0.82 (n=244)	0.07±0.01 (n=92)	49.70±77.42 (n=244)	140.88±95.86 (n=244)	10.33±25.92 (n=212)
P. adeliae	37.32±86.28 (n=119)	0.58±0.66 (n=168)	18.94±69.40 (n=258)	0.32±0.48 (n=268)	0.41±0.36 (n=289)	66.39±149.76 (n=220)	118.69±66.22 (n=198)	32.67±150.85 (n=140)
P. papua	218.91±604.66	0.66±1.04	1.78±5.75	0.64±0.78	2.60±2.38	61.28±86.09	127.55±83.87	9.08±15.04

	(n=231)	(n=291)	(n=357)	(n=385)	(n=290)	(n=365)	(n=366)	(n=325)
S. humboldti	-	3.35±3.97	37.14±14.01	5.39±6.41	0.61±0.16	139.03±65.47	236.83±243.45	-
		(n=63)	(n=63)	(n=63)	(n=63)	(n=63)	(n=63)	
S. magellanicus	-	-	17.96±24.97	0.42±0.25	1.55±2.23	-	-	-
			(n=66)	(n=66)	(n=142)			

[1]Mean values were obtained among all the biological matrices; n/d = no data available.

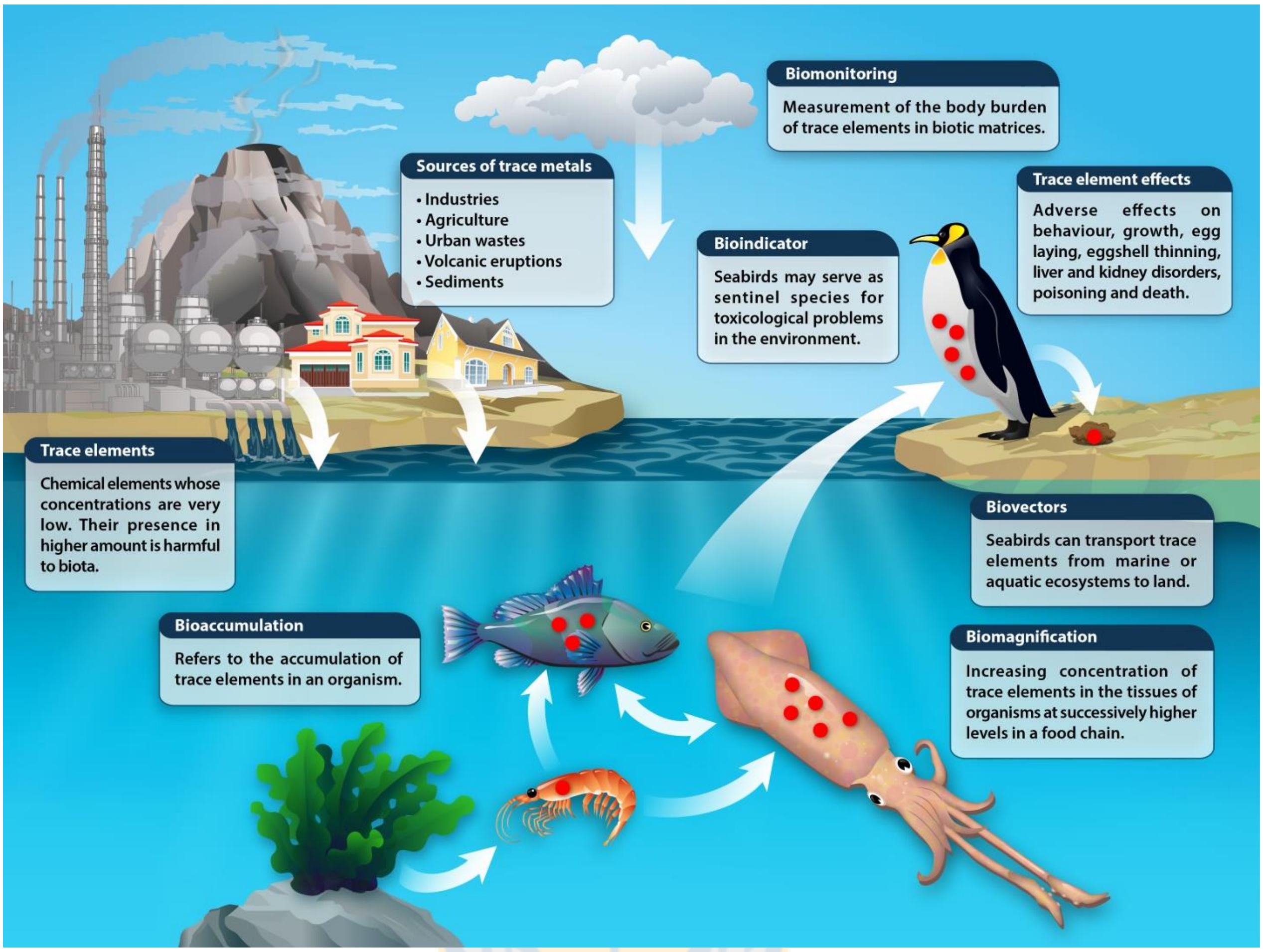

Fig 1 Sources of trace elements in the environment, bioaccumulation, biomagnification and effects on penguins. According to Newman (2015), bioaccumulation is the net accumulation of a contaminant on an organism from all sources including water, air and solid phases (food, soil, sediment, or fine particles suspended in air or water) in the environment. Biomonitoring is the use of organisms to monitor contamination and its possible effects on biota (at individual level, population, or communities) and ecosystems.

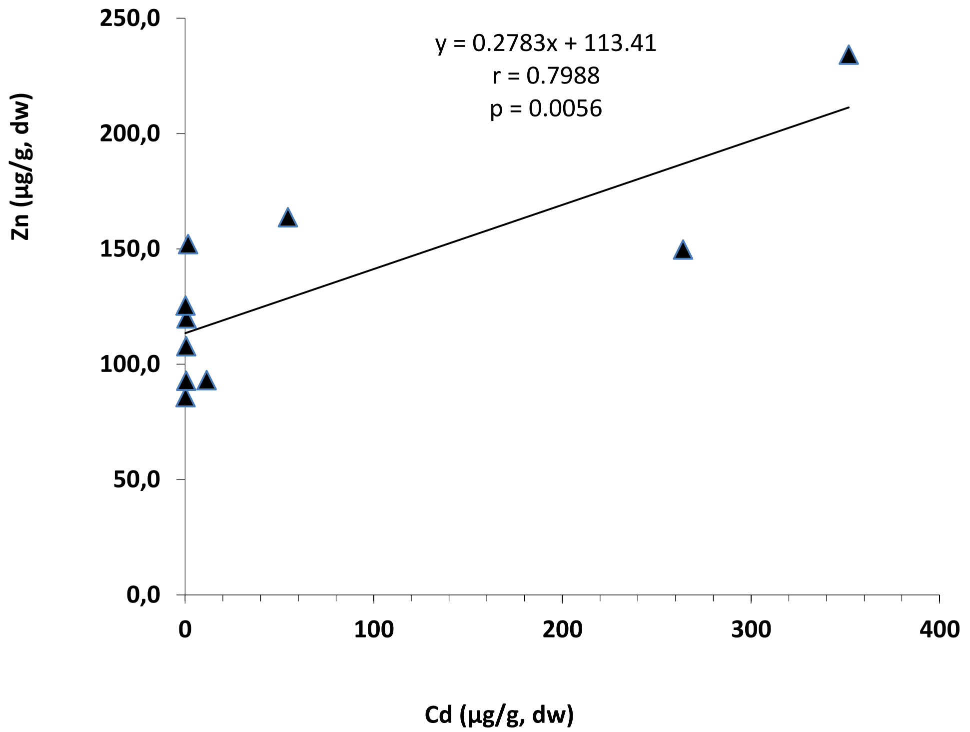

Fig 2 Relationship between renal concentrations of Cd and Zn in *Pygoscelis* penguins from the Antarctic Peninsula area (West Antarctica). Data correspond to mean values reported by Jerez et al. (2013a) and Jerez et al. (2013b), during 2007-2010.

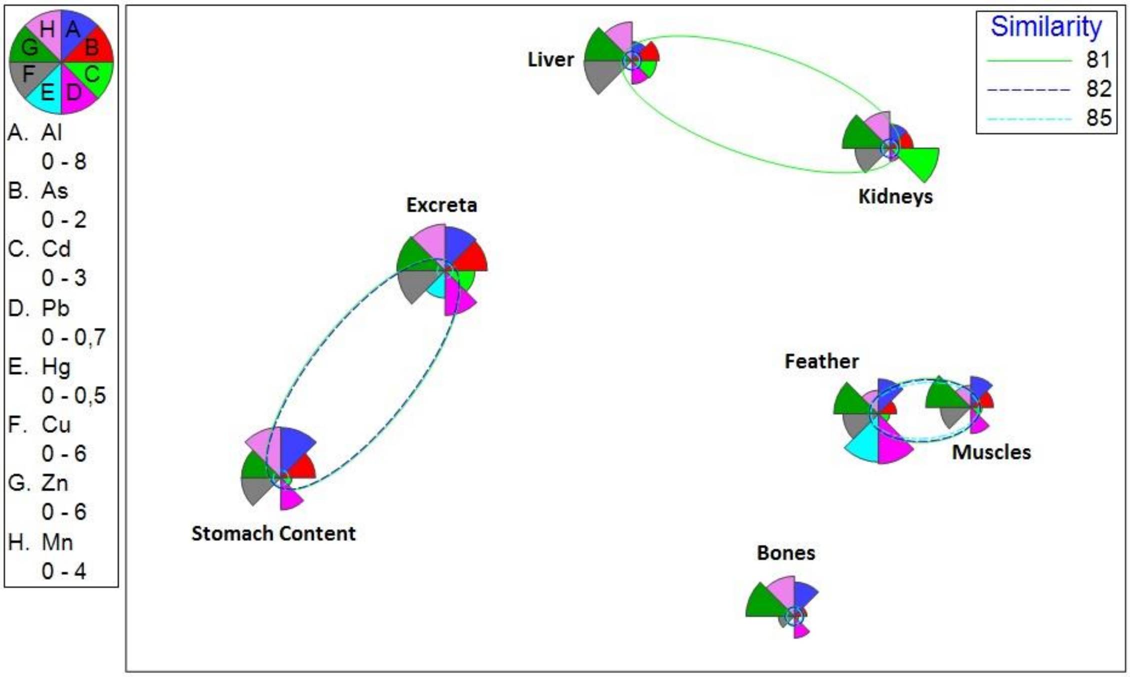

Fig 3 Bubble chart for mean concentrations of metals in different biological matrices of *Pygoscelis papua* reported from South Shetland Islands. Mean concentrations of trace elements in different biotic matrix correspond to *Pygoscelis papua* from South Shetland Islands. Data taken from Barbosa et al. (2013), Brasso et al. (2014), Celis et al. (2015b), Espejo et al. (2014), Jerez et al. (2011), Jerez et al. (2013a, b), Metcheva et al. (2006, 2010, 2011), Santos et al. (2006), Yin et al. (2008).

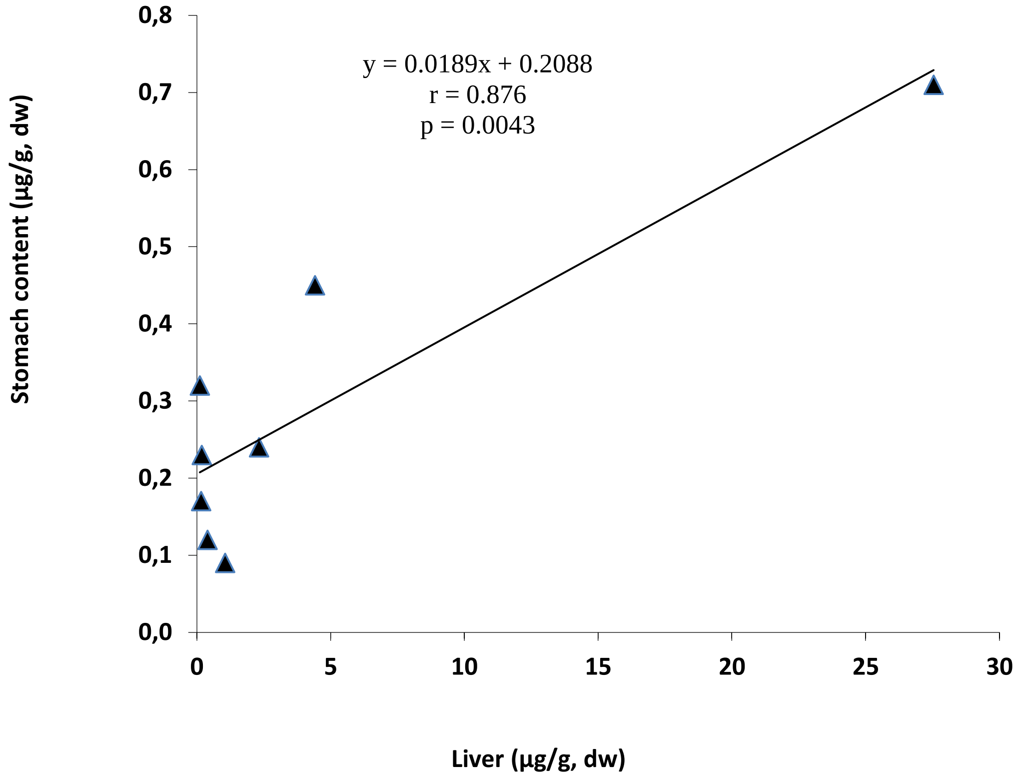

Fig 4 Relationship of Cd between the liver and stomach content for *Pygoscelis* penguins. Data correspond to mean values reported by Jerez et al. (2013a, b) from South Shetland Islands (West Antarctica) during 2007-2010.

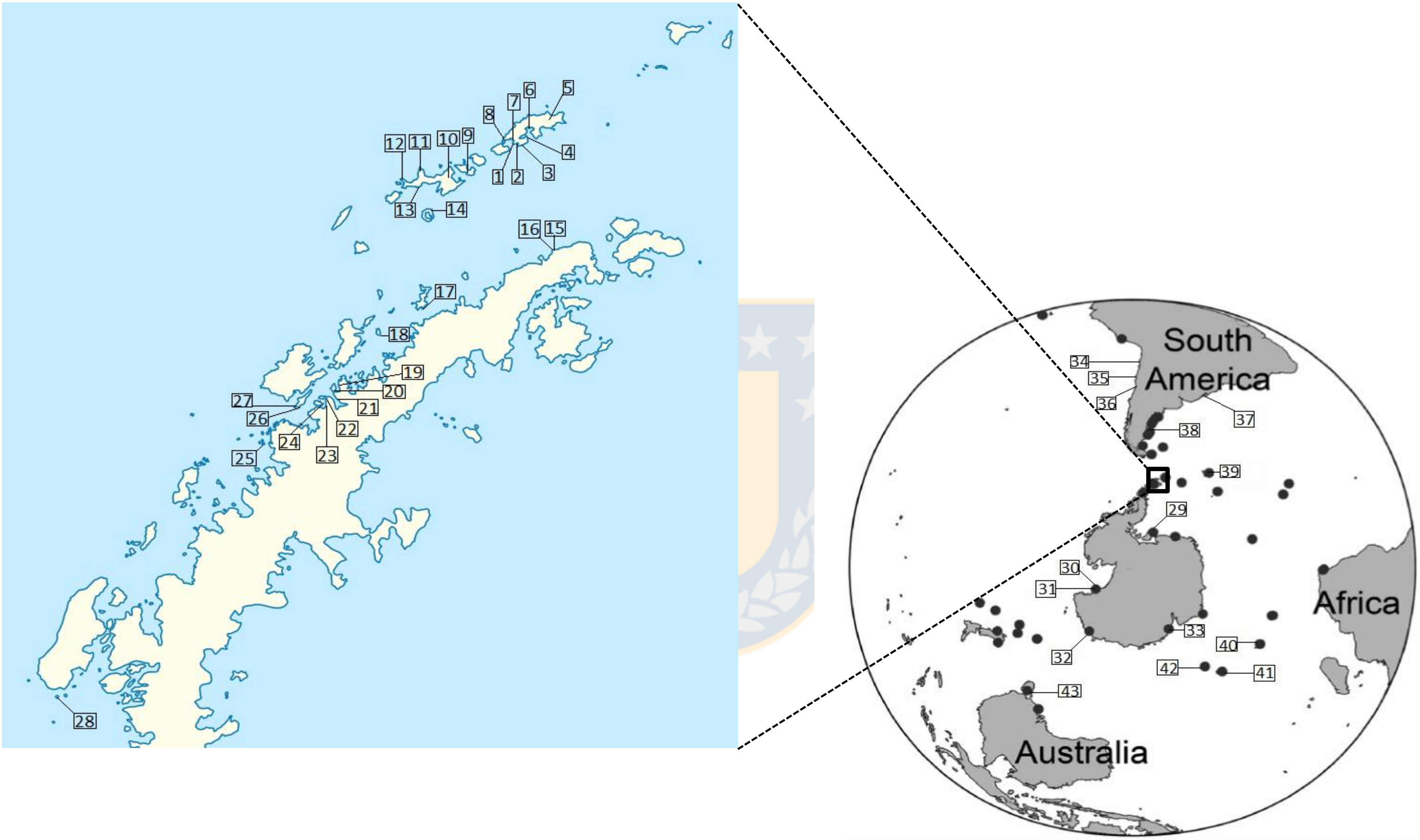

Fig 5 Geographical distribution of trace metals reported in penguins. Each number is associated with the information given in Table 11.

Capitulo III Trace element concentrations in biotic matrices of Gentoo penguins (*Pygoscelis papua*) and coastal soils from different locations of the Antarctic Peninsula

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Abstract

The aim of this work is to increase the information on trace metals in seabirds and coastal soils in the Antarctica. Concentrations (mg kg^{-1} dry weight) of Cd, Cu, Cr, Ni, Mn, Zn and Pb were determined by ICP-MS in fresh excreta and feathers of Gentoo penguins as well as in soils around the nesting sites where this species inhabits. Samples were collected in four locations throughout the Antarctic Peninsula (January 2014): O'Higgins Base, Stranger Point, Neko Harbor and Doumer Island. The highest levels of elements were found in excreta from O'Higgins Base (2.92, 266.83, 2.99, 44.75, 18.15, 1.68 and 317.92 for Cd, Cu, Cr, Mn, Ni, Pb and Zn, respectively) and Stranger Point (1.97, 222.51, 2.98, 36.62, 13.41, 1.46 and 201.18 for Cd, Cu, Cr, Mn, Ni, Pb and Zn, respectively. Similarly, the highest levels were found in feathers from O'Higgins Base (0.21, 20.89, 1.44, 1.19, 5.90, 0.63 and 64.07 for Cd, Cu, Cr, Mn, Ni, Pb and Zn, respectively) and Stranger Point (0.14, 19.65,

1.47, 1.23, 3.85, 0.60 and 64.19 for Cd, Cu, Cr, Mn, Ni, Pb and Zn, respectively). In soils, the highest levels were found in O'Higgins Base (4.31, 421.94, 64.75, 404.76, 28.13, 281.54 and 484.99 for Cd, Cu, Cr, Mn, Ni, Pb and Zn, respectively), whereas the lowest levels were found in Neko Harbor and Doumer Island. These results observed could be related to the major human presence in the northern area of the Antarctic Peninsula and large-scale transport of pollutants. The metals detected in the excreta of the Gentoo penguin can contribute to increase the contamination of coastal terrestrial ecosystems, which could also affect other living organisms.

Keywords Heavy metals; Seabirds; Penguins; Guano; Biomonitoring; Antarctic pollution.

1 Introduction

Antarctica is one of the last pristine places on the planet inhabited by a wide variety of organisms with unique ecophysiological characteristics, like penguins. It is quite necessary to investigate now baseline levels of chemical elements to monitor possible future changes in Antarctica. Heavy metal contamination is wide spread globally as a result of human activities such as oil spills, sewage, hazardous wastes, among others (Nriagu & Pacyna 1988). Due to the cumulative nature and persistence of metals, these can be found usually in water, air or soil, as well as in both flora and fauna (Zhao et al. 2006). There is evidence showing that remaining pristine regions of the planet are being affected by anthropogenic activities (Smichowski et al. 2006; Boersma 2008). Some studies have shown pollution affecting Antarctic fauna and soils, which could be linked to a raising tourist activity and research activities, some local environmental accidents and large-scale transport of pollutants (Lohan et al. 2001; Sanchez-Hernandez 2000; Santos el at. 2005; Negri et al. 2006; Espejo et al. 2014).

Even though concentrations of most trace elements in Antarctica appear to be very low, a continuous level of contamination have been noted along the Antarctic Peninsula which could be affecting some endemic species, like penguins (Yin et al. 2008; Jerez et al. 2011; Espejo et al. 2014). Antarctic ecosystems are particularly depending on anthropogenic modifications, where some characteristics of marine biota, such as narrow reproductive season, make it highly susceptible to human impact and sensitive to environmental contamination (Santos et al., 2005). Among the many ways of measuring the degree of contamination of seabirds, feathers are good biomaterials for monitoring a particular environment state by means of trace metals since metals have a high affinity for the sulfhydryl groups of the feather's structural proteins (Metcheva et al. 2006). Excreta can be used as biomonitors to study the level of heavy metals in different remote polar environments (Yin et al. 2008; Espejo et al. 2014). Both biotic matrices are not restricted by international regulations for wildlife protection, are low-cost and are easily sampled. Birds can eliminate heavy metals through excrements and feathers (Metcheva et al. 2011).

Moreover, seabirds are significant biovectors of contaminants via excreta from the ocean to the land (Michelutti et al. 2009). For that reason, excreta and feathers have been used as appropriate bioindicators to study pollution in marine ecosystems with a minimum of human intervention (Sun & Xie 2001; Ancora et al. 2002; Yin et al. 2008; Jerez et al. 2011; Celis et al. 2012).

The Gentoo penguin is a seabird at the top of the food chain of Antarctic marine ecosystems, thus playing an important role in the ecology of the coastal zones of Antarctica (Celis et al. 2012). There are some important colonies of this species that inhabit the Antarctic Peninsula. The northern of the Antarctic Peninsula concentrates many anthropogenic activities (Tin et al. 2009). Some indirect pollution (fuel combustion, waste incineration, sewage disposal, paint or accidental oil spills) or the impact of the tourism increases and scientific bases with their associated activities as plane and ship trips have been noted in the northern area of the Antarctic Peninsula (IAATO 2010; Barbosa et al. 2013). Sewage disposal, paints residues from buildings and petroleum are mentioned as the most probably sources of trace metals enrichment in areas near research stations at King George Island, although contamination levels are lower than others Antarctic research stations and industrialized regions (Santos et al. 2005). There is some evidence indicating Pb contamination caused by Antarctic scientific stations, which is linked to battery leaks and paint waste (Sheppard et al. 2000). However, the available data on the levels of heavy metals in biotic matrices of Antarctic penguins are still scarce and fragmented (Ancora et al., 2002; Metcheva et al., 2006; Yin et al., 2008; Jerez et al., 2011; Metcheva et al., 2011; Espejo et al., 2014). Soils accumulate contaminants and serve as sources of pollution to the ecosystems they are connected with, but the knowledge about heavy metals in soils is also scarce in Antarctica (Claridge et al. 1995; Sheppard et al. 2000; Webster et al. 2003; Santos et al. 2005; Bueno et al. 2011).

In Antarctica, most of the anthropogenic activities and wildlife are congregated in a small ice-free land with less than 0.34% of the continent (BAS 2004). Further studies are extremely required for identifying and preventing pollution in Antarctica, where the amount of contaminated area acquires a huge importance of the proportion of the habitat that is affected. The levels of trace metals are an important indicator of environmental quality and animal health for long-term biomonitoring in the Antarctic. Our objective is to increase the information on this issue at geographical scale investigating the levels of Cd, Cu, Cr, Pb, Mn, Ni and Zn in: i) excreta and feathers of Gentoo penguins that inhabit some important nesting sites of the Antarctic Peninsula; ii) soils in front of the penguin colonies studied here.

2 Study Site and Methodology

2.1 Sampling

During January 2014 of the austral summer, fresh biotic samples (excrements and feathers) from colonies of Gentoo penguins and soil samples from the surroundings were collected from four different locations along the Antarctic Peninsula area: O'Higgins Base (63º19'S, 57º53'W), Stranger Point (58º37'S, 62º16'W), Doumer Island (64º51'S, 63º35'W) and Neko Harbor (64º50'S, 62º33'W). In Fig. 1 are shown the studied locations. In Table 1 are indicated the geographical locations and sample sizes for each locality.

Feather samples were collected individually in polyethylene bags from adult individuals. Excreta samples were carefully taken from the ground to avoid contamination. All samples were always handled with disposable plastic gloves. Each excrement sample was collected from many individuals, spanning the length of the penguin colony, since it was not possible to evaluate metal contamination in each individual, but rather only in a group of that species. Soil samples (top 10cm) were collected at random in the marine terrace about 20 m from each penguin colony. Very clean steel containers and sealed plastic bags were also used. Soil samples were collected at random around of 20 m from each penguin colony. Once brought to the laboratory, all samples were kept in sealed plastic bags and stored in pre-cleaned aluminium foil at -4ºC for transport until their analyses.

2.2 Preparation of samples and chemical analysis

In the laboratory (accredited ISO 17025), samples were washed with distilled water (Milli-Q system, Millipore, USA), dried at room temperature and then ground and screened (24 mesh dm^{-2}). Samples were microwave digested with nitric acid, hydrochloric acid, and perchloric acid. The levels of elements in the samples were determined by mass spectrometry inductively coupled plasma (ICP-MS Perkin Elmer-Optima 800). The detection limits (mg L^{-1}) of the elements determined were as follows: 0.005 for Cd, Cu, Cr, Ni and Zn; 0.01 for Mn; 0.0025 for Pb. All measurements were carried out in triplicate, and resulting values were averaged. Non-detectable values were predicted from expected normal scores when more than 50 % of all samples showed detectable levels within each data set (Smith et al. 2007). In order to ensure quality control, a certified reference material (human hair, GBW07601) supplied by National Research Center of China was used as an internal standard in a proportion of 10 % each batch of samples.

2.3 Statistical analysis

The detected levels are presented as mean ± standard deviation in dry weight. Non-parametric statistical methodologies were used because of the assumptions of normality and homoscedasticity were not met even after the log transformation of the data. Differences among metal and trace concentrations were assessed by means of the Kruskal-Wallis non-parametric analysis of variance and Mann-Whitney U tests. Post hoc tests were carried out by means of Kruskal-Wallis analyses, using the critical differences of mean rank. A significance level of $p < 0.05$ was considered to indicate statistical significance. Data were analyzed by means of the SPSS 15.0 statistical software

3 Results and Discussion

3.1 Trace metals in feathers

Concentrations of Cd, Cu, Cr, Mn, Ni, Pb and Zn in feathers of *Pygoscelis papua* are shown in Table 2.

The feathers of Gentoo penguins showed significant differences among the different locations for several trace metals. Levels of Cu, Ni, Pb Zn in feathers of this species are significantly higher in O'Higgins Base in comparison to Doumer Island and Neko Harbor, whereas there are not significant differences between O'Higgins Base and Stranger Point. The lowest significant levels of Cr and Mn in feathers of *P. papua* were detected in Doumer Island, whereas the lowest Cd levels were found in Neko Harbor.

Trace metals found in feathers of Gentoo penguins, considering all the sampling sites together, showed the following relation: Zn > Cu > Ni > Cr > Mn > Pb > Cd. This relation of similar to these observed by Jerez et al. (2011) in *P. papua* from King George Island and by Metcheva et al. (2006) in the same species from Livingstone Island. These higher Zn levels can be related to Cd concentrations, because an increase in the Zn levels can reduce the toxic effects of Cd in animals (Goyer 1997).

The highest mean Cd levels detected in penguin feathers in the present study (0.21 mg kg^{-1}) were 7 times higher than those levels observed by Jerez et al. (2011) in Gentoo penguin feathers from King George Island, and were similar than the levels found by Ancora et al. (2002) in feathers of Adélie penguins (*Pygoscelis adeliae*) from Terra Nova Bay a decade ago. Our Cd levels were half of those levels detected by Metcheva et al. (2011) in feathers of Gentoo penguins from Livingstone Island. Cadmium is a metal with a great ecotoxicological importance, since it is directly associated with human activities (Boersma 2008). This element can be widely distributed into air, water and soil, being water the most important site for its final deposition as a soluble form (Nriagu & Pacyna 1988). Once Cd is deposited in waters it passes to the food chain (Bargagli et al. 1996). Cd levels measured in seabird feathers from other regions of the world range between 0.01 to 0.40 mg kg^{-1} d.w. (Liu et al. 2006; Burger et al. 2008; Ribeiro et al. 2009; Lucia et al. 2010).

Our highest mean Pb levels in penguin feathers were similar than those reported by Jerez et al. (2011) in Gentoo penguins and Chinstrap penguins (*Pygoscelis antarctica*), whereas are 2.8 times lower than those levels detected in feathers of Adélie penguins along the Antarctic Peninsula. Our Pb levels were 1.5 times higher than those levels found by Ancora et al. (2002) in Adélie penguin feathers from Terra Nova (Antarctica), whereas were half lower than those detected by Yin et al. (2008) in penguin feathers along West Antarctica. Our Pb levels were 2.4 times lower than those levels detected by Metcheva et al. (2011) in feathers of Gentoo penguins from Livingstone Island. Pb is released by human activities such as burning fossil fuels, solid waste incineration, paints, and accidental oil spills (Bargagli 2008). Our highest Pb levels detected seem related to the great concentration of human activities that exists in King George Island, where most scientific bases are concentrated and where there is heavy traffic of all kind of vehicles to transport tourists, scientists and support personnel (Tin et al. 2009). Our Pb levels appear to be lower than those reported in feathers of other seabirds (0.71 to 1.88 mg kg^{-1} d.w.) from the Northern Hemisphere (Burger et al. 2008; Ribeiro et al. 2009).

Regarding to Cu, the maximum mean levels found in our study in feathers of Gentoo penguins (20.89 mg kg^{-1} d.w.) were similar to those levels measured by Jerez et al. (2011) in penguin feathers along the South Shetland Islands, and those levels detected by Yin et al. (2008) in penguin feathers from different location of Antarctica. Also, our Cu levels are similar to Cu levels found by Metcheva et al. (2006) in feathers of Gentoo and Chinstrap penguins from Livingstone Island. Two decades ago, Honda et al. (1986) reported Cu levels of 14.49 mg kg^{-1} (d.w.) in feathers of Adélie penguins from the Northeast of Antarctica.

With regard to Zn, the highest mean levels found in Gentoo penguin feathers (64.19 mg kg^{-1} d.w.) were 2.3 times lower than those levels measured by Yin et al. (2008) and similar than those reported by Jerez et al. (2011) in feathers of penguins from different locations of Antarctica. Our Zn levels were 30% lower than those levels detected by Metcheva et al. (2011) in feathers of Gentoo penguins from Livingstone Island. In the current study, the maximum Zn concentrations are directly related to the highest Cd levels. The Zn is a trace metal essential for biota, and it is directly linked to Cd, in such a way that high levels of Zn may be due to reactive processes of physiological adaptation of penguins to high concentrations of Cd (Metcheva et al. 2006).

Our Ni levels detected in feathers of Gentoo penguins (5.90 mg kg^{-1} d.w.) are nearly 10 times higher of those found by Jerez et al. (2011) in feathers of the same species from King George Island, 5 times higher than those levels found in feathers of Chinstrap penguins from Ronge Island and 6.5 times higher than the levels detected in feathers of Adélie penguins from Yalour Island. Previously, a study showed Ni levels of 0.44 mg kg^{-1} (d.w.) in feathers of Adélie penguins from Northeast of Antarctica (Honda et al. 1986). Levels of Ni in feathers of several seabirds from French coast are situated between 0.90-14.10 mg kg^{-1} d.w. (Lucia et al. 2010).

The highest Mn levels were detected in Stranger Point, which are 32% lower than those levels found by Jerez et al. (2011) in feathers of Gentoo penguins from King George Island, 2.6 times lower than Mn levels found in feathers of Chinstrap penguins from Deception Island and similar than those found in Adélie penguins from King George Island. Our highest concentration of Mn is twice as lower than those reported by Honda et al. (1986) in feathers of penguins from Northeast of Antarctica. Our Mn levels were 29% lower than those levels detected by Metcheva et al. (2011) in feathers of Gentoo penguins from Livingstone Island. When comparing Mn levels in penguin feathers with Mn levels measured in other regions, we observe that the present Mn range measured (0.27-1.23 mg kg^{-1} d.w.) is lower to Mn range (0.75-2.84 mg kg^{-1} d.w.) detected in feathers of different seabirds from the Northern Hemisphere (Burger et al. 2008; Ribeiro et al. 2009).

We found the highest concentration of Cr in feathers of Gentoo penguins from Stranger Point, where Cr levels were about 20%, 5.5 and 4.3 times lower than those reported from King George Island in feathers of the same species, Chinstrap penguins and Adélie penguins, respectively (Jerez et al. 2011). Our Cr levels were 8.6 times higher than those levels detected by Metcheva et al. (2011) in feathers of

Gentoo penguins from Livingstone Island. The present Cr range measured (0.73-1.47 mg kg^{-1} d.w.) is lower or even similar than the range (1.08-1.53 mg kg^{-1} d.w.) detected in feathers of seabirds from the Northern Hemisphere (Burger et al. 2008; Ribeiro et al. 2009). Cr is linked to oil contamination (Alam & Sadiq 1993; Caccia et al. 2003), thus our highest Cr levels detected in Stranger Point and O'Higgins Base could be related to the major anthropogenic activities in these locations, as similarly noted by Jerez et al. (2011).

3.2 Trace metals in excreta

Concentrations of Cd, Cu, Cr, Mn, Ni, Pb and Zn in excreta of *Pygoscelis papua* are shown in Table 3.

The excreta of Gentoo penguins showed significant differences among the different locations for several trace metals. Levels of Ni and Pb in excreta of this species are significantly higher in O'Higgins Base in comparison to Doumer Island and Neko Harbor, whereas the levels of these chemical did not show significant differences between O'Higgins Base and Stranger Point. With respect to Cr, Mn and Zn, *P. papua* droppings showed that the levels in Doumer Island are significantly the lowest detected in this study.

The levels of trace metals found in excreta of Gentoo penguins indicated the following relation: Zn > Cu > Mn > Ni > Cr > Cd > Pb. This relation is similar to that reported by Metcheva et al. (2011) in feces of the same species from Antarctica. The same descendent order among Zn, Cu and Pb was observed by Yin et al. (2008) in Chinstrap penguin droppings from King George Island. The lower Pb levels found in penguin excreta can be explained because this metal tends to bioaccumulate mainly in bones (Teodorova et al. 2003).

The highest mean Cd levels measured in Gentoo penguin excreta were found in O'Higgins Base, and were about a half of those levels observed by Ancora et al.

(2002) in feces of Adélie penguins from Terra Nova Bay, Antarctica. In our study, the Cd levels were near twice and 3 times higher than those found by Celis et al. (2012) and Metcheva et al. (2011) in excreta of Gentoo penguins from Antarctica Peninsula, respectively. Our Cd levels are 13% lower than those Cd levels found by Espejo et al. (2014) in Gentoo penguins from Mikkelsen Harbor and Chinstrap penguins in Hydrurga Rocks. Our Cd levels are about 16 times lower than those levels found by Celis et al. (2014) in excreta of Humboldt penguins (*Spheniscus humboldti*) from the Northern of Chile.

With regard to Pb, the highest mean levels of this element were found in excreta of Gentoo penguins from O'Higgins Base (1.68 mg kg^{-1} d.w.), which are 40% lower than those Pb levels reported by Espejo et al. (2014) and about twice higher than those levels found by Celis et al. (2012) in Gentoo penguins excreta from the same location. Our Pb levels were near 4 times higher the levels measured by Metcheva et al. (2011) and 1.7 times those reported by Yin et al. (2008), all data collected from faeces of Gentoo penguins from different locations of Antarctica. In addition to that, our Pb levels overcame by about 4 times the values reported by Ancora et al. (2002) in excreta of Adélie penguins. Our Pb levels were about 6 times lower than those found by Celis et al. (2014) in Humboldt penguin excreta from the Northern of Chile. When comparing with other regions and species, our Pb levels are lower than 10 mg kg^{-1} d.w. as described in faeces from other species of birds from non-contaminated areas of the world (Beyer et al. 1997; Mateo et al. 2006; Martinez-Haro et al. 2010).

The maximum mean Cu levels found in our study were 2.7 times higher than those levels measured by Metcheva et al. (2011) in Gentoo penguin excreta, and 1.5 times lower than those reported by Yin et al. (2008) in feces of Adélie penguins and Chinstrap penguins from different locations of Antarctica. Additionally, our Pb levels are 1.3 times higher than those levels in excreta of Humboldt penguins from the Northern of Chile (Celis et al. 2014). With regard to Zn, the highest mean levels found were 2.2 times higher than those levels measured by Metcheva et al. (2011) in excreta of Gentoo penguins, whereas were 17% lower than those levels reported by Yin et al. (2008) in Adélie penguin and Chinstrap penguin droppings from different locations of Antarctica. Our Pb levels are 1.5 times lower than those levels in excreta of Humboldt penguins from the Northern of Chile (Celis et al. 2014). In the current study, the highest Zn concentration is directly related to the highest Cd level detected in droppings of Gentoo penguins at O'Higgins Base. The Zn is a trace metal essential for biota, and it is directly linked to Cd, in such a way that high levels of Zn may be due to reactive processes of physiological adaptation of penguins to high concentrations of Cd (Metcheva et al. 2006).

Our Cr, Mn and Ni levels detected in excreta of Gentoo penguins were 1.5, 3.7 and 28.5 times higher than those levels reported by Metcheva et al. (2011) in excreta of Gentoo penguins from Livingstone Island.

The highest concentrations of Cd, Cu, Cr, Mn, Ni, Pb and Zn in excreta were about 10-fold higher, compared to feathers, which is similar to the findings reported by Metcheva et al. (2011) in Gentoo penguins. The maximum ratio excreta/feathers was established for Mn (Mn_e/Mn_f = 36.4) and the minimum for Cr (Cr_e/Cr_f = 2). For Cd, Cu, Ni, Pb and Zn the following relation were calculated: Cd_e/Cd_f = 13.9; Cu_e/Cu_f = 12.8; Ni_e/Ni_f = 3.1; Pb_e/Pb_f = 2.7; Zn_e/Zn_f = 4.9. Ancora et al. (2002) reported 20-fold higher Cd level in excreta compared to feathers of Adélie penguins from Terra Nova Bay, Antarctica. The higher content of the elements in excreta indicate that probably Gentoo penguins deploy some physiological mechanisms of organism self-purification. In comparison with the rest of the chemicals, the low concentration of Pb in excreta can be explained by the strong affinity of Pb to feathers (Metcheva et al. 2011).

3.3 Trace metals in soils

Concentrations of Cd, Cu, Cr, Mn, Ni, Pb and Zn in soils around *P. papua* colonies are shown in Table 4.

The levels of metals in soils around the penguin colonies showed significant differences among the different locations studied. Levels of Cd, Cr, Mn, Ni, Pb and Zn in soils are significantly higher in O'Higgins Base in comparison to Dourmer Island and Neko Harbor. The levels of these chemicals did not show significant differences between O'Higgins Base and Stranger Point. The lowest mean level of Cu, Cr, Ni and Pb were obtained in soil samples from Neko Harbor, whereas the lowest levels of Cd, Mn and Zn were detected in soils from Doumer Island.

Levels of all seven metals were highest in soil samples from O'Higgins Base and Stranger Point, which are also the locations with the highest trace metal levels in feathers and excreta of *P. papua*. Both, O'Higgins Base and Stranger Point are sites near research stations and with major human presence. The high metal levels in soil found in the present study could be linked to anthropogenic contamination (Tin et al. 2009). There is evidence indicating Pb contamination caused by scientific stations in Antarctica (Jerez et al. 2011). On the contrary, Neko Harbor and Dourmer Island are isolated sites far from anthropogenic activities.

Currently, the available data on trace metal concentrations in abiotic matrices from Antarctica is very scarce. Some Antarctic locations adjacent to scientific bases are highly polluted (Negri et al. 2006). A recent study developed at Hope Bay located at northern of the Antarctic Peninsula, showed that the soil had high levels of Cd (47 mg kg^{-1}), Cu (2,082 mg kg^{-1}), Pb (19,381 mg kg^{-1}) and Zn (5,225 mg kg^{-1}), which were linked to majority of the soil samples presented evidences of contamination with oil, coal, alloys and feces from penguin colonies (Bueno et al. 2011). Another study described up to 92 mg kg^{-1} Cu and 8.9 mg kg^{-1} Zn in coastal soils near the research station Commandant Ferraz, King George Island (Santos et al. 2005), which may be attributed to intercontinental atmospheric transport and fuel utilized in the region (Licinio et al. 2008). Metal concentrations (As, Cd, Cu, Pb and Zn) on the land surface have been reported above background concentrations from McMurdo Station, Ross Island, Antarctica (Kennicut et al. 2010). Our Pb, Cu and Zn levels in soils are above the background values measured in Great Wall Station, King George Island (Liu et al. 2004). Soil contamination in Antarctica is a crucial issue because most of the chemicals can reach some environments as a consequence of run-off events. This may have serious implications for any biota and possible incorporation into the terrestrial food web. There is evidence showing that chemical contamination and organic enrichment reduced marine benthic ecological integrity within a few hundred meters offshore of a research station (Kennicut et al. 2010). Evidence on sediment quality shows that the incidence of benthic macroinvertebrate effects increase when concentrations of Cd > 1.2, Cu > 34, Pb > 46.7, Ni > 20.9, Cr > 81 and Zn > 150 mg kg^{-1} (Burton 2002).

3.4 Source of trace metals in Antarctic Peninsula

Volcanic activity is an important natural input of Cd (Burger & Gochfeld 2004). Local volcanism could explain the presence of this metal in the region because South Shetland Islands and northern of the Antarctic Peninsula concentrates volcanic activity (Smichowski et al. 2006). In polar environments, Cd can be also produced by up-welling of Cd-rich waters and algal blooms (Bargagli et al. 1996; Sanchez-Hernandez 2000). The abundance of Mn in sediment from some areas of the Northern of the Antarctic Peninsula (Deheyn et al. 2005) would explain our findings.

On the other hand, some anthropogenic sources can explain the presence of metals in Antarctica. High Cd and Pb levels found in Antarctica can be attributed to debris, runoff, fossil fuels combustion, shipping and sewage (Kennicutt 2003; Bargagli 2008), whereas Cr is associated with oil contamination (Alam & Sadiq 1993; Caccia et al. 2003). Zn is linked to anthropogenic sources such as mining, batteries, paints, electrical device or metallurgical industries (Tin et al. 2009). Sewage, oil spills, pesticides, solid wastes can contribute to increase Cu levels in Antarctica due to the increase on the presence of scientific stations, tourists and fishers (Tin et al. 2009). Fuel combustion, waste incineration, sewage disposal, paint, accidental oil spills, impact of the tourism increase and research facilities with their associated activities has been particularly noted in the northern area of the Antarctic Peninsula (IAATO 2010; Lynch et al. 2010; Barbosa et al. 2013). It can explain the higher trace metal levels detected in Gentoo penguins feces and feathers on Stranger Point and O'Higgins Base, both geographical locations with major human presence than Neko Harbor and Doumer Island. In agreement with our results, higher Pb levels have been found in Adélie penguin feathers from the northern Antarctic Peninsula (Jerez et al. 2011), whereas higher Pb, Cu and Zn levels have been found in Gentoo penguin excreta from the same area (Espejo et al. 2014). Elevated Pb concentrations in Antarctic soils may be a legacy of leaded fuel use or be derived from other anthropogenic sources such as paint, plumbing materials and solder (Kennicutt et al. 2010).

In addition to local pollution, some evidence has shown that trace metals can be transported by air and could be reaching polar areas of the planet (Smichowski et al. 2006). Cd levels in Antarctica could be linked to plastic industries, paints, batteries, smelters, corrosive coatings or P fertilizers, since Cd can be long-range transported atmospherically (McLaughlin et al. 1996; Burger 2008). Moreover, metals can be transported around the globe and move easily in water (Metcheva et al. 2001). Considering the increase of population and industries in countries of the Southern Hemisphere, metals could be affecting particularly the Antarctic Peninsula, the nearest area of Antarctica to South America.

4 Conclusions

The present work represents the data on the trace metals in biomaterials of Gentoo penguins and soil samples near penguin colonies collected along the Antarctic Peninsula. The levels of Cd, Cu, Cr, Mn, Ni, Pb and Zn showed great variations among the studied locations, most of them decreasing along the latitudinal gradient from North to South. Even though our trace metal levels in the biotic matrices are similar to those found previously in feathers and excreta of penguins in the region, this decrease could be related to the decrease of human presence and activities from North to South. The higher trace metal levels detected seem to be related to the great concentration of human activities that exists in King George Island and O'Higgins Base. Activities related to fuel and vehicle usage have remained in the same location for years, and therefore fuel spills frequently occur in those areas. Correlation of soil contamination and spill locations is very difficult as contaminated soil is often removed. Drainage pattern studies and monitoring of run-off events are needed to confirm this conclusion. Because of the bioaccumulation of metals in seabirds occupying highest position in the food chain, Gentoo penguins, like most biovector organisms can transfer bio-accumulated metals into terrestrial ecosystems via excreta that could be affecting some important living organisms of Antarctic coastal environments, an issue that need to be more investigated.

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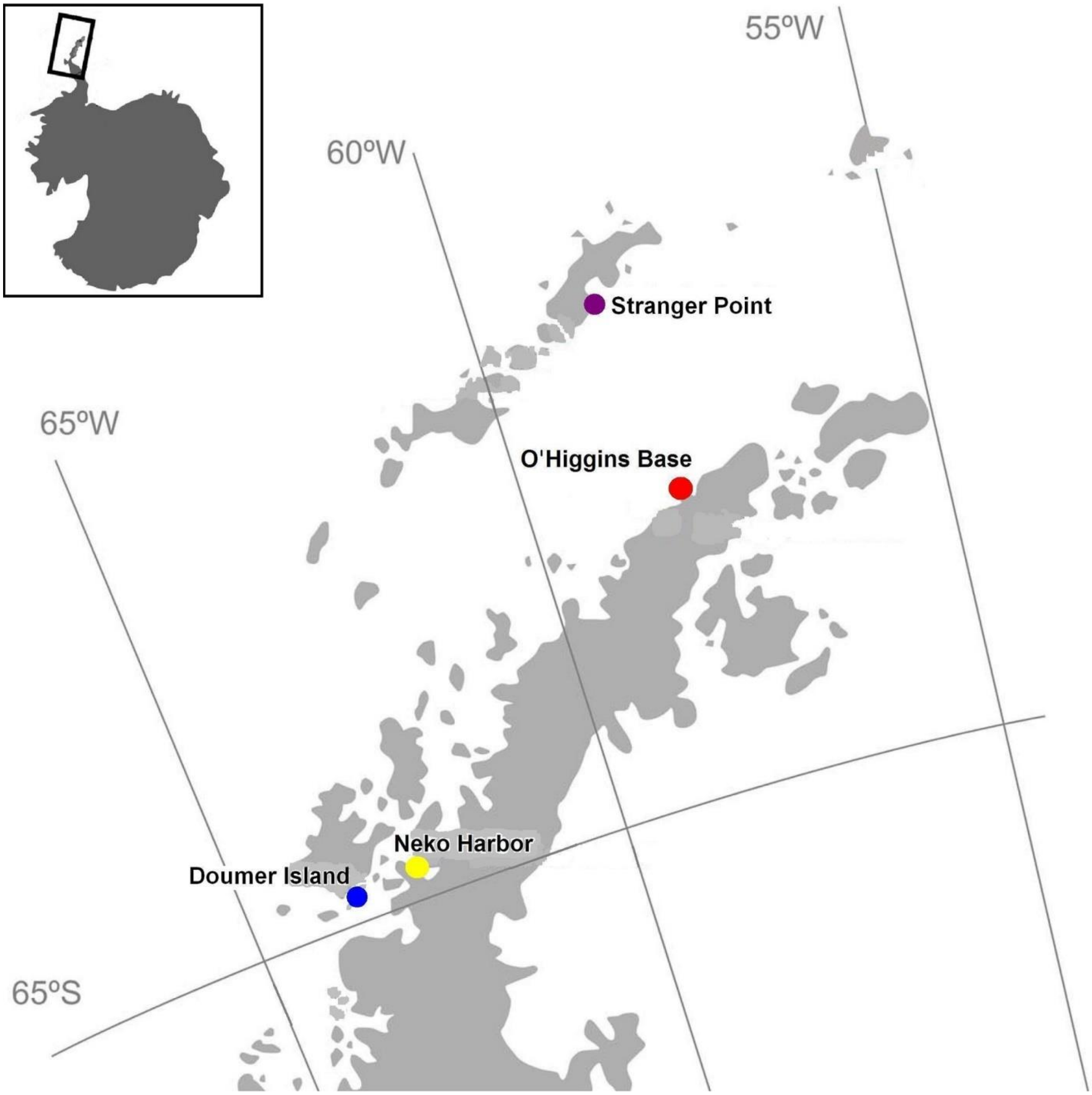

Fig. 1. Location of sampling sites along the Antarctic Peninsula.

Table 1. Sampling studied locations along the Antarctic Peninsula and sample size.

Locations	Geographical position	Sample size		
		Feathers	Excreta	Soil
O'Higgins Base	63º19'S, 57º53'W (Cape Legoupil)	10	10	10
Stranger Point	58º37'S, 62º16'W (King George Island)	10	10	10
Neko Harbor	64º50'S, 62º33'W (Andvord Bay)	10	11	19
Doumer Island	64º51'S, 63º35'W (Palmer Archipelago)	10	10	10

Table 2. Trace metal concentrations (mg kg^{-1} dry weight) in feathers of Gentoo penguins from different locations.

Locations	Cd	Cu	Cr	Mn	Ni	Pb	Zn
Neko Harbor	0.05 ± 0.07^{b}	13.74 ± 1.81^{b}	0.80 ± 0.34^{ab}	0.75 ± 0.28^{a}	1.60 ± 1.63^{b}	0.06 ± 0.04^{b}	36.89 ± 6.26^{b}
Doumer Island	0.09 ± 0.07^{ab}	14.98 ± 4.09^{b}	0.73 ± 0.36^{b}	0.27 ± 0.37^{b}	1.05 ± 1.24^{b}	0.10 ± 0.17^{b}	33.26 ± 4.04^{b}
Stranger Point	0.14 ± 0.09^{a}	19.65 ± 2.25^{a}	1.47 ± 0.82^{a}	1.23 ± 0.46^{a}	3.85 ± 2.39^{a}	0.60 ± 0.34^{a}	64.19 ± 10.67^{a}
O'Higgins Base	0.21 ± 0.28^{a}	20.89 ± 4.30^{a}	1.44 ± 0.80^{a}	1.19 ± 0.68^{a}	5.90 ± 8.23^{a}	0.63 ± 0.27^{a}	64.07 ± 10.73^{a}
	($H_{3,36}$ = 9.50)^{3}	($H_{3,36}$ = 22.44)^{2}	($H_{3,36}$ = 7.94)^{2}	($H_{3,36}$ = 18.59)^{3}	($H_{3,36}$ = 12.48)^{4}	($H_{3,36}$ = 25.08)^{4}	($H_{3,36}$ = 29.70)^{4}

Data shown are mean ± standard deviation. Different letters in the same column and biotic matrix show significant differences (^{2} $p < 0.01$; ^{3} $p < 0.001$; ^{4} $p < 0.0001$). Kruskal–Wallis H statistic values are shown in parenthesis.

Table 3. Trace metal concentrations (mg kg^{-1} dry weight) in excreta of Gentoo penguins from different locations.

Locations	Cd	Cu	Cr	Mn	Ni	Pb	Zn
Neko Harbor	1.58 ± 1.11^{b}	146.00 ± 76.17^{c}	1.90 ± 1.14^{bc}	22.43 ± 8.57^{bc}	4.44 ± 1.74^{b}	0.08 ± 0.08^{b}	142.97 ± 35.51^{bc}
Doumer Island	1.24 ± 0.25^{b}	201.54 ± 64.14^{bc}	1.67 ± 0.55^{c}	17.84 ± 13.22^{c}	4.91 ± 2.51^{b}	0.09 ± 0.10^{b}	108.74 ± 25.23^{c}
Stranger Point	1.97 ± 0.86^{ab}	222.51 ± 85.48^{ab}	2.98 ± 1.60^{ab}	36.62 ± 16.97^{ab}	13.41 ± 6.90^{a}	1.46 ± 0.49^{a}	201.18 ± 63.39^{b}
O'Higgins Base	2.92 ± 0.81^{a}	266.83 ± 42.77^{a}	2.99 ± 0.80^{a}	44.75 ± 10.67^{a}	18.15 ± 5.34^{a}	1.68 ± 0.58^{a}	317.92 ± 46.60^{a}
	($H_{3,37}$ = 16.82)[1]	($H_{3,36}$ = 12,77)[3]	($H_{3,37}$ = 15.62)[1]	($H_{3,37}$ = 19.04)[3]	($H_{3,37}$ = 27.78)[4]	($H_{3,37}$ = 30.07)[4]	($H_{3,37}$ = 28.89)[4]

Data shown are mean ± standard deviation. Different letters in the same column and biotic matrix show significant differences ([1] $p < 0.05$; [3] $p < 0.001$; [4] $p < 0.0001$). Kruskal–Wallis H statistic values are shown in parenthesis.

Table 4. Trace metal concentrations (mg kg^{-1} dry weight) in coastal soils near Gentoo penguin colonies at different locations.

Locations	Cd	Cu	Cr	Mn	Ni	Pb	Zn
Neko Harbor	2.22 ± 1.26^{b}	197.61 ± 63.96^{b}	4.74 ± 2.11^{b}	205.56 ± 91.62^{b}	1.39 ± 1.18^{b}	5.81 ± 10.08^{b}	44.08 ± 10.18^{b}
Doumer Island	1.88 ± 1.43^{b}	284.23 ± 147.15^{ab}	8.89 ± 4.25^{b}	86.96 ± 40.01^{c}	1.60 ± 0.97^{b}	8.96 ± 7.75^{b}	41.55 ± 9.06^{b}
Stranger Point	3.93 ± 1.66^{a}	389.98 ± 127.11^{a}	61.80 ± 20.22^{a}	387.57 ± 87.34^{a}	25.30 ± 9.74^{a}	244.55 ± 76.95^{a}	376.57 ± 133.15^{a}
O'Higgins Base	4.31 ± 1.50^{a}	421.94 ± 150.56^{a}	64.75 ± 21.75^{a}	404.76 ± 75.43^{a}	28.13 ± 10.34^{a}	281.54 ± 82.60^{a}	484.99 ± 181.74^{a}
	($H_{3,45}$ = 16.52)*	($H_{3,45}$ = 16.80)*	($H_{3,45}$ = 37.39)**	($H_{3,45}$ = 33.35)**	($H_{3,45}$ = 35.14)**	($H_{3,45}$ = 36.28)**	($H_{3,45}$ = 35.47)**

Data shown are mean ± standard deviation. Different letters in the same column show significant differences (* $p < 0.001$; ** $p < 0.0001$). Kruskal–Wallis H statistic values are shown in parenthesis

Capitulo IV The impact of penguins on the content of trace elements and nutrients in coastal soils of north western Chile and the Antarctic Peninsula area

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Abstract

In isolated areas without direct human impact where several species of seabirds nest, transformations affecting the soil come mainly from natural processes, such as chemical enrichment caused by seabirds. Penguins constitute an important bird biomass in the Southern Hemisphere, where they breed in colonies on different sites from one hundred to thousands of individuals. The accumulation of trace elements and nutrients in soils within two perennial colonies of Humboldt penguins (*Spheniscus humboldti*) located in north western Chile and three colonies of Adélie penguins (*Pygoscelis adeliae*) in the Antarctic Peninsula area were investigated here. Surface soil samples were collected directly from nesting sites. Control samples were taken outside the colonies within sites adjacent to the nesting areas but not affected by bird excrement. The contents of Cd, Co, Cr, Cu, Mo, Ni, Sr, V, and Zn were determined by inductively coupled plasma optical emission spectrometry. Ammonium (NH_4) and nitrate (NO_3) ions were determined colorimetrically. Extractable potassium (K) was determined by flame emission spectrometry and available phosphorus (Olsen-P) was determined by spectrophotometry. The highest concentrations of trace metals (Cd, Co, Cr, Cu, Mo, V, and Zn) and macronutrients (available N, K, and P), along with an increase in salinity and acidity levels, were found directly below the seabird colony, a situation occurring in northern Chile as well as in the Antarctic Peninsula area, highlighting the role that penguins have as bio-vectors on generating geochemical changes in different ecosystems. Some terrestrial plants and animals that live near those penguin colonies might be affected at a greater level than the organisms that live in sites similar but distant from colonies of birds. New data about the role of these species of seabirds as bio-vectors of chemical contaminants are added.

Keywords Trace metals; Heavy metals; Nutrients; Seabirds; Soil; Biotransport; Penguins.

1 Introduction

Marine birds can be important bio-vectors of trace elements and nutrient transfers from sea to land (Liu et al. 2006; Mallory et al. 2015). There is evidence showing that those animals can transport industrially produced chemicals and can provide a critical nutrient subsidy to terrestrial ecosystems (Blais et al. 2005). At the breeding sites, seabirds can negatively affect local habitats as they release most of these compounds by defecation, regurgitation, dropping food, or even mortality (Ellis 2005). Guano from seabirds is indicated as an effective vector of pollutants to soils and a significant source of biogenic substances (Liu et al. 2006; Brimble et al. 2009).

Some terrestrial habitats around colonies can be highly contaminated due to the contributions of chemicals from birds (Blais et al. 2005; Brimble et al. 2009; Zhu et al. 2014). The deposition of seabird droppings have had a significant effect on the geochemical composition of some polar terrestrial areas (Sun and Xie 2001; Xie and Sun 2008). There are studies indicating that Arctic soils next to nesting sites are strongly affected by heavy metals from gull feces (Headley 1996; Michelutti et al. 2009). The few studies from Antarctica have shown that soils had high levels of heavy metals as a consequence of contamination with oil, coal, alloys and feces from penguin colonies (Bueno et al. 2011). In any case, terrestrial biota that live near a colony of seabirds tend to ingest these chemicals at levels greater than the organisms that live in similar habitats that are distant from colonies of birds (Ligeza and Smal 2003; Choy et al. 2010).

In the Southern Hemisphere, penguins constitute an important bird biomass, where they breed in colonies on different sites from a hundred to thousands of individuals (Boersma 2008). Adélie penguins can be considered useful sentinels of the environmental changes occurring in Antarctic ecosystems, because they are seabirds occupying a high ecological position and their feeding habits are simple (Yin et al. 2008). Similarly, the Humboldt penguin is a bird also at the top of the food chain of marine ecosystems in lower latitudes, and it plays an important role in the ecology of the coastal zones of northern Chile (Celis et al. 2014). Both species can be used as well for monitoring environmental contamination over time.

Coastal soils of northern Chile are normally characterized by prevailing high temperatures and hot-cold cycles where relative humidity seldom exceeds 80% and dew point temperatures are seldom reached, typical of semi-arid environments (Rundel et al. 1996). In contrast, Antarctic soils (typically referred to as cold desert soils) are located mainly on or near the continental coastline, where typically low temperatures, low humidity, and freeze-thaw cycles are present (Aislabie et al. 2004). The impact of seabirds on soil due to the deposition of trace elements and nutrients by guano could have deeper and more persistent effects in semi-arid environments (Hutchinson 1950). On the other hand, some characteristics of Antarctica make it highly sensitive to environmental contamination (Santos et al. 2005). In both terrestrial environments, living organisms have to deal with a harsh environment, where few plants and animals have managed to survive. With the growing concerns of pollution of soils, it has become important to evaluate the levels of the chemicals measured in soils with no previous records, which can help predict possible future changes in the environment. The flow of nutrients between sea and land can be more important than the flow of energy (Polis et al. 1997). To our knowledge, studies on Adélie penguin-derived nutrients and trace elements on Antarctic soils are still rare, and none has been done in northern Chile, where there are many important colonies of Humboldt penguins. It is quite necessary to investigate now baseline levels of chemical elements to monitor possible future changes in fragile ecosystems, crucial to the planet's biodiversity. Consequently, the aim of the present study was to assess the effect of Humbodlt penguin and Adélie penguin colonies on the concentration of some trace elements and nutrients in coastal soils of the north western Chile and the Antarctic Peninsula area, respectively.

2 Study Site and Methodology

2.1 Study sites

Five perennial colonies of the seabirds were chosen in this study (Fig. 1). Two were colonies of Humboldt penguins from the north western coast of Chile: Pan de Azúcar Island (PAIs), and Chañaral Island (CHIs). Two were colonies of Adélie penguins from the south eastern coast of King George Island (South Shetland Island): Base Arctowski (Bar), and Ardley Island (AIs). One was a colony of Adélie penguins from the north western coast of Graham Land (Base O´Higgins (BOh).

The first colony of Humboldt penguins (PAIs) is located on the north of Pan de Azúcar Island, which is completely surrounded by sea. It is an oval island of 110 ha, located 2 km from the coastline and 18 km distant from Chañaral Bay. Its maximum height of ground elevation above sea level is nearly 160 m. The eastern shore of Pan de Azúcar Island sustains a rookery for Humboldt penguins and other seabirds (Rundel et al. 1996). It has one of the largest Humboldt penguin colonies in northern Chile, supporting approximately 5,000 individuals (Celis et al. 2014). There are no trees on the island.

The second colony of Humboldt penguins (CHIs) locate on the south side of Chañaral Island. It is a circular island of about 13 ha, about 7 km from the coastline and 100 km north of Coquimbo Bay. It is an isolated island supporting one of the largest Humboldt penguin colonies in northern Chile, with approximately 22,000 individuals, and it is visited by tourists (Celis et al. 2014). There are no trees on the island, only cacti and bushes, which are scattered everywhere.

The first colony of Adélie penguins (Bar) is located next to Base Arctowski, which is a permanently staffed base situated on southern King George Island (South Shetland Islands), where a large Adélie penguin colony inhabits the surrounding area. Is is also frequently visited by tourists and therefore the immediate area is impacted by humans, such as personnel, researchers, tourists, and their associated activities.

The second colony of Adélie penguins (AIs) is situated on Ardley Island (Maxwell Bay) close to the southeast end of King George Island. It is an isolated island usually inhabited by a colony of Adélie penguins. It is of particular importance for the breeding colony of Adélie penguins. Mosses and lichens predominate over the local vegetation (Zhu et al. 2014).

The third colony of Adélie penguins (BOh) is located next to Base O´Higgins, which is a permanently staffed base situated on the north western coast of Graham Land, where the number of breeding penguins usually falls below 700. The birds have established the colony next to the station, where some facilities, personnel, researchers, tourists and other associated activities may usually be observed.

2.2 Sampling

In order to assess chemical concentrations, surface soils (the top 5 cm) samples were collected in January 2016. The soil samples were collected with plastic spatulas directly from nesting sites (manured soils) and from control sites (non-manured soils, adjacent to the colonies but unaffected by birds,usually, the colonies were spread out along the cliffs and shores, making it difficult to accurately determine the distance of the control sites from the colony). From each site a cumulative soil sample of about 20 kg (total of 20 samples) was taken for analysis: 10 samples from nesting sites and 10 samples from reference sites. All samples were kept in sealed plastic bags and stored in very clean steel containers for transport until their analyses.

Typically, the texture of the Antarctic soils is mainly loamy sand or sand with abundant gravel, stones, and boulders (Bockheim 1997). The texture of coastal soils of northern Chile is typically sandy loam or sand with gravel and stones (UChile 2013).

2.3 Analytical procedure

In the laboratory the samples were kept at 4ºC prior to being analyzed. Afterwards, soil samples were air-dried, pulverized in an agate bowl, mixed and sieved (1 mm) to separate gravel (particles sizes >1 mm) and non-soil components. Next, the soil samples were dried in an oven at 40°C for 24 h, and then homogenized. Trace elements were determined by an extraction procedure suggested by Bettinelli et al., (2000). First, about 2 g of each sample was decomposed with aqua regia and hydrofluoric acid in a Teflon digestion bomb during 1 h at 120ºC. After that, bombs were opened until the samples were cool and the samples were heated again until almost dry. Then, extract recovery was performed with 0.5 M HCl. Finally, the samples were analysed in an inductively coupled plasma optical emission spectrometer (ICP-OES Perkin Elmer). All reagents used were Suprapur Merck and made with high-purity water (Milli-Q system, Millipore, USA). In order to ensure quality control a certified reference material, internal standards, blanks and duplicates after every batch of 20 samples were considered.

Nutrients were determined according to Sadzawka et al. (2006). Soil nitrate (NO_3) and ammonium (NH_4) ions were determined colorimetrically after extraction in 0.03 mol CH_3COOH dm^{-3}, using phenoldisulphonic acid and Nessler reagent, respectively. Organic C was determined by Walkley-Black wet digestion. Organic matter (OM) was determined as the percentage of organic C multiplied by 1.724. Extractable potassium (K) was determined at neutral pH in 1 N CH_3COONH_4 by flame emission spectrometry and EDTA titration. Available phosphorus (Olsen-P) was determined using a UV–visible spectrophotometer (800-900 nm). The electrical conductivity (EC) was determined with a conductivity cell by measuring the electrical resistance of a 1: 5 (soil: water) suspension. The acidity or alkalinity in soils was measured by means of pH, using the H_2O method.

2.4 Statistical analysis

Non parametric statistical methodologies were used because of the assumptions of normality and homoscedasticity were not met even after the log transformation of the data. Differences among data were assessed by means of ANOVA with Kruskal–Wallis and Mann–Whitney *U* tests. Post hoc tests were carried out for Kruskal–Wallis analyses, using the critical differences of mean rank. Spearman rank correlation coefficients were calculated among trace element and nutrient levels. The differences were considered to indicate statistical significance when $p < 0.05$. Statistical analyses were conducted using SPSS version 15.0 software.

3 Results

3.1 Trace elements

In general, the levels of Cd, Co, Cr, Cu, Mo, V, and Zn tended to be higher in the nesting sites as compared to the control sites. Particularly, most of the soils affected by Humboldt penguins (Table 1) and Adélie penguins (Table 2) showed a strong enrichment ($p < 0.05$) with Co, Cr, Mo and V.

In northern Chile, Co and Mo levels in soil of Chañaral Island from Humboldt penguin colonies were significantly 1.4 and 1.7 times higher as compared to control sites, respectively (Table 1). Similarly, the levels of Cr, Cu, V, and Zn were 3, 1.6, 3.2 and 2 times higher ($p < 0.05$), respectively, at nesting sites of Pan de Azúcar Island than those levels from control sites. Our Sr levels at nesting sites ranged from 286.25 to 467.4 mg kg^{-1}, with the highest Sr levels at Pan de Azúcar Island.

In the Antarctic Peninsula area, Cd, Cu and Zn levels were significantly (5.5, 2.2 and 2.1 times, respectively) higher in soils of Ardley Island affected by Adélie penguins as compared to control soils (Table 2). Similarly, the levels of Co were higher ($p < 0.05$) at nesting sites of Ardley Island and Base O´Higgins. Our Sr levels on nesting sites ranged from 292.2 to 1,167.5 mg kg^{-1}. The high Sr concentrations at nesting sites can be related to the high levels of Sr found in Antarctic krill (Tatur and Myrcha 1984). The levels of V were higher (p <0.05) on nesting sites of Base Arctowski and Base O´Higgins, whereas Mo levels were significantly higher at all the nesting sites studied (Ardley Island, Base Arctowski and Base O´Higgins).

Considering all the sampling sites together, the following relations among trace element levels were noted in this study: Sr >Zn >Cu >V >Cr >Ni >Co >Mo >Cd. With the exception of Zn, Cu and Sr, the average Cd, V, Ni, Cr, Co and Mo levels detected in soils of the northern Chile were 2.7, 3.8, 5, 6.4, 6.2 and 63.3 times higher, respectively, than those levels found in soils of the Antarctic Peninsula area.

3.2 Nutrient parameters

In general, the levels of OM, NO_3, NH_4, K, P and EC tend to be higher in the nesting sites as compared to the control sites. The soil enrichment in macronutrients (N, P and K), organic matter (OM), salinity (EC) and acidity at sites with increased penguin influence was noted in the islands of northern Chile (Table 3) as well as in the locations of the Antarctic Peninsula area (Table 4). Particularly, most of the soils affected by penguins showed a significant enrichment with NH_4.

In northern Chile, pH averaged 12% lower in nesting sites than pH found outside the colonies (Table 3). The contents of NO_3 and NH_4 in nesting sites of Pan de Azúcar Island were significantly higher as compared to non-manured soils, 2.7 and 114.8 times, respectively. Similarly, OM content and EC were 12 and 15 times higher (p <0.05), respectively, on nesting sites of Pan de Azúcar Island, whereas pH was 15.8% lower.

In the Antarctic Peninsula area, the highest K levels were found in the nesting sites at Ardley Island (8.51 g kg^{-1}, Table 4). The levels of NO_3 and K were significantly higher in manured soils at Ardley Island as compared to control soils (29.8 and 2.6 times, respectively). Similarly, the levels of NH_4 were 16.5, 18.7 and 3.2 times higher

(p <0.05) in nesting sites at Ardley Island, Base Arctowski and Base O´Higgins, respectively. The values of pH averaged 6.3 in manured soils, while in non-manured soils it tended to be higher (pH = 6.7). The levels of EC were higher (p <0.05) at nesting sites of Ardley Island and Base Arctowski, whereas pH levels were significantly lower at nesting sites of Ardley Island.

In general, the average levels of nutrients detected in Antarctic soils were higher (260 mg kg^{-1} for NO_3, 2 200 mg kg^{-1} for NH_4, and 8.94 g kg^{-1} for P) than those levels found in northern Chile soils (52 mg kg^{-1} for NO_3, 190 mg kg^{-1} for NH_4 and 8 g kg^{-1} for P). These nutrient contents are linked to a higher OM content found in Antarctic soils (2.8%) as compared to northern Chilean soils (1.3%), which ranged from 2 to 4.24% and 0.26 to 3.98%, respectively.

3.3 Relationship among trace elements and nutrient parameters

Soils of the north western coast of Chile showed several significant correlations among the levels of trace elements and nutrients (Table 5). The contents of Cd, Co and V had stronger correlations with K and P. Additionally, significant positive correlations were found between OM-NO_3, OM-NH_4, OM-EC, NO_3-NH_4, NO_3-P, NO_3-EC, K-NH_4, P-NH_4, NH_4-EC, K-P and K-EC.

In Antarctic soils, several significant correlations were observed among the levels of heavy metals and nutrients (Table 6). The concentrations of Cd, Cu and Mo had stronger correlations with NH_4 and NO_3, whereas the levels of Co, Sr, V and Zn are correlated with NO_3. On the other hand, the concentrations of Cr and Ni are correlated with NH_4. Moreover, significant positive correlations were found between OM-NH_4, OM-K, OM-EC, NO_3-P, NH_4-K, NH_4-EC and pH-P.

Figure 2 shows the way in which soil samples are grouped on the basis of the concentrations of the physical chemical parameters. PCA shows a similar association of NH_4 in semiarid and Antarctic soils which may be attributed to penguin excreta deposited on the ground (as was noted in Tables 5 and 6, which showed a positive correlation between organic matter and ammonium).

4 Discussion

The role of seabirds as main vectors moving chemical elements to soils has been discussed lately, because some species reach densely populated colonies and thus they can deliver significant amount of metals and macronutrients on a local scale (Ligeza et al. 2003; Ellis et al. 2006; Liu et al. 2006; Mallory et al. 2015). Previous studies of seabirds have been reported from sub-Antarctic islands (Smith, 1979), from island communities in the Gulf of California (Anderson and Polis, 1999), from semi-arid North African islets (García et al. 2002), from the Gulf of Maine (Ellis et al. 2006), and from the Artic (Blais et al. 2005; Brimble et al. 2009), which all have demonstrated that seabirds appear to form the major input for the transport of marine-derived trace elements and nutrients to different terrestrial coastal ecosystems, and soil below seabird colonies tends to be enriched with trace elements, N, P, and organic matter, while pH may decrease. Similarly, we found an increase of Cd, Co, Cr, Cu, Mo, V, Zn, OM, N, K, P, salinity, and acidity levels in soils influenced by Humboldt penguin colonies of northern Chile and Adélie penguin colonies of the Antarctic Peninsula area. In contrast, Sr and Ni levels did not significantly differ between manured soils and non-manured soils. Likewise, Brimble et al. (2008) found that Sr levels did not decrease as distance from the colony of fulmars increased. Our relationships between OM and accumulation of trace elements and nutrients in soils are in agreement with a study performed by Madrid et al. (2005), which found a strong association between the levels of some metals and the OM content in soils.

Our highest Cu, Ni, Sr and Zn levels found in non-manured Antarctic soils are 4.65, 1.70, 7.62 and 4.60 times higher, respectively, than those levels reported by Santos et al. (2005) from Admiralty Bay (King George Island). Geochemical background values of Cu at Great Wall Station (98.6 mg kg^{-1}, Liu et al. 2004), a location near Ardley Island, is quite similar to that we found in non-manured soils at Ardley Island (99.4 mg kg^{-1}). In contrast, our highest Cd, Cr, Cu, Ni, and Zn levels detected in soil at Base O´Higgins are 2.72, 7.36, 2.06, 4.02 and 2.03 times lower, respectively, than those levels found in soil at the same location two years ago (Celis et al. 2015). Extraction techniques were different, however, which can explain the temporal differences found between the levels of trace elements (Santos et al. 2005).

In general, the highest Cd, Co, Cr, Mo, Ni, and V levels detected in soils of northern Chile can be explained due to large-scale mining activities in this area (Celis et al. 2014). Many metals (Cd, Co, Cr, Cu, Mo, Ni, V, Zn) come from mining, industries and the combustion of coal and solid wastes (ATSDR 2016). Great amounts of trace elements and macronutrients can reach the sea through rivers and by surface runoff. Penguins feed almost exclusively at sea, but nest on land (Boersma 2008). Most of the metals and nutrients accumulate in the food chain, and because seabirds are usually top predators they bio-concentrate those chemicals, which are deposited in the soil by means of excretion (Liu et al. 2006; Yin et al. 2008). In northern Chile there are many mines and thermal power plants operating. On the other hand, the highest Cu and Zn levels were detected in soils of the Antarctic Peninsula area, which may be related to the large number of scientific stations and their associated facilities located on King George Island, which use fossil fuel as an energy source, thus generating significant levels of Cu and Zn (Ribeiro et al. 2011). Most research stations in Antarctica are located in the Antarctic Peninsula area, which therefore bears the greatest brunt of human activity. This is particularly true for King George Island (South Shetland Island), which has experienced an increase of tourism and its associated activities, such as plane and ship trips (Lynch et al. 2010; Barbosa et al. 2013). Fuel combustion, waste incineration, sewage disposal, paint, and accidental oil spills have been also noted in the northern area of the Antarctic Peninsula (Tin et al. 2009; Santos et al. 2005). Moreover, the higher Cu levels could be related to the high Cu levels present in Antarctic krill (Nygard et al. 2001), the main prey of Adélie penguins (Jerez et al. 2013). Some possible implications of the concentrations of trace elements in soil might be manifested in both diverse environments.

The Cd levels in this study are <22 mg kg^{-1}, an established standard level in soil for the protection of environmental and human health (CQG 1999). However, some evidence indicates that populations of bacteria and fungi in soil are adversely affected by 3 mg Cd kg^{-1} (Kobus and Kurek 1990), whereas 5 mg Cd kg^{-1} soil has been observed to reduce N mineralization by 28% (Liang and Tabatabai, 1977). The maximum Cd level detected in the soil of Pan de Azúcar Island (5.35 mg kg^{-1}, Table 1) is above those levels, which raises the possibility of negative effects on some biological and chemical soil properties.

Globally, the greatest concentrations of Co (above 120 mg kg^{-1}) in soil have been linked to industrial pollution (Barałkiewicz and Siepak 1999). The maximum Co levels we detected were in soils of northern Chile (47.42 mg kg^{-1} in Chañaral Island and 10.57 mg kg^{-1} in Pan de Azúcar Island, Table 1), an area highly impacted by mining activities (Celis et al. 2014).

The Cr levels detected in the soils impacted by Humboldt penguin colonies of the northern Chile ranged from 116 to 165.6 mg kg^{-1} (Table 1) and are above the standard Cr level in soil (87 mg kg^{-1}) for the protection of environmental and human health (CQG 1999). On the other hand, the Cr levels found in Antarctic soils influenced by Adélie penguin colonies (6.11-62.32 mg kg^{-1}, Table 2) are below that standard.

The highest Cu levels we detected (266.75 mg kg^{-1}, Table 2) are lower than those Cu levels found in polluted urban areas of the Northern Hemisphere (910 mg kg^{-1}, Tong 1990), even though they are above the standard Cu levels in soils for the protection of environmental and human health (91 mg kg^{-1}, CQG 1999).

Molybdenum background concentrations ranged between 0.2 and 6 mg kg^{-1} (He et al. 2005). The Mo levels from the two nesting sites of the northern Chile (> 11 mg kg^{-1}, Table 1) show that those soils are clearly impacted by the presence of the Humboldt penguin colonies.

The levels of Ni found on Pan de Azúcar Island and Chañaral Island were higher than those found in the Antarctica and are directly related to mining activities in northern Chile. Some studies in the Northern Hemisphere have reported an increased Ni content in soils of 250 mg kg^{-1} from areas highly polluted by galvanization plant sewage (Barałkiewicz and Siepak 1999). Some evidence shows that N mineralization can be reduced by 17% with about 300 mg Ni kg^{-1} soil (Liang and Tabatabai 1977). The highest Ni levels we detected (55.9 mg kg^{-1}, Table 1) are below the 89 mg kg^{-1} standard set in soils for the protection of environmental and human health (CQG 1999).

There is some evidence that increased levels of Sr from 950 to 8,100 mg kg^{-1} lead to a reduction in soil microbial respiration of about 54% (Margon et al. 2013). Some Antarctic soils near penguin rookeries are usually devoid of vegetation, thus their productivity depends almost exclusively on microbial activity (Tatur and Myrcha 1984). In general, the Sr levels we detected are below that range, with the exception of the Sr levels detected in manured soil at Base O´Higgins (1,167.5 mg kg^{-1}, Table 2).

The mobility of V in soils appears to be affected by redox conditions, pH, soil organic matter, and mineral composition (Gabler et al. 2009). In our study there was a significant negative correlation between pH and V (Table 4). The highest V levels we found in soils of Chañaral Island (264.6 mg kg^{-1}, Table 1) are above the standard for environmental pollution in soils (130 mg kg^{-1}, CQG 1999). A level of 255 mg kg^{-1} of V in soil can reduce nitrification by 12% at pH 7.8 (Liang and Tabatabai 1977). Moreover, soil enzymatic activity can be sensitive to V at higher than 111 mg kg^{-1} (Yang et al. 2014).

With regard to Zn, soil microbial respiration can be reduced by 21% after Zn exposure of 10 mg kg^{-1} for 8 weeks, whereas nitrification can be reduced by 24% at a Zn concentration of about 325 mg kg^{-1} (Liang and Tabatabai 1977). The highest Zn levels we detected (341.4 mg kg^{-1}, Table 2) are below the standard in soils for the protection of environmental and human health (360 mg kg^{-1}, CQG 1999).

Research done on sediment quality that there is a toxic effect threshold on benthic macroinvertebrate organisms exposed to Cd >3, Cr >100, Cu >200, Ni >61 or Zn >540 mg kg^{-1} d.w. (Burton, 2002). The metal levels detected at Pan de Azúcar Island (5.35 mg kg^{-1}, Table 1) are above the threshold value for Cd. Similarly, the levels found in Pan de Azúcar Island and Chañaral Island are above the threshold value for Cr, whereas the levels of Cu detected in Ardley Island and Base O´Higgins (Table 2) are above the corresponding threshold value. These findings indicate that Humboldt penguins and Adélie penguins are possibly causing local contamination in some terrestrial areas of northern Chile and Antarctic Peninsula area, which could affect other living organisms, an issue that needs to be further investigated.

The ornithogenic soils of Antarctic Peninsula area and northern Chile had significantly higher available P concentrations than non-manured soils. These findings agree with previous reports. The soils within penguin colonies are rich in phosphorus, as they can transport up to 20,000 tons of P annually to the land via droppings (Zhu et al. 2006). Some research has shown that organic P can reach up to to 90,000 mg kg^{-1} (d.w.) in soils impacted by penguin colonies at Ardley Island, whereas background P levels were 2,000 mg kg^{-1} (Zhu et al. 2014).

Most of the manured soils impacted by penguins in the present study showed a significant enrichment with NH_4. A study found very high N levels (~51,000 mg kg^{-1}, d.w.) in surface sediments from Devon Island (Canada) impacted by fulmar guano (Brimble et al. 2008). The highest NO_3 (896.53 mg kg^{-1}, Table 4) and NH_4 (5,845.2 mg kg^{-1}, Table 4) levels we found in manured soils are 1.7 and 6.2 times higher, respective, than those levels found in soils impacted by colonies of black cormorants (*Phalacrocorax carbo sinensis*) and grey herons (*Ardea cinerea*) from Europe (Ligeza et al. 2003). Surface soils in Adélie penguin nesting sites on King George Island are described as having high contents of C, N and P (Tatur and Myrcha 1984).

All nesting sites had significantly higher available K concentrations than non-manured soils. Although high amounts of K are neither noxious nor toxic for plants, and its uptake is generally unlimited (Zeng et al. 2000), there is evidence indicating a negative effect of K on plants, especially when soil moisture decreases (Ligeza et al. 2003). This is a typical situation that could be occurring in soils affected by Humboldt penguin colonies of northern Chile, where precipitation is very low (Rundel et al. 1996).

Soil texture is one of the main factors that determine the level of accumulation of chemicals. For example, sandy soils, unlike clay soils, have a low rate of immobilization of elements (Ligeza et al. 2003). Penguin-derived heavy metals are low mobile elements and may remain in the soil for decades (Hawke et al. 1999), thus long-term effects can be expected. Despite their high capacity to accumulate elements, soils might not retain all the chemicals delivered by seabirds, resulting in element dispersion (Ziólek and Melke 2014). The sandy soils in the Humboldt penguin and Adélie penguin colonies are probably contributing to the migration of contaminants into the biogeochemical cycles of northern Chile and Antarctica. On the other hand, the presence of high concentrations of dissolved OM in soil leachates can also enhance metal mobility, thus posing a risk to the surrounding areas (Singh 1990).

Our results indicate that penguins tend to acidify the heavily penguin-affected soils, which can be linked to the mineralization of the organic matter added by seabirds and its subsequent nitrification (García et al. 2002). Additionally, the higher salinity of soils affected by penguins could alter the soil water potential and, consequently, the nutrients to be taken up by plants (García et al. 2002). In northern Chile, the values of pH were 16.4% less in nesting sites than pH found outside the colonies. In Antarctica, the pH in the nesting sites was 11.5% lower as compared to control sites. This is probably due to the high load of organic matter, urine, and feces of the penguins (Motavalli et al. 1995; Moral et al. 2008). An increase in the concentration of H ions in the soil can generate a lower capacity to retain Ca, Mg, K and Na, which are essential elements for the majority of the plants (García et al. 2002). Over time, this could affect plant communities in semi-arid environments typical of the coast of northern Chile. Some evidence indicates that the adverse effects of trace elements depend on pH. In manured soils, the organic matter added by penguins implies a decrease in soil pH, which may increase heavy-metal availability (Srivastava and Sethi 1981). It could affect the performance of plants that have to deal with a shortage of some nutrients, and yet have to tolerate an excess of others (García et al. 2002). From a phytotoxicological point of view, the effects of Mo are generally higher in acid ($pH < 6.5$) than in neutral and basic soils ($pH > 6.5$), indicating that Mo is less toxic to higher plants grown on acid soils (McGrath et al. 2010). In contrast, some cationic metals such as Cu (Rooney et al. 2006), Ni (Rooney et al. 2007) and Co (Micó et al. 2008; Li et al. 2009) are more toxic to plants grown on basic soils.

These findings might imply some important negative impacts to fragile soils such as those of the northern Chile and Antarctica, thus adding new data about the role of Humboldt penguins and Adélie penguins as bio-vectors of chemical contaminants from different ecological systems. It is particularly relevant in those places where Adélie penguins live, because they are more agile and breed further inland than the other Antarctic penguin species do (Tatur and Myrcha 1984). The study of trace elements and nutrients levels in ornithogenic soils will improve our understanding of element cycling in semi-arid and Antarctic tundra ecosystems.

5 Conclusions

The present study adds new data on trace elements and nutrients in soils impacted by some important colonies of Humboldt penguins and Adélie penguins from two different ecological environments. Humboldt penguin colonies were studied at two semi-arid locations of the northern Chile, while Adélie penguin colonies were studied at three locations of the Antarctic Peninsula area. The expected soil enrichment in macronutrients and organic matter as well as in salinity and acidity at sites with increased penguin influence is confirmed, as are the concentrations of Cd, Co, Cr, Cu, Mo, V and Zn. All these variables were higher in areas supporting intense penguin activities. In addition to the differences in climate, the sandy soils where Humboldt penguin and Adélie penguin colonies are located have a low rate of immobilization of elements, therefore they are probably contributing to the migration of contaminants into the biogeochemical cycles of northern Chile and Antarctica. Because of the bioaccumulation of metals and nutrients in seabirds occupying the highest position in the food chain, Humboldt penguins and Adélie penguins, like most bio-vector organisms, can transfer bio-accumulated metals into terrestrial ecosystems through excreta that could be affecting some important living organisms of semi-arid soils of northern Chile and Antarctic Peninsula coastal environments. This is an issue that needs to be more deeply investigated.

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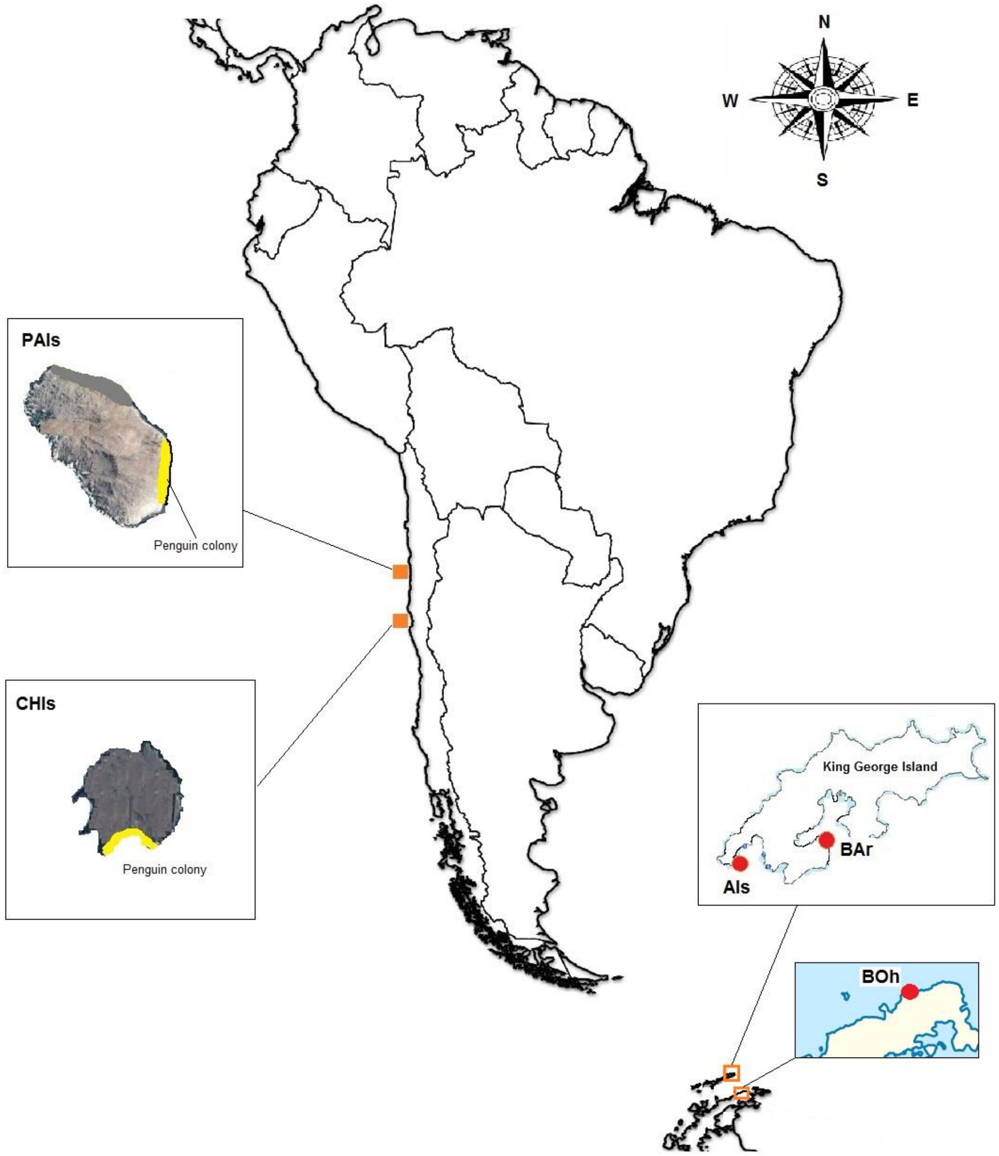

Fig. 1. Map of the sampling locations. PAIs = Pan de Azúcar Island (26º09’S, 70º40’W); CHIs = Chañaral Island (29º01’S, 71º34’W); AIs = Ardley Island (62°12′S, 58°57′W); BAr = Base Arctowski (62°09′S, 58°28′W); BOh = Base O´Higgins (63º19′S, Longitude: 57º53′W).

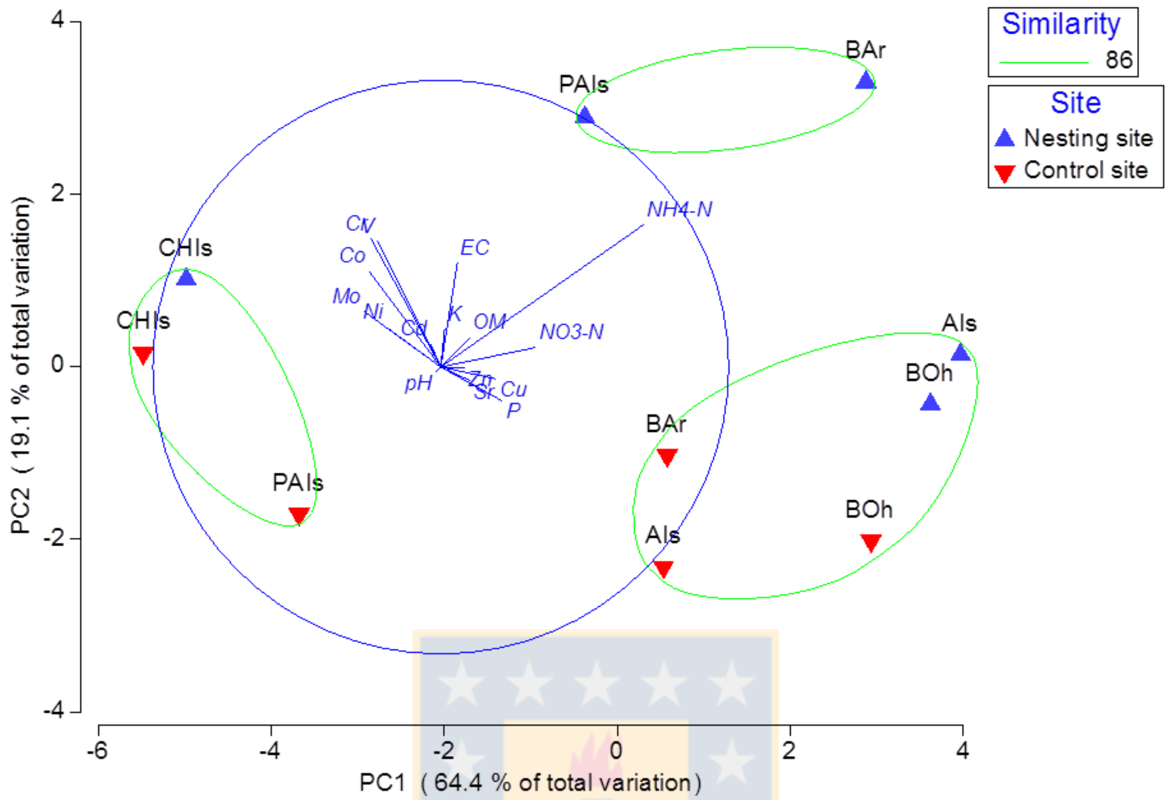

Fig. 2. Principal component analysis based on the physical chemical parameter concentrations in soil. PAIs = Pan de Azúcar Island; CHIs = Chañaral Island; AIs = Ardley Island; BAr = Base Arctowski; BOh = Base O´Higgins. For clarity and readability reasons, the center of the correlation circle has been moved from its origin (X = 0; Y = 0).

Table 1. Concentrations of trace elements (mean ± standard deviation, mg kg^{-1}, dry weight) in surface soil from northern Chile, according the collecting site (nesting and control).

	Trace elements								
Locations	**Cd**	**Co**	**Cr**	**Cu**	**Mo**	**Ni**	**Sr**	**V**	**Zn**
PAIs									
Nesting site	5.35±1.34[a]	10.57±2.79[a]	165.60±22.96[a]	35.34±9.99[a]	11.18±4.04[a]	43.74±11.54[a]	467.40±299.74[a]	65.38±22.66[a]	152.76±33.88[a]
Control site	3.66±2.96[a]	5.87±3.43[a]	54.80±57.85[b]	21.56±5.70[b]	8.13±4.79[a]	31.86±16.02[a]	300.20±181.36[a]	20.13±7.41[b]	76.20±21.19[b]
CHIs									
Nesting site	1.96±0.53[a]	47.32±4.81[a]	116.00±7.42[a]	54.74±23.03[a]	12.34±2.27[a]	55.88±5.40[a]	286.25±37.54[a]	264.60±43.24[a]	117.52±20.56[a]
Control site	1.42±0.59[a]	33.20±8.97[b]	104.64±26.97[a]	55.20±9.62[a]	7.15±0.50[b]	44.08±15.07[a]	276.60±76.78[a]	217.80±65.93[a]	98.08±11.94[a]

PAIs = Pan de Azúcar Island; CHIs = Chañaral Island. Different letters between collecting sites indicate significance at $p < 0.05$.

Table 2. Concentrations of trace elements (mean ± standard deviation, mg kg^{-1}, dry weight) in surface soil from the Antarctic Peninsula area, according the collecting site (nesting and control).

	Trace elements								
Locations	**Cd**	**Co**	**Cr**	**Cu**	**Mo**	**Ni**	**Sr**	**V**	**Zn**
AIs									
Nesting site	1.44±0.47[a]	3.17±0.37[a]	6.11±3.19[a]	214.20±9.58[a]	0.36±0.43[a]	10.73±3.07[a]	696.00±52.18[a]	15.09±10.53[a]	207.20±15.55[a]
Control site	0.26±0.06[b]	2.35±0.05[b]	4.89±0.95[a]	99.36±36.59[b]	0.03±0.02[b]	8.69±3.51[a]	376.60±268.46[a]	11.68±2.18[a]	98.76±46.59[b]
BAr									
Nesting site	0.93±0.47[a]	11.50±0.73[a]	62.32±16.96[a]	161.00±26.54[a]	0.15±0.07[a]	8.14±5.11[a]	292.20±76.16[a]	129.20±12.68[a]	96.48±4.26[a]
Control site	0.79±0.21[a]	5.06±3.55[a]	8.87±5.07[b]	116.20±35.54[a]	0.03±0.02[b]	7.04±0.91[a]	194.00±98.56[a]	41.66±36.68[b]	75.64±23.72[a]
BOh									
Nesting site	1.94±0.43[a]	1.12±0.07[a]	12.08±1.11[a]	266.75±62.47[a]	0.30±0.10[a]	10.97±2.56[a]	1,167.5±245.94[a]	18.25±3.02[a]	341.40±54.70[a]
Control site	1.58±0.30[a]	0.27±0.18[b]	8.80±0.78[b]	204.80±36.82[a]	0.05±0.03[b]	7.00±2.52[a]	899.00±107.41[a]	6.26±1.54[b]	239.26±68.00[a]

AIs = Ardley Island; BAr = Base Arctowski; BOh = Base O´Higgins. Different letters between collecting sites indicate significance at $p < 0.05$.

Table 3. Contents of physical-chemical parameters (mean ± standard deviation) in surface soil from northern Chile, according the collecting site (nesting and control).

Locations	OM %	NO_3 mg kg^{-1} (dw)	NH_4 mg kg^{-1} (dw)	available K g kg^{-1} (dw)	available P g kg^{-1} (dw)	Salinity (EC) dS m^{-1}	pH
	Physical-chemical parameters						
PAIs							
Nesting site	3.10±1.60[a]	131.4±160.78[a]	735.67±866.60[a]	15.33±4.76[a]	17.43±24.93[a]	26.12±11.28[a]	6.50±0.22[a]
Control site	0.26±0.07[b]	49.52±31.22[b]	6.41±3.27[b]	3.80±1.73[b]	5.22±2.97[b]	1.73±1.91[b]	7.72±0.30[b]
CHIs							
Nesting site	3.98±0.74[a]	21.15±21.32[a]	11.87±6.91[a]	4.39±1.98[a]	6.18±8.53[a]	3.65±1.70[a]	6.91±0.29[a]
Control site	0.83±0.31[b]	7.53±3.57[b]	5.82±3.27[a]	1.13±1.02[b]	3.21±1.90[b]	2.95±1.30[a]	7.90±0.13[b]

PAIs = Pan de Azúcar Island; CHIs = Chañaral Island. Different letters between collecting sites indicate significance at p <0.05.

Table 4. Contents of physical-chemical parameters (mean ± standard deviation) in surface soil from the Antarctic Peninsula area, according the collecting site (nesting and control).

	Physical-chemical parameters						
	OM	**NO_3**	**NH_4**	**available K**	**available P**	**Salinity (EC)**	**pH**
Locations	**%**	**mg kg^{-1} (dw)**	**mg kg^{-1} (dw)**	**g kg^{-1} (dw)**	**g kg^{-1} (dw)**	**dS m^{-1}**	
AIs							
Nesting site	3.77±0.48[a]	246.94±156.11[a]	4,570.5±3,991.8[a]	8.51±2.05[a]	12.03±8.62[a]	3.79±1.29[a]	6.19±0.15[a]
Control site	2.59±0.17[b]	8.29±7.39[b]	276.35±119.38[b]	3.34±5.71[b]	4.96±2.56[b]	1.60±0.49[b]	6.95±0.22[b]
BAr							
Nesting site	4.24±1.05[a]	103.68±29.51[a]	5,845.2±2,716.1[a]	7.21±2.66[a]	16.55±18.83[a]	17.12±7.70[a]	6.04±0.36[a]
Control site	1.36±0.37[b]	71.49±19.89[b]	311.9±70.7[b]	3.23±1.02[b]	3.66±1.68[b]	1.70±1.10[b]	6.55±0.14[b]
BOh							
Nesting site	2.58±0.36[a]	896.53±192.33[a]	1,671.74±251.09[a]	3.61±1.01[a]	13.48±26.57[a]	5.38±1.09[a]	6.03±0.30[a]
Control site	2.00±0.20[b]	223.98±47.63[b]	523.46±440.37[b]	1.33±0.79[b]	3.01±1.87[b]	3.46±1.78[a]	6.86±0.11[b]

AIs = Ardley Island; BAr = Base Arctowski; BOh = Base O´Higgins. Different letters between collecting sites indicate significance at p <0.05.

Table 5. Spearman correlation coefficient matrix of the trace elements and the physical-chemical parameters measured for the soils with the presence of Humboldt penguins in northern Chile.

	Cd	Co	Cr	Cu	Mo	Ni	Sr	V	Zn	OM	NO_3	NH_4	K	P	EC
Co	-0.59**														
Cr	0.20	0.18													
Cu	-0.30	0.65**	0.14												
Mo	-0.07	0.40	0.53*	0.21											
Ni	-0.43	0.65**	0.23	0.33	0.50*										
Sr	0.56**	-0.04	0.08	0.09	-0.26	-0.26									
V	-0.47	0.88**	0.11	0.68**	0.15	0.50*	0.06								
Zn	0.37	0.24	0.63**	0.28	0.36	0.14	0.30	0.21							
OM	0.37	0.25	0.76**	0.28	0.35	0.17	0.34	0.28	0.85**						
NO_3	0.42	0.13	0.55*	0.04	0.47	0.10	0.36	0.06	0.73**	0.80**					
NH_4	0.55	-0.04	0.66	0.03	0.42	0.11	0.38	-0.08	0.78**	0.79**	0.91**				
K	0.61**	-0.71**	0.29	-0.35	0.03	-0.40	0.09	-0.72**	0.25	0.31	0.37	0.50*			
P	0.68**	-0.50*	0.42	-0.11	0.30	-0.33	0.29	-0.47*	0.38	0.32	0.46*	0.60**	0.66**		
EC	0.39	0.10	0.72**	0.12	0.40	0.11	0.30	0.15	0.68**	0.86**	0.73**	0.74**	0.47*	0.41	
pH	-0.59**	0.16	-0.55*	-0.02	-0.35	-0.14	-0.26	0.23	-0.61**	-0.65**	-0.72**	-0.84**	-0.50*	-0.54*	-0.51*

*Significant at *p* <0.05 **Significant at *p* <0.0

Table 6. Spearman correlation coefficient matrix of the trace elements and the physical-chemical parameters measure for

	Cd	Co	Cr	Cu	Mo	Ni	Sr	V	Zn	OM	NO_3	NH_4	K	P	EC
Co	-0.29														
Cr	0.41*	0.28													
Cu	0.78**	-0.35	0.22												
Mo	0.52**	0.12	0.39*	0.62**											
Ni	0.41*	-0.10	0.12	0.31	0.24										
Sr	0.68**	-0.65**	0.18	0.83**	0.48	0.37*									
V	-0.06	0.80**	0.60**	-0.26	0.21	0.01	-0.47*								
Zn	0.65**	-0.53**	0.15	0.84**	0.53**	0.31	0.90**	-0.38*							
OM	0.15	0.24	0.12	0.22	0.45*	0.37*	0.10	0.15	0.16						
NO_3	0.51**	-0.41*	0.09	0.67**	0.38*	0.03	0.60**	-0.38*	0.69*	-0.23					
NH_4	0.37*	0.34	0.39*	0.43*	0.70**	0.37*	0.20	0.31	0.31	0.71**	0.20				
K	0.16	0.58**	0.15	0.15	0.32	0.16	-0.27	0.43*	-0.09	0.44*	-0.05	0.61**			
P	0.50**	0.78**	-0.07	0.51**	0.19	0.16	0.66**	-0.61**	0.65**	0.01	0.53**	-0.03	-0.31		
EC	0.43*	0.10	0.63**	0.43*	0.62**	0.31	0.33	0.27	0.38*	0.56**	0.34	0.80**	0.25	0.14	
pH	-0.01	-0.67**	-0.25	0.07	-0.14	0.06	0.44*	-0.55**	0.27	-0.36	0.07	-0.47*	-0.72**	0.50**	-0.34

the soils with the presence of Adélie penguins in the Antarctic Peninsula area. *Significant at p <0.05 **Significant at p <0.01

Capitulo V TROPHIC TRANSFER OF CADMIUM IN AQUATIC FOOD WEBS FROM THE WESTERN PATAGONIA AND THE ANTARCTIC PENINSULA AREA

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Articulo en Preparacion.

Abstract

In aquatic environments, Cd contamination is a great concern because this non-essential metal presents risks both for wildlife and human health. Data about the concentration and transfer of Cd in Patagonian and Antarctic aquatic food webs are crucial for assessing the impacts of this element in pristine ecosystems. Consequently, the concentration of Cd was measured in thirty two species collected in the 2014 austral summer from one locations of the Western Patagonia and two locations of the Antarctic Peninsula. The main objective of this work was to assess the relationship between Cd concentration and trophic position. In the studied trophic positions, Cd showed a positive relationship between concentration and trophic level, which suggests biomagnification of this element in macroinvertebrates. However, there was a significant dilution when higher trophic organisms were considered.

Keywords: Cadmiun, Biomagnification, Trace elements, Marine food webs, Patagonia, Antarctica

Introduction

Cadmium (Cd) biomagnification in aquatic ecosystems remains a concern because this pollutant is detrimental to wildlife and humans (Eisler, 1985). Cd is a non-essential element with no biological function, and it is classified as one of the most hazardous heavy metals (Ravera, 1984). This metal is found widely in the Earth´s crust, spread by anthropogenic activities (e.g. metallurgy, electroplating, paints, combustion of coal and oil), erosion and volcanos (Kakkar and Jaffery, 2005). Cd is well known because of its toxicity to aquatic organisms and its potential for bioaccumulation, and the concentration of this metal has been studied in several marine species (Bargagli et al., 1996). Recently, some evidence revealed a possible biomagnification of Cd in different marine food webs (Cheung and Wang, 2008; Majer et al., 2014). Moreover, some studies have found that Cd concentrations increased at higher trophic levels, depending on the site and specific factors of the species, rather than on the characteristics of the element (Ikemoto et al., 2008; Zeng et al 2013).

Antarctica is one of the most pristine places left on the planet and has not been greatly affected by anthropogenic activities. Patagonia is also considered a relative pristine environment, which has also not been much affected by human presence, though it is located in lower latitudes. However, both environments are exposed to the impact of global and local anthropogenic activities (Commendatore and Esteves, 2007; Bargagli et al., 2008). The evidence indicates that global sources can reach remote areas via atmospheric transport of persistent pollutants from lower latitudes (Lambert et al., 1990; Smichowski et al., 2006). Considering the increase in population and industrial development in countries of the Southern Hemisphere, metals could be affecting these areas in particular (Celis et al., 2015). Edible marine organisms tend to accumulate high concentrations of metals, thus affecting human health (Primost et al., 2017). It is well known that Cd can cause several deleterious effects in fish and wildlife, including humans (Eisler, 1985). In order to understand the impact of human activities on the biogeochemical cycle of Cd at local and global scales, it is necessary to assess its level in remote and unpolluted areas.

By studying the trophodynamics (the way a chemical moves through different trophic levels), the concentration of a metal can increase (biomagnification), decrease (biodilution) or even present no tendency (Luoma and Rainbow 2008). Nitrogen stable isotope ratio ($\delta^{15}N$) analysis is a very useful tool to estimate the trophic position of a consumer (Cabana and Rasmussen, 1994). Thus, it is possible to test the relationship between $\delta^{15}N$ and Cd concentration, and therefore, the possible biomagnification of this element (Majer et al., 2014). The comparison between chemical concentrations and trophic levels through the use of stable isotopes can improve our understanding of biological phenomena in aquatic environments and possible human exposure through diet, an issue that has received special attention during the last decades (Luoma and Rainbow, 2008; Lavoie et al., 2013; Walters et al., 2016).

Material and methods

From the Chilean Western Patagonia (Sector 1, Fig. 1), species of macroinvertebrates and fishes were collected near the Marchant River Mouth (44°5´S, 73°5´W). From the Antarctic Peninsula (Sector 2), species of macroinvertebrates, fishes and birds were collected at Paradise Bay (64º51´S, 62º54´W), whereas species of macroinvertebrates and birds were sampled at Cape Shirreff (62º28´S, 60º46´W), South Shetland Islands. The samples of animals were obtained manually by using a van Veen grab or by Scuba diving, during February/2014. The fishes were captured by means of harpoon and nets, and then were anesthetized with 5% benzocaine (BZ-20®, Veterquimica), slaughtered by cessation of the spinal cord, and finally muscle samples were obtained. Soft tissues of mollusks were completely extracted, while in the other macroinvertebrates the whole body was retained. All specimens were stored at -20°C until their arrival to the laboratory.

Samples were freeze-dried until dry masses were constant and then were homogenized into a fine powder using a glass mortar and pestle pre-cleaned with 2% Conrad solution (Merck) for 24 h, washed with deionized water and HCl 1M and rinsed with distilled water (Van Wyk et al. 2001). According to availability, sub-samples between 0.02 and 0.45 g of the material were subjected to microwave digestion with high purity grade (GR) nitric acid, hydrochloric acid, and perchloric acid. The concentrations of Cd were determined by means of an electrothermal atomic absorption spectrometry (ET-AAS) ZEEnit 60 (Analytik Jena, equipped with Zeeman-effect BG correction system) at the Radioisotopes Lab of the Biophysics Institute, University of Rio de Janeiro (Brazil). All measurements were carried out in triplicate and resulting values were averaged. Quality control was carried out by means of certified reference material Dolt 4 (dogfish liver), Dorm 3 and Dorm 4 (fish protein) from National Research Council of Canada to test the accuracy of the method. Our recovery results from certified material were always above 90-110%.

The stable isotopes measurements were performed by means of elemental mass spectrometer Costech 4010 interfaced with Delta XP at the Stable Isotope in Nature Laboratory, University of New Brunswick (Canada). Stable isotope measurements were reported as delta isotope δ in parts per thousand (‰) (Post et al., 2002). Two standards were used as reference materials: atmospheric nitrogen (N_2) and methylene (CH_2), both certified by the International Atomic Energy Agency (IAEA) for isotope values (Logan et al., 2008; Wassenaar et al., 2000). Additionally, two certified standards of commercially available elements, acetinilide and nicotinamide were used. Replicates of each of 10 samples were performed, and the accuracy was 0.14 ± 0.14‰ for $\delta^{15}N$. According to Post (2002), $\delta^{15}N$ value is a direct indicator of trophic levels of consumers, being positive and significant the correlation between these variables. Data for the reference material presented both relative standard deviation and agreement between observed and certified concentrations lower than 10%, while blank signals were lower than 0.2% of the mean sample signal.

The biomagnification of Cd was quantified using the trophic level (TL) according to the following equation (Lavoie et al., 2013):

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \Delta^{15}N + \lambda \quad (1)$$

where λ is the trophic level of the baseline organism (being 2 for primary consumers), $\delta^{15}N_{consumer}$ and $\delta^{15}N_{baseline}$ are nitrogen stable isotope values as part per thousand (‰) of a given consumer and the baseline organism, respectively. A trophic discrimination factor for $\Delta^{15}N$ of 3.4‰ was used for aquatic organisms (Jardine et al., 2006; Borgå et al. 2012).

Levels of Cd were $\log_{10}$-transformed to meet the assumptions of normality and biomagnification was examined using linear regressions as in Lavoie et al. (2013) using the following equations:

$$\log_{10}[Ta] = b\ \delta^{15}N + a \quad (2)$$

$$\log_{10}[Ta] = b\ TL + a \quad (3)$$

where b in equation 3 is known as the trophic magnification slope (TMS) and the antilog as the trophic magnification factor (TMF). Statistical analyses were performed using Infostat 2017 for Windows version (di Rienzo et al. 2016).

Results and Discussion

The concentrations of Cd for the studied species are summarized in Table 1. The range of Cd concentrations varied widely (from 0.0014 to 28.10 μg g^{-1}). At the Chilean Western Patagonia coast (Marchant River Mouth), the species with the highest Cd concentration were the carnivore sea star *Stichaster striatus* (3.98 μg g^{-1}). In contrast, the species with the lowest Cd levels were the benthopelagic predator fish *Merluccius australis* (0.002 μg g^{-1}). At the Antarctic Peninsula, the species with the highest Cd concentration were the carnivorous starfish *Odontaster validus* (28.10 μg g^{-1}) from Cape Shirreff, and the predator and scavenger starfish *Diplasterias brucei* (7.58 μg g^{-1}) from Paradise Bay. In contrast, the species with the lowest Cd levels were the seabird skua *Catharacta maccormicki* (0.021 μg g^{-1}) from Cape Shirreff, and the carnivore fish *Trematomus hansoni* (0.0045 μg g^{-1}) from Paradise Bay. Our highest Cd levels found in soft tissue of *Nacella concinna* on South Shetland Islands are higher than those reported previously in the same species (5.04 μg g^{-1}) on the same area (Ahn et al., 2002).

In macroinvertebrates, the Cd levels of the Antarctic Peninsula area (0.154-28.10 μg g^{-1}) were higher than those levels found at Western Patagonia (0.30-3.98 μg g^{-1}). Also, our Cd levels from Antarctica were similar to those levels reported in benthic organisms from the same area (0.20-15.6 μg g^{-1}) (Szopińska et al., 2017) and those from the Barents Sea, Northern Hemisphere (0.20-24 μg g^{-1}) (Zauke et al., 2003), although our Cd levels from Western Patagonia were lower than those levels. This is indicative of the natural enrichment of Cd in polar food chains, a phenomena typically occurring in Antarctica (Sanchez-Hernandez, 2000; Grotti et al., 2008). The concentrations of Cd in macroinvertebrates from Paradise Bay and Cape Shirreff are higher than those reported in superficial sediments (0.1-0.9 μg g^{-1}) of different

Antarctic sites (Negri et al., 2006; Ianni et al., 2009; Ribeiro et al., 2011), thus evidencing the occurrence of bioaccumulation of this element.

In general, our Cd levels in marine fish muscles are lower than those levels reported from the Northern Hemisphere (0.15-0.60 μg g^{-1}) (Elnabris et al., 2013; El-Moselhy et al., 2014) and from subantarctic Kerguelen Island (0.14-0.65 μg g^{-1}) (Jaffal et al., 2011). Our Cd levels found in muscles of fish at Paradise Bay are lower than those levels reported from Eastern Antarctica (0.1-0.2 μg g^{-1}) (Sanchez-Hernandez, 2000) and those levels reported from Terra Nova Bay (0.03-0.04 μg g^{-1}) (Szopińska et al., 2017). Our Cd concentrations found in muscle of fishes are much lower than the maximum permissible level for human consumption (0.25 μg g^{-1}) in Europe (Jaffal et al., 2011), excepting the Cd levels found in *Genypterus blacodes* (0.38 μg g^{-1}) from Marchant River Mouth, which is a commercial species. The highest Cd levels found in *Genypterus blacodes* can be explained as demersal fish tend to exhibit higher metal levels than fish living in the upper water column (e.g. *Merluccius australis*, *Salilota australis*) because they are in direct contact with the sediments and they receive a greater amount of metal concentrations from zoobenthic predators (Yi et al., 2011).

Our maximum Cd levels found in feathers of *Pygoscelis papua* at Cape Shirreff were lower than those levels reported by Metcheva et al. (2010) at Livingstone Island (0.50 μg g^{-1}). In general, Cd concentrations in bird feathers are lower than those found in seabirds of the Northern Hemisphere (0.04-1.28 μg/g) (Kim et al., 1998; Agusa et al., 2005; Mansouri et al., 2012).

Regressions of $\log_{10}$[Cd] versus trophic level from different food webs (Fig. 2) showed significant differences between the aquatic ecosystems studied. In macroinvertebrates, data from Western Patagonia, Marchant River Mouth (Fig. 2A) showed a significant positive relationship between Cd concentration and trophic level (TMS 0.66; R2 0.76; *p*-valor 0.0004). At the Antarctic Peninsula area there was a significant positive relationship between Cd concentration and trophic level at both locations, Paradise Bay (TMS 1.90; R2 0.54; *p*-valor 0.03) and Cape Shirreff (TMS

2.84 R^2 0.86, *p*-valor 0.02) (Fig. 2A). This finding indicates that there is an increase in Cd levels throughout this benthic food web. Our results are in agreement with those reported by Majer et al. (2014) in a food web from Admiralty Bay (King George Island, Antarctica), who observed an increase in Cd levels throughout benthic organisms, thus suggesting a case of biomagnification. In contrast, regressions of Cd and trophic levels for fishes and birds showed no significant relationships at any locations (Fig. 2B). Considering a more diverse food web, a significant dilution of Cd in the higher trophic levels of the food webs studied here was noted in all locations Western Patagonia, Marchant River Mouth (TMS -0.5; R^2 0.45; p-valor 0.02), Paradise Bay (TMS -0.81; R^2 0.52; p-valor 0.005) and Cape Shirreff (TMS -0.92 R^2 0.72, p-valor 0.01) (Fig. 2C). A probable explanation of Cd biodiminution across food webs is linked to a greater elimination rate of Cd concentrations in fish and birds (Nfon et al., 2009), whereas benthic organisms tend to have low elimination rates for metals (Wang, 2002). Similarly, Signa et al. (2017) also had observed Cd biodilution in fishes of the Mediterranean Sea and other places in the Northern Hemisphere (Campbell et al., 2005; Mathews and Fisher, 2008). In our case, the results clearly show that the trophic transfer of this metal is highly dependent on the species.

The slopes of the linear regressions concerning a simpler food web were similar between the Antarctic locations, and both differed from the values found at Patagonia (Table 2). No differences were detected between Antarctica and Patagonia considering a more diverse food web (Table 3). The present study revealed that there is biomagnification of Cd in macroinvertebrates. However, there was a significant dilution when higher trophic organisms (like fishes and birds) were considered.

Finally, it is needed to take caution in interpreting our experimental results. They are indicating a need for knowing more deeply about the mechanisms of trophic transference of Cd in marine environments. This is relevant, since it has been shown that the lower species of the trophic chain could be most affected by increasing Cd levels, rather than the higher organisms. Human health can be at risk due to consumption of seafood (Primost et al., 2017). Thus, more research is needed in order to know which species are really most affected by the increase of the Cd levels in still pristine aquatic environments.

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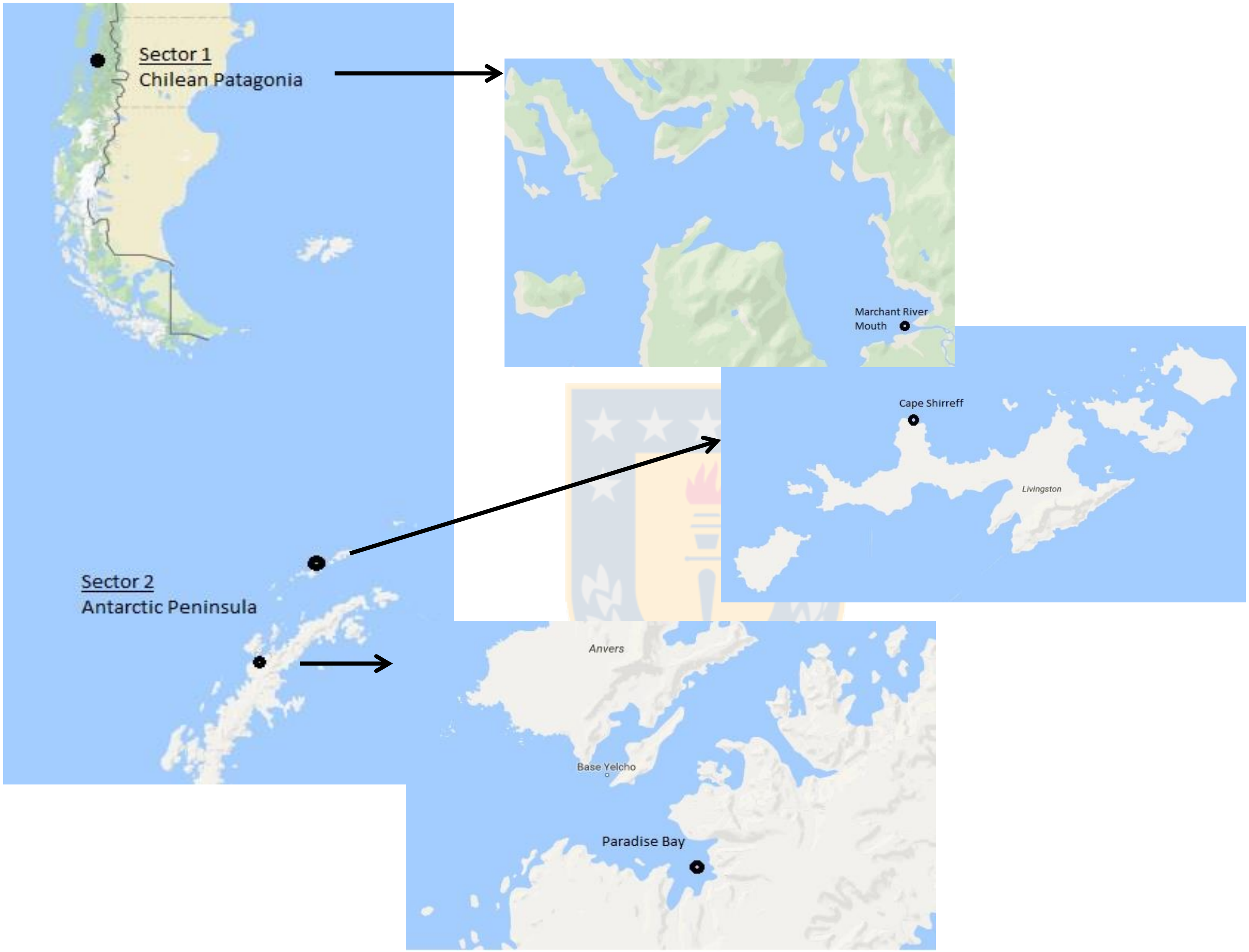

Fig. 1. Locations of study marine food webs in Chilean Western Patagonia (Sector 1) and Antarctic Peninsula area (Sector 2).

Table 1. Concentration (µg g^{-1} dry weight) of Cd, $\delta^{15}N$ values (‰), and trophic level (TL) in animals of different locations from the Chilean Western Patagonia coast (CP) and Antarctic Peninsula area (AP). Data presented as mean ± standard deviation. *Baseline species.

Location	**Group**	**Species**	**N**	**Sample**	**$\delta^{15}N$(‰)**	**TL**	**Cd (µg g^{-1})**
Marchant River M. (CP)	Macroinvertebrate	*Stichaster striatus**	3	Soft tissue	12.92 ± 0.29	3.68 ± 0.09	3.98 ± 0.53
		Aulacomya ater	3	Soft tissue	9.69 ± 0.49	2.73 ± 0.14	1.59 ± 1.54
		Hemigrapsus granulosus	3	Whole body	8.16 ± 0.97	2.28 ± 0.28	0.302 ± 0.089
		Loxechinus albus	3	Soft tissue	10.54 ± 0.70	2.98 ± 0.20	0.528 ± 0.056
	Fish	*Eleginops maclovinus*	3	Muscle	13.58 ± 0.52	3.87 ± 0.15	0.053 ± 0.078
		Genypterus blacodes	3	Muscle	16.29 ± 0.25	4.67 ± 0.07	0.38 ± 0.47
		Macruronus magallanicus	1	Muscle	12.56	3.57	0.016
		Merluccius australis	1	Muscle	14.69	4.20	0.002
		Salilota australis	1	Muscle	16.14	4.62	0.004
		Schroederichthys chilensis	2	Muscle	15.53 ± 0.43	4.44 ± 0.13	0.136 ± 0.153
Paradise Bay (AP)	Macroinvertebrate	*Diplasterias brucei**	2	Soft tissue	7.25 ± 0.01	2.86 ± 0.004	7.58 ± 2.21
		Chorismus antarcticus	1	Soft tissue	7.60	2.97	6.77
		Chorismus antarcticus	3	Whole body	7.37 ± 0.30	2.90 ± 0.09	0.80 ± 0.27
		Nacella concinna	3	Soft tissue	5.06 ± 0.74	2.22 ± 0.22	5.08 ± 4.40
		Euphausia superba	3	Whole body	5.83 ± 0.95	2.45 ± 0.28	0.26 ± 0.16
		Haplocheira sp	3	Whole body	6.78 ± 0.02	2.73 ± 0.01	1.92 ± 0.26
	Fish	*Harpagifer antarcticus*	3	Muscle	11.55 ± 0.58	4.13 ± 0.17	0.006 ± 0.0055
		Trematomus bernacchii	1	Muscle	11.79	4.20	0.007
		Trematomus hansoni	2	Muscle	11.64 ± 0.81	4.16 ± 0.24	0.0045 ± 0.0002
	Seabird	*Catharacta maccormicki*	3	Feather	11.38 ± 0.87	4.08 ± 0.26	0.042 ± 0.018
		Pygoscelis papua	3	Feather	10.49 ± 4.40	3.82 ± 1.29	0.078 ± 0.038
Cape Shirreff (AP)	Macroinvertebrate	*Diplasteria brucei**	1	Soft tissue	6.59	0.06	1.03
		Macroptychaster sp	1	Soft tissue	6.62	0.07	0.154
		Nacella concinna	3	Soft tissue	8.54 ± 0.09	0.63 ± 0.03	17.73 ± 9.54
		Odontaster validus	1	Soft tissue	7.91	0.45	28.10
	Seabird	*Pygoscelis Antarctica*	1	Feather	15.66	2.73	0.036
		Pygocelis papua	1	Feather	12.51	1.80	0.109
		Catharacta maccormicki	3	Feather	13.88 ± 2.58	2.21 ± 0.76	0.021 ± 0.005

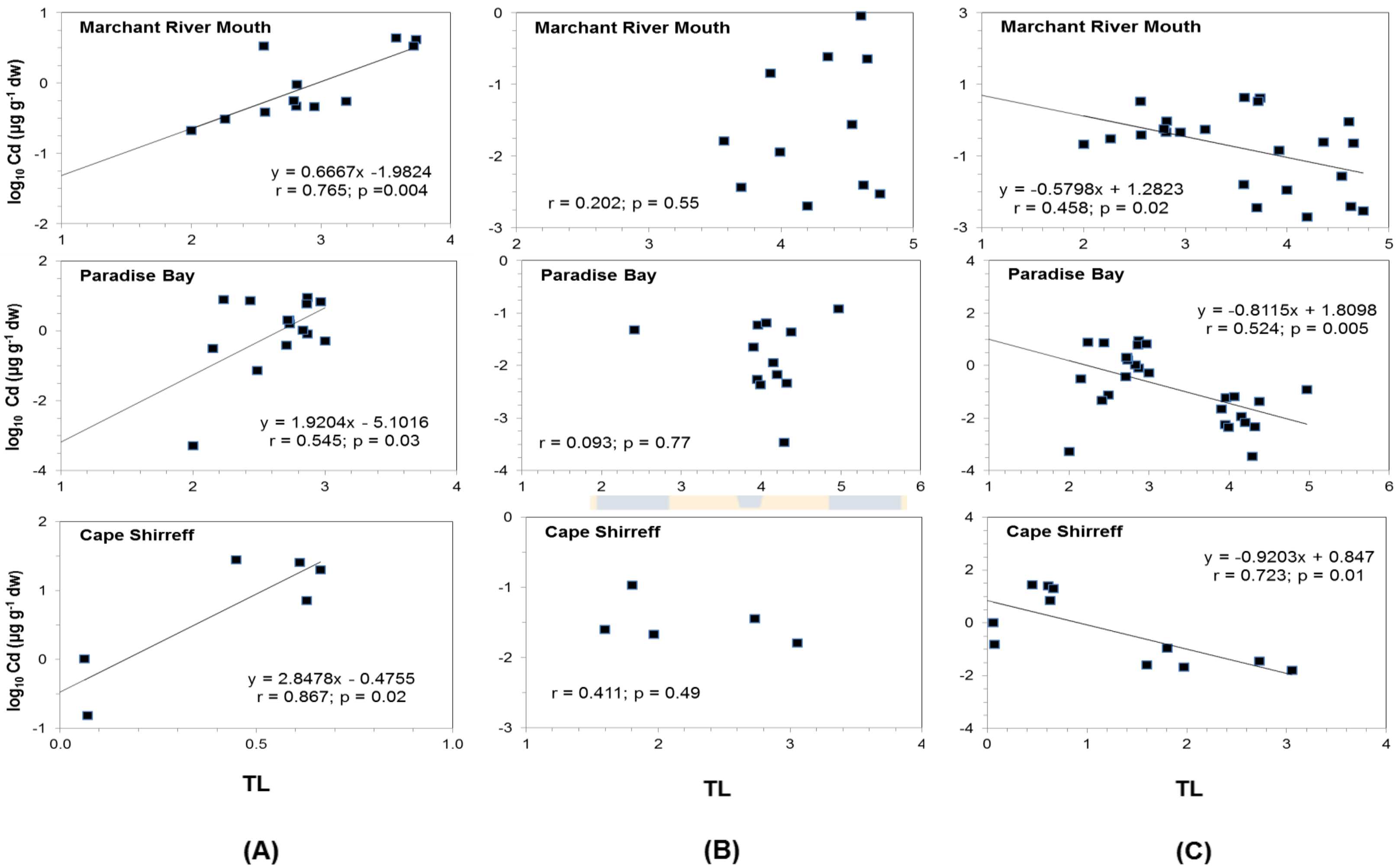

Fig. 2. Regressions of logCd versus trophic level (TL) for (A) a simpler food web (data of macroinvertebrates); (B) data of fishes; (C) a more diverse food web (data of the whole food web) in Western Patagonia (Marchant River Mouth), and Antarctic Peninsula area (Paradise Bay and Cape Shirreff).

Capitulo VI BIOMAGNIFICATION OF TANTALUM THROUGH DIVERSE AQUATIC FOOD WEBS

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Abstract

Tantalum (Ta) is a Technology-Critical Element (TCE) that is growing in global demand because of its use in electronic and medical devices, capacitors, aircraft, and hybrid cars. Despite its economic relevance, little is known about its environmental concentrations and the trophodynamics of Ta in aquatic food webs have not been studied. Invertebrates and fishes from coastal marine food webs representing different climatic zones in northwestern Chile, western Chilean Patagonia, and the Antarctic Peninsula were sampled and analyzed for Ta. The trophic level (TL) of species was assessed with nitrogen stable isotopes ($\delta^{15}N$), and carbon stable isotopes ($\delta^{13}C$) were used to trace energy flow in the food webs. Levels of Ta varied among taxa and sites, with the highest values found in fishes (0.53 – 44.48 ng g^{-1}dry weight) and the lowest values found in invertebrates (0.11 – 7.80 n ng g^{-1}dry weight). The values of $\delta^{13}C$ ranged from -11.79 to -25.66 ‰. Ta biomagnified in all four aquatic food webs, with slopes of log Ta versus TL ranging from 0.16 to 0.60. This has important implications as little is known about its potential toxicity and there may be increased demand for TCEs such as Ta in the future.

Keywords Tantalum, Biomagnification, Trophic Transfer, Aquatic Ecosystems, Technology-Critical Elements, Marine Organisms, Stable Isotopes.

Introduction

Tantalum (Ta) is a rare transition element that is highly corrosion-resistant and stable at high temperatures,[1, 2] and it is increasingly used in technology related to renewable energies, electronics, the automotive and aerospace industries, and biomedicine.[3, 4] World production of Ta has increased over the last two decades, although its extraction remains low (ca. 1000 metric tons per year) when compared to other elements.[5] Although Australia, Brazil, Canada, Ethiopia and Nigeria have produced Ta, countries such as Burundi, Congo and Rwanda (65% of global production since 2014) have used it to finance illegal military operations during civil wars, dubbing it a “conflict mineral”.[5, 6] Nonetheless, it is estimated that new uses for Ta will increase global demand and production[4] but its environmental concentrations and fate are poorly characterized.[7]

Published data on Ta levels in the environment are scarce, focusing mainly on mineralogical analysis and then abiotic matrices,[7] with only a few reports on Ta in aquatic animals. Ascidians (*Styela plicata*) from Japanese waters had 100-410 mg g^{-1} dw Ta[8] whereas marine organisms from coastal areas of southern England ranged from 0.009 in mollusks to 2.3 mg g^{-1} dw in crustaceans.[9] Chebotina et al. [10] reported the bioconcentration of Ta from water to phytoplankton ($>10^1$) and zooplankton ($>10^7$). Despite evidence of Ta bioaccumulation in aquatic organisms, the factors affecting its concentrations in different species have not been examined.

Metals such as mercury, persistent organic pollutants and organotin compounds are known to biomagnify in diverse aquatic food webs to levels in upper-trophic-level fish that may pose a risk to fish consumers and the fish themselves.[11-13] The trophic level (TL) of species is estimated from $\delta^{15}N$ and frequently used to provide a measure of the relative trophic position of organisms within food webs.[11] Levels of contaminants are regressed against TL to understand whether they biomagnify and these relationships can be compared among ecosystems differing in species composition, physical and chemical characteristics, and climatic zones.[11, 12]

There is a lack of knowledge on the concentrations of Ta in biota and whether this element biomagnifies through aquatic food webs.[7] This is important to address because of the likely increased use of Ta and the potential risk it may pose from dietary exposures.[13] The objectives of the present study were to determine the concentrations of Ta and the relative trophic level of aquatic organisms from marine coastal food webs across three climatic zones in Antarctica and Chile. The results show for the first time that there is an increase in Ta concentrations with increasing trophic level, and that its biomagnification occurred at sites differing in their physical and biological characteristics.

Material and methods

Field collections

During the austral summer of 2015, four marine ecosystems with different climatic conditions were sampled in the following regions of the southern hemisphere (Figure 1): northwestern coast of Chile (Sector A), with a tropical hyper-desertic climate;[14] western Chilean Patagonia (Sector B) with a climate classified as template hyper-oceanic;[14] and the Antarctic Peninsula area (Sector C), which is classified as a cold desert.[15] In northwestern Chile, samples were obtained from Pan de Azúcar Bay (26°09´S, 70°40´W). In Chilean Patagonia, samples were obtained from two sites: the first was off of La Leona Island (44°1´58"W, 73°7´56"W) and the second was at the mouth of the Marchant River (44°5´15"S, 73°5´6"W). In Antarctica, samples were obtained from Fildes Bay (62°12´S, 58°58´W).

Fishes and invertebrates were collected from each of the locations by SCUBA to ensure the collection of the selected species, as well as to minimize any impacts of sampling. At Pan de Azúcar Bay in northwestern Chile, 8 species of macroinvertebrates and 6 species of fishes were collected (N = 61; Supplementary Table S1). In Chilean Patagonia, 4 species of macroinvertebrates and 3 species of fishes were collected at the mouth of the Marchant River (N = 31), and 4 species of macroinvertebrates and 3 species of fishes were sampled at La Leona Island (N = 28; Supplementary Table S2). At Fildes Bay in Antarctica, 9 of both macroinvertebrate and fish species were sampled (N = 55; Supplementary Table S3). Fish were anaesthetized with BZ-20 (Veterquimica), sacrificed by severing the spinal cord, and sampled for muscle tissues. Soft tissues of mollusks were collected and whole bodies of other macroinvertebrates were retained. All specimens were stored at -20°C until processed in the laboratory.

Laboratory analyses

Individual fish muscle and soft invertebrate tissues were freeze-dried until dry masses were constant and then were homogenized into a fine powder using a glass mortar and pestle pre-cleaned with 2% Conrad solution (Merck) for 24 h, washed with deionized water and HCl 1M and rinsed with distilled water.[16] Sub-samples (0.2 g) were placed into 50 mL Teflon beaker with 5 mL of ultrapure nitric acid and heated (at 110ºC) until almost dry (about 3 h). Then 5 mL of ultrapure nitric acid and 1 mL of hydrogen peroxide were added and the mixture was heated again to near dryness (about 3 h). The residue was dissolved in 5 mL of 1% ultrapure nitric acid, filtered with glass fiber filter® (<0.45 µm), and then transferred to a centrifuge tube. This digestion and filtration were repeated four times so as to obtain a final volume of 25 mL. Total Ta was determined by mass spectrometry coupled with a plasma inductor (ICP-MS, NexION-350D, Perkin Elmer) at the Environmental Health Science Laboratory, Toyo University (Japan).

To ensure the quality of the Ta measurements, a seven-point calibration curve was made and a median response factor used to calculate sample concentrations. The detection limits and quantification limits were 0.0019 and 0.036 ng g^{-1} dw respectively ng g^{-1}dw for each batch of samples calculated as 3X and 5X the standard deviation of the blanks (n = 12). A certified reference material (CRM) for Ta in biological materials is not available. Instead a Custom Claritas PPT Grade Tantalum for ICP-MS (CLTA9-1BY) by SPEXertificate (n = 12) and Multi-element Calibration Standard 5 by Perkin Elmer (n = 12) were used. The internal standard was In (stable isotope of indium, standard atomic weight 115). Triplicates of every 10^{th} sample were analyzed and the accuracy was 0.28 ± 0.29 ng g^{-1} for Ta (n = 54). All Ta values are expressed on a dw basis.

Quantification of Stable Isotopes

Tissues (1 mg) were analyzed for carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) isotopes using an elemental mass spectrometer Costech 4010 interfaced with Delta XP at the Stable Isotopes in Nature Laboratory (SINLAB) at the University of New Brunswick (Canada). The stable isotope measurements were reported in delta notation (δ) and in parts per thousand (‰). Two reference materials, N-2 (n = 6) and CH-7 (n = 6), both certified by the International Atomic Energy Agency (IAEA) for isotope values[15, 16] were used as well as certified standards of commercially available elements, Acetanilide (n = 18) and Nicotinamide (n = 18). In addition, three laboratory working standards, bovine liver (n = 18), muskellunge muscle (n = 42) and protein (n = 18) were used and they had an average deviation of 0.03‰ from the long term values. Replicates were performed of every 10th sample and the accuracy was 0.2 ± 0.17‰ for $\delta^{13}C$ and 0.14±0.14‰ for $\delta^{15}N$ (n=24).

The raw $\delta^{15}N$ values were adjusted by subtracting the average $\delta^{15}N$ of primary consumers from each site, thus obtaining $\delta^{15}N_{adj}$ values.[17, 18] Raw and lipid-adjusted $\delta^{13}C$ data were used to ensure organisms were energetically linked and details are given in the SI file (Table S7−S12 and Figure S1). Consumer $\delta^{15}N$ values were also converted to trophic levels (TL) according to the following equation:

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \Delta^{15}N + \lambda \quad (1)$$

where, λ is the trophic level of the baseline organism, herein 2 for primary consumers. $TL_{consumer}$ is the trophic level of a given consumer, and $\delta^{15}N_{consumer}$ and $\delta^{15}N_{baseline}$ are raw $\delta^{15}N$ values of a given consumer and the baseline organism for each site (See tables TS7, TS8 and TS9). A trophic discrimination factor for $\delta^{15}N$ ($\Delta^{15}N$) of 3.4‰ was used as in Lavoie et al.[11]

Data analysis

Levels of Ta were $\log_{10}$-transformed to meet the assumptions of normality and biomagnification was examined with linear regressions as in Lavoie et al.[11] and Yoshinaga et al.[19] using the following equations:

$$\log_{10}[Ta] = b\ \delta^{15}N_{adj} + a \quad (2)$$

$$\log_{10}[Ta] = b\ TL + a \quad (3)$$

where b in equation 2 is known as the trophic magnification slope (TMS) and the antilog of the slope in equation 3 as the trophic magnification factor (TMF). Analysis of Covariance (ANCOVA) was used to determine whether Ta biomagnification was significantly different in the four food webs. Statistical analyses were performed using JMP from SAS.

Results and Discussion

The trophic levels of species sampled in northwestern Chile showed TL ranged between 2.21 ± 0.16 and 4.81 ± 0.11; *Crucibulum scutellatum* had the lowest TL and *Hemilutjanus macrophthalmus* had the highest TL. In contrast, in Chilean Patagonia at the Marchant River Mouth *Fissurella* spp. (2.48 ± 0.69) had the lowest trophic level and *Graus nigra* (4.69 ± 0.06) had the highest trophic level. At Leona Island, *Aulacomya ater* (2.21 ± 0.13) and *Pinguipes chilensis* (4.23 ± 0.27) were the species with the lowest and highest TL, respectively. Finally from the Antarctic Peninsula, the TL values ranged from 2 (*Cnemidocarpa verrucosa*) to 4.13 (*Pagothenia borchgrevinki*).

Across all three climatic regions, Ta levels in macroinvertebrates were consistently lower than those in fishes (SI file Tables S4−S6). In macroinvertebrates from the northwestern coast of Chile, the lowest mean Ta level was in sea snails (*Crucibulum scutellatum*, 0.17 ng g^{-1}), a benthic grazer, whereas the highest was in sea stars (*Forcipulatida spp.*, 0.83 ng g^{-1}), a benthic predator. In fishes, the lowest and highest mean Ta levels were found in *Pinguipes chilensis* (a benthic-pelagic predator, 2.09 ng g^{-1}) and *Trachurus symetricus murphyi* (a pelagic predator, 2.86 ng g^{-1}), respectively, and they were 12.3 to 17.6 times higher than the lowest levels found in macroinvertebrates from this location. In western Chilean Patagonia at Marchant River, mean Ta levels ranged from 1.05 ng g^{-1} in the filter-feeding mollusk (*Aulacomya ater*) to 1.51 ng g^{-1} in crabs (*Cancer coronatus*), benthic predators. In fishes, mean Ta was 2.08 ng g^{-1} in *Genypterus chilensis* (a benthic predator) and 2.48 ng g^{-1} in *Eleginops maclovinus* (a benthic-pelagic predator), over two times higher than those in mollusks from the same location. Similarly for La Leona Island, the lowest Ta levels were found in macroinvertebrates, and ranged from 0.23 ng g^{-1} in mollusks (*Aulacomya ater*,), to 0.37 ng g^{-1} in mollusk (*Concholepas concholepas*), a filter-feeder. In fishes from this location, the lowest and highest Ta levels were in *Panguipes chilensis* (0.61 ng g^{-1}) and *Sebastes capensis* (1.84 ng g^{-1}, a benthic predator), respectively. Finally, from the Antarctic Peninsula, the lowest and highest mean Ta levels of all macroinvertebrates were in sea urchin (*Abatus agassizii*, 0.43

ng g^{-1}), which is a benthic filter-feeder, and starfish (*Odontaster validus*, 7.8 ng g^{-1}), a predator. Fishes from Antarctica had the highest Ta of all sites examined herein, with mean levels ranging from 2.23 ng g^{-1} in *Pagothenia hansoni* to 14.0 ng g^{-1} in *Notothenia kempi*, both are benthic predators.

For those taxa collected at several sites, the levels of Ta varied but not consistently across the climatic gradient. More specifically, *Aulacomya ater* had Ta levels at Marchant River Mouth that were 4.5 and 2 times lower than the levels found at nearby La Leona Island and the most northerly site Pan de Azúcar Bay, respectively. In contrast, *Fissurella* spp. had lower Ta levels at Pan de Azúcar Bay (0.31 ng g^{-1}) than at Marchant River Mouth (1.4 ng g^{-1}). Finally, *Concholepas concholepas* showed similar Ta levels of 0.26 ng g^{-1} at Pan de Azúcar Bay and 0.38 ng g^{-1} at La Leona Island; similarly, *Genypterus chilensis* had Ta levels of 1.83 ng g^{-1} and 2.08 ng g^{-1} from La Leona Island and Marchant River Mouth, respectively. Although nothing is known about the dynamics of Ta in organisms and the factors that affect its uptake and storage, these data suggest that site specific factors may be relevant in determining its environmental fate.

It is possible to make only limited comparisons of the Ta levels in marine species from Chile and Antarctica to data from other regions. Ta values in the current study are much lower than those reported in macroinvertebrates from Southern England (ranging from 0.1 to 2 ppm dw),[9] and those in the ascidian *Styela plicata* collected off the coast of Japan (between 100 to 410 ppm dw).[8] It was not possible to find other studies on Ta in fishes.

Trophic transfer of Ta

In general, in all the food webs studied here (Tables S7−S9 and Figure S1), fishes had $\delta^{13}C$ values that were between those of the macroinvertebrates, indicating reliance on both pelagic and benthic energy sources, as observed in temperate lake food webs.[20] Ta levels increased with the TL of the organisms, showing biomagnification of this element (Table S13 and Figure S2). The TMS ranged from 0.05 at Marchant River Mouth in Chilean Patagonia to 0.18 at Fildes

Bay in Antarctica (Table 1 and Figure 2). The slopes of log Ta versus TL were significantly different across sites (site * TL, $p < 0.001$) and translated into TMF values of 2.29 in northwestern Chile, 2.00 and 1.45 at the sites in Chilean Patagonia and 3.98 in Antarctica, indicating that Ta does not consistently biomagnify across sites and that the highest trophic transfer of Ta occurred at the coldest latitude. Indeed, the slope for Antarctica was significantly higher than for all other sites and this may be because these marine food webs are simple and clearly defined on the basis of benthic and pelagic populations, which are strongly coupled with each other.[21] The Ta biomagnification slopes for the food webs of Pan de Azúcar Bay and La Leona Island fell between those of the Marchant River and Antarctica sites and were not statistically different from one another ($p = 0.46$). In contrast, Marchant River Mouth had the lowest TMF and a slope that was significantly lower than those at all other sites ($p < 0.012$). The lower biomagnification of Ta may be the result of the large inputs of nutrients and other elements from the river to the coast[22, 23] that in turn affect the bioaccumulation and trophic transfer of this element, as has been observed for Hg[11]. It was not possible to find either laboratory or field studies on Ta trophodynamics for comparison. The TMF values observed for Ta were mostly lower than those reported for TBT (from 3.88 to 4.62)[13]. Also, our log Ta versus $\delta^{15}N_{adj}$ slopes were lower than those reported in a global review of total Hg (0.21 for polar, 0.22 for temperate, 0.16 for tropical systems) and MeHg (0.21 for polar, 0.26 for temperate and 0.14 for tropical systems) in marine environments[11], suggesting that Ta biomagnifies to a lesser extent than for Hg.[11] The exception was for Ta in the Fildes Bay food web, which had a TMS in the same range of polar areas and slightly higher than tropical. Overall, Ta biomagnified regardless of the latitude and composition of the marine food web, which could be a characteristic of this particular element, as with Hg.[11] We recommend Ta biomagnification be examined in other diverse food webs to develop a broader understanding of how ecosystem characteristics affect the fate of this element.

There is no published information on Ta toxicity in aquatic animals. In mammals, Ta_2O_5 inhalation can cause bronchitis and interstitial pneumonitis.[24] So far, there is a general consensus that Ta does not play a biological role[25] but it is unclear whether the biomagnification of Ta observed herein poses a risk to upper-trophic-level consumers. This becomes important considering that the production and use of Ta will likely increase with the growing demand for new technologies and, as such, is an issue that needs more investigation.

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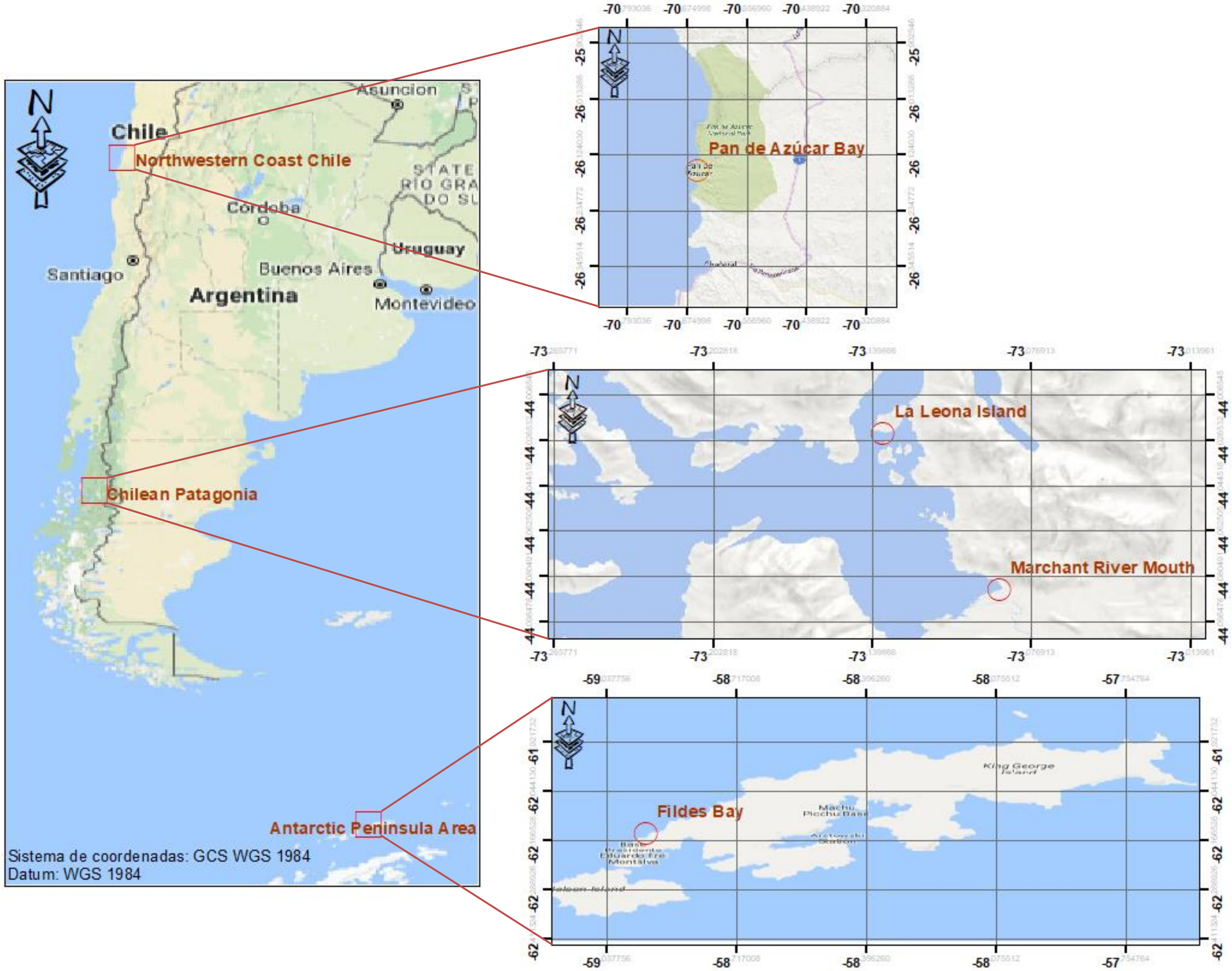

Figure 1. Map of the locations of the marine coastal food webs sampled in 2015 in northwestern Chile, Chilean Patagonia and Antarctica.

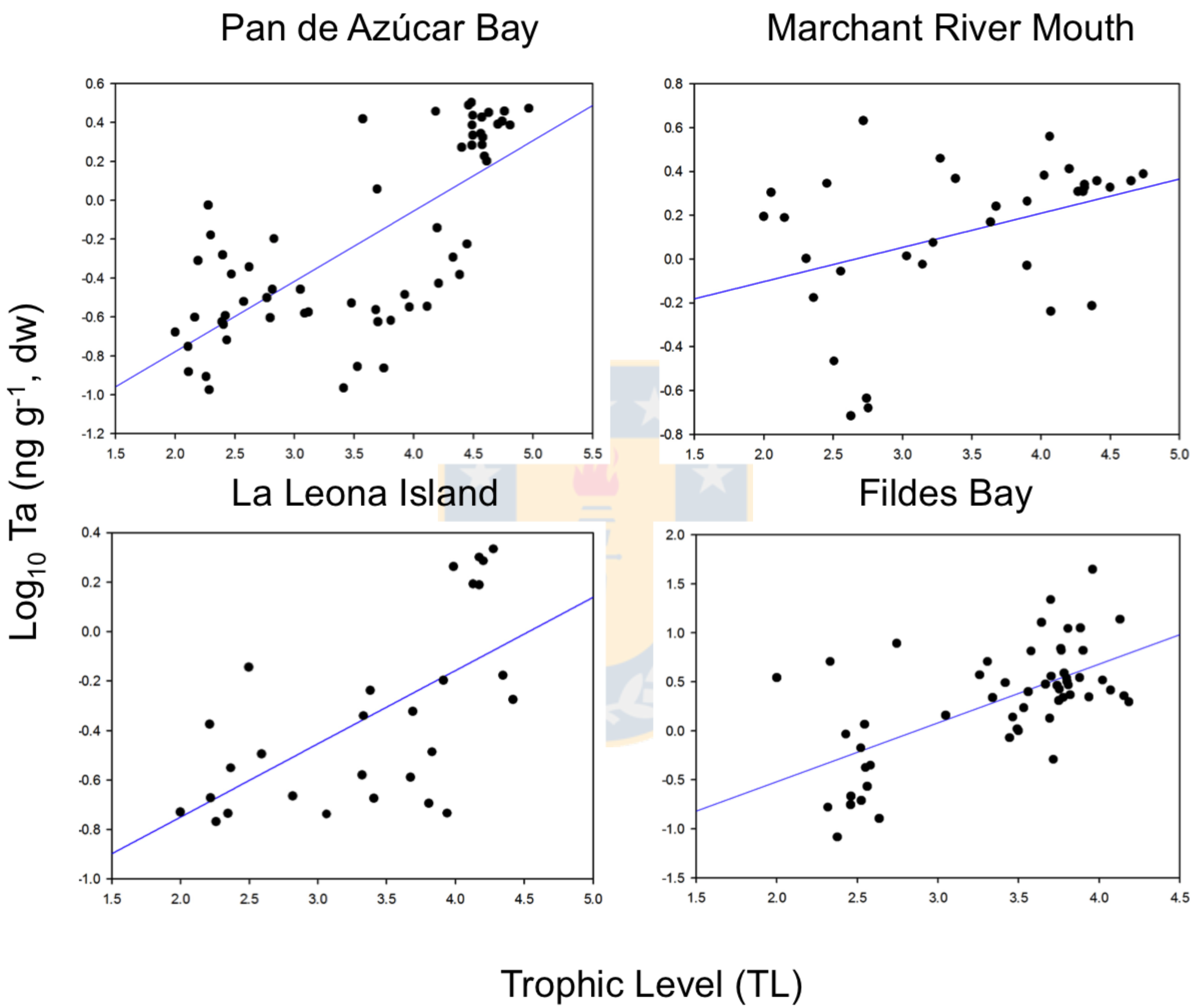

Figure 2. Regressions of log Ta versus trophic level (TL) for fishes and macroinvertebrates collected from coastal, marine food webs in northwestern Chile (Pan de Azucar), Chilean Patagonia (Marchant River Mouth and La Leona Island), and Antarctica (Fildes Bay). dw: dry weight

Table 1. Regressions of $\log_{10}$Ta versus TL for fishes and invertebrates collected from coastal sites in northwestern Chile, Chilean Patagonia, and Antarctica (see Figure 2). Letters indicate significant differences among Trophic Magnification Slopes (TMS)

Sector	Location	Slope	Intercept	R^2	*p*-value	N
A	Pan de Azúcar Bay	$0.36^a \pm 0.04$	-1.50 ± 0.17	0.52	<0.0001	61
B	La Leona Island	$0.30^a \pm 0.07$	-1.34 ± 0.25	0.39	0.0004	28
	Marchant River Mouth	$0.16^b \pm 0.07$	-0.42 ± 0.23	0.15	0.02	34
C	Fildes Bay	$0.60^b \pm 0.10$	-1.72 ± 0.35	0.39	<0.0001	55

A = Northwestern Coast of Chile; B = Western Chilean Patagonia; C = South Shetland Island (Antarctic Peninsula area).

Supplementary Information to

BIOMAGNIFICATION OF TANTALUM THROUGH DIVERSE AQUATIC FOOD WEBS

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Table S13. Regressions of log10Ta versus δ15Nadj for fishes and invertebrates collected from coastal sites in northwestern Chile, Chilean Patagonia, and Antarctica (see Figure S2). Slope equals Trophic Magnification Slope (TMS) as in Lavoie et al. (2013)

Figure S1 Relationships between stable isotopes of δ^{15}Nadj and δ^{13}CLip (‰) from different food webs studied.

Figure S2. Regressions of logTa versus $\delta^{15}N_{adj}$ for fishes and macroinvertebrates collected from coastal, marine food webs in northwestern Chile (Pan de Azucar), Chilean Patagonia (Marchant River Mouth and La Leona Island), and Antarctica (Fildes Bay).

Text S1. Supplemental methods

Laboratory Analyses

The lipid content of subsamples was determined according to the methods of Bligh and Dyer (1959)[1] with modifications by Smedes (1999)[2]. These data were then used to correct the raw δ^{13}C for % lipids using the equation in Post et al. (2007)[3].

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Text S2. Food web structures

Two different groups of organisms were found within the food web of Pan de Azúcar Bay (Supplementary Table S7; Fig. S1). The first group consisted of lower-trophic-level organisms, with *Crucibulum scutellatum* having the lowest (0.73‰) and *Fissurella* spp. having the highest (3.77‰) $\delta^{15}N_{adj}$ values. The second group consisted mainly of fishes and some macroinvertebrates, with *Concholepas concholepas* having the lowest (6.3‰) and *Hemilutjanus macrophthalmus* having the highest (9.55‰) $\delta^{15}N_{adj}$ values. Most of the species exhibited $\delta^{13}C_{Lip}$ values between -15.25 to -16.75‰, except *Forcipulatida* spp. (-10.52‰) and *Fissurella* spp. (-20.26‰).

The food webs from the Western Chilean Patagonia showed different groups of organisms at the two locations studied (Supplementary Table S8; Fig. S1). In Marchant River Mouth, *Fissurella* spp. had the lowest $\delta^{15}N_{adj}$ value (1.63‰), whereas *Graus nigra* had the highest value (9.16‰). Here, most of the species showed $\delta^{13}C_{Lip}$ values ranging from -15.22 to -18.55‰. At Leona Island, most of the species exhibited $\delta^{15}N_{adj}$ values between 2.27 to 7.57‰, except *Aulacomya ater* (0.70‰). The values of $\delta^{13}C_{Lip}$ ranged from -13.44 to -15.09‰, with the exception of *Aulacomya ater* (-17.41‰).

At Fildes Bay there were also two different groups of organisms within the food web (Supplementary Table S9; Fig. S1). The first was a group of lower-trophic-level organisms, with *Nacella concinna* having the lowest $\delta^{15}N_{adj}$ value (1.48‰) and *Parborlasia corrugatus* having the highest value (5.03‰). The second group consisted mainly of fishes, with *Notothenia rossi* having the lowest $\delta^{15}N_{adj}$ value (6.03‰) and *Pagothenia borchgrevinki* having the highest (7.24‰). An exception was noted with *Cnemidocarpa verrucosa,* which was completely separated from the rest with an δ^{15}Nadj value of zero. Most of the species exhibited $\delta^{13}C_{Lip}$ values between -10.01 to -19.58‰, except *Cnemidocarpa verrucosa* (-24.53‰).

Table S1. Biological samples collected by group of species from the Northwestern Coast of Chile.

Location	Group	Specie	N	Sample collected
Pan de Azúcar Bay	Macroinvertebrate	*Crucibulum scutellatum*	5	Muscle
		Aulacomya atra	5	Muscle
		Loxechinus albus	5	Muscle
		Tegula atra	5	Soft tissue
		Fissurella spp.	5	Soft tissue
		Forcipulatida spp.	5	Soft tissue
		Concholepas concholepas	5	Soft tissue
		Cancer edwardsii	5	Soft tissue
	Fish	*Trachurus symetricus murphyi*	1	Muscle
		Cheilodactylus gayi	5	Muscle
		Cojinova medusa	5	Muscle
		Thyrsites atun	*1*	Muscle
		Pinguipes chilensis	5	Muscle
		Hemilutjanus macrophthalmus	4	Muscle

Table S2. Biological samples collected by group of species from two locations of the Western Chilean Patagonia.

Location	Group	Specie	N	Sample collected
Marchant River Mouth	Macroinvertebrate	*Fissurella* spp.	5	Muscle
		Aulacomya ater	5	Muscle
		Hemigapsus crenulatus	5	Soft tissue
		Cancer coronatus	7	Whole body
	Fish	*Eleginops maclovinus*	5	Muscle
		Genypterus chilensis	2	Muscle
		Graus nigra	2	Muscle
		Salmo trutta	1	Muscle
		Salmo salar	2	Muscle
La Leona Island	Macroinvertebrate	*Aulacomya ater*	5	Muscle
		Cosmasterias lurida	5	Soft tissue
		Concholepas concholepas	4	Muscle
		Trophon geversianus	5	Soft tissue
	Fish	*Genypterus chilensis*	1	Muscle
		Sebastes capensis	5	Muscle
		Panguipes chilensis	3	Muscle

Table S3. Biological samples collected by group of species from the Antarctic Peninsula area.

Location	Group	Specie	N	Sample type
Fildes Bay	Macroinvertebrate	*Cnemidocarpa verrucosa*	1	Soft tissue
		Nacella concinna	5	Soft tissue
		Abatus agassizii	3	Soft tissue
		Odontaster validus	1	Whole body
		Sterechinus nemayeri	5	Whole body
		Glyptenotus antarcticus	3	Whole body
		Actinia (unidentified)	5	Soft tissue
		Diplasterias brucei	1	Soft tissue
		Parborlasia corrugatus	3	Soft tissue
	Fish	*Notothenia rossi*	4	Muscle
		Notothenia coriiceps	6	Muscle
		Trematomus scotti	6	Muscle
		Pagothenia borchgrevinki	1	Muscle
		Pagothenia hansoni	2	Muscle
		Notothenia kempi	4	Muscle
		Notothenia sp.	1	Muscle
		Trematomus newnesi	2	Muscle
		Pagothenia brachysoma	2	Muscle

Table S4. Concentration of Ta (mean ± standard deviation) in aquatic organisms from the Northwestern Coast of Chile (ng/g, dry weight).

Location	Group	Specie	Concentrations
Pan de Azúcar Bay	Macroinvertebrate	*Crucibulum scutellatum*	0.17 ± 0.07
		Aulacomya ater	0.37 ± 0.32
		Loxechinus albus	0.51 ± 0.09
		Tegula atra	0.34 ± 0.17
		Fissurella spp.	0.31 ± 0.04
		Forcipulatida spp.	0.83 ± 1.09
		Concholepas concholepas	0.26 ± 0.02
		Cancer edwardsii	0.47 ± 0.16
	Fish	*Trachurus symetricus murphyi*	2.86
		Cheilodactylus gayi	2.22 ± 0.60
		Cojinova medusa	2.35 ± 1.00
		Thyrsites atun	2.20
		Pinguipes chilensis	2.09 ± 0.39
		Hemilutjanus macrophthalmus	2.68 ± 0.28

Table S5. Concentrations of Ta (mean ± standard deviation) in aquatic organisms from the Western Chilean Patagonia (ng/g, dry weight).

Location	Group	Specie	Concentrations
Marchant River Mouth	Macroinvertebrate	*Fissurella* spp.	1.40 ± 0.55
		Aulacomya ater	1.05 ± 1.81
		Hemigapsus crenulatus	1.25 ± 0.55
		Cancer coronatus	1.51 ± 1.05
	Fish	*Eleginops maclovinus*	2.48 ± 0.27
		Genypterus chilensis	2.08 ± 0.06
		Graus nigra	2.36 ± 0.12
		Salmo trutta	2.03
		Salmo salar	2.20 ± 0.12
La Leona Island	Macroinvertebrate	*Aulacomya ater*	0.23 ± 0.11
		Cosmasterias lurida	0.34 ± 0.22
		Concholepas concholepas	0.38 ± 0.17
		Trophon geversianus	0.29 ± 0.12
	Fish	*Genypterus chilensis*	1.83
		Sebastes capensis	1.84 ± 0.28
		Pinguipes chilensis	0.61 ± 0.07

Table S6. Concentrations of Ta (mean ± standard deviation) in aquatic organisms from the Antarctic Peninsula area (ng/g, dry weight).

Location	Group	Specie	Concentrations
Fildes Bay	Macroinvertebrate	*Cnemidocarpa verrucose*	3.48
		Nacella concinna	1.22 ± 2.17
		Abatus agassizii	0.43 ± 0.25
		Odontaster validus	7.8
		Sterechinus nemayeri	0.43 ± 0.44
		Glyptenotus antarcticus	1.33 ± 0.40
		Actinia (unidentified)	2.35 ± 1.03
		Diplasterias brucei	5.09
		Parborlasia corrugate	0.97 ± 0.10
	Fish	*Notothenia rossi*	3.07 ± 0.28
		Notothenia coriiceps	5.78 ± 7.86
		Trematomus scotti	8.00 ± 3.31
		Pagothenia borchgrevinki	13.70
		Pagothenia hansoni	2.23 ± 0.06
		Notothenia kempi	14.04 ± 20.38
		Notothenia sp.	11.07
		Trematomus newnesi	2.8 ± 0.68
		Pagothenia brachysoma	3.04 ± 0.55

Table S7. Isotopes of nitrogen and carbon from the food web at Pan de Azúcar Island (Northwestern Coast of Chile). Values (mean ± standard deviation) reported in parts per thousand (‰).

Location	**Group**	**Specie**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C_{Lip}$**	**$\delta^{15}N_{adj}$**	**TL**
Pan de Azúcar Bay	Macroinvertebrate	*Crucibulum scutellatum**	-18.94 ± 2.21	13.10 ± 0.55	-18.32 ± 2.31	0.73 ± 0.55	2.21 ± 0.16
		Aulacomya atra	-19.73 ± 0.44	13.28 ± 0.45	-19.36 ± 0.43	0.91 ± 0.45	2.27 ± 0.13
		Loxechinus albus	-16.15 ± 1.88	13.71 ± 0.56	-15.25 ± 1.84	1.34 ± 0.56	2.39 ± 0.16
		Tegula atra	-16.84 ± 0.88	14.68 ± 0.58	-16.75 ± 0.87	2.31 ± 0.58	2.68 ± 0.17
		Fissurella spp.	-21.17 ± 4.34	16.14 ± 0.81	-20.26 ± 4.74	3.77 ± 0.81	3.11 ± 0.24
		Forcipulatida spp.	-11.79 ± 1.26	17.78 ± 0.45	-10.52 ± 0.74	5.41 ± 0.45	3.59 ± 0.13
		Concholepas concholepas	-16.24 ± 0.72	18.67 ± 0.62	-15.86 ± 0.83	6.30 ± 0.62	3.85 ± 0.18
		Cancer edwardsii	-16.28 ± 0.32	19.88 ± 0.60	-15.35 ± 0.66	7.51 ± 0.60	4.21 ± 0.18
	Fish	*Trachurus symetricus murphyi*	-18.99	19.79	-18.78	7.42	4.18
		Cheilodactylus gayi	-17.01 ± 0.33	20.84 ± 0.23	-15.30 ± 1.18	8.47 ± 0.23	4.49 ± 0.07
		Cojinova medusa	-18.48 ± 0.61	21.05 ± 0.43	-17.46 ± 1.18	8.68 ± 0.43	4.55 ± 0.13
		Thyrsites atun	-16.80	21.08	-16.28	8.71	4.56
		Pinguipes chilensis	-16.29 ± 0.53	21.09 ± 0.15	-15.60 ± 1.19	8.72 ± 0.15	4.57 ± 0.04
		Hemilutjanus macrophthalmus	-16.76 ± 0.24	21.92 ± 0.38	-15.73 ± 0.61	9.55 ± 0.38	4.81 ± 0.11

* Baseline species

Table S8. Isotopes of nitrogen and carbon from two locations of the Western Chilean Patagonia. Values (mean ± standard deviation) reported in parts per thousand (‰).

Location	**Group**	**Specie**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C_{Lip}$**	**$\delta^{15}N_{adj}$**	**TL**
Marchant River Mouth	Macroinvertebrate	*Fissurella* spp.*	-16.36 ± 0.74	8.55 ± 2.33	-16.27 ± 1.14	1.63 ± 2.33	2.48 ± 0.69
		Aulacomya ater	-17.91 ± 0.20	9.20 ± 0.35	-17.32 ± 0.26	2.28 ± 0.35	2.67 ± 0.10
		Hemigapsus crenulatus	-15.20 ± 2.18	9.92 ± 1.20	-15.22 ± 2.24	3.00 ± 1.20	2.88 ± 0.35
		Cancer coronatus	-18.50 ± 6.70	12.80 ± 2.48	-18.55 ± 6.72	5.87 ± 2.48	3.73 ± 0.73
	Fish	*Eleginops maclovinus*	-16.92 ± 1.39	13.18 ± 1.63	-16.53 ± 1.11	6.26 ± 1.63	3.84 ± 0.48
		Genypterus chilensis	-16.42 ± 0.06	15.09 ± 0.47	-16.51 ± 0.11	8.16 ± 0.47	4.4 ± 0.14
		Graus nigra	-16.23 ± 0.45	16.08 ± 0.21	-16.19 ± 0.45	9.16 ± 0.21	4.69 ± 0.06
		Salmo trutta	-17.24	14.64	-17.14	7.71	4.27
		Salmo salar	-17.10 ± 0.78	14.95 ± 0.24	-16.83 ± 0.84	8.03 ± 0.24	4.36 ± 0.07
La Leona Island	Macroinvertebrate	*Aulacomya ater**	-18.59 ± 0.23	8.88 ± 0.43	-17.41 ± 0.21	0.70 ± 0.43	2.21 ± 0.13
		Cosmasterias lurida	-17.45 ± 2.66	10.44 ± 0.94	-13.94 ± 1.73	2.27 ± 0.94	2.67 ± 0.28
		Concholepas concholepas	-15.24 ± 0.17	12.80 ± 0.14	-14.79 ± 0.71	4.63 ± 0.14	3.36 ± 0.04
		Trophon geversianus	-14.60 ± 0.53	14.26 ± 0.37	-14.15 ± 0.59	6.08 ± 0.37	3.79 ± 0.11
	Fish	*Genypterus chilensis*	-14.94	14.93	-14.50	6.76	3.99
		Sebastes capensis	-15.45 ± 0.24	15.63 ± 0.19	-15.09 ± 0.55	7.45 ± 0.19	4.19 ± 0.06
		Pinguipes chilensis	-14.37 ± 1.43	15.75 ± 0.93	-13.44 ± 0.51	7.57 ± 0.93	4.23 ± 0.27

*Baseline species

Table S9. Isotopes of nitrogen and carbon from the Antarctic Peninsula area. Values (mean ± standard deviation) reported in parts per thousand (‰).

Location	**Group**	**Specie**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C_{Lip}$**	**$\delta^{15}N_{adj}$**	**TL**
Fildes Bay	Macroinvertebrate	*Cnemidocarpa verrucosa* *	-25.66	4.19	-24.53	0	2
		Nacella concinna	-15.43 ± 0.99	5.67 ± 0.37	-13.73 ± 0.70	1.48 ± 0.37	2.44 ± 0.11
		Abatus agassizii	-19.75 ± 3.31	5.95 ± 0.21	-17.20 ± 2.62	1.77 ± 0.21	2.52 ± 0.06
		Odontaster validus	-16.16	6.71	-15.28	2.53	2.74
		Sterechinus nemayeri	-12.59 ± 3.03	6.79 ± 1.83	-10.01 ± 3.56	2.60 ± 1.83	2.77 ± 0.54
		Glyptenotus antarcticus	-12.86 ± 1.81	8.33 ± 2.34	-11.33 ± 3.25	4.14 ± 2.34	3.22 ± 0.69
		Actinia (unidentified)	-20.13 ± 1.85	8.62 ± 0.56	-17.41 ± 1.44	4.44 ± 0.56	3.31 ± 0.16
		Diplasterias brucei	-13.95	8.63	-13.63	4.45	3.31
		Parborlasia corrugatus	-17.41 ± 1.07	9.21 ± 0.10	-15.52 ± 0.23	5.03 ± 0.10	3.48 ± 0.03
	Fish	*Notothenia rossi*	-20.83 ± 1.99	10.21 ± 0.31	-19.58 ± 2.39	6.03 ± 0.31	3.77 ± 0.09
		Notothenia coriiceps	-20.46 ± 1.34	10.51 ± 0.83	-19.23 ± 1.34	6.32 ± 0.83	3.86 ± 0.24
		Trematomus scotti	-20.94±0.93	10.27±0.32	-18.95±2.23	6.08±0.32	3.79±0.09
		Pagothenia borchgrevinki	-18.58	11.42	-18.73	7.24	4.13
		Pagothenia hansoni	-20.20±0.32	10.87±0.91	-19.06±0.31	6.68±0.91	3.96±0.27
		Notothenia kempi	-20.37±1.84	10.21±0.53	-18.57±3.45	6.02±0.53	3.77±0.16
		Notothenia sp.	-20.02	10.33	-18.74	6.14	3.81
		Trematomus newnesi	-19.34±0.07	10.72±0.48	-18.71±1	6.53±0.48	3.92±0.14
		Pagothenia brachysoma	-19.31±1.09	10.22±0.11	-17.73±1.23	6.03±0.11	3.77±0.03

* Baseline species

Table S10. Lipid content (mean ± standard deviation) in aquatic organisms from the Northwestern Coast of Chile.

Location	Group	Specie	Lipid (%)
Pan de Azúcar Bay	Macroinvertebrate	*Crucibulum scutellatum*	8.39 ± 2.53
		Aulacomya atra	6.45 ± 0.6
		Loxechinus albus	10.5 ± 2.53
		Tegula atra	4.26 ± 1.24
		Fissurella spp.	10.61 ± 4.87
		Forcipulatida spp.	13.38 ± 5.44
		Concholepas concholepas	6.5 ± 1.78
		Cancer edwardsii	10.79 ± 3.84
	Fish	*Trachurus symetricus murphyi*	5.26
		Cheilodactylus gayi	16.81 ± 8.23
		Cojinova medusa	11.44 ± 4.65
		Thyrsites atun	7.62
		Pinguipes chilensis	8.86 ± 7.51
		Hemilutjanus macrophthalmus	11.51 ± 2.84

Table S11. Lipid content (mean ± standard deviation) in aquatic organisms from the Western Chilean Patagonia.

Location	Group	Specie	Lipid (%)
Marchant River Mouth	Macroinvertebrate	*Fissurella* spp.	4.35 ± 3.15
		Aulacomya ater	8.15 ± 3.12
		Hemigapsus crenulatus	3.47 ± 1.88
		Cancer coronatus	3.29 ± 0.8
	Fish	*Eleginops maclovinus*	6.62 ± 2.84
		Genypterus chilensis	2.92 ± 1.31
		Graus nigra	3.95 ± 0.01
		Salmo trutta	4.37
		Salmo salar	5.72 ± 0.84
La Leona Island	Macroinvertebrate	*Aulacomya ater*	12.63 ± 1.78
		Cosmasterias lurida	30.66 ± 7.59
		Concholepas concholepas	7.05 ± 4.92
		Trophon geversianus	7.15 ± 1.16
	Fish	*Genypterus chilensis*	7.04
		Sebastes capensis	6.35 ± 2.55
		Pinguipes chilensis	10.79 ± 7.26

Table S12. Lipid content (mean ± standard deviation) in aquatic organisms from the Antarctic Peninsula area.

Location	Group	Specie	Lipid (%)
Fildes Bay	Macroinvertebrate	*Cnemidocarpa verrucose*	12.25
		Nacella concinna	16.71 ± 4.26
		Abatus agassizii	23.24 ± 5.79
		Odontaster Validus	10.4
		Sterechinus nemayeri	23.46 ± 7.62
		Glyptenotus antarcticus	15.39 ± 11.04
		Actinia (unidentified)	24.55 ± 9.54
		Diplasterias brucei	6.03
		Parborlasia corrugatus	18.2 ± 7.19
	Fish	*Notothenia rossi*	13.17 ± 4.53
		Notothenia coriiceps	13.11 ± 4.96
		Trematomus scotti	18.89 ± 18.24
		Pagothenia borchgrevinki	2.45
		Pagothenia hansoni	12.36 ± 4.81
		Notothenia kempi	17.49 ± 14.96
		Notothenia sp.	13.43
		Trematomus newnesi	8.46 ± 7.16
		Pagothenia brachysoma	15.74 ± 1.08

Table S13. Regressions of $\log_{10}$Ta versus δ^{15}Nadj for fishes and invertebrates collected from coastal sites in northwestern Chile, Chilean Patagonia, and Antarctica (see Figure S2). Slope equals Trophic Magnification Slope (TMS) as in Lavoie et al. (2013)

Sector	Location	Slope	Intercept	R^2	*p*-value	N
A	Pan de Azúcar Bay	0.11 ± 0.01	-0.78 ± 0.08	0.52	<0.0001	61
B	La Leona Island	0.09 ± 0.02	-0.75 ± 0.11	0.39	0.0004	28
	Marchant River Mouth	0.05 ± 0.02	-0.11 ± 0.11	0.15	0.02	34
C	Fildes Bay	0.18 ± 0.03	-0.52 ± 0.15	0.39	<0.0001	55

A = Northwestern Coast of Chile; B = Western Chilean Patagonia; C = South Shetland Island (Antarctic Peninsula area).

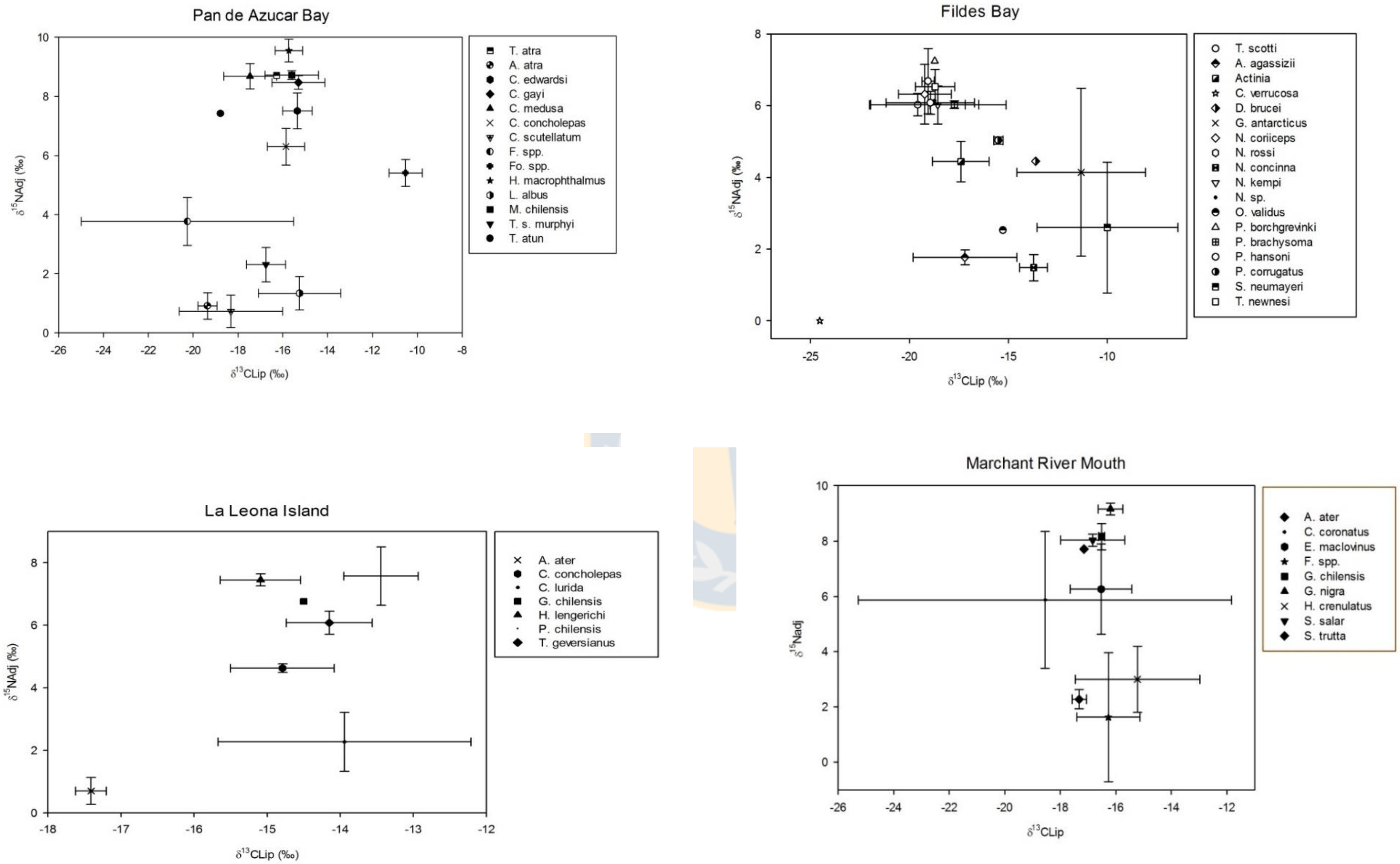

Figure S1. Relationships between stable isotopes of $\delta^{15}N_{adj}$ and $\delta^{13}C_{Lip}$ (‰) from different food webs studied.

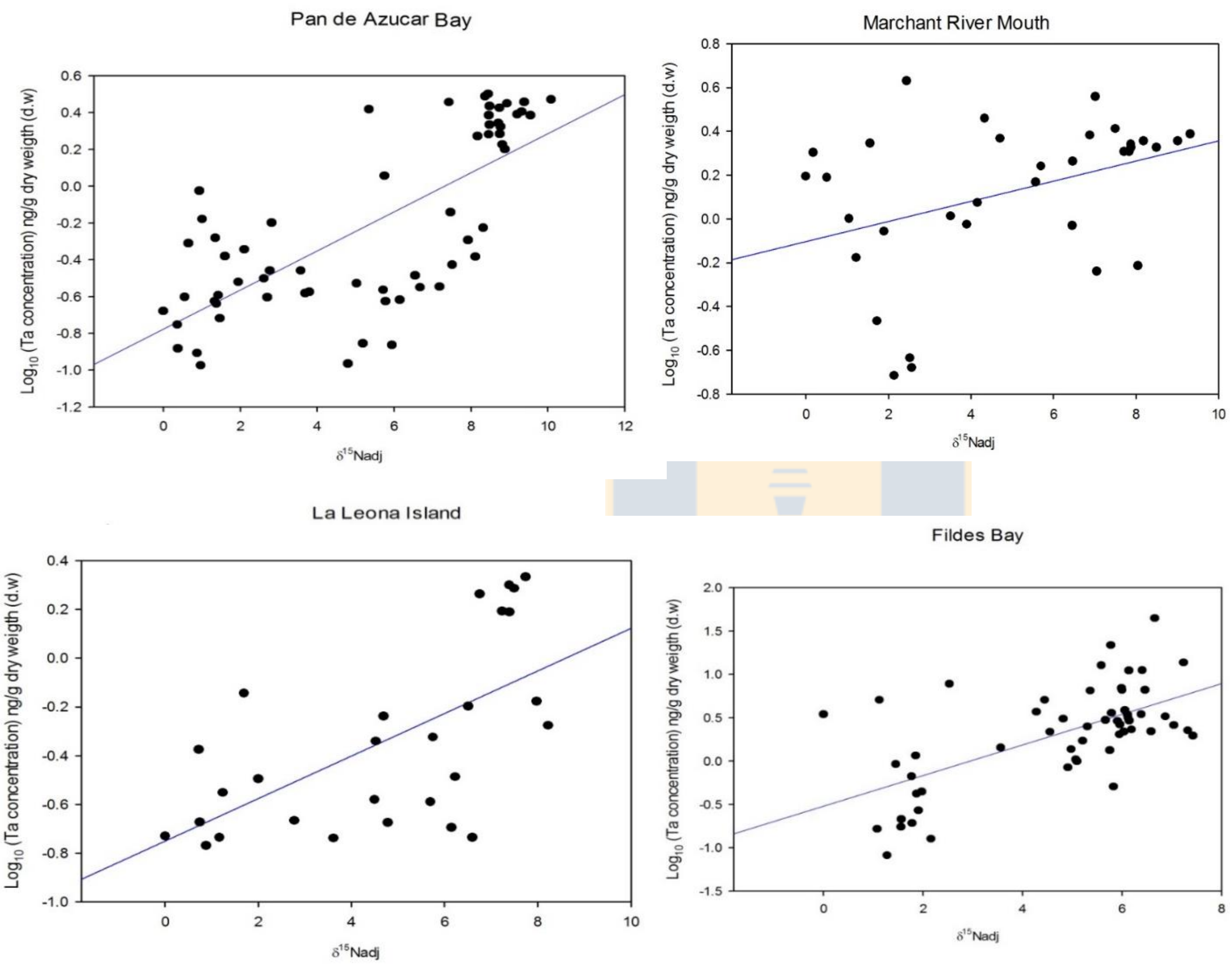

Figure S2. Regressions of logTa versus $\delta^{15}N_{adj}$ for fishes and macroinvertebrates collected from coastal, marine food webs in northwestern Chile (Pan de Azucar), Chilean Patagonia (Marchant River Mouth and La Leona Island), and Antarctica (Fildes Bay).

Capitulo VII DISCUSIÓN GENERAL

En términos de aves marinas, la revisión bibliográfica se pudo tener una visión global de las investigaciones existentes sobre elementos traza en pingüinos. En efecto se observó que la mayoría de las investigaciones de elementos traza en pingüinos reporntan niveles de Al, As, Cd, Cu, Hg, Mn, Pb y Zn. El metal más estudiado es Pb, mientras que Al es el menos informado. Otros metales como Co, Cr, Fe o Ni han sido muy poco estudiados (Jerez et al. 2013; Szopińska et al. 2016). En cuanto a los elementos no mencionados anteriormente no ha habido reporte, haciendo necesaria la incorporación de otros metales para asi poder evaluar posibles cambios en el futuro.

De las 18 especies de pingüinos a nivel global, sólo se han reportado oligoelementos en 11 de ellos (*P. papua*, *P. antarctica*, *P. adeliae*, *Aptenodytes forsteri*, *A. patagonicus*, *Spheniscus magellanicus*, *S. humboldti*, *Eudyptes chrysolophus*, *E. chrysocome, E. minor y E. moseleyi*). La mayoría de los estudios sobre concentraciones de oligoelementos en pingüinos se han centrado en el género *Pygoscelis*, principalmente en pingüinos papúa, seguidos de pingüinos Adélia y finalmente pingüinos barbijo. Por lo que información sobre metales de las especies faltantes es crucial: *Eudyptes pachyrhynchus*, *Eudyptes moseleyi*, *Eudyptes sclateri*, *Eudyptes robustus*, *Eudyptes schlegeli*, *Spheniscus demersus*, *Spheniscus mendiculus*.

Las matrices biológicas de pingüinos más estudiadas son plumas y luego excretas, seguidas de hígado, riñones, huesos, músculos y contenido estomacal. Por otro lado, son escasos los estudios llevados a cabo para medir elementos traza en sangre y órganos internos como corazón, testículos, bazo o cerebros de pingüinos son escasos (Bargagli et al. 1998; Finger et al. 2015; Metcheva et al. 2010; Metcheva et al. 2011).

La mayoría de los estudios de concentraciones de elementos traza en pingüinos se han llevado a cabo en la Antártica e islas subantárticas, lo que evidencia la falta de datos desde otras áreas donde también viven pingüinos, como Australia, Sudáfrica, América del Sur y las Islas Galápagos. Es sorprendente encontrar estudios principalmente en la Antártica, ya que los investigadores requieren una implementación adecuada y una firme determinación de trabajar bajo condiciones climáticas extremas. Tal vez la necesidad de viajar a regiones remotas y escasamente exploradas es más importante que el simple deseo de realizar investigaciones en lugares más poblados con climas más templados, donde las especies de pingüinos amenazados podrían estar más expuestas a contaminantes al estar en áreas con mayor presencia humana.

El mayor número de los estudios sobre pingüinos se han centrado en medir los niveles de exposición en diferentes matrices bióticas. La concentración de metales en los tejidos y órganos de los pingüinos puede tener una gran importancia toxicológica. En humanos, las enfermedades relacionadas con la deficiencia de oligoelementos esenciales son bien conocidas (Nordberg y Nordberg 2016). Se necesitan más estudios con biomarcadores para evaluar los riesgos reales asociados con los niveles de estos contaminantes en ambientes polares con baja diversidad ecológica, que pueden aumentar la ocurrencia de enfermedades con consecuencias negativas para la salud de las poblaciones de pingüinos (Boersma 2008).

Poco se sabe sobre la interacción de los metales que podrían activar ciertos mecanismos de desintoxicación del organismo de los pingüinos. Se sospecha que el Selenio podría jugar un papel importante en los procesos de desintoxicación de Hg. El estudio con especies en cautiverio podría ser una buena alternativa para evaluar los mecanismos fisiológicos de estas especies a una determinada concentración de metales, bajo un ambiente controlado (Falkowska et al. 2013).

Por su parte, las aves marinas pueden ser importantes **biovectores** de elementos traza y transferencias de nutrientes de mar a tierra (Liu et al. 2006; Mallory et al. 2015). Existe evidencia de que esos animales pueden transportar productos químicos producidos industrialmente, brindando asi un subsidio nutricional crítico a los ecosistemas terrestres (Blais et al. 2005). El guano de las aves marinas es un vector efectivo de contaminantes en los suelos y una fuente importante de sustancias biogénicas (Liu et al. 2006; Brimble et al. 2009). Algunos hábitats terrestres alrededor de las colonias pueden estar altamente contaminados debido a las contribuciones de los productos químicos que acarrean las aves marinas (Blais et al. 2005, Brimble et al. 2009, Zhu et al. 2014).

En la presente investigación se reportan los datos sobre los metales traza en biomateriales de pingüinos papua (plumas y excretas) y muestras de suelo cerca de las colonias de pingüinos recolectados a lo largo de la Península Antártica. Los niveles de Cd, Cu, Cr, Mn, Ni, Pb y Zn mostraron grandes variaciones entre los lugares estudiados, la mayoría de ellos disminuyendo a lo largo del gradiente latitudinal de Norte a Sur. Aunque nuestros niveles de ETs en las matrices bióticas son similares a los encontrados anteriormente en plumas y excretas de pingüinos en la región, esta disminución podría estar relacionada con una menor presencia humana y las actividades antropogénicas en los secotres mas apartados de la Península Antártica. Los niveles más altos de metales traza detectados parecen estar relacionados con la gran concentración de actividades humanas que existe en la Islas Rey Jorge y Base O'Higgins. De igual manera, para todas las localidades estudiadas, la mayoría de las concentraciones de ETs se manifestaron en aquellos suelos más cercanos a las colonias, lo cual podría estar implicaría que los pingüinos papua, como la mayoría de los organismos biovectores, pueden transferir metales bioacumulados a los ecosistemas terrestres a través de excrementos que podrían afectar a algunos organismos vivos importantes de los ambientes costeros antárticos.

Debido a los resultados anteriormente presentados, se abordó el estudio del biotransporte de ETs y nutrientes en suelos impactados por algunas colonias importantes de pingüinos de Adélia en tres localidades del área de la Península Antártica. A su vez, éstas fueron comparadas con colonias de pingüinos de Humboldt en dos localidades semiáridas del norte de Chile. Se confirmó el enriquecimiento esperado del suelo en macronutrientes y materia orgánica, así como en salinidad y acidez en sitios con mayor influencia de pingüinos, junto con Cd, Co, Cr, Cu, Mo, V y Zn. Todas estas variables fueron más altas en áreas que soportan actividades intensas de pingüinos. Sin embargo, se observó un incremento significativo para Cd, Co, Cu, Mo y Zn en colonias de pingüino Adélia que habitan en Isla Ardley, y Mo y V en colonias de pingüino Adélia que habitan en las cercanías de la Base Arctowski. Finalmente, en las colonias de pingüino Adíela que habitan en las cercanías de la Base O´Higgins presentaron un incremento significativo de Co, Mo y V. Esto confirma que para Cd, Co, Cu, Mo, V y Zn se cumple la hipótesis II de la presente investigación, no siendo ésta una regla general para todos los elementos. Por ello, se sugiere una aprobación parcial de la hipótesis, aunque hay que tener en presente que de igual forma para todos los elementos las concentraciones fueron mayores en suelos impactados por las colonias de pingüinos). Esta transferencia de elementos traza bioacumulados a los ecosistemas terrestres a través de excrementos podrían afectar a ciertos organismos que viven en los ambientes costeros de la Península Antártica y de suelos semiáridos del norte de Chile. Este es un problema que necesita ser más profundamente investigado. Las futuras investigaciones deben focalizarse en estudiar los posibles efectos que podría tener los organismos que habitan en estos suelos (microorganismos, plantas o animales) los que podrían estar expuestos a mayores concentraciones de ETs que los normalmente presentes en la Antártica. Además, este incremento de concentraciones podría ser una vía indirecta de exposición para los huevos y polluelos de pingüinos. Por otra parte, debe estudiarse si existe una movilidad de estos contaminantes vía escorrentía superficial o si estos suelos constituyen un sumidero de estos elementos.

La **transferencia trófica de elementos traza** ha sido un tema relevante de investigación en los últimos años. Se sabe que los metales como el mercurio (Hg) y los contaminantes orgánicos persistentes (COPs) se biomagnifican en diversas redes tróficas acuáticas, lo que suponer un riesgo para los consumidores de productos marinos y para los organismos marinos. La transferencia trófica de contaminantes se evalúa cada vez más utilizando índices de isótopos de nitrógeno ($\delta^{15}N$), ya que proporcionan una medida continua de la posición trófica relativa dentro de las redes tróficas (Lavoie et al. 2013, Walters et al. 2016). Los niveles de contaminantes son evaluados en conjunto con la señal de $\delta^{15}N$ para entender si se biomagnifican y estas relaciones se pueden comparar entre ecosistemas que difieren en la composición de especies, características físicas y químicas y zonas climáticas (por ejemplo, Lavoie et al. 2013, Walters et al. 2016).

La **transferencia trófica de cadmio**, es aún un tema de debate científico. Algunos autores (Majer et al. 2014) han reportado biomagnificación del Cd en ocho especies bentónicas en Bahía Admiralty (Antártica) colectadas en el verano austral 2005/2006. Ellos observaron una relación positiva entre la concentración de Cd y el nivel trófico de las especies estudiadas, lo que sugiere una posible biomagnificación de este metal. Otros autores han reportado biomagnificación de Cd en la trama trófica rocosa intermareal en aguas occidentales de Hong Kong (Cheung y Wang 2008).

Por otra parte, otros autores han encontrado que, si bien existe un incremento de las concentraciones de Cd, este no llega a ser significativo, lo cual sugiere que no hay relación entre el nivel trófico y el metal. Se ha encontrado que organismos acuáticos de los humedales del delta del rio Amarillo (China) presentan un aumento de la concentración de Cd a mayor nivel trófico, pero no presentan una transferencia trófica significativa (Cui et al. 2011). Por otra parte, en la bahía de San Francisco - Estados Unidos se reportó un enriquecimiento progresivo de Cd entre diferentes niveles tróficos de la trama trófica (Croteau et al. 2005). En el artico, muestras de riñones de mamíferos árticos han presentaro mayor concentración de Cd que otras especies, pero este incremento es significativo (Dehn et al. 2006).

Por el contrario, fue reportado una posible biodilución en la trama trófica bentónica del estuario del rio Pearl en el sur de China (Zeng et al. 2013).

En la presente investigación, se observó biomagnificación de Cd en las tramas tróficas Antárticas desde bahía Paraíso (TMS = 1.90; R^2 = 0.54; p = 0.03) y cabo

Shirreff (TMS = 2.84 R^2 = 0.86, p = 0.02), al considerar sólo fueron considerados especies de macroinvertebrados. Al comparar esta transferencia trófica con similares especies de la Patagonia Chilena se observó que, en la desembocadura del río Marchant hay una biomagnificacion de Cd (TMS = 0.66; R^2 = 0.76; p = 0.0004). Las pendientes de magnificación trófica (TMS, por su sigla en inglés) fueron mayores en las dos localidades Antárticas estudiadas al compáralas con la pendiente observada en la desembocadura del río Marchant en la Patagonia Chilena. Lo anterior, reafirma la hipótesis 1 presentada en esta investigación. Por su parte, al incluir peces en el estudio de transferencia trófica de Cd, se observó una biodilución en las dos localidades antárticas estudiadas: Bahía Paraíso (TMS = -0.81; R^2 = 0.52; p = 0.005) y Cabo Shirreff (TMS = -0.92; R^2 0.72: p = 0.01). Igual fenómeno se observó al comparar con la localidad de la Patagonia Chilena. En la desembocadura del rio Marchant existe una biodilución de Cd (TMS = -0.5; R^2 = 0,45; p = 0,02). Presentando las localidades Antárticas un mayor TMS al ser comparado con la localidad de la Patagonia Chilena. Esto vuelve a reafirmar la hipótesis 1 presentada en esta investigación.

Los resultados de esta investigación concuerdan con lo reportado por Signa et al. (2017) en el Mar Mediterráneo. La transferencia trófica de Cd es especie específica, pues al considerar sólo macroinvertebrados se observa hay biomagnificación, pero al incluir los peces en el análisis de transferencia trófica se observa hay biodilucion. Sin embargo, aún falta por comprender los factores que influyen y los posibles efectos que se podrían presentar tras el incremento de las contracciones a mayor nivel trófico en macroinvertebrados y cuáles son los factores determinantes que influyen en la posterior disminución de los niveles de Cd en especies superiores. Es por ello que las futuras investigaciones debieran focalizarse en comprender mejor los factores especie-específicos que podrían influir en la transferencia trófica del Cd.

La transferencia trófica de Ta, es un tema nuevo. A nuestro entender los resultados presentados en esta investigación son el primer reporte sobre la transferencia trófica del Ta. Falta concer mas acerca de las concentraciones de Ta en la biota y su transferencia trófica a través de las redes alimentarias acuáticas (Filella et al. 2017). Esto es crucila debido al costante aumento en el uso de Ta en nuevas tecnologías, lo que supone un riesgo potencial debido a exposiciones dietárias (Kales y Goldman 2002).

Nuestros resultados mostraron que hubo biomagnificacion de Ta en la bahía Fildes de la Península Antártica (TMS = 0,18; R^2 = 0,39; p = <0,0001). Igual fenómeno se observó en las dos localidades estudiadas en la Patagonia Chilena: desembocadura del río Marchant (TMS = 0,04; R^2 = 0,12; p = 0,05) e isla La Leona (TMS = 0,09; R^2 = 0,39; p = 0,0004). Similarmente, también se observó biomagnificacionde Ta en Bahía Pan de Azúcar en la costa norte de Chile (TMS = 0,11; R^2 = 0,52; p = <0,0001). Los resultados muestran por primera vez que hay un aumento en las concentraciones de Ta a niveles tróficos crecientes, situación que se dio en todos los sitios. Al observar los valores de TMS de las diferentes localidades, se observa que éstos fueron mayores en la Antártica, similar fenómeno que fue observado para Cd (al considerar solo macroinvertebrados), lo que viene a reafirmar para ambos elementos la hipótesis 1 planteada en la presente investigación.

No fue posible encontrar estudios de laboratorio o de campo sobre la trofodinámica del Ta. La pendiente de aumento trófico observada en las localidades estudiadas fueron menor que el promedio de las pendientes informadas en una revisión global de Hg total y MeHg en ambientes marinos (Lavoie et al. 2013). En general, Ta se biomagnifica independientemente de la ubicación y la composición de las especies de la red trófica, lo que podría ser característico de este elemento en particular, como ocurre con Hg (Lavoie et al. 2013). Esto difiere de lo que se observa para Cd, Cu, Zn, elementos que dependen del sitio y la especie (Ikemoto 2008, Zeng et al. 2013, Majer et al. 2014). Por ser este uno de los primeros reportes de biomagnificación de Ta en ecosistemas acuáticos, se requiere complemetar con más información para poder comprender el por qué Ta se biomagnifica, y cuáles serían sus implicancias. Las futuras investigaciones deben abocar sus esfuerzos en poder comprender cuales son las vías por las que Ta se biomagnifica y si ésto tendría alguna implicancia fisiológica.

Es importante mencionar que nuestros estudios de transferencia trófica de Cd y Ta se centraron en ecosistemas acuáticos con muestras de macroinvertebrados y peces. Es necesario conocer qué ocurre en otros niveles tróficos y otros tipos de ecosistemas, y si algunos factores fisicoquímicos (como la clorofila, el carbono orgánico disuelto, el fósforo total) podrían influir en la transferencia trófica, como se observó con Hg (Lavoie et al. 2013). Por su parte, hay que tener presente que para la identificación de las tramas tróficas fue usó el análisis de isotopos estables de nitrógeno en los diferentes tejidos de los organismos colectados. Actualmente se propone que el análisis de isotopos estables de nitrógeno en aminoácidos podría ser una técnica más precisa de estimar los niveles tróficos (Hetherington et al. 2017).

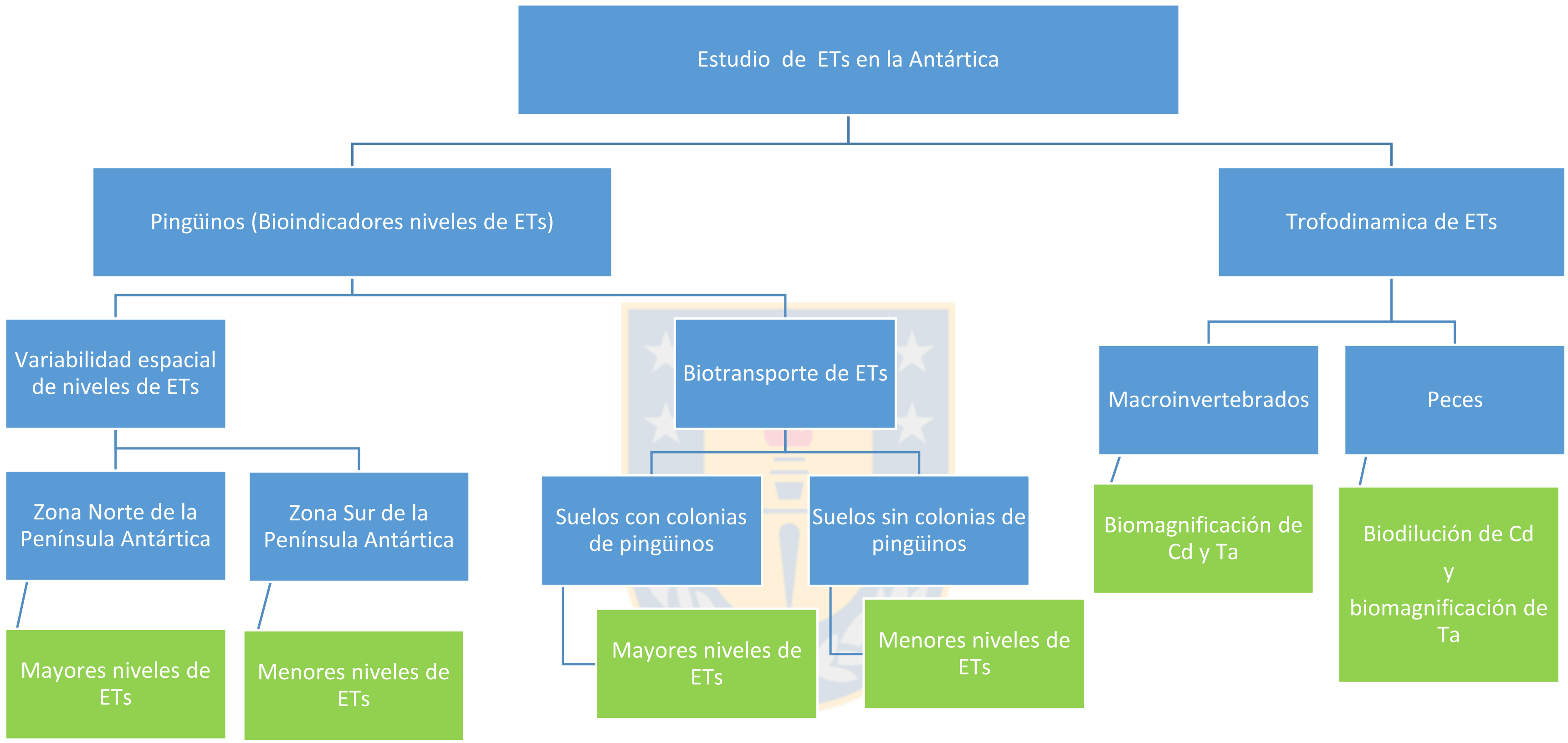

Figura 1. Mapa conceptual de los resultados y conclusiones de la presente investigación sobre la base de la estructura del presente trabajo (Figura1, introduccion recuadros en azul).

En esta investigación se reafirma el hecho de que los pingüinos son excelentes bioindicadores de contaminación química, pues tienen particulares hábitos para bucear y pasar parte de su ciclo de vida en tierra, sumado al hecho de que estos animales constituyen la mayor biomasa de los océanos australes. Esto los hace particularmete útiles para estudios de biomonitoreo ambiental de ETs. Los niveles de ETs plumas, excretas y suelos colectados en colonias de pingüinos papúa mostró que hay mayores concentraciones de ciertos ETS en la zona septentrional de la Península Antártica. Esta parte del territorio -antartico está más más cercana al Continente Sudamericano y es allí donde se concentra la mayor actividad antropogénica local.

El estudio del biotransporte arrojó mayores concentraciones de ETS en los suelos donde nidificación los pingüinos (aunque no son estadísticamente significativos para todos los elementos estudiados), lo cual sugiere un biotransporte especie-sitio específico. No obstante, no hay que descartar que pudiese existir algún grado de afectación biológica por este aumento de las concentraciones en los organismos que allí cohabitan. Adicionalmente, es posible que los ETs estén retornando al mar vía escorrentía e ingresando nuevamente a la trama trófica.

El estudio de la trofodinámica de ETs, demostró que el Cd se biomagnifica en invertebrados y presenta biodilución al incluir peces en la trama trófica. Por su parte, se descubrió que Ta se biomagnifica. Esto se contrapone con la creencia de que el Hg era el único elemento que presenta relación trófica positiva, concepto que aún esta muy arraigado en la comunidad científica global. Esto abre toda una nueva línea de investigación para evaluar si otros ETs presentan un aumento con la posición trófica y las implicancias que podrían tener en los organismos.

Si bien en la presente investigación se abordaron tres temas en particular, variabilidad espacial, biotransporte y transferencia trófica de ETs (Figura1), aún faltan más estudios para poder dilucidar los ciclos ecológicos de los elementos traza y la influencia del hombre en la Antártica, asi como a nivel global.

CONCLUSIONES GENERALES

El estudio sobre elementos traza en **pingüinos** es aún un tema emergente que precisa mayores estudios en diferentes elementos, especies, matrices biológicas, localidades además de estudiar los posible efectos y mecanismos fisiológicos de estos elementos en pingüinos. Estos estudios presentan la limitante que los pingüinos son especies protegidas lo que impide realizar metodología tradicionales para el estudio de la exposición y efectos como lo son los bioensayos. Esto conlleva un mayor desafío en poder replantear nuevas metodologías para poder contestar estas interrogantes y poder llegar a un trabajo de síntesis sobre este tema.

El **biotransporte** de ETs mediante aves marinas tiende a incrementar las concentraciones de elementos en ecosistemas costeros de la Antártica y de la costa norte de Chile. Es importante conocer si estos suelos actúan como sumidero de estos elementos o podrían movilizarse a otros compartimentos, ya sea entrando en los ciclos ecológicos terrestres de los organismos que viven cerca de las colonias, o bien reingresando a los ecositemas acuáticos mediante erosión y escorrentía superficial.

La **transferencia trófica de ETs** ha llamado la atención en los últimos años por conocer la trofodinámica de diferentes elementos, tales como Cd. Nuestra investigación mostró una biomagnificación de Cd en tramas tróficas inferiores y biodilución a mayores niveles tróficos. Sin embargo, son necesarios más estudios para poder conocer si éste es un patrón común en todos los ecosistemas. Lransferencia trófica de Ta, constituye un verdadero hallazgo ya que se observó biomagnificación de este elemento tanto en la Antártica como en los otros ecosistemas estudiados. Sin duda ésto amerita más estudios para poder entender los mecanismos ecofisiológicos y posibles implicancias de este incremento de concentraciones a mayor nivel trófico, el cual cobra mayor relevancia al ser Ta un elemento que ha visto incrementado su uso con nuevas tecnologías.

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