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Biomonitoreo ambiental: absorción de metales tóxicos a través del pelo de diferentes órdenes de mamíferos terrestres.

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PRESENTA:

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **26 de julio de 2019**, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **RENDÓN LUGO ALINA NASHIELY** con número de cuenta **95286493** con la tesis titulada: **"Biomonitoreo ambiental: absorción de metales tóxicos a través del pelo de diferentes órdenes de mamíferos terrestres"**, realizada bajo la dirección de la **DRA. LIVIA SOCORRO LEÓN PANIAGUA:**

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Sin otro particular, me es grato enviarle un cordial saludo.

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A mi hijo, a quien amo profundamente

CONTENIDO

Resumen

La contaminación ambiental es uno de los problemas más graves que amenazan la biodiversidad y la salud humana. Entre la gran cantidad de contaminantes ambientales generados y dispersados por las actividades humanas, los metales tóxicos son uno de mayor preocupación por su potencial tóxico y su persistencia en el ambiente. El biomonitoreo es una serie de herramientas fundamentales frente a esta problemática para la evaluación y control de los contaminantes en el ambiente. El uso de diferentes matrices biológicas como el pelo, para la determinación de metales contaminantes presenta muchas ventajas para la detección temprana y localizada de estos elementos. En México, a pesar de la gran contaminación por metales que existe, el uso del pelo para su biomonitoreo es muy incipiente. En el primer capítulo se llevó a cabo una revisión que muestra los avances del estudio del pelo y las características que lo han convertido en una herramienta ampliamente empleada para el biomonitoreo en todo el mundo. En el segundo capítulo se realizó un análisis experimental para establecer si metales en solución como el cadmio, plomo y cobre pueden permear hacia las capas más internas del pelo de cinco especies de mamíferos terrestres para finalmente precisar si los elementos que se depositan externamente pueden penetrar hasta las capas internas y confundirse con aquellos elementos depositados de manera endógena, es decir, que llegaron al pelo a través de la sangre que irriga al folículo piloso y fueron depositados en la hebra del pelo durante su crecimiento. Las muestras tratadas se analizaron por microscopía electrónica de barrido. No se detectaron metales depositados en las capas internas de ninguna muestra de pelo después de realizar los análisis químicos elementales con los detectores del microscopio. En el tercer capítulo se analizaron con la misma técnica de miscroscopía electrónica, muestras de pelo de mamíferos colectados en la región de la presa Endhó, Hidalgo, conocida por sus altos niveles de contaminantes de diferente naturaleza, entre los que se incluyen metales tóxicos. Los análisis químicos elementales se llevaron a cabo tanto en los folículos como en las hebras de cada muestra para diferenciar entre los elementos depositados exógenamente (en la cutícula del pelo) de aquellos de origen endógeno (en el folículo). Se encontró una gran cantidad de metales tóxicos depositados externamente y algunos metales de interés toxicológico como cromo y níquel, depositados en los folículos. *Desmodus* rotundus, *Miotys californicus* y *Mus musculus* resultaron ser las especies cuyo pelo contenía la mayor cantidad de metales exógenos y endógenos. No se encontraron metales de mayor potencial tóxico como plomo, cadmio y mercurio. Sin embargo, este estudio muestra la utilidad del pelo para la detección de metales tóxicos en al ambiente y de la microscopía electrónica como técnica que da información complementaria a la obtenida con las técnicas de química analítica tradicionales.

Abstract

Environmental pollution is one of the most serious problems that threaten biodiversity and human health. Among the large amount of environmental pollutants generated and dispersed by human activities, the toxic metals are one of the most concern because of their toxic potential and their persistence in the environment. Biomonitoring is a series of essential tools to address this problem of the evaluation and control of contaminants in the environment. The use of different biological matrices such as hair, for the detection of contaminant metals presents many advantages for the detection of early and localized of these elements. In Mexico, despite the high metal contamination exists, the use of hair for biomonitoring is very incipient. In the first chapter we conducted a review that shows the progress of the study of the hair and the features that have become a tool widely used for the biomonitoring around the world. In the second chapter we performed an experimental analysis to determine if metals in solution such as cadmium, lead and copper can permeate into the inner layers of the hair of five species of terrestrial mammals to finally determine the elements that are deposited externally can penetrate to the inner layers and be confused with those elements deposited endogenously, that is to say, who came into the hair via the bloodstream to the hair follicle and were deposited in the strand of the hair during its growth. The treated samples were analyzed by scanning electron microscopy and were not found metal deposited in the inner layers of any sample of hair, after performing the chemical analysis elemental detectors of the microscope. In the third chapter we analyzed with the same technique of electron microscopy, samples of hair of mammals collected in the region of the dam Endhó, Hidalgo, known for its high levels of contaminants of different nature, including toxic metals. Elementary chemical analysis were carried out both in the follicles as in the strands of each sample to differentiate between the elements deposited exogenously (in the hair cuticle) than those of endogenous origin (in the follicle). We found a large amount of toxic metals deposited externally, and some metals of toxicological interest such as chromium and nickel, which are deposited in the follicles. *Desmodus rotundus*, *Miotys californicus* and *Mus musculus* were the species whose hair contained the largest amount of exogenous and endogenous metals. No metals with higher toxic potential as lead, cadmium and mercury were found in the samples. However, this study shows the usefulness of hair in the detection of toxic metals in the atmosphere and the use of the electron microscopy as a technique that gives complementary information to that obtained with the traditional techniques of analytical chemistry.

INTRODUCCIÓN GENERAL

Las emisiones de elementos metálicos al ambiente causadas por las actividades humanas son uno de los problemas más graves de deterioro ambiental que se enfrentan actualmente y se han convertido en un tema central dentro de las ciencias ambientales (Lee y Lehmden, 1973; Callender, 2003; Reis *et al.* 2010).

Los elementos metálicos pueden dividirse en dos categorías: esenciales y no esenciales. Dentro de los primeros se encuentran elementos como hierro (Fe), cobre (Cu), zinc (Zn), manganeso (Mn) y níquel (Ni), los cuales son necesarios en cantidades traza por los organismos vivos. Los segundos, son elementos como el arsénico (As), aluminio (Al), cadmio (Cd), plomo (Pb) y mercurio (Hg), no tienen función biológica y al ser liberados al ambiente no pueden ser degradados como los contaminantes orgánicos y por lo tanto se acumulan originando graves daños a la biodiversidad (Ho y El-Khaiary, 2009; Chiarelli y Roccheri, 2014; Gall *et al.*, 2015; Xin *et al.*, 2015; Borghesi *et al.*, 2016). Ambos tipos de elementos pueden actuar como disruptores de procesos biológicos vitales al interactuar con diferentes tipos de biomoléculas. La diferencia principal radica en que los metales esenciales se convierten en tóxicos cuando se encuentran en cantidades excesivas, mientras que los no esenciales son tóxicos incluso a bajas concentraciones (Tchounwou *et al.*, 2012; Gall *et al.*, 2015).

Fuentes naturales de metales pesados

Los metales pesados ocurren de manera natural en el ambiente y son liberados en cantidades variables por diferentes procesos que se realizan en la litósfera. Se presentan formando parte de diferentes compuestos con una gran variación entre sus propiedades químicas, y muy raramente pueden encontrarse como elementos puros (Duffus, 2002).

Los afloramientos de roca madre por procesos de meteorización son la fuente principal de metales pesados en el suelo (Alloway, 2013; Brown, 1999; Marsden y Rainbow, 2004; Bradl, 2005; Sharma y Agrawal, 2005; Martin, 2012; Stankovik *et al.*, 2014). Estos procesos también determinan la composición química de las aguas superficiales, cuyos elementos metálicos dependerán de las rocas que formaron el suelo por donde fluyen (Bradl, 2005, Zhou *et al.*, 2008; Stankovik *et al.*, 2014).

La actividad volcánica y los movimientos tectónicos también incorporan elementos como Pb, cobalto (Co), rubidio (Rb), estroncio (Sr) y vanadio (V) en cantidades traza (Bradl, 2005). La atmósfera puede contener metales pesados por los aerosoles provenientes de los volcanes, los incendios forestales, polvos minerales, sales marinas y en algunos casos más aislados por fuentes extraterrestres (Bradl, 2005). Sin embargo, estas emisiones a la atmósfera son de corto tiempo de vida, al contrario de los metales acumulados en suelos y océanos, que se mantienen aun cuando la fuente de emisión haya desaparecido (Ayres, 1992).

Las emisiones naturales de metales pesados pueden llegar a ocasionar grandes daños a la biota local e incluso ser transportados a través de la atmósfera a grandes distancias y sumarse a las cantidades de contaminantes emitidos por fuentes antropogénicas (Tchounwou *et al.,* 2012).

Fuentes antropogénicas de metales pesados

La gran cantidad de aplicaciones de los metales han ido acompañadas por los efectos tóxicos sobre las poblaciones que los explotan. En la actualidad, a pesar del conocimiento sobre las propiedades de los metales pesados y de sus compuestos, así como de su potencial tóxico; la sobreexplotación minera y la enorme demanda de metales para la agricultura siguen causando la emisión y dispersión de grandes cantidades de contaminantes a través de la atmósfera y el agua (Athar y Vohora, 1995; Callender, 2003). Además de la alta demanda de metales y metaloides que involucra a estas dos actividades económicas primarias, los estándares actuales de vida demandan también grandes cantidades de metales pesados para uso industrial, médico, doméstico y tecnológico (Cuadro 1).

El biomonitoreo es actualmente una herramienta fundamental para la detección de emisiones de estos contaminantes, por medio de un organismo o una parte de éste para la medición, evaluación y finalmente el control de su liberación y dispersión (Preston 1975; Martin, 2012). El uso de organismos animales en el biomonitoreo provee información valiosa sobre los contaminantes metálicos en el ambiente, sus concentraciones y efectos en ecosistemas acuáticos y terrestres; ya que presentan diferentes grados de sensibilidad y despliegan distintas respuestas toxicológicas colocándolos en un papel central en la ecotoxicología (Martin, 2012; Moore y Ramammorthy, 2012). Su objetivo principal es la identificación y la eliminación de las posibles fuentes de exposición a los metales pesados para evitar así la pérdida de biodiversidad y el deterioro ambiental que origina este tipo de contaminación. Además, permite observar las variaciones de contaminantes y sus efectos en el tiempo, mapear la distribución geográfica de las regiones más afectadas por la contaminación y probar la efectividad de las medidas de protección al ambiente (Needham *et al.*, 2007; Paschal, 2007; Esteban y Castaño, 2009).

Los organismos vertebrados en el biomonitoreo

Los vertebrados se exponen a los contaminantes ambientales principalmente por ingestión tanto de agua como de alimento, inhalación y absorción cutánea (Limic y Valkovic, 1987; Goullé *et al.*, 2005; Xu *et al.*, 2006) RESPETAR EL ORDEN CRONOLÓGICO QUE ESTÁS UTILIZANDO. La presencia de metales pesados en el ambiente origina severos problemas en los organismos vertebrados de tipo nervioso, endócrino, reproductivo, morfológico, histológico e incluso la muerte (Battaglia *et al.*, 2005; Tellez y Merchant, 2005; Sánchez-Chardi *et al.*, 2009; Grillitsch y Schiesari, 2010).

Las detecciones de metales tóxicos en vertebrados se llevan a cabo mediante el análisis de sangre, orina, y en tejidos como hígado, riñones, intestino, gónadas, glándulas adrenales, bazo, páncreas y músculo (Kraus, 1989; Sánchez-Chardi *et al.*, 2009). El uso de biomarcadores como metalotioneínas,

estrés oxidante, genotoxicidad y alteraciones inmunológicas también se ha extendido en la evaluación de los efectos tóxicos en la fauna de vertebrados (Maes *et al.*, 2005).

También puede ocurrir depósito tanto endógeno como exógeno en apéndices epidérmicos como uñas (Jenkins, 1979), escamas (Kaur, 1998; Jones y Holladay, 2006), plumas (Furness, 1993; Mora, 2003; Borghesi *et al.*, 2016) y pelo (Combs *et al.,* 1982; Limic y Valkovic, 1987; Mora *et al.*, 2000; Tobin, 2005; Schramm, 2008), los cuales pueden usarse para la detección de contaminantes metálicos con la ventaja de ser métodos no invasivos.

Mamíferos en el biomonitoreo

Los mamíferos han desempeñado un papel muy importante dentro del biomonitoreo de metales tóxicos y existe una gran cantidad de trabajos que evalúan los efectos tóxicos de diferentes clases de metales y sus efectos en distintas especies. Los órganos más usados para los análisis de metales son hígado, riñón y músculo (Mohallal y Younes, 2015). El pelo es una matriz no invasiva que ha sido ampliamente usada ya que presenta varias ventajas al ser de fácil obtención, almacenamiento, manejo y en muchos estudios se ha probado que los contenidos metálicos encontrados en pelo reflejan la magnitud de esos mismos metales en los órganos internos (Jacob *et al.*, 1978; Kales y Christiani, 2005). Además, las variables que pueden modificar la incorporación de metales al pelo son las mismas que modifican la toxicocinética en los organismos animales en general como son los hábitos de forrajeo (Hickey *et al.,* 2001) la edad (Ikemoto *et al.,* 2004), sexo y talla (Combs *et al*., 1982).

El pelo ha sido considerado como la característica morfológica y diagnóstica más importante de los mamíferos. Es un apéndice epidérmico con una estructura morfológica relativamente sencilla, constituida básicamente por cuatro elementos: la médula, que es la parte más interna y formada por columnas de células queratinizadas; la corteza, capa media que rodea a la médula; los gránulos de pigmento, que le confieren color al pelo y se encuentran inmersos en la corteza y la médula; y la cutícula, que es la capa más externa y que está constituida por placas de células dispuestas en escamas que toman diversa formas en los diferentes grupos de mamíferos (Hausman, 1920; 1924; 1930; Stoves, 1942; Noback, 1951).

Los pelos más externos de los mamíferos o de protección, conocidos como pelos de guardia han sido ampliamente utilizados en las ciencias biológicas, la arqueología y la medicina forense ya que son los más largos, resistentes y no presentan daños incluso cuando se encuentran en contenidos estomacales (Baca y Sánchez-Cordero, 2004).

Los avances en el estudio del pelo o tricología, han documentado otras características físicas del pelo, como es la afinidad química hacia los elementos metálicos, los cuales son absorbidos fácilmente por las fibras pilosas. La capacidad del pelo para absorber metales tóxicos ha sido atribuida mayoritariamente al azufre, el cual se encuentra en la corteza y la cutícula como constituyente del aminoácido cisteína, que

conforma a la queratina. Esta característica ha permitido el uso del pelo como biomonitor con el cual se pueden analizar los metales tóxicos a los que se expone la fauna silvestre como consecuencia de las actividades humanas (Hinners *et al*., 1974; Combs *et al*., 1982; Cargnello *et al., 1995;* McLean *et al*., 2009; Noguchi *et al.,* 2012).

Las investigaciones del pelo para su uso en el biomonitoreo se han centrado en la detección de metales tóxicos principalmente en los seres humanos y en especies individuales de mamíferos (Burger y Gibbons, 1998*;* Afridi *et al*., 2006; Schramm, 2008). Sin embargo, no existen estudios en México en los que se analice la capacidad de absorción de metales en el pelo y se use éste para la detección de estos elementos en regiones contaminadas del país.

En el presente trabajo, despúes de hacer una revisión sobre los avances en estudios del pelo enfocados al biomonitoreo (Capítulo I), se analizó experimentalmente la absorción de tres metales en solución para conocer su permeabilidad hacia las capas internas del pelo y poder determinar si los metales depositados externamente pueden atravesar las capas de escamas y llegar hasta la corteza y la médula (Capítulo II). Finalmente, se analizaron muestras de pelo de mamíferos colectados en la región de la presa Endhó, Hidalgo, la cual presenta uno de los casos más graves de contaminación en Mexico (Capítulo III). Dichas muestras fueron analizadas por microscopía electrónica de barrido para la caracterización de los metales presentes tanto en la cutícula como en los folículos y saber si la fauna se ha expuesto a los metales tóxicos presentes en la presa y en los cultivos irrigados con sus aguas.

Cuadro 1. Los principales usos y fuentes de los metales y metaloides con mayor importancia toxicológica de acuerdo con Järup (2003).

Metal/Metaloide	Usos/fuentes	Fuente
Pb	Pigmentos, acumuladores, baterías, municiones y balas, recubrimiento de cables, plomerías caseras, soldaduras, protección contra radiación, cristalería, cerámica, emisiones vehiculares.	Naja y Volesky (2009)
Cd	Pigmentos, plásticos, baterías plata-cadmio, aleaciones, humo de cigarro.	Volesky (1990) Sharma y Agrawal (2005) Järup (2003)
	Vegetales de hoja verde, hongos, granos y semillas.	Tchounwou *et al. (*2012)
	Celdas solares, reactores nucleares, galvanoplastia.	Athar y Vohora (1995)
Hg	Baterías, amalgamas dentales, termostatos, fungicidas, pescados y mariscos, productos farmacéuticos.	Tchounwou *et al.(*2012)
As	Herbicidas, rodenticidas, insecticidas, fungicidas, componentes electrónicos, medicina veterinaria.	Bradl (2005) Athar y Vohora (1995)

	Preparaciones medicinales, aleaciones con plomo para balas, pirotecnia, pinturas textiles, pigmentos.	Moore y Ramammorthy (2012)

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Capítulo I

BIOMONITOREO AMBIENTAL: AVANCES EN EL ESTUDIO DE LA ABSORCIÓN DE METALES TÓXICOS A TRAVÉS DEL PELO

INTRODUCCIÓN

El pelo es un apéndice epidérmico compuesto por células queratinizadas, ampliamente estudiado desde finales del siglo XVIII y principios del siglo XIX. Se ha considerado como la característica diagnóstica más importante de los mamíferos ya que se presenta por lo menos en alguna etapa de su vida y en ambos sexos, además de tener características y propiedades que lo hacen útil en estudios de diversas áreas de las ciencias biológicas (Hausman, 1920, 1930; Arita y Aranda, 1987; Teerink, 2003).

La resistencia y estabilidad del pelo y todos sus componentes, así como la facilidad para su obtención y manejo lo han convertido en un excelente elemento para la investigación biológica, forense, arqueológica y sobre contaminación ambiental. En este último caso, el pelo ha sido reportado como un tejido con una gran capacidad de absorción de partículas como los metales pesados, cuyas cantidades en las fibras pilosas están relacionadas con la concentración de estos contaminantes en el ambiente, razón por la cual ha sido ampliamente utilizado en el biomonitoreo ambiental (Combs *et al.,* 1982; Limic y Valkovic, 1987; Tobin, 2005; Schramm, 2008).

La capacidad del pelo de absorción de metales tóxicos se ha empleado en la detección de la exposición de los organismos a la contaminación ambiental (Medvedev, 1999; Ivanov *et al.* 2012). Sin embargo, estos estudios se han enfocado principalmente a seres humanos y no han sido ampliamente explorados en la mayoría de las especies de mamíferos silvestres y México no es la excepción, ya que en nuestro país no existen estudios de este tipo dirigidos a la fauna nativa. Aunado a esto, se trata de un método no invasivo y de detección temprana de la exposición de los organismos a ciertos contaminantes como son los metales pesados y tóxicos, cuya concentración en el pelo se ha encontrado correlacionada a la concentración en los órganos internos en fauna silvestre y seres humanos (Burger *et al*. 1994; Medvedev, 1999; Watanabe *et al.* 1996; Ikemoto *et al.* 2004; Vermeulen *et al.* 2009).

LOS METALES TÓXICOS EN EL AMBIENTE

Fuentes naturales y antropogénicas de metales tóxicos.

Los metales tóxicos son elementos que se encuentran de manera natural en el ambiente. Las fuentes naturales incluyen erupciones volcánicas, incendios y la erosión (Callender, 2003). No obstante, las actividades humanas han ocasionado la emisión de enormes cantidades de metales tóxicos y sus compuestos al ambiente, originando serios daños en los ecosistemas y en la salud humana (Lee y Von Lehmden, 1973; Callender, 2003; Reis *et al.* 2010).

Los metales tóxicos son aquellos elementos metálicos que no son esenciales para la vida, y por lo tanto no tienen función biológica, ocasionando daños severos a la salud cuando son ingeridos o inhalados (Rodrigues *et al.* 2008; Onuwa *et al.* 2012).

Los problemas ecológicos ocasionados por contaminación con metales tóxicos no se reducen a las emisiones de elementos no esenciales, algunos minerales esenciales (como el cobre, zinc, selenio y manganeso) también pueden causar daños a la biodiversidad y a la salud humana cuando son ingeridos en grandes cantidades (Nageeb y Soltan, 2005; Reis *et al.* 2010), así como algunos metaloides, entre los que se encuentra el arsénico, elemento altamente tóxico en bajas concentraciones (Goullé *et al.* 2005).

Las fuentes principales de origen humano que liberan elementos esenciales y no esenciales al ambiente incluyen la quema de combustibles, procesos de galvanización, fabricación de pilas, uso de fertilizantes, fungicidas y pesticidas, fundidoras, minería, fábricas de papel, pinturas y porcelana. Entre los elementos tóxicos más comunes que se liberan como producto de estas actividades se encuentran el cadmio, plomo, mercurio, cobalto, cromo, níquel y arsénico. Los efectos nocivos que ocasionan en el organismo y a bajas concentraciones son bien conocidos. Algunos de ellos incluyen daños irreversibles a órganos como hígado y riñones, sistema nervioso, inhibición de síntesis de proteínas, anemia, cambios hormonales que afectan la reproducción y cáncer (Di Paolo y Casto, 1979; Ivanov *et al.* 2012; Onuwa *et al.* 2012).

CARACTERÍSTICAS DEL PELO: MORFOLOGÍA, ESTRUCTURA BIOQUÍMICA Y PROPIEDADES DE ABSORCIÓN.

Morfología.

El pelo está constituido por tres capas principales: la parte más interna, llamada médula, conformada por los remanentes de células queratinizadas, organizadas en columnas; la corteza, que es la capa intermedia, compuesta por células en forma de huso y que además contiene los gránulos de pigmento que confieren el color al pelo y la cutícula, la región externa, constituida por placas de células dispuestas en escamas (Hausman, 1920, 1924, 1930; Stoves, 1942; Noback, 1951). Los análisis de estas estructuras microscópicas del pelo, han llevado a conocer las diferencias morfológicas de las células medulares (figura 1A) y de las escamas que conforman la cutícula de las distintas especies de mamíferos (figura 1B), razón por la cual el pelo ha sido ampliamente utilizado para la identificación de géneros, e incluso de especies (Arita y Aranda, 1987; Amman *et al.* 2002; Baca y Sánchez-Cordero, 2004).

Figura 1A. Tipos de médula. Simple: a) ovada, b) elongada, c) aplanada; Compuesta: d) ovada, e) aplanada. Modificado de Debelica y Thies (2009).

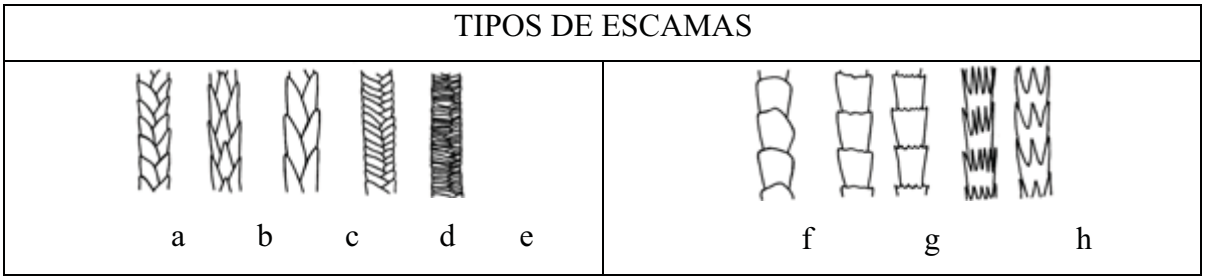

Figura 1B. Tipos de escamas. Imbricadas: a) ovada, b) acuminada, c) elongada, d) crenada, e) aplanada; Coronales: f) simple, g) serrada, h) dentada. Modificado de Debelica y Thies (2009).

La cutícula, además de contribuir con la capacidad aislante y la protección mecánica del pelo (Chernova, 2003), presenta características morfológicas que le permiten atrapar algunas partículas como granos de polen. Las escamas que la constituyen están en algunas especies de mamíferos, muy abiertas o separadas en los bordes de la hebra del pelo formando un ángulo con éste, a diferencia de otras que se encuentran muy cerradas, y son una importante adaptación en algunos mamíferos como los murciélagos nectarívoros que pueden, de esta manera, transportar una gran cantidad de polen que queda atrapado entre las escamas (Howell y Hodgkin, 1976; Meyer, 2002).

Estructura bioquímica y propiedades de absorción.

Las proteínas que constituyen la fibra capilar son la queratina y la tricohialina. Esta última está implicada en el proceso de queratinización que ocurre durante la biogénesis del pelo, además de formar parte de la región medular de éste (Birbeck y Mercer, 1957; Alibardi, 2004a, b). La queratina es una proteína rica en cisteína y otros aminoácidos como arginina, histidina, lisina y glicina (Block, 1939). La cisteína se caracteriza por tener grupos sulfhidrilos, moléculas que, al contener azufre, presentan una gran afinidad por los metales (Hinners *et al*. 1974; Combs *et al*. 1982; Cargnello *et al. 1995;* Aryal *et al. 2006a,b;* McLean *et al*. 2009; Noguchi *et al.* 2012). Sin embargo, a pesar de que tradicionalmente se ha atribuido al azufre la gran afinidad química hacia los metales, otras moléculas se han sugerido como las implicadas en la unión de elementos metálicos al pelo, ya que en algunos estudios se ha sugerido que los puentes metal-azufre no son estables (Hinners *et al*. 1974).

El grupo carboxilo es otra de las moléculas que se han considerado afines a los elementos metálicos, debido principalmente a los cambios en la capacidad de absorción del pelo cuando cambia el pH, el cual modifica la cantidad de aniones carboxílicos que pueden unirse a los cationes metálicos (Bate, 1966; Hinners *et al*. 1974; Patil *et al.* 2012).

En general, se ha encontrado que existe una gran afinidad entre los aminoácidos y los metales. Estas interacciones se han demostrado en experimentos con aminoácidos como lisina y glicina, que también forman parte de la estructura capilar (Dou *et al*. 1999ab; Selvakannan *et al.* 2003; Wangoo *et al.* 2008).

Los gránulos de melanina que están inmersos en la corteza del pelo también tienen propiedades muy importantes, ya que además de dar color al pelo, absorben selectivamente diferentes tipos de toxinas, incluyendo elementos metálicos. Esta propiedad ha sido considerada como una importante adaptación que permitió que los seres humanos habitantes de las zonas costeras y que consumían principalmente pescado, no se intoxicaran con las sustancias nocivas que los peces acumulan naturalmente en sus órganos (Kintz y Villain, 2005; Tobin, 2008).

Los elementos químicos y moléculas con afinidad hacia los metales tóxicos ya mencionados presentes en el pelo, dan una idea de cómo ocurren tanto el depósito exógeno como el endógeno de diferentes sustancias. El azufre que forma parte de la queratina de la cutícula, así como los grupos carboxilos, atraen elementos y moléculas que se encuentran en la atmósfera y en el sustrato terrestre, proceso conocido como depósito exógeno. Por otra parte, el depósito endógeno ocurre cuando algunas toxinas se incorporan al pelo cuando éste se está desarrollando, a través del flujo sanguíneo que irriga el folículo piloso (figura 3) (Beernaert *et al.* 2007; Schramm, 2008; Mclean *et al.* 2009). En este proceso los gránulos de pigmento formados por los melanocitos del folículo, absorben toxinas para posteriormente incorporarse a la corteza del pelo (Kintz y Villain, 2005; Tobin, 2008).

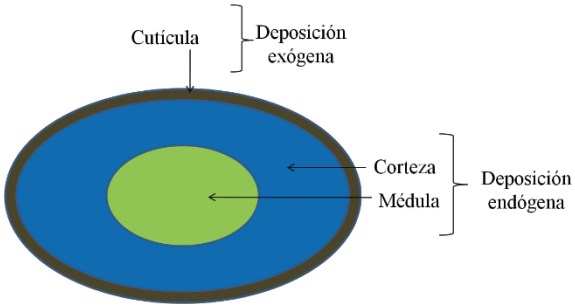

Figura 3. Esquema de corte transversal del pelo y los tipos de depósito que ocurren en cada una de sus capas.

MÉTODOS

Se analizaron 92 artículos sobre la determinación de la exposición a metales tóxicos a través del pelo de diferentes mamíferos silvestres, de granja u humanos.

RESULTADOS Y DISCUSIÓN

USO DEL PELO HUMANO PARA LA EVALUACIÓN DE EXPOSICIÓN A METALES TÓXICOS

Los estudios del pelo para la detección de contaminantes y toxinas han estado enfocados en gran medida a la salud humana. La exposición a contaminantes presentes en el ambiente de carácter antropogénico, ha sido evaluada en diferentes tipos de muestras biológicas como sangre, orina y el pelo (Budtz-Jorgensen *et al. 2004;* Kales y Christiani, 2005). La emisión de estas sustancias a la atmósfera, a la tierra y a los ecosistemas acuáticos se ha visto reflejada en la presencia de metales tóxicos (Jenkins, 1979; Goullé *et al.* 2005; Onuwa *et al.* 2012) y contaminantes orgánicos (Schramm, 2008) en el pelo de personas que viven en grandes urbes, zonas industriales, agrícolas y regiones mineras (Jenkins, 1979; Combs *et al.* 1982; Onuwa *et al.* 2012).

La contaminación ocasionada por la minería ha sido documentada y evaluada en diferentes regiones del mundo, evidenciando los graves problemas que origina a la salud humana y de los ecosistemas (Palheta y Taylor, 1995; Pereira *et al.* 2004, 2006). La contaminación del agua en la selva Amazónica es un ejemplo de los problemas ambientales que ha originado la minería, particularmente del oro, que ha expuesto tanto a comunidades rivereñas como a mamíferos de la zona a grandes cantidades de mercurio, mismas que se han evaluado en sangre, orina y pelo de seres humanos, vacas y cerdos, en los que se encontraron grandes cantidades del metal además de individuos con síntomas característicos de la intoxicación por mercurio (Palheta y Taylor, 1995). Otros estudios en Brasil han llegado a conclusiones similares con la reserva de que el pelo es mejor para la detección de ciertos metales como el mercurio (Rodrigues *et al.* 2008).

La presencia de metaloides como el arsénico en aguas subterráneas en el sudeste asiático también se ha reflejado en el pelo y uñas de personas que consumen el agua contaminada (Gault *et al.* 2008), lo que muestra la utilidad del pelo para la detección de toxinas con orígenes diferentes. Los procesos de extracción del agua pueden contaminarla con arsénico, el cual está presente en la pirita del sustrato de las aguas subterráneas, tal como se encontró en Bengala y en la India, donde además del arsénico se determinó cadmio, selenio y mercurio en escamas dérmicas, uñas y pelo de los habitantes de la región (Gautam *et al.* 2004).

La exposición ocupacional es otro de los riesgos que se ha evaluado usando muestras de pelo, además de sangre y orina (Afridi *et al.* 2006; Olmedo *et al.* 2010). Los hallazgos muestran que el pelo es

una buena alternativa para la detección de riesgos de contaminación en trabajadores por el tiempo de contacto con metales (Mehra y Juneja, 2005; Gil *et al.* 2011) y por exposición accidental (Hagedorn-Gotz *et al.* 1977).

El riesgo relacionado a las ocupaciones laborales, a pesar de que se investiga en términos de salud humana, está completamente relacionada con los daños a las especies y al ambiente en general. Las actividades económicas que originan daños a la salud como la extracción de minerales, fabricación de pilas, fármacos, pinturas, papel, aparatos electrónicos, entre otros, han originado también serias alteraciones al ambiente. Es por esto que cuando se detectan sustancias nocivas en pelo humano y otro tipo de muestras, producto de estas actividades, se debe también analizar el daño al medio ambiente para poder tomar medidas preventivas y de control de emisión de contaminantes.

EVALUACIÓN DE LA PRESENCIA DE METALES TÓXICOS EN PELO DE DIFERENTES ESPECIES DE MAMÍFEROS.

Estudios en mamíferos terrestres.

La contaminación ambiental provocada por la liberación de innumerables toxinas al ambiente ha sido una de las grandes preocupaciones en materia de conservación que se tienen en la actualidad. En este ámbito el pelo es una de las mejores alternativas para la detección de amenazas sin la necesidad de ser invasivos para la toma de muestras de la fauna.

1) Animales de granja

La literatura reporta que la presencia de metales tóxicos y otros elementos metálicos como cobre y zinc se remonta a la época de los años 50 (Cunningham y Hogan, 1958). Muchos de estos trabajos se concentran en el análisis del pelo de ganado por la relación existente entre la contaminación del forraje y los suelos, así como por los riesgos a la salud humana derivados del consumo de carne y otros productos contaminados (Combs, 1987; Milhaud y Mehennaoui, 1988; Nandi, *et al.* 2005; Rashed y Soltan, 2005; Patra *et al.* 2006, 2007). La utilidad del pelo se ha visto reflejada en la detección de cadmio, cobalto y plomo en el pelo de vacas, ovejas, alpacas y caballos, y en la correlación positiva entre las cantidades presentes en sangre y en pelo de estos metales (Dorn *et al*. 1974; Patra *et al.* 2006), demostrando que la fibra pilosa es un tejido de gran utilidad para el biomonitoreo continuo de la salud de la fauna, los ecosistemas y la evaluación de riesgos a la salud.

El estado nutricional de animales de granja también puede evaluarse a través del análisis de metales esenciales presentes en el pelo como el cobre y el zinc (Combs *et al.* 1982; Combs, 1987; Milhaud y Mehennaoui, 1988). Dichos elementos, aun cuando presentan una función biológica y son importantes para la vida, también representan un problema ambiental, ya que se necesitan a nivel de trazas y el

incremento de sus concentraciones puede tener repercusiones serias en la salud de animales, seres humanos y en los ecosistemas (Milhaud y Mehennaoui, 1988). Los metales como el zinc y el cobre también son liberados a la atmósfera generalmente formando compuestos sumamente tóxicos que contaminan el agua y el suelo de la misma manera que lo hacen los metales tóxicos (Rogowska *et al.* 2009). Ambos tipos de metales se bioacumulan en plantas (Raskin *et al.* 1994), iniciando así su camino a través de las redes tróficas, representando un enorme riesgo para la fauna silvestre y el ganado.

A pesar de que una gran cantidad de análisis se realizan con pelo de guardia dorsal, también se ha empleado lana para la detección de metales (Hawkins y Ragnarsdóttir, 2009) por la gran afinidad química. Dicha afinidad ha sido estudiada para conocer mejor estas propiedades de absorción para su aplicación en biorremediación. Patil *et al. (*2012) utilizaron lana molida (milled cashmere guard hair) para probar su afinidad hacia iones metálicos como Cr^{6+} y Zn^{2+}, encontrando una gran utilidad para remover metales tóxicos del agua.

2) Fauna silvestre.

El pelo se ha utilizado para la detección de metales tóxicos en una gran variedad de especies de diferentes órdenes de mamíferos silvestres. Los metales tóxicos más comunes en estas especies son el Pb y el Hg, mientras que los metales esenciales son el Zn y Cu (tabla 1).

Tabla 1. Especies de mamíferos usados para detección de metales tóxicos en el pelo.

Taxa	Metal pesado	Metaloide	Metal no pesado	Metal esencial	Autor
1Orden Didelphimorphia					
Didelphis virginiana	Pb, Cd, Cr		Hg, Mn		Burger *et al.* 1994.
2Orden Erinaceomorpha					
Erinaceus europeus	Cd, Co, Cr, Pb	As	Ag, Al, Ni	Cu, Fe, Zn	D'Havé *et al.* 2006, 2007; Vermeulen *et al.* 2009.
3Orden Chiroptera					
Myotis lucifugus					Hickey *et al.* 2001.
M. leibii					
M. septentrionalis					
Eptesicus fuscus					
6Orden Rodentia					
Rattus rattus	Cr	As			Pereira *et al.* 2006.
Mus spretus	Cr	As			
Ctenomys talarum	Pb			Zn, Fe, Cu	Schleich *et al.* 2010.
Spermophilus beecheyi	Pb, Cd, Co	As	Sb, Al, Hg. Mo, Ni	Ca, Mg, Cu, Fe, K	Hubbart, 2012.
Meriones persicus	Cr, Ti	As		Cu, Mn, Fe	Khazaee *et al.* 2016.
7Orden Carnivora					
Vulpes vulpes	Pb, Cr, Ni		Ni, Al	Zn, Cu, Ca	Filistowicz *et al.* 2011.
Felis pardalis			Hg		Mora *et al.* 2000.
Lynx rufus			Hg		Cumbie, 1975.

Canis latrans			Hg		Huckabee *et al.* 1973.
Procyon lotor			Hg	Se	Cumbie, 1975; Clark *et al.* 1986.
Callorhinus ursinus	Pb		Sb		Ikemoto *et al.* 2004.
Pusa caspica	Pb		Sb		
Pusa sibirica	Pb		Sb	Zn	
Phoca sibirica					Watanabe *et al.* 1996.
Phoca bitulina			Hg		Nöel *et al.* 2016.
8Orden Artiodactyla					
Sus scrofa			Hg		Sobanska, 2005.
Rangifer tarandus			Hg		Duffy *et al.* 2005.
Alces alces gigas	Pb		Mn	Ca, Mg, Cu, Fe, K, Zn, Na	Flynn *et al.* 1975.

La capacidad para acumular metales en el pelo o en los órgamos internos está determinada por múltiples factores relacionados con la fuente de la que provengan los metales y con mecanismos de regulación interna. Los hábitos alimenticios también infuyen en el grado de exposición de los organismos, por ejemplo, aquellos que consumen animales hiperacumuladores estarán consumiendo grandes cantidades de metales, como es el caso de los insetívoros. Este fenómeno de bioacumulación, afecta no solo a las poblaciones de organismos expuestos por la ingesta de contaminantes, también la dinámica ecosistémica se ve afectada debido a la biomagnificación, la cual implica el paso de metales a través de la cadena alimenticia hacia niveles tróficos superiores con una concentración cada vez mayor (Cheruiyot *et al.* 2013; Gall *et al.* 2015)

Los análisis de pelo de mamíferos acuáticos sugieren un importante nivel de contaminación en los hábitats marinos y de agua dulce. En pinípedos, se han encontrado metales como Cd, Pb, Fe, Mn, Cu y Hg no solo en este sino en riñón, pulmón, bazo e hígado (Watanabe *et al.* 1996). El pelo, así como tejidos de riñón, pulmón, bazo e hígado de foca del lago Baikal (*Phoca sibirica*) fueron analizados y se encontraron metales pesados Cd y Pb, además de otros metales esenciales y no esenciales como hierro, manganeso, cobre y mercurio. Este último elemento resultó ser el mejor caso para el biomonitoreo con pelo (Watanabe *et al.* 1996). Resultados similares se encontraron en foca del caspio (*Pusa caspica*) y en oso marino ártico (*Callorhinus ursinus*), que presentaban vanadio, manganeso, plata, selenio y mercurio en el pelo (Ikemoto *et al.* 2004). Los resultados que han arrojado los análisis del pelo de estas especies demuestran la utilidad y confiabilidad del pelo para la detección de contaminantes en ambientes acuáticos que amenazan a las especies que habitan en ellos y que además, implican el salto de estos contaminantes hasta el ser humano a través de productos de consumo que provienen de estas regiones.

Implicaciones de las características biológicas en el uso del pelo como biomonitor

Las diferencias biológicas de las especies se han sugerido como factores que influyen en la absorción de los elementos metálicos, por lo que es imprescindible considerar estos aspectos particulares al escoger a las especies para poder explotar con esto los diferentes potenciales en el biomonitoreo (Pereira *et al.* 2006; Schleich, *et al.* 2010; Hubbart, 2012). Las variables que pueden modificar la incorporación de metales al pelo incluyen los hábitos de forrajeo (Hickey *et al.* 2001), la edad (Ikemoto *et al.* 2004), sexo, peso y tamaño del organismo (Combs *et al*. 1982). Sin embargo, dichas variables deberían ser más investigadas, ya que existen estudios en donde se sugiere que éstas no tienen efecto en el depósito de elementos en el pelo (Beernaert *et al.* 2007; McLean *et al.* 2009).

Los roedores son el orden de mamíferos terrestres más usado, ya que son indicadores de contaminación muy localizada y reciente en el tiempo, lo que puede resultar de gran utilidad para la evaluación de riesgo (ver tabla 1). Sin embargo, son pocas las especies cuyo pelo se ha utilizado para evaluar la exposición a metales tóxicos, a pesar de que, al ser consumidos por una gran variedad de especies carnívoras, la detección de estos elementos en ellos refleja un riesgo para la biota en general.

La microscopía electrónica como técnica de análisis elementales

Las técnicas espectrométricas son las más empledas en biomonitoreo por su capacidad de detección de elementos traza (Yamashita, 1996; Goullé *et al.* 2005; Beernaert *et al.* 2007; McLean *et al.* 2009). La microscopía electrónica en cambio, se ha utilizado en los análisis morfológicos de la cutícula y de la médula (Chernova, 2002; 2003), dejando a un lado la capacidad de análisis elemental por medio de sus detectores de rayos x característicos (EDS por sus siglas en inglés), los cuales proveen un análisis cualitativo, en el que se muestra qué elementos se encuentran en la muestra analizada, y un análisis cuantitativo, que arroja los porcentajes en los que se encuentra cada uno de los elementos detectados (Goldstein *et al.* 1992). En la figura 4 se muestran los elementos detectados con la técnica de rayos X característicos por microscopía electrónica, en la que se observan los componentes elementales del pelo como el azufre, carbono y nitrógeno en *Canis latrans*, mientras que en la figura 5 se muestra el mismo tipo de análisis, pero en el que se detectaron otros elementos que se depositaron en el pelo de la misma especie.

La gran ventaja que ofrece la microscopía electrónica sobre otras técnicas empleadas para la detección de metales tóxicos, es que muestra la localización exacta de los elementos en una muestra, permite el mapeo de elementos, la repetición de los análisis (no es una técnica destructiva) y la posibilidad de medición de las partículas contaminantes que se unieron al pelo, cuyo tamaño está relacionado directamente con la fuente de la que proviene la sustancia contaminante (Lee y von Lehmden, 1973). Además, los gránulos de pigmento se pueden observar mediante estas técnicas y llevar a cabo el análisis

elemental (EDS) de manera puntual en cada uno de éstos, ya que son estructuras relacionadas con la uníon química de metales al pelo para establecer si existen metales unidos a estas estructuras que no correspondan a sus elementos constituyentes naturales. Es una técnica no destructiva que ofrece párrafo inconcluso

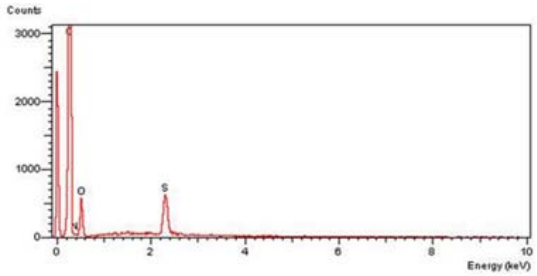

Figura 4. Espectro de EDS que muestra los elementos que constituyen el pelo.

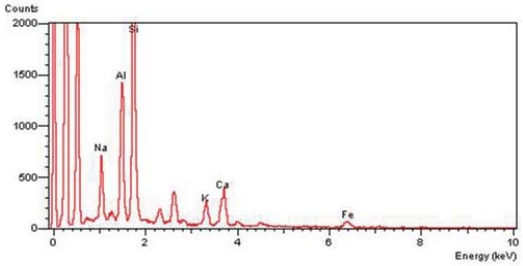

Figura 5. Espectro de EDS que muestra elementos depositados en el pelo de *Canis latrans*.

La información precisa que se obtenga sobre el origen de los elementos tóxicos en pelo, proporciona la oportunidad de establecer medidas más concretas de prevención y control de la emisión de contaminantes con las que se disminuyan las amenazas a la salud ambiental y humana, y se informe a la población de las zonas aledañas a las fuentes de contaminantes sobre los riesgos sanitarios.

CONCLUSIONES

Importancia del uso del pelo como biomonitor para la conservación.

La contaminación ambiental es una de las grandes amenazas a la biodiversidad y a la salud humana que se viven actualmente (McNeely, 1992). Los metales tóxicos, aun cuando se encuentran en los ecosistemas por fenómenos naturales, incrementan de manera desmedida sus niveles en la atmósfera, la tierra y los ecosistemas acuáticos debido a las actividades humanas (Medvedev, 1999; Calender, 2003). Las plantas, al tener la capacidad de incorporar a sus tejidos los metales que se encuentran en el agua y el suelo (Raskin *et al,* 1994), exponen a la fauna herbívora a la ingesta de metales tóxicos que comenzarán así su paso a través de los niveles tróficos (Dunlap *et al.* 2007). Esto representa una fuerte amenaza para una gran cantidad de especies, más aún si se considera que la exposición a éstas también ocurre por la ingesta de agua contaminada y el depósito exógeno de partículas suspendidas en el aire que los mamíferos ingieren al acicalar sus pelajes (Pereira *et al.* 2006; Schleich, *et al.* 2010; Hubbart, 2012). Como

consecuencia, las condiciones patológicas originadas por la presencia de metales tóxicos en los órganos causan cambios importantes en los patrones reproductivos que pueden mermar seriamente las poblaciones de mamíferos (DiPaolo y Casto, 1979; Ivanov, 2012), principalmente si se trata de especies amenazadas.

El pelo ofrece, bajo este panorama de amenazas a la biodiversidad, una manera eficaz, de fácil obtención y manejo, de bajo costo, no invasiva y de detección temprana de la presencia de metales tóxicos en el ambiente y en los órganos internos de las especies (Jacob *et al.* 1978; Kales y Christiani, 2005; D'Havé *et al*. 2006; Vermeulen *et al*. 2009). Incluso ha resultado útil en la detección de otro tipo de contaminantes, como los compuestos organoclorados presentes en los agroquímicos (Schramm, 2008). Se trata por lo tanto de una estructura muy útil para la evaluación de riesgos a la biodiversidad que puede ayudar al establecimiento de mejores estrategias de conservación.

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Capítulo II: Permeability of hair to cadmium, copper and lead in five species of terrestrial mammals and implications in biomonitoring (Artículo de requisito)

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Permeability of hair to cadmium, copper and lead in five species of terrestrial mammals and implications in biomonitoring

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Abstract The capacity of mammal hair to absorb toxic metals and its utility in biomonitoring has been broadly studied. Though these metal-binding properties has generally been attributed to the sulphur contained in cysteine, an amino acid that forms part of keratin, there are not many experimental studies that analyze the role of sulphur in the external deposition of potentially toxic metallic elements in order to better understand the potential of hair in biomonitoring and generate better tools for differentiating between internal and external deposition of contaminants. In this study, an experimental analysis is carried out using a scanning electron microscope on hairs of five terrestrial mammal species (*Peromyscus furvus*, *P. maniculatus*, *Glossophaga soricina*, *Artibeus jamaicensis* and *Marmosa mexicana*) treated with cadmium, copper and lead salts. We quantified absorbed metals as well as natural elements of the hair by energy dispersive X-ray spectroscopy (EDS) to analyze using simple statistics the role of sulphur in the absorption Cd, Cu and Pb. Given the lack of studies comparing the mechanisms of deposition of metal elements among different orders of Class *Mammalia*, external morphology was considered to be an important factor in the deposition of metallic particles of Cd, Cu and Pb. Bat species (*Glossophaga soricina*, *Artibeus jamaicensis*) showed a high concentration of particles in their scales, however, no between-species differences in metal absorption were observed, and during the exogenous deposition metal particles do not permeate the medulla. These results suggest that the sulphur in hair itself cannot bind metals to hair cuticle and that hair absorption capacity depends on a variety of factors such as aspects of hair morphology.

Keywords Mammalian hair · Metal deposition · Electron microscope · Absorption

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Introduction

The emission of toxic metals to the environment due to human activities forms part of the serious problem of environmental deterioration we currently face. Toxic metals are elements that have no biological function, and while they occur naturally, economic, domestic, medical and technological applications have led to their dispersal in the environment, exposing wildlife and humans to their harmful health effects (Callender 2003; Bencko 2005; Tchounwou et al. 2012).

Monitoring has been defined as the repeated observation of the presence of chemical or biological elements using standardized methods in determined units of time and space (Van der Oost et al. 2003; Torres et al.

2008). Biomonitoring is monitoring which is based on the sampling and analysis of tissues and fluids of an organism to evaluate exposure to such elements (Zhou et al. 2008), and is a fundamental tool for the measurement and assessment of exposure of humans and the rest of the biota to environmental pollutants (Preston 1975; Needham 2008; Esteban and Castaño 2009). Its main objective is the identification and elimination of the possible sources of heavy metal exposure to avoid the loss of biodiversity and environmental deterioration that this type of contamination generates. In addition, it allows the observation of the variation in contaminants and their effects over time, geographic mapping of regions most affected by pollution, and testing of the effectiveness of environmental protection measures (Paschal 2008; Needham et al. 2007; Esteban and Castaño 2009). For this reason, biomonitoring studies have an advantage over soil and water studies (Zhou et al. 2008; Tellez and Merchant 2015), which do not provide integrated information including current and predicted future effects on the equilibrium of ecosystems.

The use of animals in biomonitoring provides valuable information about metallic pollutants in the environment, their concentrations and effects on terrestrial and aquatic ecosystems, since they present different degrees of sensitivity and display different toxicological responses, giving them a key role in ecotoxicology (Martin 2012; Moore and Ramammorthy 2012). However, obtaining samples such as urine or blood can be invasive for wildlife, such that other matrices like hair have been presented as a valuable alternative that provides reliable results for the detection of potentially toxic metals in wildlife whose concentration is related to the concentration of those contaminants in the environment (Limic and Valkovic 1987; Tobin 2005; Kales and Christiani 2005; Rashed and Soltan 2005; Schramm 2008). Additionally, concentration of pollutants in hair and other tissues like blood in wildlife also depends on the particular chemical species. When pollutants are released and dispersed, they undergo speciation processes that can modify their mobility and bioavailability in the environment, as well as the capacity of the organisms to accumulate and excrete them (Brown et al. 1999). Bioavailability is the beginning of any toxicological process and depends on ability of the chemical to contact and interact with an organism through specific receptors and pathways (Katayama et al. 2010).

Hair is an epidermal appendage characteristic of mammals and has a simple morphology composed of three main layers: the medulla, which is the innermost layer and is composed of columns of keratinized cells; the cortex, the middle layer which surrounds the medulla; pigment granules, which confer color to the hair and are immersed in the cortex and medulla; and the cuticle, which is the external layer and is made up of plates of cells arranged like scales which take a diversity of shapes in different groups of mammals (Hausman 1920, 1924, 1930; Stoves 1942; Noback 1951; Amman et al. 2002; Tobin 2005). The cuticle contains a large amount of sulphur, to which the ability of the hair to absorb metals is attributed (Hinners et al. 1974; Combs et al. 1982; Cargnello et al. 1995; Aryal et al. 2006a, b; McLean et al. 2009; Noguchi et al. 2012).

The medulla and cuticle contain little or no sulphur, but both layers are embedded with pigment granules, which confer color to the hair as well as being able to selectively bind metal elements. For this reason, they have been widely studied in toxicology, but experimental studies which effectively describe their potential in biomonitoring are still needed. Thus, even though the hair's ability to absorb metals has been attributed principally to the amount of sulphur contained in the keratin that conforms it (Block 1939), the use of techniques that have not been previously employed for the detection of contaminants in hair, as well as statistical analyses, would clarify the role of sulphur in the binding of metals to the cuticle and differentiate between mechanisms of external deposition from those of internal deposition that do not directly involve this element. As such, in this work, a scanning electron microscope was used to analyze the adhesion of toxic metals to the hair cuticle of mammals from three different orders, submitted to a treatment of metal salts in solution. We also quantified the percentage of metals adhered and the elements constituting the hair using characteristic X-rays to analyze whether there was a correlation between the metals adhered to the hair and the sulphur contained by the hair.

Methods

Hair samples

Samples of dorsal guard hairs were taken from specimens deposited in the "Alfonso L. Herrera" Zoological Museum of the Facultad de Ciencias, UNAM. These

samples were from three individuals each from five species in three genera of terrestrial mammals: *Peromyscus furvus*, *P. maniculatus*, *Glossophaga soricina*, *Artibeus jamaicensis* and *Marmosa mexicana*.

Laboratory procedures

Treatment of hair samples

A saturated solution (1 M to enhance adherence) of copper II nitrate, lead II nitrate, and cadmium II acetate salts were prepared, following Tan et al. 1985, who found that hair absorption capacity depends on the concentration of solution.

Hair samples were washed with pure ethanol to remove accumulated dust and oils. Once dried, samples of 20 hairs were placed into Eppendorf tubes and 1 mL of metal salt solutions was added to each tube inside an extraction hood. Thus, the hairs from each individual were subjected to three treatments: cadmium, copper, and lead salts. We considered very important to analyze toxic metals with different chemical behavior in mammal hair. Cd and Pb were selected because they are both well documented in mammals, especially bats, as elements that have no biological function, exhibit high chemical affinity to mammal hair, and are currently a serious environmental problem. On the other hand, copper is a necessary element in trace amounts for living organisms and has little affinity to hair cuticle (Noguchi et al. 2012; Hernout et al. 2016). The metal salts selected are highly soluble in water, allowing a simple experiment with mammal hair.

The samples were removed from the solutions after 2 months and were washed with distilled water to remove the excess solution, for mounting on sample holder with carbon tape for examination with a scanning electron microscope.

Scanning electron microscope

Imaging and hair type classification Imaging was carried out using a JEOL JSM-5900LV scanning electron microscope to classify cuticle types for each of the species and observe and analyze the presence of metal particles for each treatment. The hair region considered was the middle region at its widest part, or shield. Cuticle morphology was determined according to Hausman (1920).

Energy dispersive X-ray spectroscopy Before the hair elemental analysis, an Oxford (Isis model) energy dispersive X-ray spectroscopy (EDS) detector was calibrated with a standard sample of known composition, to determine the detection limit and the percentage of error of the detector.

In X-ray microanalysis (EDS), precision refers to the irreducible minimum error. In accordance with Friel (1994), precision is given by the following equation:

$$\frac{\Delta C}{C(\%)} = \frac{2.33}{nN^{1/2}}\ (100)$$

where

C	concentration as a fraction
N	average counts (X-ray events)
n	number of analysis points
2.33	k e^2

After calibration, several analyses were run on control samples of guard hairs that were not treated with metal salts to determine their elemental composition.

In order to quantify the percentage of each element (sulphur, carbon, Cd, Cu and Pb) present in the cuticle of treated hair of each species, a characteristic X-ray analysis was carried out using an Oxford detector (Isis model) of the same microscope as above, on an 8 μ^2 area of the spatula region of each sample (the minimum area necessary to perform this analysis is about 2 μ^2; Friel 1994). A characteristic X-ray is produced when an electron beam strikes an electron of the sample, producing a displacement that leaves vacancy in the electron shell, which will be filled by an electron of a higher electron shell, resulting in the emission of an X-ray photon. This X-ray energy emission is unique to each element and is represented by a characteristic peak on the EDS spectrum (Goldstein et al. 1992).

Ultramicrotome sections Samples of *G. soricina* hair treated with cadmium II acetate salt for 6 months were embedded in LR White resin and polymerized in an ultraviolet chamber for 24 h in order to determine whether absorption occurs within the innermost hair layers. Then, the resin-embedded hair samples were cross-sectioned using a Leica UC6 ultramicrotome for observation under the scanning electron microscope. We carried out this analysis in only one of our study species because this was the only species in which resin

successfully penetrated the hair and was cross-sectioned without breaking.

Statistical analyses

A multiple linear regression with hypothesis test was performed in R (R Core Team 2013) to test whether the amount of sulphur and carbon in the hair are explanatory variables related functionally to each of the metals absorbed.

We considered β_S and β_C the respective estimators of the variables sulphur (S) and carbon (C).

As such, the null hypothesis is the following:

H_0: $\beta_S = \beta_C = 0$ (this hypothesis indicates that the variables in question are not explanatory and thus are removed from the model)

The alternative hypothesis is:

H_1: At least one of β_S *and* β_C differs from zero. As such, the corresponding variables are explanatory in the model. If both differ from zero, this indicates that both variables are explanatory in the model.

Criteria:

If $p \leq \alpha$, the null hypothesis H_0 is rejected.
If $p > \alpha$, the null hypothesis H_0 is not rejected.

Results

Scanning electron microscopy

Images from a total of 45 dorsal guard hair samples treated with metal salts were obtained from three individuals of each species (*A. jamaicensis*, *G. soricina*, *P. furvus*, *P. maniculatus* and *M. mexicana*). The images of the hairs from all five species showed metal particles adhered to the cuticle. The morphological analysis was necessary to relate morphology to metal presence, since as we had suspected, a large number of metal particles were trapped by the edges of the scales (Figs. 1 and 2). Cuticle morphology was classified following Hausman (1920) in two groups: coronal scales (Figs. 1, 2 and 3), whose edges extend and separate from the shaft, and imbricate scales (Figs. 4 and 5), whose edges are completely flush with the surface of the hair shaft. The brightest parts of the photograph correspond to metal particles adhered to the hair.

The following images show lead particles adhered to the hairs of *G. soricina* (Fig. 2), *P. furvus* (Fig. 3) and *M. mexicana* (Fig. 4). Coronal scales can be seen in *G. soricina* and *A. jamaicensis* and present a high concentration of metals at their edges, while in *P. furvus*, *P. maniculatus* and *M. mexicana*, the closed, imbricate scales do not trap metals around their edges.

The calibration of the EDS detector using a standard sample of known composition, confirmed a precision of ± 2% and a detection limit of 0.1%, and subsequently, the elemental analysis in control samples determined the presence of expected elements (C, S and O) as well as the lack of any metallic element in the guard hair.

The elemental analysis was carried out on an 8 μ^2 area of the middle region (distal portion) of the 45 samples. With this analysis, we detected the presence of the three metals with which the hairs were treated, despite washing with distilled water following the treatment, which suggests some affinity between the metals and the chemical components of the hair tissue. In addition, in all cases, each of the test metals was detected, even in regions where no particles were visible.

In each of the microanalyses (EDS), we obtained a spectrum whose peaks corresponded with each element in the sample, as shown in Fig. 6. We also carried out quantitative analysis of the percentage in which each of the metals was found. In addition to the metals in each treatment, (lead, cadmium, copper), we obtained the percentages of carbon, oxygen, and sulphur, which are the elements that make up the hair tissue.

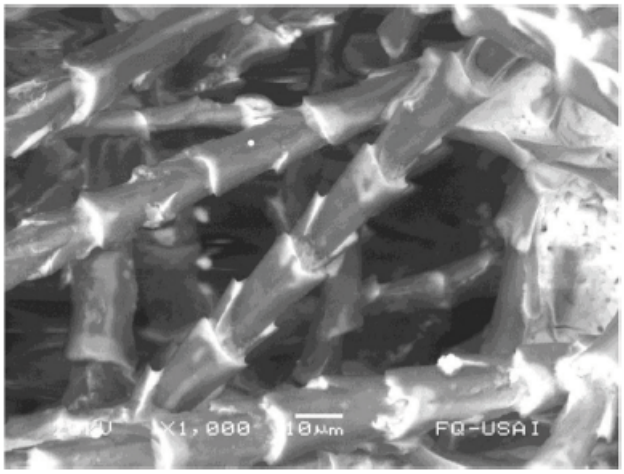

Fig. 1 *Glossophaga soricina* hair with cadmium II acetate

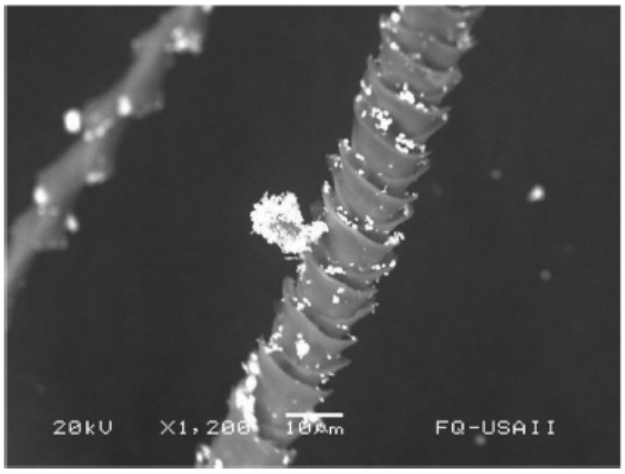

Fig. 2 *Artibeus jamaicensis* with cadmium II acetate

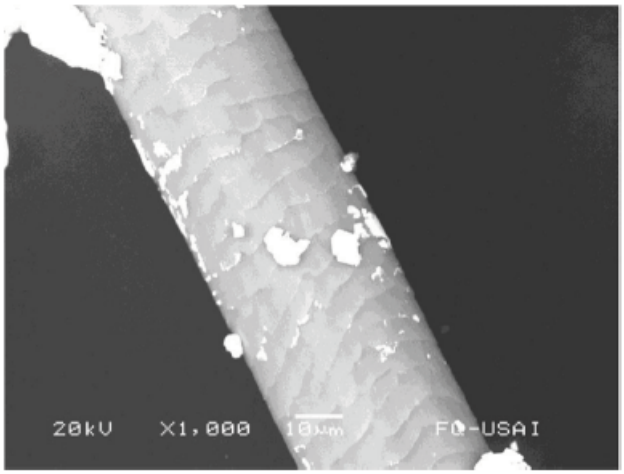

Fig. 4 *P. furvus* hair with lead

Ultramicrotome sections

The ultramicrotome sections of hairs of *G. soricina* treated with cadmium salts for 6 months that were analyzed by scanning electron microscopy presented only two small areas with cadmium (Fig. 7). These small regions (marked with red arrows) were in the cortex, layer in which a pigment granule with cadmium was found.

The EDS analysis was repeated around the perimeter of the hair to search for cadmium in the cuticle. However, no cadmium was detected, despite the long duration of the cadmium acetate treatment which was meant to allow greater permeability of the metal through the hair. Some of the zones in which microanalysis was carried out are indicated with white boxes. In these zones the microscope detector found only naturally occurring components of the hair (carbon and oxygen). It is worth mentioning that the hair of the order Chiroptera does not possess a medulla, such that a large hole can be seen in the middle of the section (Fig. 7).

Statistical analyses

The multiple regression analysis took into account the quantities of sulphur and carbon associated with each metal found in the samples. The characteristic X-ray detector quantifies the percentages of each element found.

Average atom percentages The average percentages of the elements found in the samples are shown in Tables 1, 2 and 3. It can be observed that the element that was most absorbed was cadmium, followed by lead and least, copper. Between species, *G. soricina* showed the highest percentages of copper and cadmium, while *M. mexicana* had the lowest percentage of these elements. However, there was no consistent trend in the

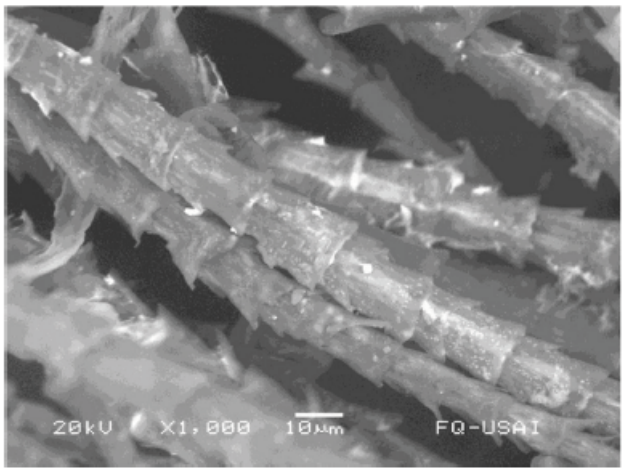

Fig. 3 *G. soricina* hair with lead

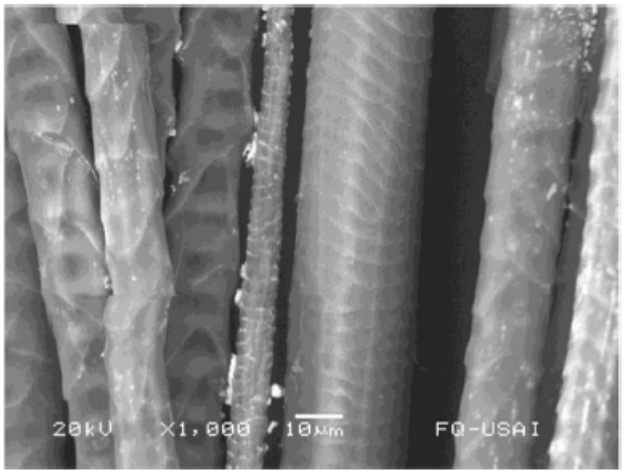

Fig. 5 *M. mexicana* hair with lead

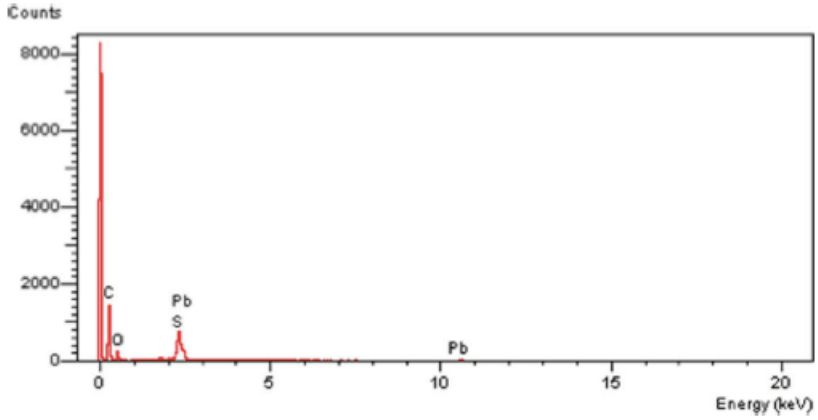

Fig. 6 Spectrum obtained from hairs of *P. maniculatus* treated with lead

absorption of metals among species. On the other hand, the concentrations of naturally occurring hair elements were found in the expected proportions, with carbon found in the highest proportion (75 to 87.4%), followed by oxygen (10.5 to 22.7%), and finally sulphur in lower proportions (1.3 to 1.6%). This confirms the reliability of electron microscopy for quantifying the elemental composition of a sample.

Multiple linear regression From the analysis yielded by the program,

$\beta_S = 0.4716$ with an associate p value of 0.064087.

At a significance threshold of $\propto = 0.1$, which corresponds to 90% confidence, $p \leq \propto$.

The null hypothesis is rejected, and it can be argued that the variable S is explanatory at a percentage of nearly 50%.

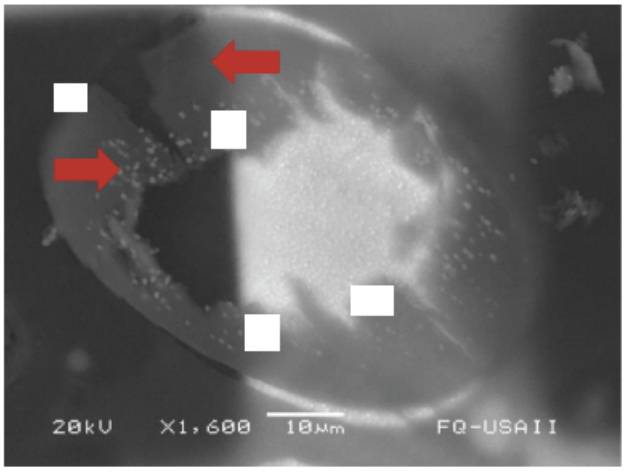

Fig. 7 Cross-section of *G. soricina* hair. Red arrows indicate parts where cadmium was detected, while white boxes indicate regions in which microanalysis was carried out

Analogously, for the variable C it is small, and given that its negative value is inverse, it has little explanatory power.

According to this analysis, the variable S is the most significant for explaining the hair's capacity to absorb metals.

Discussion

Mammal hair has been used for biomonitoring in different regions in the world in wild (D'Havé et al. 2006; Filistowicz et al. 2011) and domestic species (Nandi et al. 2005; Rashed and Soltan 2005; Patra et al. 2007). In both cases, metal elements like Pb and Cd have been detected in mammal hair in regions near mines (Pereira et al. 2004) and industrial waste (Patra et al. 2006). Rodents are considered the most useful species since they can indicate localized and recent pollution over time and reflect a risk for biota in general as they are consumed by many carnivorous species that can be poisoned with the toxic metals presents in their prey (Schleich et al. 2010; Hubbart 2012).

Mammal hair has many advantages for biomonitoring, including being easy to collect, transport and store, and it is a noninvasive matrix for toxic metal monitoring in wildlife. However, absorption of metals in hair can be affected by biological aspects like age or sex. In addition, it can be difficult differentiate between endogenous and exogenous deposition, which involve different structural elements for metal binding like sulphur in first case, and melanosomes in the second (Schramm 2008).

The chemical affinity between sulphur and metallic elements is well documented in the literature (Hinners et al. 1974; Combs et al. 1982; Cargnello et al. 1995; Aryal et al. 2006a; McLean et al. 2009; Patil et al. 2012).

Table 1 Percentage of each element in the samples treated with copper

	Copper	Sulphur	Carbon	Oxygen
General	0.52	1.51	81.13	16.81
G. soricina	0.83	1.46	78.07	19.62
A. jamaicensis	0.38	1.53	87.46	10.56
P. furvus	0.62	1.61	75.04	22.72
P. maniculatus	0.53	1.38	87.22	10.86
M. mexicana	0.23	1.60	77.87	20.28

However, the low correlation coefficients between sulphur and toxic metals in each treatment did not show the expected trend, despite the 2-month-long hair treatment. Noguchi et al. 2012 performed an experiment in which human hair was placed in a treatment of different metal solutions for 12 h by shaking in a reciprocating shaker. We considered that two months without shaking should be sufficient for the metal-hair reaction because of the well-documented hair-metal affinity and useful properties of hair for water treatment due to the said affinity (Patil et al. 2012).

This could suggest that the external deposition of toxic elements on hairs plays a minor role, though it should also be considered that chemical affinity is a multifactorial phenomenon, in which temperature, pH and particle size all play important roles in the absorption of metals in hair (Kar and Misra 2004; Monier et al. 2010). On the other hand, linear regression analyses can only establish whether there is a linear relationship between variables, just as its name indicates, and cannot determine whether variables which are not related can be described by other, non-linear, statistical models (Daniel and Cross 2010).

In addition, some of the works that report a strong affinity between keratin and metals have used hair that has been ground, or even subjected to digestion, considerably increasing the available reaction surface between hair molecules and metallic ions (Taddei et al. 2003; Monier et al. 2010; Patil et al. 2012). This has important implications for biomonitoring, since if whole hairs reduce the possibility of chemical bonding with toxic elements in the environment, the presence of considerable amounts of toxic metals found on strands could indicate greater internal deposition. As such, it is important to carry out experimental analyses to understand the absorption of metals on the external hair fibers as well as its reach into the inner fibers.

Internal deposition has been attributed to strong affinity between the pigment granules or melanosomes and metals, which are deposited on the inside of the hair while it is forming in the follicle and the metals which are in the bloodstream selectively bind to the melanosomes (Larsson 1993; Mars and Larsson 1999; Pereira et al. 2004; Bencko 2005; Hong and Simon 2007). Notwithstanding, it would be useful to analyze whether nanometer-sized particles are absorbed and migrate from the outside of the hair inward.

With respect to cuticle morphology, the images suggest that the scale type in each order can influence the hair's mechanical capacity to trap particle, similar to findings of pollen in the hair scales of bats (Howell

Table 2 Percentages of each element present in the samples treated with cadmium

	Cadmium	Sulphur	Carbon	Oxygen
General	1.40	1.62	75.48	21.43
G. soricina	3.35	2.26	73.48	20.90
A. jamaicensis	1.89	1.49	75.02	21.60
P. furvus	0.73	1.54	74.99	22.73
P. maniculatus	0.77	1.01	78.21	20.00
M. mexicana	0.51	1.81	75.72	21.95

Table 3 Percentages of each element present in samples treated with lead

	Lead	Sulphur	Carbon	Oxygen
General	0.72	1.57	85.20	12.48
G. soricina	0.39	1.22	79.82	18.49
A. jamaicensis	0.61	2.12	90.73	6.52
P. furvus	1.12	1.32	80.73	16.82
P. maniculatus	0.75	1.28	90.53	7.43
M. mexicana	0.72	1.93	84.19	13.14

and Hodgkin 1976). This makes the hair of bats a good option for biomonitoring (Hickey et al. 2001) and for the analysis of external deposition of contaminants under natural conditions. In addition, the capacity for coronal scales to trap particles can have important repercussion on the ingestion of toxic metals in some species of nectarivorous bats such as *G. soricina*, which have a well-known behavior of licking their fur (Howell and Hodgkin 1976). This could lead to increased ingestion of metallic contaminants in species exposed to a pollution source that ingest metals in food and water as well as particles lodged in their fur. The opposite would be true in the many species of mammals which have primarily imbricate scales on most of their fur, except for the first third of the strand, which generally presents coronal scales (Hausman 1920). Imbricate scales, which were observed in species like *P. furvus* and *M. mexicana*, are closed, and thus do not allow metal particles to be trapped on the strand as were observed in the two chiropteran species. This could limit the adhesion of metals to the hair and the ingestion particles when these species clean their fur.

The elemental analysis of the ultramicrotome sections did not show the expected concentrations of cadmium, which was practically absent from the cuticle, despite being treated with cadmium salts for 6 months. This could mean that the bond between the metals and the cuticle is based on weak electrostatic bonds that were dissolved when exposed to the resin used to embed the hair. With respect to the two small zones of the cortex where cadmium was detected, we must be cautious in our interpretation, since we do not have sufficient evidence that the hair was permeable enough to allow the metal to pass through the cuticle layer to the cortex. It is also possible that during the sectioning process, the blade of the ultramicrotome dragged metals to this region of the hair. Further analyses are necessary to rule out this possibility, since although the permeability of hair has been documented, this is often only after chemical treatment has modified the cuticle morphology so that particles can permeate the hair shaft (Tobin 2008). Noguchi et al. 2012 found with a synchrotron micro X-ray fluorescence analysis that absorption in hair treated with metal salts only occurs on the surface of hair, and metals cannot penetrate into the internal layers.

In general, our results suggest a weak functional relationship between sulphur and amount of metals absorbed, unlike carbon, which according to our analysis does not influence the absorption of metals in hair. This suggests that while sulphur has a strong affinity for metals, the hair's ability to trap particles depends on a variety of factors rather than a single variable, and that the sulphur which makes up the keratin in the hair has a weak electrostatic attraction to metals. As such, using hair from mammal species with imbricate scales in conjunction with a thorough washing protocol would eliminate exogenously deposited metals (Hawkins and Ragnarsdóttir 2009) and allow the determination of only the metals in the environment that have been deposited endogenously in the hair of native fauna; in other words, only metals which have been consumed and deposited in the internal layers of the hairs after travelling through the bloodstream and being incorporated into the hair from the follicle, and therefore represent a greater threat.

Electron microscopy is a powerful tool that allows the observation of particles deposited on the cuticle as well as analysis of elemental composition with great precision. However, it has been used mainly for morphological analysis of hair. Important research has been done with SEM on hair structure in different orders of mammals (Chernova 2002, 2003; Teerink 2003), but despite having great potential, to our knowledge EDS SEM has not been previously used for detection of toxic metals in hair, and this is the first study on the

application of SEM and EDS in hair absorption properties analysis. SEM has the advantage of being a non-destructive technique like inductively coupled plasma mass spectroscopy, so samples can be reexamined, and it allows the analysis of small sample areas (Goldstein et al. 1992). Though chemical analyses of hair are traditionally carried out using spectrometric techniques (Yamashita 1996; Onuwa et al. 2012), analysis using electron microscopy allows the observation of the micro and ultrastructure of the hair for detecting and measuring contaminant particles and the hair's permeability to them. More widely used analytical chemistry techniques, such as inductively coupled plasma mass spectroscopy, have detected toxic metals like Cd, Pb and Cu in hair samples of wild mammal species, even when present in a few ppm (Burger et al. 1994). Though in SEM there is a relatively higher detection limit of 1000 ppm (0.1%) and accuracy is very difficult to measure since it is the sum of all error factors, like operational, instrumental and specimen errors (Goldstein et al. 1992; Friel 1994), if electron microscope operations factors are selected and developed with care, SEM EDS can be used to obtain a high quality quantitative analysis.

Conclusion

Even though hair has been used as a useful material for biomonitoring, it can be difficult to differentiate between endogenous and exogenous deposition. The results of the morphological and elemental analyses made possible by scanning electron microscopy allow the observation, detection and quantification of particles adhered to the cuticle to determine whether there has been external deposition in the samples. This analysis shows that during exogenous deposition of Cd, Cu and Pb on hair, metal particles do not permeate the medulla, such that if metal particles are found adhered to the melanosomes or immersed in the medulla, it can be inferred that they came from ingestion and deposition in the hair from the follicle during the development of the hair.

Experimental studies offer a broader perspective on hair's absorption capacity and hair-metal affinities. Examples include the research developed by Tan et al. 1985 and Noguchi et al. 2012, who found high affinity with toxic metals like Pb, and low affinity with essential metals like Cu, after treating human hair with metal salts in solution. In addition, they emphasize the usefulness of hair for the removal of heavy metal pollutants from water, and the advantages of hair for assessment of human exposure to metallic pollutants, respectively. Nevertheless, it would be interesting to analyze the hair's permeability to particles smaller than those detected by the scanning microscope using finer techniques such has transmission electron microscopy, in order to clarify whether nanoparticles can pass though the cuticle to the internal layers.

Finally, a great diversity of mammals and ecological conditions, along with morphological differences in hair among species, makes this a good biomonitoring tool, which along with simpler analysis techniques offers a reliable, non-invasive, and easily managed alternative for the detection of toxic metals in the environment.

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Capítulo III: Mammal hair for biomonitoring of heavy metals by means of EDS-SEM

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Internal and external deposition of heavy metals in guard hair of five terrestrial mammals localized in Endhó watershed at Hidalgo, Mexico.

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Abstract

In this study we investigate the presence of toxic metals in hair of mammals from the Endhó watershed in the state of Hidalgo, Mexico, a region with severe environmental pollution. Sampling was carried out in localities around the watershed to collect guard hairs from mammals of the region in order to determine the internal and external deposition of toxic metals that have been found in water, soil and crops. The elements present in guard hairs and follicles were analyzed using energy dispersive x-ray spectroscopy (EDS) of scanning electron microscopy (SEM), a technique that has not been previously used for biomonitoring toxic metals. The bat species *Myotis californicus* and *Desmodus rotundus* and the rodent *Mus musculus* were the species with the greatest number of metals deposited in hair shafts and follicles. Follicles (internal deposition) showed less deposition of elements than external deposition in all species. Nickel and chromium were the most toxic elements found. Further studies are necessary to establish wildlife's exposure to toxic metals pollutants.

Key words: Biomonitoring, hair, heavy metals, metal pollution, scanning electron microscopy.

Introduction:

Toxic metal pollution is a serious environmental problem that humanity is currently facing. Toxic metals occur naturally, but their domestic, medical and technological applications have led to their release and spread in the environment (Callender, 2003; Reis *et al.* 2010). The lack of suitable management and efficient recycling measures to reduce the demand for toxic elements and keep them within closed cycles in the anthroposphere leads to progressive ecosystem deterioration through the damage they cause to organisms (Ayres, 1992; Abbasi *et al.*, 2015).

The drainage systems surrounding large cities frequently receive a variety of these pollutants through wastewater, causing serious environmental problems in the (often distant) regions where wastewater eventually ends up. The use of untreated wastewater from large cities for irrigation of arid zones is a common practice that leads to the accumulation of many toxic materials in crops and flora, thus entering the the food chain (Burger, 2007; Dickin *et al.* 2016; Hanjra *et al*. 2012). Consequently, animals that consume contaminated plants can accumulate essential and non- essential metal elements that vary in toxicity potential. Essential metals are those that are necessary, in trace amounts, for certain

biological functions, but become toxic at higher concentrations. Non-essential metals have no biological function and are toxic even at low concentrations (Tchounwou *et al.*, 1012; Gall *et al.*, 2015). Accumulation of either type of metal can be detected by analyzing internal organs or other tissues or substances, such as blood or urine. The use of organs or tissues to evaluate individuals' exposure to pollutants is defined as biomonitoring; this is a fundamental tool for assessing, identifying, and eliminating exposure to toxic metals (Preston, 1975; Needham, 2008; Zhou *et al*., 2008).

In addition to the use of organs, epidermal appendages like mammal hair are reliable bioindicators for assessing exposure to toxic metals. The high metal affinity to hair is well known. Metals bind to the cuticle scales of hairs when the metals are located outside the body (external deposition), and/or the internal layers of hair when metals consumed by an organism travel through the blood stream and are deposited in follicles during hair growth (internal deposition) (Flache *et al.*, 2018). Hair is therefore a useful biomonitoring tool for detecting exposure to toxic metals in regions with high levels of pollutants, like the Mezquital Valley, Hidalgo, Mexico. This region has been seriously affected by pollution due to industrial activities and the use of the Endhó watershed as an endpoint for wastewater from Mexico City (Siemens *et al.* 2008; Guedrón *et al.* 2014; Montelongo-Reyes *et al.* 2015). This watershed is located within the Mezquital Valley, a semiarid region with a large amount of agricultural activity facilitated by irrigation using wastewater from Mexico City which is stored in the Endhó dam reservoir. However, the use of this unthreatened wastewater has caused the accumulation of large amounts of pollutants that have affected human and ecosystem health. Among the many pollutants contained in this water are toxic heavy metals like cadmium, lead and mercury (Lüneberg *et al.*, 2018). For this reason, the objective of this study was to determine which heavy metals are deposited externally (on hair cuticles) and internally (in hair follicles) of mammals inhabiting the area around the Endhó watershed to characterize their exposure to heavy metals. The analyses were carried out using an energy dispersive x-ray spectroscope attached to a Scanning Electron Microscope, a novel technique in biomonitoring that provides useful information that is not available using classic analytic techniques.

Methods

Hair samples

Sampling was performed in the Mezquital Valley, Hidalgo, Mexico around the Endhó watershed (figure 1), which is surrounded by agricultural land and a very few highly conserved areas. The captured species were *Mus musculus*, *Peromyscus difficilis* and *Heteromys irroratus* of Order Rodentia, and *Myotis californicus* and *Desmodus rotundus* of the Order *Chiroptera.* We sampled dorsal guard hairs from each individual. Dorsal guard hair samples were plucked from the root and were placed

in polypropylene bags and maintained at room temperature. Species and sex were visually determined and the capture location was recorded with GPS.

The individuals captured were deposited in the mammal collection of "Alfonso L. Herrera" Zoological Museum of the Facultad de Ciencias, UNAM, except *Mus musculus.* As a negative control, hair samples were taken from a specimen of *Heteromys pictus* from the region of Chamela, Jalisco, Mexico where there is no known heavy metal exposure, from the same mammal collection. as in a previous study (Rendón-Lugo *et al.* 2017), it was confirmed that the elemental composition of these hairs consisted only of carbon, nitrogen, oxygen and sulfur, the elements that conform hair shafts and follicles.

Scanning electron microscopy (SEM)

Sample preparation

Guard hair samples from ten individuals (five rodents and five bats) were chosen for SEM analysis. The vampire bat is particularly prone to toxic metal exposition because it feeds on livestock blood (Becker *et al.* 2017), so four of the five bat specimens were from this species. The selected samples correspond to one individual of *Heteromys irroratus*, one of *Myotis californicus*, two individuals of *Peromyscus difficilis*, two of *Mus musculus* and four individuals of *Desmodus rotundus.*

Hair samples were mounted in carbon stubs and adhered with conductive carbon paint without any prior treatment or washing protocol in order to observe the externally deposited particles. No coating was used to avoid masking the metallic elements of the sample during EDS microanalysis. Hair follicle samples were prepared in the same way as hair shafts, but were examined first under an optical microscope to ensure that the entire follicle was present.

Imaging

Samples were observed in a scanning electron microscope JEM7600F equipped with an Oxford energy dispersive spectroscope to classify cuticle types and analyze the presence of metal particles adhered to the cuticle and perform the elemental chemical analysis via EDS. Cuticle morphology was determined according to Hausman (1920). The hair region considered in all samples was the first middle, or proximal region, from the root to the shield, which is the widest part of the hair shaft located in the middle region and has a typical scale morphological pattern.

Energy dispersive X-ray spectroscopy (EDS)

Energy dispersive x-ray spectroscopy (EDS) is a semiquantitative analysis that determines the elemental composition of a sample based on characteristic peaks in the EDS spectrum. By this technique in not possible to determinate the stoichiometry of the compounds presents in the sample. EDS analysis was performed with an Oxford detector (Inca model), attached to the scanning electron microscope in

order to determine which metallic elements were deposited on the hair cuticle. The analyses were carried out on the cuticle of five dorsal guard hair of 10 individuals of the five species captured at the study site to determine the presence of metallic elements. The whole hair shaft was examined, but with particular emphasis on regions where the SEM revealed particles adhered to the hair shaft. In order to characterize elements that were internally deposited, EDS was carried out on follicles from each of the five species in both longitudinal and cross-sectional orientations.

Results

The SEM images of the guard hair samples from all ten individuals show species-typical cuticle morphology and particles adhered to the hair shaft. The cuticle morphology differs between bat and rodent species. Bat hairs have coronal scales, which traps particles under the scales, as can be seen at low relatively magnification (Figure 2). Clearly, the contrast differences in the SEM micrograph indicates the presence of heavier metal particles adhered mainly to the coronal scales zones. Conversely, rodent hairs have imbricate scales which are closer together and therefore do not trap as many particles. Even so, it is possible to observe, small bright particles on smooth zones of the hair (Figure 3). As well, SEM images of follicle samples of five species were obtained. In figures 4 and 5 it can be seen follicle micrographs of *M. musculus and M. rotundus* respectively.

Hair Shaft Energy dispersive X-ray spectroscopy (EDS) analysis (external deposition)

The EDS X-ray analysis of control specimens showed, as expected, only carbon (C), oxygen (O), nitrogen (N) and sulphur (S) which are the natural constituent elements of hair fibers (Hinners *et al*. 1974; Combs, 1987; Harkey, 1993; Cargnello *et al. 1995;* Aryal *et al. 2006[a], 2006[b];* McLean *et al*. 2009; Noguchi *et al.* 2012). These elements where detected in all hair samples in each analysis carried out of all species.

Moreover, all ten specimens from the contaminated study site showed the presence of metal elements (Table 1). Among the metallic elements detected, iron (Fe), copper (Cu), chromium (Cr) and gold (Au) are heavy metals (i.e. density greater than 5 gcm^{-3}; Jaishankar *et al.* 2014). The most common metallic elements found were Ca and Fe, which was present in all specimens. Non-metal elements (P, Si and Cl) were found in hair samples of some species. Compounds such as organochlorides are present in pesticides meanwhile, Si and P compounds are present in most of soil., On the other hand, transition metals like Mn is present in *M. Muscullus* and Ti was found in *P. Difficilis* samples, as it is possible to observe in table 1

The elements detected by EDS in each specimen are shown as an x-ray spectrum (Figure 6 shows the spectrum obtained in a sample (that) of *Myotis californicus*), in which each peak corresponds to one element detected on the hair shaft. The rodent *H. irroratus* had the fewest elements deposited in hair cuticule, while *Mus musculus* shows a greater amount of heavy elements detected by EDS, including chromium. The bats *M. californicus* and *D. rotundus* show additional elements, including toxic metals like chromium.

Follicle EDS analysis by lineal mapping (internal deposition)

The control follicle has the same non-metal elements that were found in follicle samples as well as the metals Na, Al, K, Fe, which were found in follicles of the analyzed species. On the other hand, fewer elements were found in follicles than on hair shafts (table 2).

Elements that were frequently found on the hair shafts (Mg and Mn) were not found in any follicle from those pecimens, while (and) Si was found only in follicles from the control sample. Calcium, another element found in all the hair shafts, was detected in follicles only in *P. difficilis* and *M. musculus.*

M. californicus, *D. rotundus* and *M. musculus*, were the three species with the greatest number of elements detected in hair shafts which also had the highest number of elements in follicle samples. Further, nickel was detected in these follicles' species, but was not detected in any other follicle.

A lineal mapping analyses of follicle cross-sections (figure 7) yielded nearly identical results to longitudinal analyses (figure 8), except that some heavy metals like Cr, Ni and Zr, were detected only in one of the two EDS lines. A higher magnification of the lineal concentration plot obtained by EDS is shown in figure 8b. The plot shows the presence of a large amount of carbon. The collected x-rays characteristic of carbon increases in the area of the sample holder, behavior that is consistent because a carbon tape was used to stick the follicle. A great amount of sulfur and oxygen is also present in the follicle but there are heavier elements such Cl, Na and even Ni, Al and K.

The EDS mapping where it shows the plot concentration of each element separately through a line over the sample is observed in figure 9. In this case, a magnified pattern of elemental concentration throughout the cross-section follicle can be observed, for the particular case of *M. musculus*. The cross-section and longitudinal lineal mapping analyses throughout of follicle show a homogeneous concentration distribution pattern of K, but Al and Ni show an inhomogeneous distribution, as it is possible to observe in the individual plots. The other elements present concentration peaks in different parts of the follicle. In the longitudinal analysis (along the follicule axis), the same metals, Al, K and Ni were detected. In *D. rotundus* spectra (figure 10), Cu and K are distributed homogeneously throughout the follicle, but Al and Ni shows two peaks of concentration. The same pattern is repeated in lengthwise analysis in Cu, Ni and Al. The elements with peaks of concentration are the natural constituents of keratin, that is to say, C, S

and O. The pattern of homogeneous elemental concentration in follicles can be observed in general in Fe, K, Al, Na in all samples, as well as heterogeneous concentration pattern of organic elements. Only heavy metals like Zr in *P. difficilis* and Ni in *M. californicus* present peaks of concentration. It should be noted that in control samples, all the elements are distributed very heterogeneously in the follicle.

Discussion

The use of untreated wastewater for agricultural irrigation in the Mezquital Valley of Hidalgo over the past 100 years has led to serious environmental deterioration, making this one of the most polluted regions of Mexico (Ortega-Larrocea *et al.* 2001; Montelongo-Reyes *et al.* 2015; Contreras *et al.* 2017). Towns surrounding the Endhó watershed have been polluted with a variety of pharmaceutical (Siemens *et al.* 2008*)*, chemical (Mireles *et al.* 2004; Guédron *et al.* 2014) and biological pollutants (Chávez *et al.* 2011; Contreras *et al.* 2017). This situation has threatened human health; this has attracted the attention of the media, who have reported on the seriousness of the pollution by interviewing people that inhabit the towns surrounding the Endhó dam reservoir. These anecdotal reports of pollution have been supported by scientific research in water quality and biological studies, which have shown the presence of many toxic metals at concentrations above the legal limits (Casanova *et al*. 2007; Montelongo-Reyes *et al.* 2015). In this sense, hair analysis provides a reliable and non-invasive tool for assessing heavy metal exposure in both wildlife and human populations. Mammal hair is easy to collect, handle, transport and analyze, and provides information about long term exposition to heavy metals (Schramm, 2008; Arain *et al.* 2015). On the other hand, EDS element detection using SEM is a powerful but little used instrument for detecting heavy elements in biomonitoring and histopathological research (Scimeca *et al.* 2018). Hair analysis with SEM has focused mainly on morphology (Chernova, 2002; Teerink, 2003).

The observations of mammal hair using SEM have shown the capacity of chiropteran hair scales to trap particles like pollen (Howell and Hodgkin, 1976). On the other hand, imbricate scales lay flatter against the shaft (Hausman, 1920; Short, 1978; Chernova, 2002), without leaving space for large particles to get trapped. In this study, this was evidenced by a larger number of particles trapped in hairs with coronal scales (bats), making bat hair a particularly useful sampling tool for detecting the external deposition of heavy metals in the environment. However, numerous particles were found throughout the hair shaft, not only wedged between the edges of scales. This is likely due to adhesion of the metal particles to the large amount of sulphur in the cuticle, whose chemical affinity for metals is well documented (Hinners *et al.* 1974; McLean *et al.* 2009), and has allowed a successful assessment of wildlife exposure to toxic metals (Mehra and Thakur, 2016).

EDS microanalysis detected the elements which constitute the molecular structure of hair fiber itself (S, C, O and N) in all samples. While not particularly interesting, the consistency of this result is evidence of the reliability of EDS analysis.

Elemental composition analysis using EDS-SEM detectors is much less frequently used than other techniques for biomonitoring. Detection of metallic elements is most often analyzed using spectrometric analytical techniques like Inductively coupled plasma mass spectrometry (Goullé *et al.* 2005; Mikulewicz et al. 2013; Pozebon *et al.* 2017). These analytical techniques have allowed the detection of metallic and semi-metallic elements in hair of mammals including *Rattus rattus, Mus spretus* (Pereira *et al.* 2006), *Ctenomys talarum* (Schleich, *et al.* 2010), *Spermophilus beecheyi* (Hubbart, 2012), *Vulpes vulpes* (Filistowicz *et al.* 2011), *Didelphis virginiana* (Burger *et al.* 1994), *Erinaceus europaeus* (D'Havé *et al.* 2006, 2007; Vermeulen, 2009), *Alces alces gigas* (Flynn *et al.* 1975), *Felis pardalis* (Mora *et al.* 2000), *Rangifer tarandus,* (Duffy *et al.* 2005), *Rangifer tarandus* (Pacyna *et al.* 2018) , *Lynx rufus* (Cumbie, 1975), *Procyon lotor* (Cumbie, 1975; Clark *et al.* 1989), *Sus scrofa* (Sobańska, 2005), *Myotis lucifugus, M. leibii, M. septentrionalis* and *Eptesicus fuscus* (Hickey *et al.* 2001).

Despite the precision and accuracy of this technique, EDS-SEM provides some important advantages for biomonitoring and pollution research. EDS-SEM is a non-destructive technique that allows repeated analysis of the same sample to detect the exact location on the sample where an element was found, and it is very reliable for elemental identification in rough samples (Miler and Mirtic, 2013). In addition, during SEM observations, heavier elements look brighter and have higher contrast than the natural hair elements (Goldstein *et al.* 1992). This allows a quick visual screening of particles that may contain toxic metallic elements, which can then be specifically examined with the EDS detector to determine the elemental composition of this observed particles. This was the case of chromium in *M. californicus*, *M. musculus* and *D. rotundus*, present in particles adhered to the scales. EDS spectrum also shows a concentration pattern of an element detected in the EDS graphs, such as it can be observed in EDS spectrums of follicles, whose lines representing an element, shows peaks in specific spots where the elemental concentration is higher.

In general, all the species presented the same elements, except *H. irroratus*, with only a few elements present in the cuticle. A species' molt pattern has been suggested to influence metal accumulation in hair (Wenzel *et al.* 1993; Zarrintab and Mirzaei, 2017).). This species generally presents two molts per year in the months of february; march, june, august and september (Dowler and Genoways, 1978). Since we sampled in X, the hairs we sampled were likely newly grown, such that hairs had not had time to accumulate the metals detected in the other species.

More elements were deposited externally than internally in all individuals. This was expected because the incorporation of metallic elements into the follicle depends on many regulatory processes in the entire

body of an organism, while the cuticle is exposed to all the elements present in the environment (Hinners *et al.* 1974). External deposition and binding of metals to the cuticle is principally due to the sulfur content of hair keratin, which has a strong chemical affinity for metals. However, not all elements found on the external surface of the hair are necessarily of exogenous origin. Combs (1987) mentions that externally deposited minerals can have an endogenous origin due to the contact of the hair shaft to the sebaceous and sweat glands, which have some elements like Ca, P, Mn and Zn. All these elements except Zn were found on hair shafts and may have been of endogenous origin.

Nickel and chromium were the metals with the greatest toxicological potential found in this work. Hair has been found to be a good biomarker of chromium in rodents (Pereira *et al*. 2006), while nickel is rarely found in mammal hair (Filistowicz *et al.* 2011). Though these metals have been considered essential elements, their biological demand is limited to a very low concentration before they become toxic (Tchounwou *et al.* 2012). Nickel is a heavy metal that has been found in water and sediment of the Endhó watershed (Vázquez-Alarcón *et al.* 2001), and in the case of bats, *D. rotundus* (Greeenhall *et al.* 1971) and *M. californicus* (Krutzsch, 1954) drink water directly, which can expose them to nickel accumulated in the watershed and wastewater.

Chromium particles were found in the cuticle scales of the bat species and in *Mus musculus,* but only in the follicle of *Myotis californicus*. Nevertheless, it cannot rule out possibility of the presence of this metal in minor concentrations in other follicles, since it was found in several particles deposited in cuticle of these three species.

The rodent species with the greatest number of elements detected with EDS was, as expected, *Mus musculus,* due to the omnivorous diet and adaptation to disturbed habitats (Phifer-Rixey and Nachman, 2015). These biological characteristics can expose some rodents to the ingestion of a large number of pollutants and make them good candidates for biomonitoring (Khazaee *et al.* 2016). In addition, the characteristic home range of *Mus musculus* allows the characterization of pollutants that is highly localized in time and space (León *et al.* 2007). Finally, the cohabitation of this species with humans in highly anthropized environments (Mikesic and Drickamer, 1992) makes hair sampling in this species a good candidate for biomonitoring of pollutants in contact with human populations.

Follicle samples:

Hair follicles are immersed below the skin in the epidermal epithelium and in contact with the bloodstream through a network of blood vessels that transfers chemical elements to the growing hair (Harkey, 1993). For this reason, elements present in the follicle are deposited endogenously, from food

that may contain toxic heavy metals. The same three species had the highest number of metals both on the cuticle and in the follicle. As in external deposition, the habit of drinking water can expose the bats *M. californicus* and *D. rotundus* directly to the heavy metals of the watershed and wastewater (Greenhall *et al.* 1971; Krutzsch, 1954), making these species more vulnerable to pollution, which may explain the presence of nickel in both bat species and chromium in *M. californicus* follicles.

Nickel was found in follicles of three species in trace amounts, and even though the detection limit in SEM is about 0.1%, that is to say, 1000 ppm (Goldstein et al. 1992; Friel, 1994), this heavy metal cannot be discarded because is present in three samples of individuals that inhabit the same polluted region and has been found in water and crops analysis of Endhó dam (Vázquez-Alarcón *et al.* 2001). Though nickel is considered an essential metal, its biological functions are not well understood (Schroeder *et al.,* 1961; Cempel and Nikel, 2006). Despite is not present in *P. difficilis* and *H. irroratus*, cannot be discarded because this element has been found in water analysis of Endhó dam. Vázquez-Alarcón *et al.* (2001), found nickel in crops and water of Endhó dam used to irrigate crops and mentioned the necessity of assessments in order to reduce the exposition of nickel and other toxic metals like cadmium and lead. These two metals have been found in other studies in Tula river and Endhó dam.

The fact that more metals were detected in *D. rotundus* and *M. californicus* may also be influenced by their diet which is relatively high in the food chain and thus more vulnerable to biomagnification. *D. rotundus* is a hematophagous species (Wimsatt, 1969) that feeds on blood of the cattle present in the region which consume large quantities of grass exposed to the polluted water of Tula river and Endhó watershed, and *M. californicus* is insectivorous, consuming large quantities of insects (Krutzsch, 1954) which frequently bioaccumulate toxic metals (Ravera, 2001; Cheruiyot *et al.*, 2013). Both bath species may be involved in biotransfer processes of toxic metals through food chain, that begins with the bioaccumulation of metals in plants and their transfer to primary consumers (Zhuang *et al*. 2010; Gall *et al*. 2015). This can produce biomagnification of toxic metals in higher trophic levels, making higher-level consumers more exposed to the toxic effects of some metals (Jæger *et al.*, 2009; Cheruiyot *et al.*, 2013).

Conclusion

The relative ease of collecting, storing, and analyzing mammal hair are advantageous qualities for biomonitoring of heavy metals in polluted regions. Nevertheless, further histopathological studies are needed to understand the effects of exposure to the pollutants presents in the Endhó watershed on the wildlife that inhabit one of the most polluted regions in Mexico.

The comparison between elements in follicles and cuticle makes it possible to distinguish which elements are deposited only externally and which are actually absorbed by an organism that is exposed to high

levels of pollution. The fact that fewer heavy metals were detected in the hair follicle that externally demonstrates that not all the heavy metals to which the mammal hair is exposed are deposited internally, and electron microscopy techniques allow us to observe these differences. The biological differences between species seems to be of great importance in the exposure to environmental pollutants, like intake of certain food and drinking water, and for that reason species selection is crucial for providing environmental information on wildlife exposure to pollutants like heavy metals.

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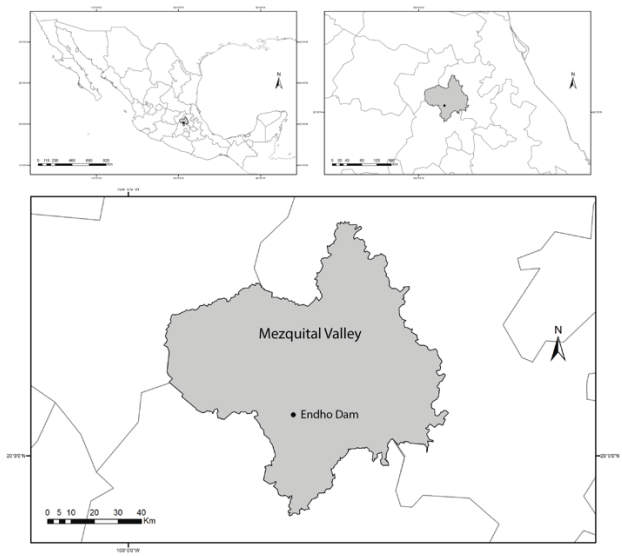
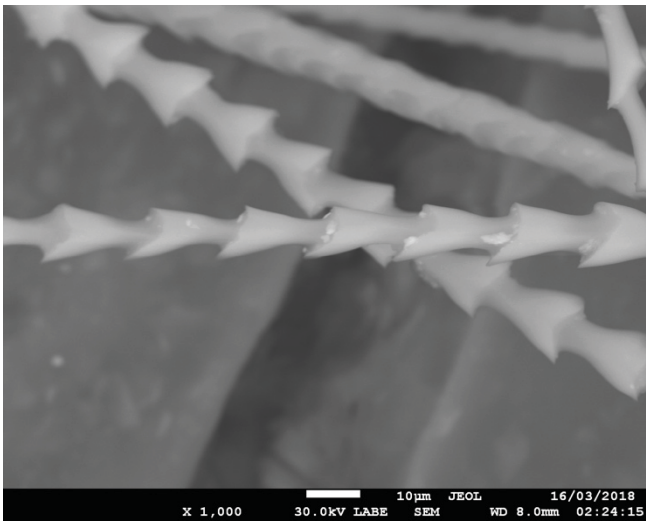
	Nonmetals			**Transition Metals**		**Metals**								
	P	Si	Cl	Mn	Ti	Na	Al	K	Ca	Mg	**Fe**	**Cu**	**Cr**	**Au**
P. difficilis	x		x		x	x	x	x	x		x			
H. irroratus			x					x	x		x			
M. californicus	x	x	x			x	x	x	x	x	x		x	
M. musculus	x	x		x		x	x		x	x	x		x	
D. rotundus	x	x	x		x	x	x	x	x	x	x	x	x	x

Table 1. Chemical elements found in each species (heavy metals are in bold).

	Non-metals			**Metals**								
	P	Si	Cl	Na	Al	K	Ca	**Fe**	**Cu**	**Cr**	**Ni**	**Zr**
Control sample	X	X	X	X	X	X		X				
P. difficilis	X		X			X	X					X
H. irroratus	X		X	X	X	X						
M. californicus	X		X	X	X			X		X	X	
M. musculus	X		X	X	X	X	X		X		X	
D. rotundus	X		X	X	X	X			X		X	

Table 2. Chemical elements found in each follicle species (Heavy metals in bold).


Mezquital Valley
Endho Dam
N
0 5 10 20 30 40
Km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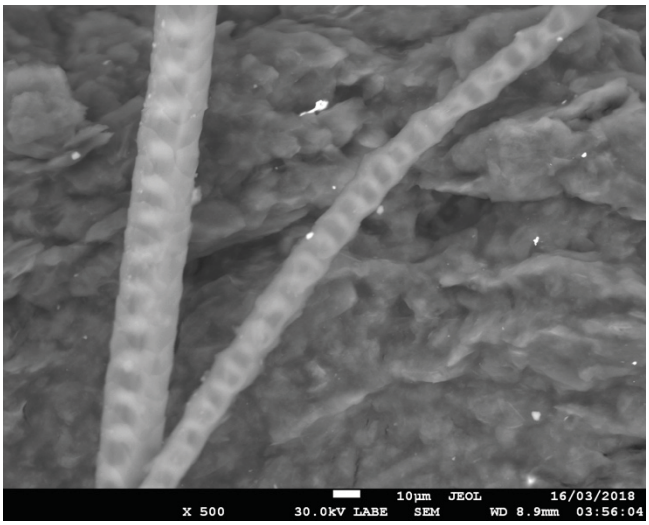
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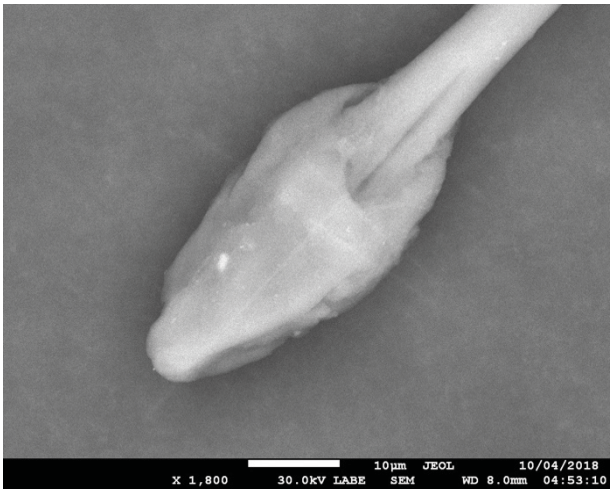
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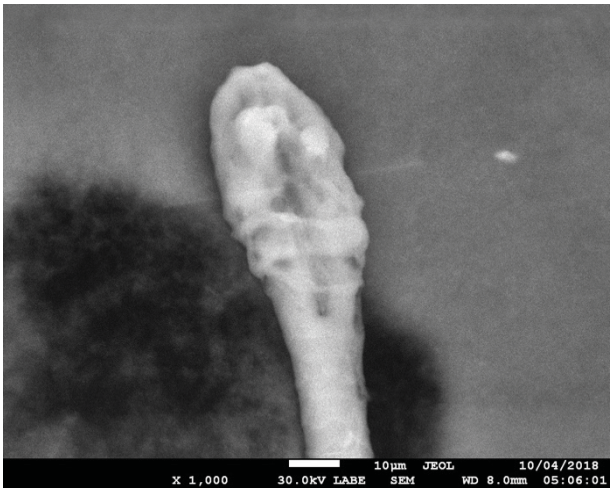
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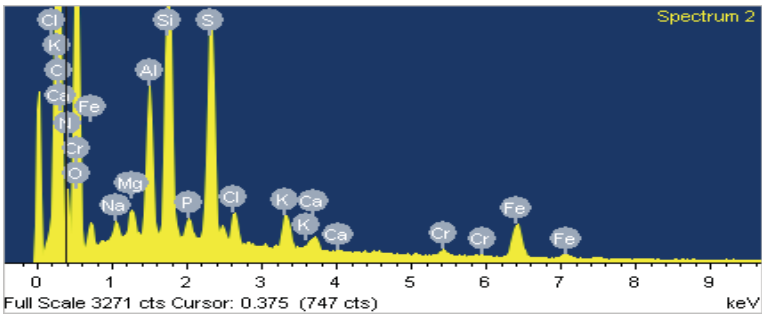
Spectrum 2
Cl
K
Ca
Fe
Ti
Sr
O
Mg
Na
Al
Si
P
S
Cl
K
Ca
K
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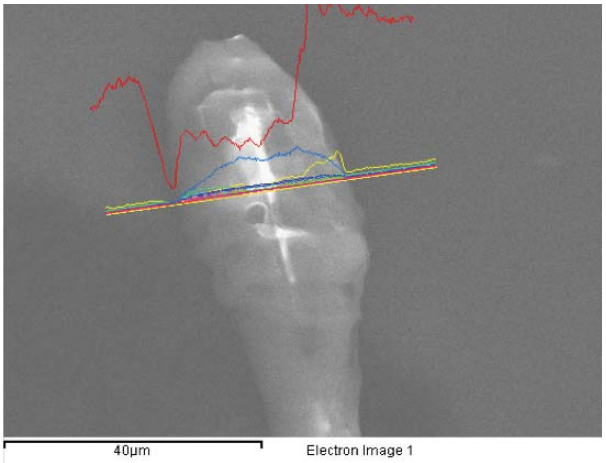
Cr
Cr
Fe
Fe
0 1 2 3 4 5 6 7 8 9
Full Scale 3271 cts Cursor: 0.375 (747 cts)
keV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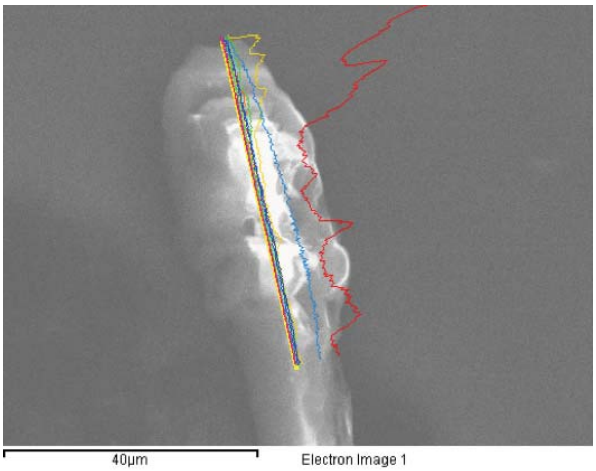
Electron Image 1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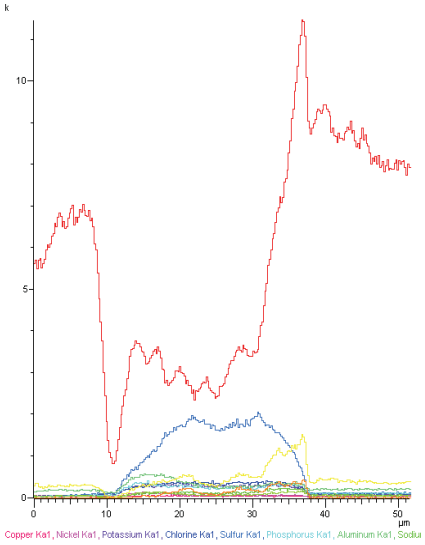
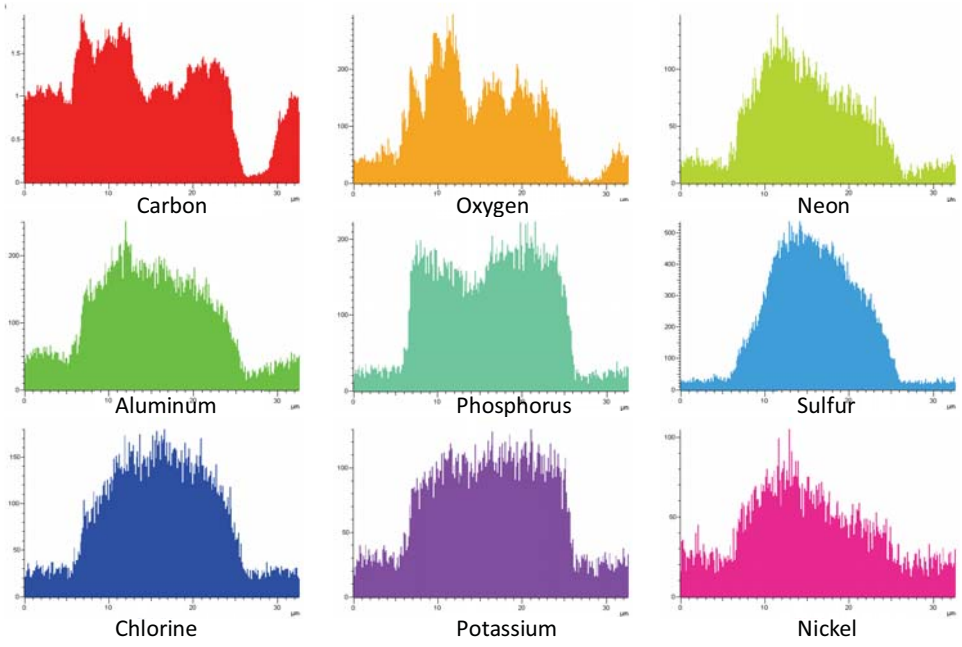
Electron Image 1


k
10
5
0
0
10
20
30
40
50
µm
Copper Ka1, Nickel Ka1, Potassium Ka1, Chlorine Ka1, Sulfur Ka1, Phosphorus Ka1, Aluminum Ka1, Sodiu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Oxygen
Neon
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Phosphorus
Sulfur
Chlorine
Potassium
Nickel

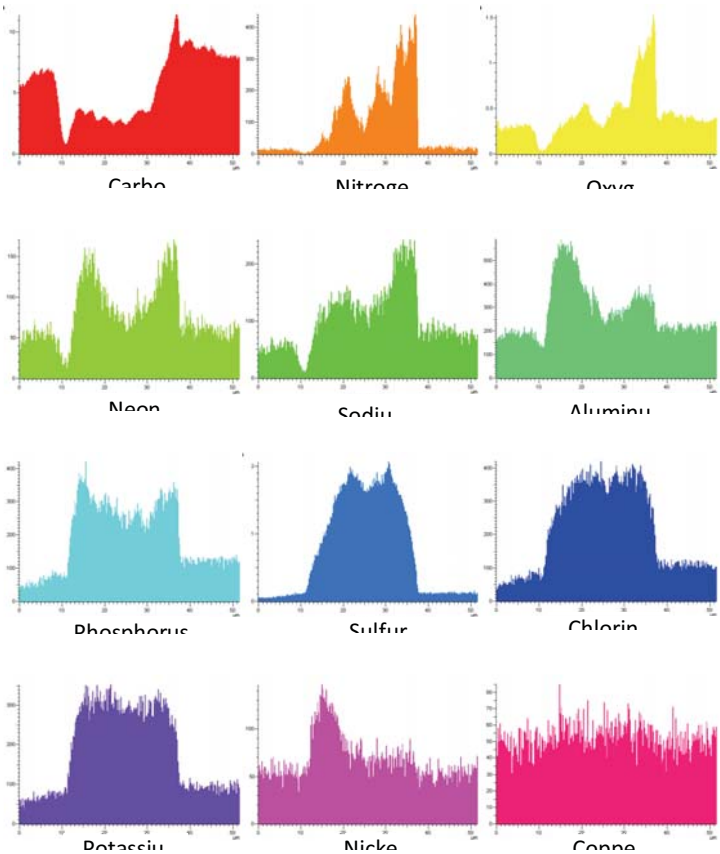

Figure 1. Map of the study area located in Hidalgo, Mexico.
Figure 2. *M. californicus* guard hair.
Figure 3. *P. difficilis* guard hair.
Figure 4. *M. musculus* follicle.
Figure 5. *D. rotundus* follicle.
Figure 6. EDS spectrum of *M. californicus.*
Figure 7. Cross-sectional energy dispersive x-ray spectroscopy analysis of a *D. rotundus* follicle (colored lines represent each element found).
Figure 8. Longitudinal energy dispersive x-ray spectroscopy analysis of a *D. rotundus* follicle.
Figure 8b. Magnified energy dispersive x-ray spectroscopy analysis of a *D. rotundus* follicle.
Figure 9. Energy dispersive x-ray spectroscopy spectra of each metal found in the cross-sectional analysis of a *M. musculus* follicle.
Figure 10. Energy dispersive x-ray spectroscopy spectra of each metal found in the cross-sectional analysis of a *D. rotundus* follicle.

Discusión general

La contaminación es uno de los problemas más graves de deterioro ambiental que enfrenta actualmente la humanidad, causante de severos daños ecológicos que comprometen la salud y la seguridad alimentaria. Entre la gran cantidad de contaminantes liberados y dispersados por las actividades humanas, los metales tóxicos constituyen uno de los contaminantes de mayor preocupación tanto por sus efectos en los organismos como por su persistencia en el ambiente. Ante esta problemática, el biomonitoreo ambiental es una herramienta fundamental para la detección y la evaluación de la contaminación ambiental (Preston 1975; Martin, 2012; Siddig *et al*. 2016). Se distingue del monitoreo tradicional con agua o aire porque usa un organismo o una parte de él para la evaluación, medición y control de los contaminantes en el ambiente, permitiendo así la obtención de una mayor cantidad de información acerca de los posibles efectos ecológicos derivados de las interacciones entre las especies expuestas y los demás organismos tanto en ecosistemas terrestres como acuáticos (Zhou *et al.* 2008; Tellez y Merchant, 2015). Además, el uso de ciertos tejidos biológicos de fácil obtención y no invasivos como el pelo, hacen mucho más eficiente la detección de contaminantes en el ambiente, sus efectos y distribución.

El pelo es una estructura cuya composición molecular presenta una gran afinidad hacia los elementos metálicos y metaloides, incluyendo aquellos que se consideran esenciales para determinadas funciones biológicas y los elementos no esenciales y de mayor importancia toxicológica como Pb, Cd y Hg (Hinners *et al*. 1974; Combs *et al*. 1982; Cargnello *et al.* 1995; McLean *et al*. 2009; Noguchi *et al.* 2012). Además, su fácil obtención y manejo han incrementado su uso como herramienta en análisis ambientales, médicos y de riesgo ocupacional (Combs, *et al.,* 1982; Limic y Valkovic, 1987; Caroli et al. 1992; Tobin, 2005; Schramm, 2008), tal como se muestra en el Capítulo I, donde se hace una revisión del papel del pelo en el biomonitoreo de metales tóxicos en seres humanos, mamíferos silvestres y domésticos. La diversidad de especies en las que se han encontrado metales esenciales y no esenciales pone de manifiesto la gran vulnerabilidad de la fauna silvestre ante contaminación por metales. Mamíferos que habitan diferentes ecosistemas, desde hábitats acuáticos hasta terrestres y que ocupan distintas posiciones dentro de las redes tróficas han sido expuestos a compuestos metálicos que pueden bioacumularse en el pelo y varios órganos como hígado y riñón. Esto muestra la urgencia de medidas de control que disminuyan la liberación y dispersión de contaminantes metálicos que están ampliamente distribuidos en todo el mundo. Las zonas más afectadas son aquellas que están junto a lugares donde se llevan a cabo actividades económicas como la minería y cercanas a zonas industriales. Sin embargo, la alta demanda de metales para uso doméstico, médico, etc. junto al mal manejo de residuos para la reutilización de materiales incrementan enormemente la contaminación (Ayres, 1992). En el caso de la afectación a la salud humana,

la exposición ambiental en humanos no es la única vía de entrada de metales, sino que se incluye el riesgo ocupacional que vulnera a los trabajadores de la industria y la minería (Afridi *et al.* 2006; Gil *et al.* 2011) y la ingesta de metales a través de los productos de origen animal como carne y leche, cuya contaminación proviene de los suelos y que se transfiere al alimento de los animales de granja (Combs, 1987; Milhaud y Mehennaoui, 1988; Nandi, *et al.* 2005; Rashed y Soltan, 2005; Patra *et al.* 2006).

La revisión del Capítulo I permite entender la gravedad de los metales tóxicos dentro de la enorme problemática de la contaminación ambiental, así como todos los avances en el estudio del pelo que derivaron en su utilización como herramienta para biomonitoreo.

Sin embargo, se siguen mencionando las mismas desventajas en el uso de esta matriz biológica, a pesar de que cada vez se hacen más comunes los análisis dentro de las áreas ambientales, Una de estas desventajas es la dificultad para diferenciar el depósito externo e interno. Los protocolos propuestos de lavado intentan limpiar todas las impurezas externas para así poder determinar mediante técnicas de química analítica, qué elementos fueron depositados en las fibras capilares de manera endógena, es decir, a través de la ingesta de agua o alimento contaminados con metales tóxicos. No obstante, algunos autores han observado que ciertos métodos de lavado además de limpiar la cutícula, se llevan también partículas de origen endógeno y que se encontraban depositadas en las capas más internas del pelo. Por otra parte, el depósito externo puede verse afectada por la presencia de metales en las glándulas sudoríparas y las glándulas sebáceas, las cuales pueden depositar elementos metálicos en la superficie del pelo cuando este se encuentra en crecimiento, por lo que no todos los elementos presentes en la cutícula son de origen exógeno (Combs, 1987). Ambos fenómenos, en los que puede confundirse los dos tipos de depósito de metales en el pelo (endógena y exógena) tienen implicaciones importantes en la interpretación de los resultados.

La parte experimental del proyecto (Capítulo II) permitió conocer la permeabilidad de elementos metálicos en solución hacia las capas que constituyen el pelo con el fin de conocer mejor el papel del depósito externo de metales y sus implicaciones en el biomonitoreo.

Aún cuando el pelo se reconoce como una fibra porosa, los poros corresponden en realidad a la separación existente entre las escamas de la cutícula que determinan el paso de partículas hacia las capas internas. Algunos factores como el pH, humedad del aire y altas temperaturas pueden modificar esta separación, volviendo al pelo más poroso (Robles-Velasco et al. 2009). Sin embargo, se carece de información suficiente sobre la permeabilidad de metales tóxicos de origen exógeno en el pelo de especies silvestres sometidas a estas condiciones ambientales. La información que se ha generado sobre los factores químicos y físicos que modifican la porosidad del pelo es en su mayoría sobre pelo humano debido al interés cosmetológico para probar sustancias que pueden abrir las escamas y así permitir la entrada de partículas que modifican el color. Sin embargo, podría ser complicado que en la naturaleza se dieran

condiciones de estrés químico o físico en el pelo de un mamífero para que las escamas puedan abrirse al grado de permitir el paso de partículas hasta la corteza y médula en suficiente cantidad para ser detectadas por las técnicas analíticas comúnmente usadas. En Rendón-Lugo *et al*. (2017) después de someter los pelos a tratamientos de sales metálicas en solución de plomo, cadmio y cobre durante seis meses, no se encontraron partículas de ningún metal al interior del pelo. Esto sugiere que existe una baja permeabilidad de partículas metálicas detectables por microscopía electrónica de barrido (SEM, por sus siglas en inglés) cuando no se usan agentes químicos que incrementen la porosidad, incluso en especies con escamas coronales a lo largo de toda la hebra del pelo como en el caso de los murciélagos. Sin embargo, la presencia de partículas de escalas nanométricas no podría ser descartada y tendría que ser detectadas mediante otras técnicas microscópicas.

La falta de valores de referencia de concentraciones en el pelo que permitan determinar con claridad en qué casos los organismos están expuestos a la contaminación ambiental es otra de las desventajas del uso del pelo en el biomonitoreo. Algunos autores mencionan la utilidad de los análisis histológicos para reconocer daños derivados de la presencia de metales en diferentes órganos. No obstante, esto se contrapone con la ventaja del pelo de ser poco invasivo, por lo que deberían de analizarse las propuestas de valores de referencia que ya han hecho algunos autores (Caroli *et al*. 1992; Goullé *et al. 2005*; Liang *et al*. 2017).

La microscopía electrónica como técnica complementaria para la evaluación de contaminantes en el ambiente.

La microscopía electrónica de barrido es una herramienta que brinda información muy importante sobre la localización exacta de los metales encontrados tanto en la superficie del pelo como en el folículo. Esta información complementa a los datos de concentración tan exactos obtenidos mediante las técnicas de química analítica más usadas para la detección de elementos traza de mayor interés toxicológico como Pb, Cd, Hg y As, con las cuales se pueden detectar partes por millón de estos elementos pero no se puede saber exactamente en que región del pelo se depositaron. La información visual que provee la microscopía electrónica de barrido resulta muy relevante para conocer qué estructuras están involucradas en la unión química de metales y cuáles son importantes para la excreción de estos elementos a través del pelo, específicamente en el folículo, considerado un mini órgano en el que ocurren muchos de los procesos biológicos de los organismos y que está en contacto directo con todos los elementos de la sangre al ser irrigado por los vasos sanguíneos. Los análisis elementales por microscopía electrónica de barrido, como los que se llevaron a cabo en el estudio de caso que constituye el Capítulo III, permiten también

conocer las diferencias en los patrones de concentración entre metales esenciales y no esenciales depositados en el folículo.

Este capítulo muestra el trabajo de muestreo de pelo de guardia dorsal de mamíferos de la región de Endhó, Hidalgo y los análisis mediante EDS-SEM de estas muestras tanto en el folículo como en las partículas depositadas en la cutícula a lo largo de cada hebra, para caracterizar y diferenciar los metales depositados de manera endógena y los de origen exógeno. Con esto se puede observar que la exposición exógena incluye una mayor cantidad de elementos que el depósito endógeno y que, además, existen elementos de mayor interés toxicológico como el Cr, que pueden encontrarse tanto en partículas adheridas a la cutícula como en el folículo. Los análisis no mostraron la presencia de metales de mayor interés toxicológico como Pb o Cd, pero su presencia no puede ser descartada ya que se han encontrado en análisis de hortalizas y peces de la región. Sin embargo, sería necesario el análisis de las muestras por medio de técnicas espectrométricas capaces de detectar elementos traza con mucha mayor precisión.

Aún cuando ambas técnicas (EDS-SEM y técnicas espectrométricas) dan información relevante sobre los metales depositados en una muestra de pelo, la toxicidad de un elemento depende en gran medida de la especie química en la que se encuentre y no solo de la concentración. Por esto, el conocer los metales tóxicos a los que está expuesta la biota en una región representa la primera parte del biomonitoreo, el cual deberá continuarse con otro tipo de análisis que permitan establecer el riesgo de la fauna por la exposición a determinados compuestos para finalmente establecer medidas efectivas para disminuir el impacto de estos metales en el ambiente.

Problemática de contaminación en la presa Endhó.

La presa Endhó se ha convertido desde hace décadas en repositorio de las aguas residuales de la ciudad de México, ocasionando una severa contaminación que ha comprometido severamente la salud de los habitantes de las localidades asentadas en los alrededores de la presa. Los alcances de esta contaminación no solo se limitan a la salud humana de los lugareños y a la biodiversidad de la región sino también a las regiones del país donde se comercializan las hortalizas que se producen en los campos irrigados con el agua de la presa (Siemens *et al.* 2008; Guedrón *et al.* 2014; Montelongo-Reyes *et al.* 2015). Es por esto que esta región debe de considerarse como una zona prioritaria para el biomonitoreo ambiental, que permita conocer más a fondo la bioacumulación y biotransferencia de contaminantes desde la presa hacia los organismos de diferentes niveles tróficos.

También resulta prioritario establecer medidas que permitan la disminución progresiva de los niveles de contaminantes en la presa, los cuales incluyen no solo metales pesados, sino compuestos orgánicos persistentes, bacterias, contaminantes emergentes como analgésicos, etc. Una medida necesaria para evitar la dispersión de más contaminantes en la zona es el tratamiento de las aguas residuales destinadas

para la irrigación de cultivos, así como de nuevas estrategias en la ciudad que disminuyan la liberación de tantos desechos al drenaje, como sería una mejora en la recuperación y reciclaje de muchos materiales. Esto no solo podría disminuir progresivamente los niveles de agentes químicos potencialmente tóxicos, también disminuiría la gran cantidad de basura que llega a la presa y que se hace más evidente durante la época seca, cuando sus niveles están muy bajos y deja al descubierto la enorme cantidad de deshechos plásticos que viajan desde el sistema de drenaje de la ciudad y quedan depositados en la presa.

CONCLUSIONES

-El pelo es una estructura que por la gran afinidad química hacia elementos metálicos y su fácil obtención y manejo es un tejido muy apropiado para el biomonitoreo.

-A pesar de que no existe ningún organismo o parte de él que sea ideal en el biomonitoreo de contaminantes, la gran cantidad de mamíferos distribuidos en los diferentes ecosistemas del mundo, así como los diferentes papeles ecológicos que juegan en cada región representan distintas opciones con las que se puede determinar qué muestras de pelo son las más adecuadas para alcanzar los objetivos propuestos en el biomonitoreo de diversas regiones.

-La microscopía electrónica es una técnica poco aprovechada en biomonitoreo ambiental. Sin embargo, junto con las técnicas analíticas usadas para la caracterización de metales tóxicos se puede obtener información muy valiosa sobre la localización exacta y los patrones de concentración de los metales hallados en las muestras analizadas.

-Existen regiones en el país como la presa Endhó y los pueblos rivereños alrededor de esta que son prioritarios para establecer medidas de mitigación de la contaminación ambiental, cuyos efectos han deteriorado drásticamente la salud humana. Aunque la persistencia de los metales en el ambiente y su toxicidad los convierte en contaminantes de gran importancia toxicológica, la presa contiene todo tipo de contaminantes que también deben considerarse dentro de los planes de manejo, como microorganismos patógenos, compuestos orgánicos persistentes y contaminantes emergentes como analgésicos.

- El papel de la presa como repositorio de los desechos de la Ciudad de México así como fuente de agua para la irrigación de cultivos pone de manifiesto la gravedad de los problemas generados por la falta de tratamiento de las aguas residuales antes de ser usadas para tales fines. Esto es de crucial importancia para no generar otra región en México con una contaminación de tales proporciones y para mejorar las condiciones actuales de la presa y sus alrededores.

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