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

A. N. Rendón-Lugo · P. Santiago · I. Puente-Lee · L. León-Paniagua

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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

A. N. Rendón-Lugo · L. León-Paniagua (✉)
Museo de Zoología "Alfonso L. Herrera", Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, A. P. 70-399, D. F, 04510 Coyoacán, Mexico
e-mail: llp@ciencias.unam.mx

P. Santiago
Instituto de Física, UNAM, Mexico City, Mexico

I. Puente-Lee
Facultad de Química, UNAM, Mexico City, Mexico

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.

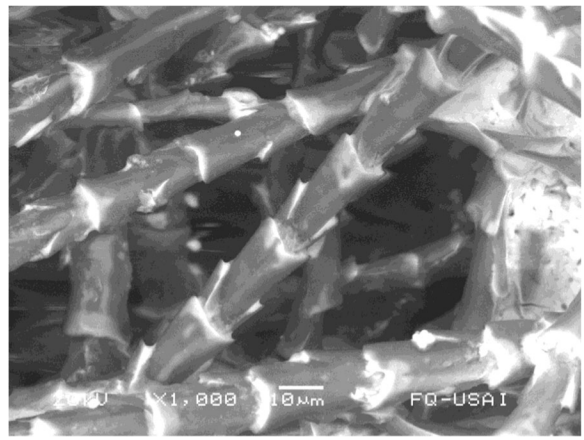

Fig. 1 *Glossophaga soricina* hair with cadmium II acetate

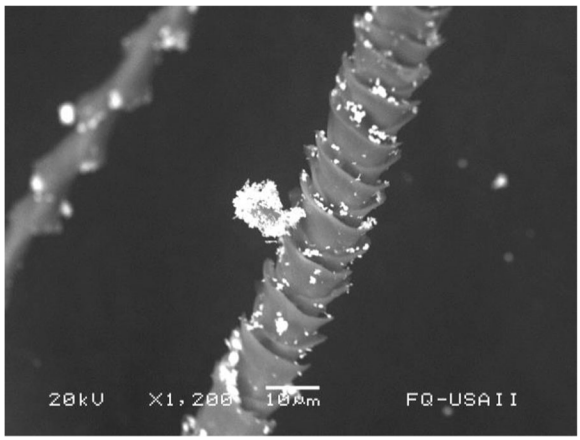

Fig. 2 *Artibeus jamaicensis* with cadmium II acetate

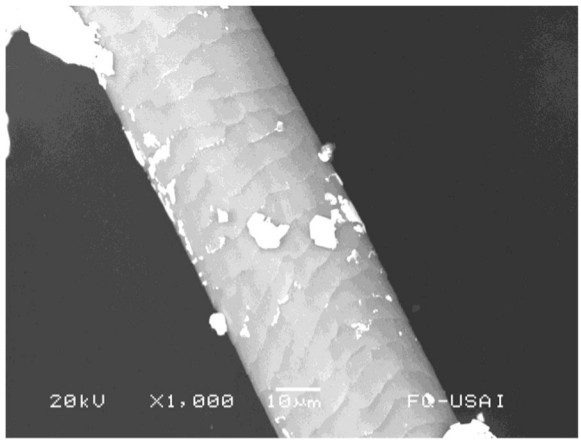

Fig. 4 *P. furvus* hair with lead

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

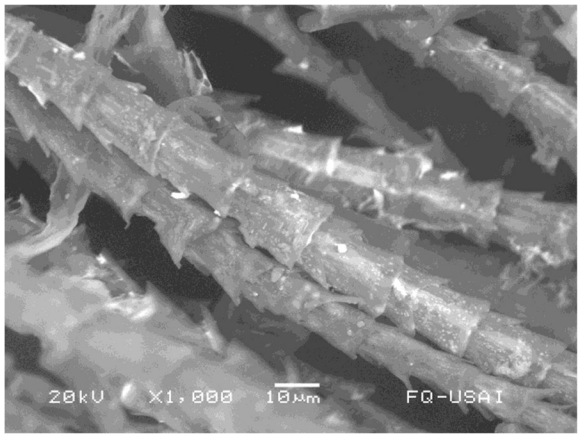

Fig. 3 *G. soricina* hair with lead

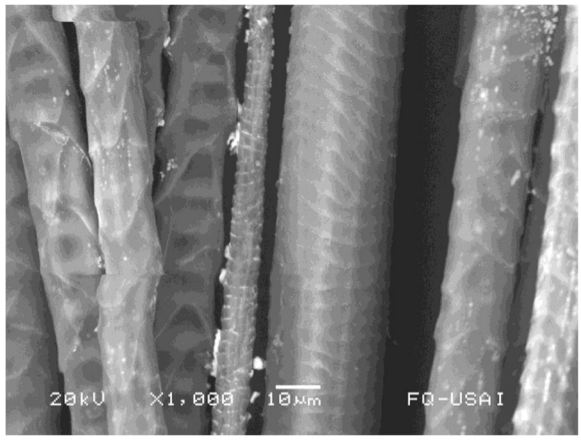

Fig. 5 *M. mexicana* hair with lead

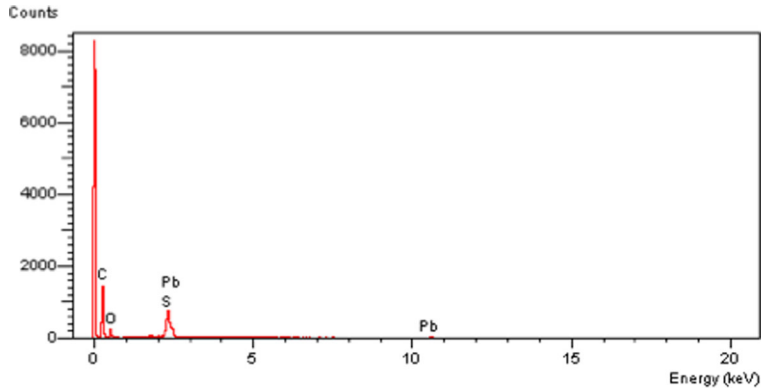

Fig. 6 Spectrum obtained from hairs of *P. maniculatus* treated with lead

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%.

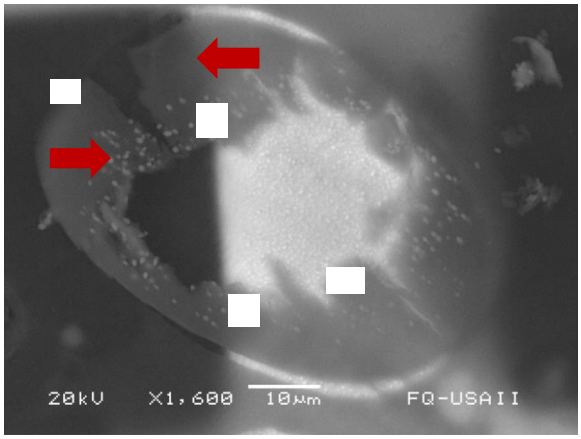

Fig. 7 Cross-section of *G. soricina* hair. Red arrows indicate parts where cadmium was detected, while white boxes indicate regions in which microanalysis was carried out

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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