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Analysis of lead pollution levels within an urban ecosystem using the cestode *Hymenolepis diminuta* and its rat hosts as bioindicators

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Abstract

The overall goal of this study was to use the *Rattus* spp./*Hymenolepis diminuta* model to assess environmental lead pollution in different landscape units of an urban ecosystem. Rats of the genus *Rattus* were collected from three shanty towns and three residential neighbourhoods of the city of Buenos Aires. Concentrations of lead in the livers of wild rats and in their parasite *H. diminuta* were measured using inductively coupled plasma mass spectrometry (ICP-MS). The landscape unit and tissue type had a significant effect on lead concentration, being higher in residential neighbourhoods as well as in *H. diminuta* tissue. Nevertheless, no significant differences were found for the mean lead concentration in livers between uninfected and infected rats. Since the available information describing heavy-metal pollution within the city of Buenos Aires is scarce, the results of this study allow us to update data about the extent of biologically available lead contamination. Considering that rats and *H. diminuta* are distributed worldwide, this monitoring system for lead pollution might be applied successfully in other urban ecosystems.

Introduction

Increasing industrialization and urbanization, swift and often unplanned, has been accompanied by the extraction and distribution of mineral substances from natural deposits (Brenner & Schmid, 2014), leading to a rapid increase in anthropogenic toxic metal emissions and, consequently, in increased air and water pollution levels (Moore *et al.*, 2003; Stankovic & Stankovic, 2013). Lead is one of the most abundant heavy metals on Earth and may affect every organ in the body, mainly nervous, excretory and circulatory systems (ATSDR, 2007). Lead is found in several compounds and alloys, and it is used in several industrial processes, including the production of batteries, paint, cables, petrol, ceramics, electronics and plastics (Meyer *et al.*, 2008). The distribution of lead in the environment varies among and within countries, depending on historical and current uses of this metal. Elevated contaminant levels in the environment are not necessarily indicative of adverse effects. Only a fraction of the metal in the environment is bioavailable for potential intake by the biota and exertion of adverse effects within the receptor organism (Baker *et al.*, 2003).

To determine the risk of exposure to heavy metals is often a complex task, but the use of animal species as bioindicators offers a potentially simple solution for the difficulty of measuring bioavailability and summarizing complex patterns of contamination in the environment (Sures, 2004; Vidal-Martínez & Wunderlich, 2017). Bioindicators of accumulation are biological monitors that accumulate a pollutant in their tissues without significant adverse effects, and are therefore used to measure the amount of a pollutant that is biologically available (Beeby, 2001; Vidal-Martínez & Wunderlich, 2017). In recent years, intestinal parasites of vertebrates have received increasing attention as indicators of heavy metals, mainly acanthocephalans of the orders Moniliformida (Scheef *et al.*, 2000; Sures *et al.*, 2000; Torres *et al.*, 2011; Teimoori *et al.*, 2014) and Echinorhynchida (Siddall & Sures, 1998; Schludermann *et al.*, 2003; Sures & Reimann, 2003; Thielen *et al.*, 2004), and cestodes of the order Cyclophyllidea (Sures *et al.*, 2003; Eira *et al.*, 2005; Jankovská *et al.*, 2010a; Torres *et al.*, 2010, 2011). These helminths have the ability to bioconcentrate different metals at concentrations surpassing that of their hosts (Sures *et al.*, 2002, 2003; Torres *et al.*, 2004; Al-Quraishy *et al.*, 2014; Teimoori *et al.*, 2014). However, few studies involving helminths as environmental quality indicators have been conducted in terrestrial habitats (Vidal-Martínez & Wunderlich, 2017).

In urban environments, the *Rattus* spp./*Hymenolepis diminuta* system was the first model to be used for detection of lead pollution involving a cestode (Sures *et al.*, 2002, 2003;

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Al-Quraishy *et al.*, 2014; Čadková *et al.*, 2014). This system is widely distributed, easy to collect and identify, especially in urban ecosystems (Feng & Himsworth, 2014; Hancke & Suárez, 2016), meeting some of the criteria set for a good bioindicator (Beeby, 2001). Most of the studies using this model have been based on comparing sites with similar environmental characteristics but different lead concentrations.

In the urban matrix of the city of Buenos Aires (Argentina), two landscape units can be distinguished as being associated with housing quality and urban planning: residential neighbourhoods with urban services such as garbage removal, sanitation networks, electricity and plumbing; and shanty towns with precarious houses and an inadequate supply of basic urban services (Fernández *et al.*, 2007; Cavia *et al.*, 2009). These characteristics affect the establishment and proliferation of animal populations. Previous studies conducted in Buenos Aires city have found that *Rattus rattus* is the dominant species in residential neighbourhoods, while *Rattus norvegicus* is the most abundant in shanty towns (Cavia *et al.*, 2009). At the same time, differences in environmental conditions that characterize each of these landscape units also alter the parasite burden in rats. According to Hancke & Suárez (2016), the abundance of *H. diminuta* within the urban matrix is highest in shanty towns, possibly due to a greater abundance of intermediate hosts.

The determination of lead concentration in the *Rattus* spp./*H. diminuta* system in the city of Buenos Aires would help to infer the bioavailability of this metal in different landscape units of this city. Although studies of heavy-metal pollution have been performed in the city of Buenos Aires, they took place in specific areas and were not followed up over time (Lavado *et al.*, 1998; Ratto *et al.*, 2004, 2006; Smichowski *et al.*, 2004; López *et al.*, 2006; Perelman *et al.*, 2006). The overall goal of the present study was to use the *Rattus* spp./*H. diminuta* model as a tool to assess environmental lead pollution in shanty towns and residential neighbourhoods of the city of Buenos Aires.

Materials and methods

Study area

Fieldwork was conducted in the city of Buenos Aires, Argentina (34°37′S, 58°24′W). This city covers an area of 200 km^2 with 2,890,151 inhabitants (INDEC, 2010). The climate is temperate with a mean annual temperature of 17.4°C and mean annual precipitation of 1146 mm. The matrix of the city is composed of buildings, houses and paved streets, with internal patches formed by parks, green spaces and shanty towns (Cavia *et al.*, 2009).

Rodent trapping

Rodents were collected from surveys carried out as part of a rodent control programme in the city of Buenos Aires from 2003 to 2006. Sampling was conducted in six sites of two different landscape units: three shanty towns and three residential neighbourhoods. Rodents were captured using live cage traps as described by Cavia *et al.* (2009). Traps were placed inside houses and in their yards, in stores or factories, and were monitored every morning for four consecutive days. Captured animals were sacrificed with anaesthesia, identified, sexed, measured and weighed. All animals were fixed in formaldehyde and a week later preserved in 70% ethanol and stored in the collection of the Laboratory of Urban Rodent Ecology of the Buenos Aires University. The intestines of all adult *Rattus* spp. (17.5–26.0 cm) were reviewed for the isolation and identification of *H. diminuta*. Parasites and a sample of the liver of each parasitized rat were isolated for lead determination, and were stored in 10% formaldehyde that had been tested for heavy-metal content before its use.

Analytical procedure

Lead extraction from rat livers and *H. diminuta* specimens was performed according to a modification of the methodology proposed by Torres *et al.* (2011). About 200 mg of liver and the whole parasite biomass found in a host were each placed in a 10-ml flask with 2 ml of nitric acid (Suprapur, Merck, Buenos Aires, Argentina). The samples were gradually heated using an infrared lamp for 5 h. After the digestion, samples were diluted in distilled water to a volume of 10 ml. Lead determinations were carried out using inductively coupled plasma mass spectrometry (ICP-MS). Analytical blanks were prepared under the same conditions. The detection limits of the measurements (mean ± 3 SD of blanks) were 3.05 µg/l. To prevent inaccuracies in the determination of lead based on wet weight in parasites and tissues, the concentrations of heavy metals were applied to dry weights, according to Jankovská *et al.* (2010a). However, to compare our data with those obtained by other authors, wet weight conversion factors were calculated for parasites and livers (µg/g wet $\text{weight}_{\text{parasite}}$ = µg/g dry weight × 0.23 and µg/g wet $\text{weight}_{\text{liver}}$ = µg/g dry weight × 0.29, respectively).

Statistical analysis

To study the factors affecting lead concentration in rats and *H. diminuta* in the city of Buenos Aires, a general linear mixed model (GLMM) was performed. Lead concentration in rat liver and parasites was considered as a response variable, whereas tissue (liver or parasite), landscape units, sex, weight of rats and dry weight of parasites, and their interactions, were explanatory variables. Sampling sites were included as a random factor. Since lead concentrations were not normally distributed, and had variance heterogeneity, data were Ln-transformed. Results were considered to be statistically significant when $P < 0.05$. Data were analysed using the gls and lme functions of the nlme R-packages interfaced by *InfoStat* Statistical Software version 2015 (Di Rienzo *et al.*, 2015).

The relation between lead concentration in parasites and in the host's liver was calculated using the bioconcentration factor proposed by Sures *et al.* (1999), as the ratio of the mean metal concentrations in the parasite and the host liver ($C_{[\text{parasite}]}/C_{[\text{liver}]}$). This was calculated for each landscape unit separately.

To study the effect of *H. diminuta* on lead concentration in the host, a Student's *t*-test was performed to compare the lead concentrations in livers between infected and uninfected rats. To avoid external or environmental effects, only rats trapped in one of the shanty towns were used. All the statistical analyses were performed using *InfoStat* Statistical Software version 2015 (Di Rienzo *et al.*, 2015).

Results

Sixty-five rats were captured and 22 of them were infected with *H. diminuta*. Fifty per cent of the captured animals in shanty towns ($n = 36$) were parasitized, while only 14% ($n = 29$) were infected in residential neighbourhoods (table 1).

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Table 1. Number of rats infected with *Hymenolepis diminuta* and mean values of lead concentration in rat livers and parasites, relative to landscape unit (different letters indicate significant differences, $P < 0.05$). All concentrations are presented as μg/g dry weight.

		Rats examined		Lead concentration (μg/g dry weight) (mean ± SD)	
Landscape unit	Rats infected with *H. diminuta*	*R. rattus*	*R. norvegicus*	*H. diminuta*	Rat liver
Residential neighbourhood	4	29	–	55.22 ± 29.70^A	10.58 ± 5.27^B
Shanty town	18	–	36	12.50 ± 13.27^a	3.99 ± 2.21^b

The mean lead concentration of *H. diminuta* was highest in residential neighbourhoods (table 1), where the specimen with highest lead level was also found (79.6 μg/g dry weight). The lowest mean lead concentration was detected in livers of rats from shanty towns (table 1), where the specimen with lowest lead level was also found (1.5 μg/g dry weight).

The GLMM analysis showed that landscape unit and tissue type have a significant effect on lead concentration, being higher in residential neighbourhoods, and higher in *H. diminuta* tissue (df = 4, $F = 9.55$, $P = 0.037$ and df = 37, $F = 22.84$, $P < 0.0001$, respectively).

When comparing lead levels in *H. diminuta* and rats, the bioconcentration factor revealed a fivefold higher lead level in *H. diminuta* compared to host livers from residential neighbourhoods, while in shanty towns this relationship was 3:1.

Finally, no significant differences were found between the mean lead concentration in livers of uninfected rats ($n = 11$) and infected rats ($n = 6$; $t = -0.72$, df = 15, $P = 0.480$) in animals trapped in one of the shanty towns.

Discussion

The results of this study show the feasibility of using bioindicators to characterize heavy-metal pollution in urban ecosystems when environmental data are scarce. Lead concentration in *H. diminuta* and in its hosts, the rats, was higher in residential neighbourhoods compared to shanty towns. Although data of environmental lead contamination in this study area were not available, the results obtained so far allowed us to hypothesize the possible causes for the observed patterns. In Argentina, the principal sources of lead in cities are service pipes, peeling paint and industry (Mattalloni *et al.*, 2014). Since shanty towns lack urban basic services and the industries in Buenos Aires city are more likely to be located within residential neighbourhoods, a higher exposure to lead sources was expected in these latter landscape units. Rat tissues have been widely studied as bioindicators of environmental pollution, and positive correlations between environmental and tissue lead concentrations have been widely reported (Way & Schroder, 1982; Ceruti *et al.*, 2002; Nakayama *et al.*, 2013; Bortey-Sam *et al.*, 2016).

In the city of Buenos Aires, a clear spatial segregation was described for rat species, *R. rattus* and *R. norvegicus* (Cavia *et al.*, 2009). This may restrict the extrapolation of our results, because it is difficult to differentiate whether differences in lead concentration in parasites and hosts are due to the effect of differences in environmental lead levels between landscape units, or to the effect of behavioural differences between rat species. *Rattus rattus* is a better climber and builds nests out of artificial materials, allowing this species to be dominant in residential areas, while the abundance of *R. norvegicus* is correlated with the presence of plant cover and water in the environment, enabling them to be successful in shanty towns (Cavia *et al.*, 2009; Feng & Himsworth, 2014). This could lead to differences between rodent species in the rates of contact with different types of lead pollution sources. However, despite the behavioural differences between both host species, we believe that our results did reflect the conditions of lead pollution in each landscape unit. Higher lead levels were detected in rats and parasites from residential neighbourhoods compared to shanty towns, in agreement with the greater presence of lead sources, as mentioned previously.

Parasites are widely recognized as indicators of anthropogenic pollution. In some cases, the presence or absence of parasite species is associated with chemical contamination of the environment, while other parasites have the ability to accumulate trace metals from the environment in their tissues (Sures, 2004; Vidal-Martínez & Wunderlich, 2017). According to Thielen *et al.* (2004), parasites without a digestive tract, such as cestodes, are able to concentrate more metals than host tissues. In the present study, the concentration of lead in *H. diminuta* was significantly higher than in rat livers, and the bioaccumulation factor obtained in the residential neighbourhoods revealed that *H. diminuta* can accumulate lead up to five times the level accumulated by the host´s liver. This value is lower than that obtained by Sures *et al.* (2003) and Al-Quraishy *et al.* (2014) in natural conditions for the rat/*H. diminuta* system. It has been noted that parasites accumulate heavy metals much faster than their host, but a maximum is reached approximately after 4 weeks. Therefore, the bioaccumulation factor would tend to be smaller in chronic exposure situations (Oyoo-Okoth *et al.*, 2012; Čadková *et al.*, 2014). Additionally, methodological differences in tissue digestion could explain the results between different studies. Here, the whole biomass of parasites was digested for lead determination, while other researchers have taken only a sample of parasites. It has been mentioned that metals do not have a homogeneous distribution inside the parasite (Riggs *et al.*, 1987; Vijayalakshmi *et al.*, 2003; Horáková *et al.*, 2017). This could affect the value and accuracy of bioaccumulation factor determination.

In this study, no effect of parasitism with *H. diminuta* was found on the lead concentration of the host liver. In agreement with the results of Sures *et al.* (2003) for the same host–parasite model, and Jankovská *et al.* (2010a) for red foxes (*Vulpes vulpes*) infected with *Mesocestoides* spp., no difference in lead concentration was observed between parasitized and non-parasitized rats. However, other studies have reported an effect of parasitism on heavy-metal uptake of the host. Jankovská *et al.* (2010b) found significant differences between parasitized and non-parasitized animals in an experimental study of lead accumulation in a sheep–cestode system (*Moniezia expansa/Ovis aries*). According to Čadková *et al.* (2014), the toxicokinetics in a host body depends on the lead concentration to which it is exposed. However, and consistent with our results, for similar lead exposure levels no significant differences were found in lead concentration between parasitized and non-parasitized animals. This is reasonable because it is possible that cestodes take the lead attached to bile, which had been absorbed by the host previously and then excreted into the duodenum via the hepatic cycle (Sures *et al.*, 2003; Čadková *et al.*, 2014).

Considering that information describing heavy-metal pollution within the city of Buenos Aires is scarce, the results of this study allow us to update the data about the degree of lead contamination. The rats captured in the two landscape units were exposed to biologically available lead, showing higher concentrations in the residential neighbourhoods than in shanty towns. Considering that rats and *H. diminuta* are distributed worldwide, this monitoring system for lead pollution could be an effective tool for application in other urban ecosystems throughout the world.

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of relevant national and institutional guidelines on the care and use of animals (National Law 14346).

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