



Are soft tissues of urban rats good indicators of exposure to heavy metal pollution? A study conducted in one of the most polluted basins of Buenos Aires, Argentina

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Abstract The overall goal of this work was to analyze the relationship between the concentration of lead in the kidney, liver, and stomach contents of rats captured in a polluted urban basin and the concentration of this metal in the environment, meteorological factors, and different intrinsic characteristics of the individuals. To this end, we determined the concentration of lead in the kidney, liver, and stomach contents of 133 Norway rats (*Rattus norvegicus*) captured in the Matanza-Riachuelo River Basin (Buenos Aires, Argentina). This basin is one of the most severe cases of water pollution in Latin America and metals like lead represent the most common chemical pollutants. Rats were trapped in nine sites with different soil and water lead concentrations, between 2014 and 2015. A positive relationship was found between lead concentration in the liver and kidney of *R. norvegicus* and the environmental concentration of this metal in water and soil. Although the remaining variables analyzed were also related to the lead concentration in soft tissues, they did not affect the association between the lead concentration in soft tissues and that in the environment. The lead concentration in the stomach contents was not related to any of the variables

analyzed. Our results constitute the first study in an urban basin with a gradient of environmental lead concentration and suggest that the quantification of lead in the kidney and liver of rats can be used as an indicator of exposure to this metal within a large city.

Keywords Lead · Biomonitoring · Sentinel species · *Rattus norvegicus* · Polluted urban basin · Health

Introduction

In the last few decades, the pressure from the activities of urban populations has been intense and the presence of toxic substances in the environment has become a global problem (Stankovic and Stankovic 2013). The increasing industrialization and urbanization have been accompanied by the extraction and distribution of mineral substances from natural deposits (Brenner and Schmid 2014), a fact that has led to a rapid increase in anthropogenic toxic metal emissions and, consequently, to an increase in the air, soil, and water pollution levels (Moore et al. 2003; Stankovic and Stankovic 2013). The pollution of urban basins represents a global problem, because these areas are inhabited by millions of people who live in precarious conditions, with unhealthy sanitary practices and exposed to high levels of environmental pollution that put their health at risk (Adekola and Mitchell 2011; Armengol et al. 2017; Bernhardt and Gysi 2013; Bukit 1995; D'Arrigo et al. 2011).

Lead is one of the most abundant and toxic metals on Earth and cannot be transformed to any harmless form

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(Sanín et al. 1998). This metal can affect every organ in the body, mainly the nervous, excretory, and circulatory systems, and is able to cause encephalopathy and other associated signs such as ataxia, coma, seizures, and hyperirritability and can lead to death (ATSDR 2007). Although the use of lead in recent years has been drastically reduced worldwide, the number of deaths due to exposure to this metal is still rising, reaching more than a million of people in 2017 (IHME 2019; Meyer et al. 2008; WHO 2018).

The increased levels of metal pollutants in the environment are not necessarily indicative of a hazard to human life. Only a fraction of these is bioavailable for potential intake by the biota and hence, only this fraction can exert adverse effects within the receptor organism (Baker et al. 2003). Thus, elucidating the risk of exposure to heavy metals is often a complex issue. In this sense, the use of animal species as sentinels offers a potentially simple solution to measure the bioavailability of metals and assess the complex contamination within an environment (Sures 2004; Timbrell 1998; Vidal-Martínez and Wunderlich 2017). The selection of an appropriate sentinel species must have the following criteria: high abundance in the study area, a minimum immigration rate, a restricted or small home range, and a high life expectancy that allows long-term research of the exposure to contaminants (O'Brien et al. 1993).

In urban ecosystems, Norway rats (*Rattus norvegicus*) fulfill the basic requirements as sentinel species for biomonitoring and ecotoxicological studies (Bortey-Sam et al. 2016; Ceruti et al. 2002; Getz et al. 1977; McLean et al. 2009; Mouw et al. 1975; Nakayama et al. 2013; Way and Schroder 1982). These rats are strongly associated with urban ecosystems, live close to humans, usually have small home ranges and an omnivorous diet, being waste its main food source (Davis et al. 1948; Feng and Himsworth 2014; Traweger et al. 2006). This species has a remarkable capacity of adaptation to a wide range of environments and resources, but marginal urban environments of low incomes are often more prone to rat infestations because they tend to have abundant harborages and food sources for rats (Cavia et al. 2009; Easterbrook et al. 2005; Feng and Himsworth 2014; Fernández et al. 2007).

One of the indicators most used to estimate the exposure to heavy metal pollutants in mammals is the concentration of contaminants in different soft tissues (liver, kidney, lung, brain, and muscle). Lead

concentrations in the kidney and liver of *R. norvegicus* have been used for comparative studies of lead concentration in natural reserves, rural and urban environments, and results have shown significant differences between sites with contrasting levels of contamination with this metal (Mouw et al. 1975; Way and Schroder 1982). However, the contaminant levels in animal tissues might also be affected by other internal factors, such as the age and sex of the specimen, and external factors such as seasonal variations. In mammals, the lead concentrations in soft tissues have been shown to vary with internal factors like size and/or age (Bortey-Sam et al. 2016; Way and Schroder 1982; Zarrintab and Mirzaei 2017). Regarding external factors, seasonal variations have been observed but not always with consistent results (Janiga et al. 2012; Tataruch and Kierdorf 2003), while the influence of rainfall and ambient temperature has not yet been evaluated. In addition, very few studies have analyzed the relationship between these variables and lead concentration in *R. norvegicus* and, to our knowledge, there are no studies of this species in an urban basin.

Thus, the overall goal of this study was to determine the relationship between the concentration of lead in the kidney, liver, and stomach contents of *R. norvegicus* rats captured in a polluted urban basin and the concentration of this metal in the environment, meteorological factors, and different intrinsic characteristics of the individuals, to assess the potential use of lead accumulation in soft tissues of *R. norvegicus* as an indicator of exposure to this metal.

Materials and methods

Study area

This study was carried out in the Matanza-Riachuelo River Basin (MRRB), located within the Province of Buenos Aires (90%) and the city of Buenos Aires (10%), Argentina. The MRRB represents the oldest and one of the most severe cases of water pollution in Latin America. This is the most industrialized and urbanized area of the country, with a population of more than 4,000,000 inhabitants and 16,000 industries (Mendoza et al. 2015).

The climate in the area is subtropical with 23.9 °C of maximum average temperature in January, 10.4 °C of minimum average temperature in July, and an annual

rainfall of approximately 1200 mm. The dominant soils near the alluvial plain of the rivers and streams are classified as Arguidolls, Natraquolls, and Natraqualfs, and the vegetal communities are often dominated by typical species of grasslands and humid meadow (De Siervi et al. 2005; Breuste and Faggi 2015). The water-courses of the MRRB are subject to multiple types of contaminants from agricultural and urban runoff, industrial effluents, sewage treatment plants, and domestic garbage dump leaching (Biruk et al. 2017; Magdaleno et al. 2008). Metals, like lead, represent the most common chemical pollutants in the basin (Mendoza et al. 2015; Ronco et al. 2008). The MRRB is topographically divided into three sub-areas: the lower, middle, and upper basins. The matrix of the lower part of the MRRB, which is the closest area to the mouth in the Río de La Plata, is formed by shanty towns and industries, with parks and open green areas forming patches. The main industries located in the lower basin are tanneries, cold storage warehouses, and metallurgical and chemical industries. This area shows the highest population and industrial densities of the entire basin and, in 2013, was characterized as one of the ten most polluted sites in the world by the Green Cross Switzerland (Bernhardt and Gysi 2013). The middle basin is less urbanized and industrialized than the lower basin, and thus has lower pollution levels (ACUMAR 2009). Finally, the upper basin is mainly surrounded by agricultural and cattle breeding areas (ACUMAR 2009).

Most of the people who live in the MRRB reside in houses with precarious and inadequate supply of basic urban services, many of which are located on the banks of the rivers (de Pietri et al. 2011; Defensor del Pueblo de la Nación 2003; Fundación Ciudad 2002). Studies carried out by ACUMAR, the agency in charge of the implementation of the Environmental Sanitation Plan in the MRRB, showed that, between 2012 and 2015, more than 10% of the population living in 14 out of the 16 neighborhoods of the MRRB had higher values of lead in blood than those considered safe for health (ACUMAR 2018b).

Rodent trapping

Rats were trapped in four sites of the lower basin (S1, S2, S3, and S4) and in five sites of the middle basin (S5, S6, S7, S8, and S9) on the coast of the Riachuelo and Matanza rivers or their tributaries, distanced from each other by a minimum distance of 2 km (Fig. 1). All

capture sites were located within or close to a shanty town or marginal settlement (the maximum distance was 200 m in site 1). The population density of these shanty towns ranged from a maximum of 360 inhabitants per hectare in site 2 to a minimum of 73 inhabitants per hectare in site 7. Each site was visited in the winter and summer during two consecutive years (2014 and 2015).

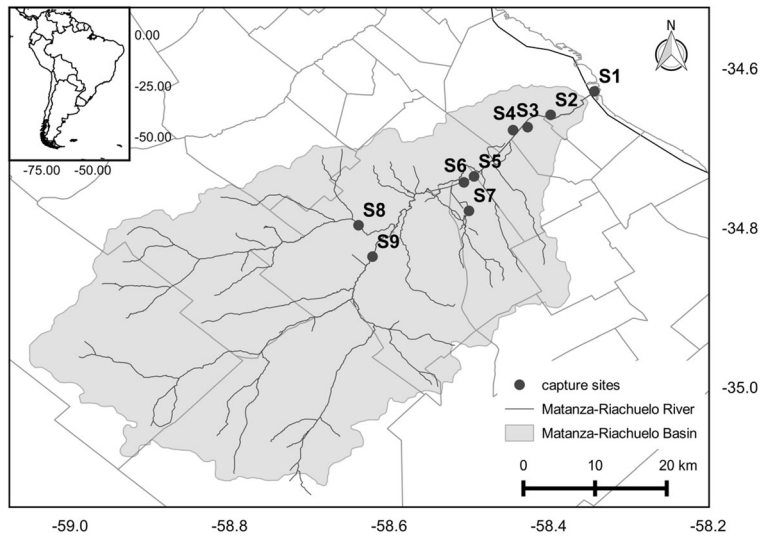
A total of 133 *R. norvegicus* rats were captured using live cage traps as described in Cavia et al. (2009). Table 1 details the number of rats captured relative to the sampling site, season, and year. Traps were placed and monitored every morning during four consecutive days. The rats captured were anesthetized with ketamine and acepromazine (40 mg/kg and 5 mg/kg, respectively), identified taxonomically, sexed, measured, and weighed. The age of each rat was calculated by paired eye-lens weight according to Hardy et al. (1983). To quantify lead concentration, samples of liver, kidney, and stomach contents were obtained from each specimen. All samples were deep-frozen at $-20\text{ }^{\circ}\text{C}$ and lyophilized for subsequent metal analysis.

All the rats captured were fixed in formaldehyde, preserved in 70% ethanol a week later, and stored in the collection of the Ecology of Urban Rodents Research Unit at the University of Buenos Aires. Both in the field and in the laboratory, rats were handled strictly following biosafety norms and according to National Law no. 14346 of animal care.

Lead determination in liver, kidney, and stomach contents

Samples (0.5–1 g dry weight) of liver, kidney, and stomach contents were analyzed for lead content. Samples were placed in Teflon vessels with 7 ml HNO_3 (Merck, Suprapur) and 1 ml H_2O_2 (Panreak). All the materials used in the digestion process were thoroughly acid-rinsed. Each sample was mineralized in a microwave oven (Milestone ETHOS labstation with easyWAVE) at $200\text{ }^{\circ}\text{C}$ for 30 min. After their complete digestion, samples were diluted with Milli-Q water and then analyzed in an inductively coupled plasma mass spectrometer (Perkin Elmer - Sciex, ELAN DRC-e). The analytical procedure was checked using bovine liver as standard reference material (BCR-185R, Institute for Reference Materials and Measurements, European Commission). Analytical blanks were prepared under the same conditions to determine the detection limit ($\text{DL} = \text{mean} + 3\text{SD}$) (Siddall and Sures 1998).

Fig. 1 Location of sampling sites in the Matanza-Riachuelo River Basin (Buenos Aires, Argentina): sites 1 to 4 (S1, S2, S3, and S4) were located in the lower basin, whereas sites 5 to 9 (S5, S6, S7, S8, and S9) were located in the middle basin



The percentage of lead recovery in the reference material was $93.1 \pm 3.3\%$, while the DL was $2.68 \mu\text{g l}^{-1}$ ($0.02 \mu\text{g g}^{-1}$). The lead concentrations in tissues and stomach contents are presented as microgram per gram dry weight. To compare our data with data reported in wet weight, the lead content can be recalculated using the following conversion factors of dry weight to wet weight: 0.34 for the liver tissue and 0.27 for the kidney tissue. These values were obtained by calculating the average moisture percentage of 10 samples of each of the tissues not used in the analysis.

Table 1 Number of rats (*Rattus norvegicus*) captured relative to the sampling site, season, and year. Sites indicated as “–” are those where no rodent sampling was carried out

	Site	2014		2015	
		Summer	Winter	Summer	Winter
Lower basin	S1	5	13	6	1
	S2	2	–	–	7
	S3	8	5	11	3
	S4	4	5	7	0
Middle basin	S5	2	6	6	11
	S6	11	0	–	–
	S7	4	0	–	–
	S8	–	5	4	1
	S9	–	5	0	1

Lead determination in soil

Between 3 and 5 soil samples of 15 cm depth, free of plants and leaves, were taken at each sampling site during each rodent trapping events in those transects where rats were captured. The samples were labeled and stored at room temperature in plastic bags.

For lead determination, the soil samples were homogenized and a subsample of 0.35 g was randomly taken from each. Microwave-assisted wet digestion of the samples was performed in two steps: first, a program of 20 min with 8 ml HNO₃, 5 ml ClH, and 1 ml H₂O₂ at 210 °C; second, 2 ml HF and 5 ml H₃BO₃ were added to the solution obtained and heated at 160 °C for 15 min. After complete digestion, samples were diluted with Milli-Q water and analyzed in an inductively coupled plasma mass spectrometer (Perkin Elmer - Sciex, ELAN DRC-e). Three standard reference materials were used for quality assurance of soil analyses: NIST SRM 2709 - San Joaquin Soil; NIST SRM 2711 - Montana II Soil; and NIST SRM 2586 - Trace Elements in Soil Containing Lead From Paint. The percentages of lead recoveries in the reference materials were $121 \pm 5\%$, $110 \pm 1\%$, and $115 \pm 2\%$, respectively.

Lead determination in water

Since 2008, ACUMAR has been carrying out a Surface Water and Sediment Quality Monitoring Program,

which includes quarterly sampling and measuring of more than 50 parameters considered representative of surface water quality, including physicochemical parameters, metals, organic compounds, and hydrocarbons, in 38 sites located in the MRRB (ACUMAR 2017). Thus, the lead concentration in water at each sampling site was characterized based on that obtained from the ACUMAR database (ACUMAR 2018a). The selected ACUMAR sampling points and dates were those that were closest to the date (between 0 and 51 days) and location (between 0 and 2000 m) of the rodent capture events.

Meteorological factors

The daily temperature and precipitation records from the study area were provided by the National Meteorological Service. The average of medium, minimum, and maximum temperature and accumulated rainfall 3, 7, and 30 days prior to each rodent capture event were calculated.

Statistical analyses

To analyze differences in lead concentrations among the three tissues analyzed, a Generalized Linear Mixed Model (GLMM) was performed using lead concentration as the response variable and the type of tissue (liver, kidney, or stomach contents) as the explanatory variable. The capture site and ID of specimens were included as random factors. Gamma distribution of the errors was assumed and a logarithmic function was used as a link for the response variable. Tukey's test was performed to make a posteriori comparisons.

The relationship between the lead concentration in each tissue of *R. norvegicus* (liver, kidney, and stomach contents) and environmental lead concentration (concentration of lead in soil and water), meteorological factors (temperature and precipitation), and rat characteristics (sex, head-body length, body weight, and age) was analyzed using GLMMs. A Gamma distribution of errors was assumed and a logarithmic function was used as a link for the three response variables (lead concentration in liver, kidney, and stomach contents). The variable year (2014 or 2015), capture season (summer or winter), environmental lead concentration, meteorological factors, and rat characteristics and their interactions were considered as explanatory variables in the

analysis, while the capture site was considered as a random factor. The Variance Inflation Index (VIF) was calculated to estimate the multivariate correlation between the variables and only those with a VIF lower than 5 were used so that the variables that enter in the model do not provide redundant information (Davis et al. 1986; Zuur et al. 2010). The criterion to select the best model was the one with the lowest Akaike's information criterion (AIC) and the smallest number of variables (for models with a change in AIC lower than 5).

The analyses were performed with the program R 3.4.3 (R Core Team 2017), using the lme4 package for the GLMM (Bates et al. 2015; Bates et al. 2017) and the multcomp library (Hothorn et al. 2008) to perform Tukey's test.

Results

The highest mean concentration of lead in *R. norvegicus* was detected in stomach contents, being 22 times higher than that in the liver and 4 times higher than that in the kidney, while the mean lead concentration in the kidney was 4 times higher than that in the liver (P values < 0.001, < 0.001, and < 0.001, respectively; Table 2). The concentration values of lead in the stomach ranged between 0.02 and 450 $\mu\text{g g}^{-1}$. The maximum value of lead concentration for the kidney was 24.5 $\mu\text{g g}^{-1}$, whereas that for the liver was 10.4 $\mu\text{g g}^{-1}$. Also, eight samples of the kidney exceeded the value of 15 $\mu\text{g g}^{-1}$ and two samples of the liver had values greater than 5 $\mu\text{g g}^{-1}$, values considered indicative of lead poisoning for small mammals (Ma 1996).

Regarding the environmental lead concentration, the sites located in the lower basin had, on average, higher values of lead in the soil than those located in the middle basin. In the case of the lead concentration in water, there was no clear trend, with the highest values found in sites 3 and 6 from the lower and middle basin, respectively (Fig. 1). Table 2 details the average lead concentrations in rat tissues and in water and soil samples for each capture site.

The lead concentration in rat tissues (kidney and liver samples) and the lead concentration in water and soil at the capture site were found to be positively associated (P values < 0.05 for all associations; Figs. 2 and 3). Besides, the lead concentration in the kidney of rats captured in winter was higher than that of rats captured in

Table 2 Average of lead concentration in rat tissues, water, and soil samples for each capture site

Capture site		Lead concentration				
		L (<i>n</i> , 133) ($\mu\text{g g}^{-1} \pm \text{SD}$)	K (<i>n</i> , 133) ($\mu\text{g g}^{-1} \pm \text{SD}$)	SC (<i>n</i> , 80) ($\mu\text{g g}^{-1} \pm \text{SD}$)	W (<i>n</i> , 24) ($\text{mg l}^{-1} \pm \text{SD}$)	S (<i>n</i> , 24) ($\text{mg kg}^{-1} \pm \text{SD}$)
Lower basin	S1	1.2 \pm 1.3	7.3 \pm 9.3	60.4 \pm 136.7	0.01 \pm 0.003	390.2 \pm 18.9
	S2	2.9 \pm 1.7	12.8 \pm 4.7	17.06 \pm 17.6	0.003 \pm 0.0	681.4 \pm 0.0
	S3	1.2 \pm 1.1	6.0 \pm 6.6	13.4 \pm 11.2	0.04 \pm 0.02	141.5 \pm 24.0
	S4	0.5 \pm 0.5	3.4 \pm 3.1	25.6 \pm 46.7	0.01 \pm 0.02	161.4 \pm 35.7
Middle basin	S5	1.3 \pm 2.1	7.2 \pm 6.3	22.7 \pm 42.0	0.01 \pm 0.004	170.2 \pm 40.3
	S6	0.1 \pm 0.04	0.1 \pm 0.05	3.55 \pm 4.24	0.04 \pm 0.0	22.1 \pm 0.0
	S7	0.3 \pm 0.2	0.1 \pm 0.04	0.68 \pm 0.74	0.03 \pm 0.0	58.8 \pm 0.0
	S8	0.5 \pm 0.6	3.1 \pm 1.9	14.7 \pm 13.6	0.01 \pm 0.00	68.7 \pm 43.7
	S9	0.1 \pm 0.1	1.3 \pm 0.9	5.9 \pm 2.7	0.005 \pm 0.001	13.6 \pm 19.2
Total		1.0 \pm 1.4 ^c	4.8 \pm 5.5 ^b	21.8 \pm 58.9 ^a	0.02 \pm 0.02	200.5 \pm 168.8

L, liver; K, kidney; SC, stomach contents; W, water; S, soil. All values for tissues and soil were expressed in dry weight. Different letters indicate significant differences (P value < 0.001)

summer (P value < 0.001; Fig. 2), while the lead concentration in the liver was positively associated with the age of the rats and greater in rats captured in 2015 than in those captured in 2014 (P value < 0.05; Fig. 3). However, because no significant interactions were detected, these variables did not affect the relationship found between lead concentration in rat tissues and the lead concentration in the environment. Neither the remaining variables related to the rat characteristics nor the meteorological variables showed a significant relationship with the lead concentration in the kidney or liver. Probably, the fact that seasonality includes the meteorological variables analyzed in this study could explain why they did not contribute to explaining the changes in the concentration of lead in these organs in the final model. Finally, the lead concentration in the stomach content was not related to any of the variables analyzed.

Discussion

The results of the present study showed a positive relationship between the lead concentration in soft tissues (liver and kidney) of *Rattus norvegicus* and the environmental concentration of this metal in water and soil. Therefore, our results allow us to confirm that the quantification of lead in the kidney and liver of rats can be used as an indicator of exposure to this metal. In accordance with these results, studies carried out in *R. norvegicus* and other wild rodents have found a higher lead concentration in animals from contaminated sites than in those from less contaminated sites (Al Sayegh Petkovšek et al. 2014; Hermoso de Mendoza García et al. 2008; Jankovská et al. 2008; Kålås et al. 2000; Lunardelli et al. 2018; Pereira et al. 2006; Way and Schroder 1982). Our results suggest that the lead concentration in soft tissues of *R. norvegicus* is sensitive

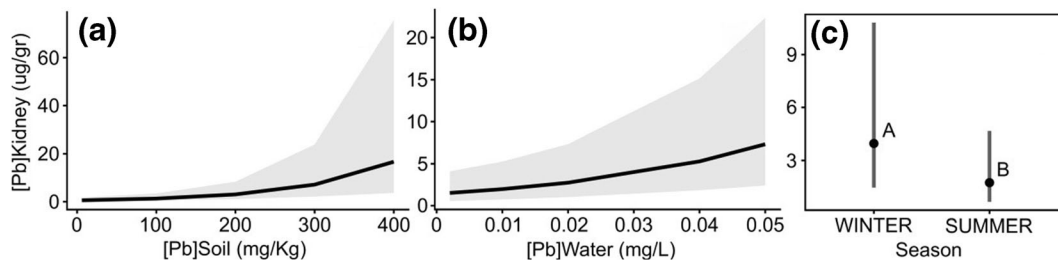
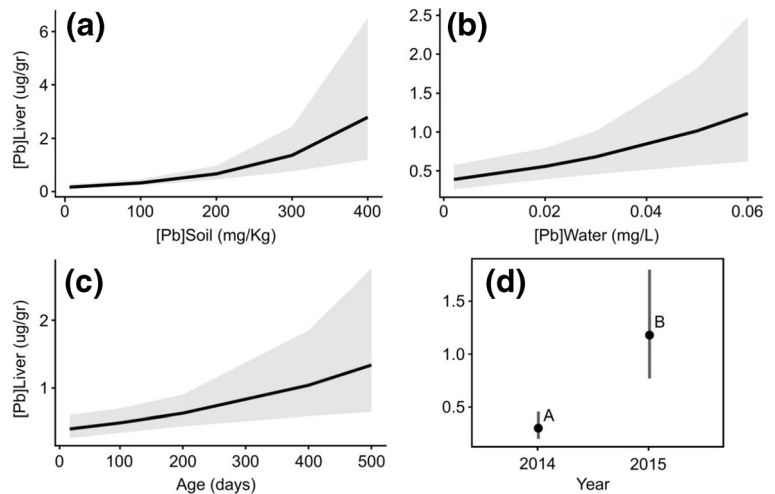


Fig. 2 Relation between kidney lead concentration and the explanatory variables selected in the best model: **a** soil lead concentration (P value < 0.001); **b** water lead concentration (P value <

0.001); and **c** season of capture of individuals. Gray areas and lines indicate the confidence interval of the lines (**a** and **b**) or points (**c**). Different letters indicate significant differences (P value < 0.001)

Fig. 3 Relation between liver lead concentration and the explanatory variables selected in the best model: **a** soil lead concentration (P value < 0.001); **b** water lead concentration (P value < 0.05); **c** age of individuals (expressed in days) (P value < 0.001); and **d** year of capture of individuals. Gray areas and lines indicate the confidence interval of the lines (**a–c**) or points (**d**). Different letters indicate significant differences (P value < 0.05)



to detect changes in a lead concentration gradient and not only in radically opposite situations of contamination.

The determination of the level of accumulation of metals in soft tissues is important for the diagnosis of poisonings caused by exposure to them (Johnson et al. 1978; Komarnicki 2000; Ma 1989; Sharma and Shupe 1977). Ma (1996) reported that clinical signs of intoxications with lead in mammals appear to be associated with lead levels above $15 \mu\text{g g}^{-1}$ in the kidney and above $5 \mu\text{g g}^{-1}$ in the liver (dry weight) because these values may lead to structural and functional tissue damages. In this study, 8% of the rats captured in the MRRB exceeded the threshold values for at least one of these two organs, indicating that the lead levels to which rats were exposed are high enough to produce tissue damage in the individuals. Other studies in small mammals have found that concentrations of lead even lower than those referred above can induce histopathological changes in the kidney and liver (Ceruti et al. 2002; Damek-Poprawa and Sawicka-Kapusta 2003; Pereira et al. 2006). This suggests that the risk due to exposure to this metal in the MRRB could be even greater.

According to Ma (1989), one of the main consequences of chronic exposure of animals to lead is that they exhibit a higher average lead concentration in the kidney than in the liver. Indeed, in the present study, we detected higher concentrations of lead in the kidney than in the liver, which suggests that rats in the MRRB have been exposed to a chronic lead contamination.

The concentrations of lead found in this study in both soft organs and stomach contents of *R. norvegicus* were highly variable within the same site and even within the same rodent trapping event. This variability has also been observed in the human populations residing in the MRRB, where wide variations in blood lead levels were detected in people living in the same neighborhood (and sometimes even in the same family) (Miller 2014). Possibly, the wide variations of these data could be associated with the age, sex, nutritional status, life history, and genetic variability of each individual, as well as with sources of lead exposure in the environment and exposure times to the pollutants. In this aspect, different studies have shown contradictory results. For example, in the present study, one of the factors that explained the levels of lead concentration in the liver was the age of the rats. Similarly, Ceruti et al. (2002), Way and Schroder (1982), and Garcia et al. (2011) showed that the lead accumulation in several tissues, especially in the kidney, liver, and lungs, was positively correlated with rodent age. However, in contrast, other studies performed in small mammals found no relationship or even a negative relationship between lead concentration in tissues and age (Damek-Poprawa and Sawicka-Kapusta 2003; Marcheselli et al. 2010; Zarrintab and Mirzaei 2017). Several authors have suggested a greater gastrointestinal absorption of lead in younger individuals, which could explain the inverse relationship between age and metal concentration (Kostial et al. 1978; Zarrintab and Mirzaei 2017). Other explanations relate

the lead concentration in an organism with the home range size, which covariates with the age of the individual (Burger et al. 2007; Zarrintab and Mirzaei 2017). In situations where contamination is not homogeneously distributed, larger specimens (which have a larger home range) are more likely to spend more time feeding in areas where they obtain food with different levels of contamination. On the other hand, Janiga et al. (2012) suggested that the lead concentration in liver tissue is often assumed as a stable detoxification mechanism to prevent toxic effects in the rest of the organism and does not show a clear bioaccumulation pattern with age.

Regarding seasonal variations, Janiga et al. (2012) and Massányi et al. (2003) found that the season had an effect on the lead concentration in soft tissues in snow voles and brown hares, respectively. These authors indicated that these differences were due to a higher lead intake during winter feeding due to changes in the food available for consumption. Our results showed changes between seasons for the lead concentration in the kidney but not for that in the stomach contents or liver. It is important to note that the stomach contents only indicate the ingestion of lead from the last hours prior to capture, while the average life time of this metal in soft tissues, such as the kidney and liver, ranges between 3 and 4 weeks (Mykkanen et al. 1979; Scheuhammer 1991). Therefore, the differences found between seasons in the kidney reflect a higher exposure to lead contamination in winter, possibly related to changes in the feeding behavior or in the home range of these animals or in the availability of different foods between winter and summer.

Conclusions

We consider that this study represents an important contribution regarding the use of *R. norvegicus* as a sentinel species to assess the risk of lead exposure because it constitutes the first study carried out in an urban basin with a gradient of environmental lead concentration. We observed that, although variables such as life history, microhabitat use, and meteorological factors must be considered, they did not affect the association between the lead concentration in soft tissues and that in the environment. Thus, we consider that *R. norvegicus* is a good sentinel species of lead contamination and suggest that further studies should consider environmental lead bioavailability to confirm the potential

application of this species as an indicator of exposure to this metal within larger basins.

Finally, studies in this type of environment involve several problems such as high crime rates, theft of traps, and periodic flooding, all of which make it difficult to obtain an optimal sample size. Although more studies involving other heavy metals and biomarkers are needed, the results here obtained indicate that the environmental lead present in the MRRB is bioavailable and that individuals living here are chronically exposed to lead concentrations capable of causing toxic effects. It is also important to note that rats and humans do not explore the environment in the same way and the entryways of lead into the body are not equal. As rats live in burrows and are closely associated with water sources, this could result in a greater intake of lead contained in the soil and water. Anyway, considering that the MRRB is a highly populated area whose population lives on the floodplain of rivers and presents a disfavored socioeconomic situation residing in precarious housings with a lack of access to health centers, this work highlights the potential and neglected health risk to which people are exposed to. Therefore, finding an optimal biological indicator to assess the risk of heavy metal contamination on health in urban areas worldwide is important to provide useful information to policy makers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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