Mercury, lead and cadmium concentrations in *Talpa occidentalis* and in their digeneans of the genus *Ityogonimus*

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Abstract

Many parasites living in aquatic ecosystems are useful indicators of environmental health. However, information is yet scarce with respect to helminth parasites of vertebrates living in terrestrial ecosystems as monitoring tools for toxic element environmental pollution. The present study evaluates the suitability of the model *Talpa occidentalis* / *Ityogonimus* spp. as a bioindicator system for mercury (Hg), lead (Pb) and cadmium (Cd) contamination in agricultural soils from Asturias (Spain). Thirty-six *T. occidentalis* specimens (14 infected by *Ityogonimus* spp.) were analyzed. The highest mean levels of Hg and Pb were found in *Ityogonimus* individuals (20.9 and 12.4 µg g^{-1} wet weight, respectively). Considering renal and hepatic concentrations in *T. occidentalis*, bioaccumulation factors of *Ityogonimus* for Hg were 83.7 and 58.6 respectively, whereas concerning Pb bioaccumulation factors were 38.2 and 82.9, respectively. No bioaccumulation was detected in *Ityogonimus* in the case of Cd. More studies involving digenean parasites of small mammals are needed, especially when biomonitoring environmental toxic element pollution in terrestrial ecosystems. The present results support the above-mentioned model as a suitable biomonitoring system to evaluate environmental Hg and Pb contamination in terrestrial non-urban Iberian habitats. Similar models involving other species (*Talpa* spp. / *Ityogonimus* spp.) might be used in a much wider geographical range.

Keywords: Pollution; parasites; Talpidae; biomonitoring model; terrestrial ecosystems

Introduction

Toxic elements are widespread in the environment and can be responsible for negative effects in the biota. In environmental impact studies, certain free-living organisms provide valuable information about the chemical state of their environment through their bioaccumulation capacities. The use of small mammals to evaluate these effects is yet limited despite their proven relevancy to predict environmental risk [1-3]. On the other hand, several studies have evidenced that some helminth parasites are able to accumulate much more trace elements than their respective definitive host. Therefore, parasites are useful monitors for environmental health since they reveal the availability of contaminants, even if environmental concentrations are expected to be low, while tolerating high contaminant burdens. However, most of the helminth / host models tested for this purpose (involving several vertebrates) have been performed in freshwater habitats [4]. Contrarily, in terrestrial systems, only a few studies have tested this capacity mostly involving cestodes and/or acanthocephalan parasites of small mammals [5-10]. Concerning terrestrial digeneans, to date only two models involving larger mammals have been evaluated, using cattle (porcine and bovine) and buffaloes infected by *Fasciola* specimens [11, 12]. This lack of information motivates the need for further studies on parasitological models involving digeneans that may reflect small-scale differences in heavy metal contamination in terrestrial ecosystems.

The present study evaluates for the first time a model involving a small mammal and their adult terrestrial trematodes (*Talpa occidentalis* / *Ityogonimus* digeneans) in order to determine its potential usefulness as a biomonitoring tool for mercury (Hg), lead (Pb) and cadmium (Cd) under natural field conditions.

Materials and methods

Moles of the genus *Talpa* are strictly subterranean mammals, which are widely distributed throughout the western Palearctic region from the Iberian Peninsula to Siberia. The genus *Talpa* involves nine species that present an irregular distribution [13]. In fact, *T. caeca* and *T. europaea* present a wide distribution in Europe whereas *T. occidentalis* is restricted to the Iberian Peninsula, *T. romana* to Italy and *T. stankovici* to the Balkans (see Nicolas et al. [14] for more information on the evolutionary history of Talpidae in Europe). The Iberian mole, *Talpa occidentalis* Cabrera, 1907 (Eulipotyphla: Talpidae) is a fossorial small mammal that feeds on invertebrates (mainly earthworms and insect larvae) categorized as a Least Concern species by the IUCN that inhabits only in Portugal and Spain [15].

Ribas and Casanova [16] concluded that only three digeneans (*Combesia macrobursata*, *Ityogonimus lorum* and *I. ocreatus*) are exclusive parasites of *Talpa*. The genus *Ityogonimus* includes only three species: *I. lorum*, *I. ocreatus* and *I. scalopi*. Species of the genus *Ityogonimus* are Brachylaimidae digenids belonging to the Ityogoniminae subfamily that includes parasites of birds and mammals, using terrestrial molluscs as intermediate hosts [17]. *Ityogonimus scalopi* parasitizes Nearctic moles of the genus *Scalopus*. *Ityogonimus lorum* and *I. ocreatus* frequently infect *T. occidentalis*, *T. europaea* and *T. romana* in the Palearctic but mixed infections were evidenced for the first time in *T. occidentalis* by Adalid et al. [18].

All analyzed *T. occidentalis* individuals were collected in Asturias, in the northern maritime facade of Spain, at small agricultural plots (mainly apple orchards used in the production of cider). All individuals (36 adult specimens of *T. occidentalis*) were accidentally trapped with snap traps (Topcat®, Switzerland) placed in galleries during vole pest control campaigns from February 2011 to February 2012. They were dissected using stainless steel instruments, and scanned for *Ityogonimus* intestinal specimens. Samples of around 150 mg (wet weight, w. w.) of kidney and liver of each specimen were collected and stored in glass vials (frozen at -20ºC). Specimens of *Ityogonimus* (both *I. lorum* and *I. ocreatus*) were also stored in the same form until element analysis. The analyzed *T. occidentalis* specimens were grouped in two subsamples of 14 moles infected with mixed *Ityogonimus* spp. (38.88% of prevalence) and 22 moles uninfected by these digeneans. Each sample was digested in Teflon vessels with 2 mL HNO_3 (Merck, Suprapur) and 1 ml H_2O_2 (Panreac) at 90ºC in an oven and left overnight. All materials used in this process were thoroughly acid-rinsed. After digestion, samples were diluted with 30 ml of Milli-Q water. The whole process was standardized and validated at the CCiTUB ("Centres Científics i Tecnològics de la Universitat de Barcelona"). Blanks and standards (National Research Council, Canada) were used to obtain the detection limits as well as the accuracy of the results. Total concentrations of all elements were quantified by inductively coupled plasma mass spectrometry (ICP-MS, Perking Elmer Elan 6000). The detection limits (mean blank value plus three standard deviations) were always inferior to one ng ml^{-1} and the accuracy values were always higher than 95% for all three elements. Concentrations refer to wet weight tissues. Bioaccumulation factors (BF) for elements in the digeneans (*Ityogonimus* spp.) were calculated as [Element $_{parasite}$] / [Element $_{host\ tissue}$] as proposed Sures et al. [19]. In several cases datasets did not present a normal distribution and variances for some groups were significantly different even after log-transformation. Thus, Mann Whitney U tests and Kruskal−Wallis tests with the post-hoc Dunn’s test were used instead of parametric tests. All tests were performed in Prism 5, and a minimum significance level $P < 0.05$ was applied in all tests.

Results

Mercury, lead and cadmium mean concentrations in hepatic and renal tissues of both infected and uninfected subsamples of *T. occidentalis* as well as in *Ityogonimus* specimens are represented in Figure 1. Mercury, lead and cadmium mean concentrations (±SE) along with their median and range are presented in Table 1. Comparing infected with non-infected hosts, no significant differences were detected between median concentrations of the analysed toxic elements considering either kidney or liver tissues (see Table 1, Mann-Whitney tests' results). The highest mean concentration of Hg and Pb were found in two individual samples of *Ityogonimus* (73.9 and 45.1 µg g^{-1} w. w., respectively). Contrarily, the highest value of Cd (14.9 µg g^{-1} w. w) was recorded in renal tissue of one *T. occidentalis* uninfected by flukes.

The mean Hg concentration found in the digeneans was nearly 84-times higher than that found in renal tissues and 59-times higher than Hg concentrations in hepatic tissues of infected hosts (Table 2). In fact, significantly higher median Hg concentrations were found ($H=24.6$, $P<0.0001$) in the parasites in comparison to renal and hepatic tissues of infected hosts (Dunn's test: kidney, $p<0.0001$; liver, $p=0.0002$) although no significant differences were found between renal and hepatic tissues of infected hosts (Dunn's test, $p>0.9999$).

The mean Pb concentration found in *Ityogonimus* was 38-times higher than that found in renal tissue and 83-times higher than that found in hepatic tissues of their hosts (Table 2). Significantly higher median Pb concentrations were found ($H=31.2$, $P<0.0001$) in the parasites in comparison to renal and hepatic tissues of infected hosts (Dunn's test: kidney, $p=0.0012$; liver, $p<0.0001$) although no significant differences were found between renal and hepatic tissues of infected hosts (Dunn's test, $p>0.9999$).

The mean Cd concentration found in *Ityogonimus* was only 1.3-times higher than that found in hepatic tissues of their hosts (Table 2). No significant differences were thus found when comparing median Cd concentrations in parasites, renal and hepatic tissues of infected hosts ($H=3.18$, $P=0.2038$). However, when comparing the Cd median concentration exclusively between *Ityogonimus* and host liver values, the Cd concentration was in fact significantly higher in the parasite than in hepatic tissue of infected hosts (see Table 1, Mann Whitney test, U=56; P=0.0278).

Discussion

Several studies aiming to investigate terrestrial environmental quality have mainly been done on expectedly polluted areas or after environmental accidents [3, 7, 20]. However, scarce attention has been paid to areas of high ecological or economic importance that may also be subject to several types of chronic low direct or indirect anthropogenic chemical stress, making them less suitable for wildlife or human activities [8, 21, 22]. In this context, the field of Environmental Parasitology focusing on parasites as indicators of environmental contamination seems to be useful for monitoring both environmental health and anthropogenic impacts [4].

The role of mercury accumulation in terrestrial food webs is often neglected and studies on aquatic and marine environments are preponderant when compared to terrestrial ecosystems. In this sense, there are not many works quantifying levels of Hg in small or medium terrestrial mammals. The prevalent routes of Hg intake in terrestrial mammals include inhalation but ingestion is usually the most important pathway, therefore, being strongly influenced by preferences and food selection. Moles feed on invertebrates, especially earthworms [23], which constitute a very important part of the total biomass of the soil fauna and play an important role in the heavy metal intake of moles due to its demonstrated ability to efficiently accumulate these elements from soils [24]. In fact, earthworms represent the most important link in trophic pathways of contaminants from soil to predators representing serious risks of secondary poisoning of vertebrate predators due to biomagnification [25, 26]. Although more attention has been paid to earthworm bioaccumulation of several elements other than mercury, they are also known to accumulate this element [27]. Also, the uptake of Hg is influenced by the presence of organic matter and different conditions of the soil [28].

There are several possible origins of toxic elements bioavailable to small mammals. Mercury-containing compounds were widely used as fungicides for seeds and often used in conjunction with other organic insecticides; consequently seed-eating small or medium mammals were particularly exposed to hazardous Hg concentrations [29]. Bull et al. [30] analyzed bank voles (*Clethrionomys glareolus*) from England and reported renal and hepatic concentrations of mercury (80 and 60 ng g^{-1} w. w., respectively) in a control area whereas levels near a polluted area amounted to 350 and 150 ng g^{-1} w. w., respectively. More recently, Eira et al. [6] analyzed the model *Oryctolagus cuniculus* / *Mosgovoyia ctenoides* in Portugal and reported concentrations of 80 and 40 ng g^{-1} w. w. in kidney and liver of these lagomorphs and a concentration of 40 ng g^{-1} w. w. in their intestinal cestodes. These authors concluded that the relatively high renal level of mercury in rabbits of Dunas de Mira (Portugal) might have been related with the focalized and long-lasting use of organomercury fungicides in a large flower production facility in the area. In the present study, our results indicate that Hg contamination in the sampled area (soil, sediment, water) or particularly in mole prey may be quite high, since internal tissues of *T. occidentalis* presented mean concentrations of 231 and 405 ng g^{-1} w. w. (respectively kidney and liver). On the other hand, the high bioaccumulation factors produced by the concentrations observed in the analyzed *Ityogonimus* specimens confirm the role of the model *T. occidentalis* / *Ityogonimus* spp. as a very promising bioindicator system for mercury contamination.

Mean concentrations of lead in kidney and liver of *T. occidentalis* both in uninfected individuals and in those infected by *Ityogonimus* were around 325 and 150 ng g^{-1} w. w., respectively. The concentration of Pb was much higher in *Ityogonimus* specimens (12373.6 ng g^{-1} w. w.), which implies high Pb bioaccumulation factors (38.2 and 82.9 respect to their host kidney and liver). The lead values reported here are comparatively high with respect to values obtained (60 and 160 ng g^{-1} w. w. in kidney and liver, respectively) in *O. cuniculus* in Portugal [6]. Although the intake of this toxic element may again be related with the different type of food items ingested by moles (mostly earthworms) and wild rabbits (different types of vegetation), Eira et al. [6] pointed out that their study area was used for game and possible shot injuries prior to rabbits' death could explain the high Pb concentrations in the analyzed wild rabbits. The hypothesis that hunting ammunition is one of the wildlife sources of Pb contamination has been suggested in several mammals mostly because of ingestion of lead pellets [31]. This could also be one of the reasons for the elevated Pb concentration in *T. occidentalis* in the present study. However, the ingestion of earthworms living in Pb contaminated soils should account for most of the metal intake by moles since bioaccumulation of Pb by earthworms is well-documented [32].

In the study performed by Sures et al. [5] in two polluted areas adjacent to the city of Cairo (Egypt), the model *Rattus norvegicus* / *Hymenolepis diminuta* was investigated to test the suitability of this system as an indicator for Pb pollution under field conditions. The concentrations of lead were always inferior to 100 ng g^{-1} w. w. in liver and around 200 ng g^{-1} w. w. in kidney. Despite the surely different diets between rodents in large urban areas and moles in agriculture areas, Pb concentrations in tissues of *T. occidentalis* in an agricultural area with very low human population pressure are higher than lead levels found in an omnivorous small mammal inhabiting a very large city. Similarly, Torres et al. [7] evaluated the model *Apodemus sylvaticus* / *Skrjabinotaenia lobata* in a dumping site near Barcelona (Spain) where the field mouse (*A. sylvaticus*) presented kidney and liver lead concentrations of 144.8 ng g^{-1} w. w. and 23.1 ng g^{-1} w. w., respectively, which were much lower than the values reported in the present study. These models involving small sized cestodes such as *H. diminuta* or *S. lobata* in expectedly polluted sites were shown to be promising tools for biomonitoring lead contamination [5, 7]. However, the model proposed in the present study is probably a much better small mammal/helminth Pb biomonitoring tool for the evaluation of low contamination in certain areas, since no other model analyzed until now produced higher lead bioaccumulation factors.

In general, cadmium levels reported in the present study are much higher than renal and hepatic concentrations reported by Eira et al. [6] in *O. cuniculus* from Portugal. Taking into consideration that rabbits and moles feed on very different prey items, which might account for the different concentration levels in each host species, it is still difficult to infer about the origin of this apparent higher contamination in the present study area. However, considering the presence of small agriculture fields, the contamination levels observed in the present study might result from the application of phosphate fertilizers, which usually represent a direct input of Cd into arable soils [33-36].

No significant differences were found between median renal and hepatic concentrations of Cd in *T. occidentalis* infected by *Ityogonimus* in comparison to the respective medians in not infected individuals. Despite the relatively high levels of cadmium in *T. occidentalis* tissues, the median concentration observed in *Ityogonimus* was still significantly higher than the Cd median concentration in their host's liver (infected moles). It is, however, clear that *Ityogonimus* sp. do not bioaccumulate Cd up to values such as those shown by other models involving small mammals and their cestodes [8]. Thus, based on the present results, it is not possible to confirm the model *T. occidentalis* / *Ityogonimus* as a promising bioindicator for environmental Cd contamination.

Moles of the genus *Talpa* are widely distributed throughout the western Palearctic region. As referred earlier, Ribas and Casanova [16] concluded that only three digeneans (*Combesia macrobursata*, *Ityogonimus lorum* and *I. ocreatus*) are exclusive parasites of *Talpa*. *Ityogonimus lorum* and *I. ocreatus* frequently infect *T. occidentalis, T. europaea* and

T. romana in the Palearctic. Thus, the model proposed in the present study involving digeneans of *T. occidentalis* might be reproduced throughout Europe using other *Talpa* spp. / *Ityogonimus* spp. models. Notwithstanding, information about the mechanisms responsible for trace element uptake by helminths is scarce and, in the future, more attention should be paid to other digenean parasites of small mammals when biomonitoring environmental contamination in terrestrial ecosystems.

Concluding remarks

The mercury and lead bioaccumulation factors reported in the present study for *Ityogonimus* are very high considering the theoretically low level of contamination at the sampling area. Therefore, the model *Talpa* spp. / *Ityogonimus* spp. is a promising biomonitoring system to evaluate environmental mercury and lead contamination in several terrestrial non-urban habitats where both genus are present. Future research should focus on evaluating mercury and lead in soil, water, and mole prey (mainly earthworms) from the study area.

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Fig. 1 Mercury, lead and cadmium mean concentrations in hepatic and renal tissues of both infected (+) and uninfected (-) subsamples of *T. occidentalis* as well as in *Ityogonimus.* Error bars indicate Standard Error.

Graphics were produced in PRISM 5 and joined in Photoshop CS6 in tiff format

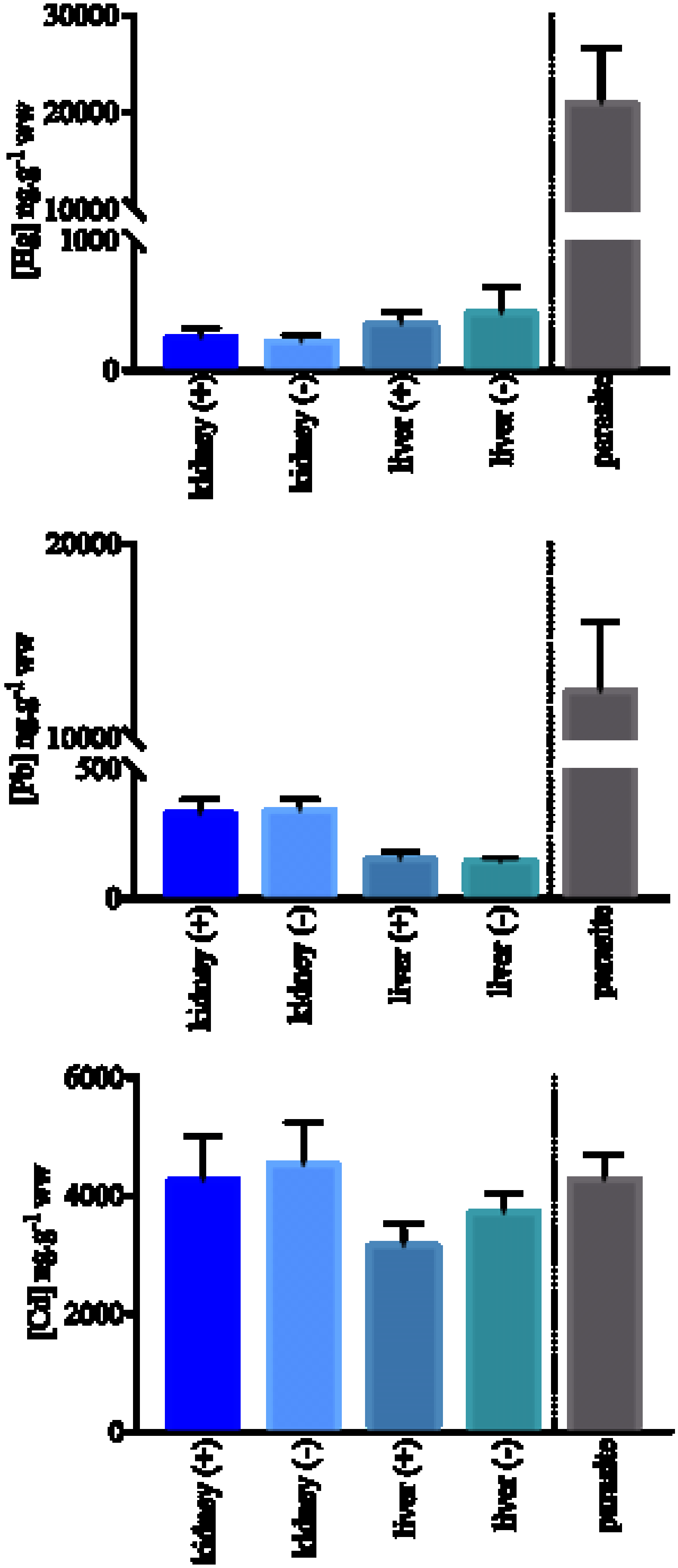
Table 1 Mercury, lead and cadmium concentrations (ng g^{-1} w. w.) in renal and hepatic tissues of *T. occidentalis* infested (+) and non-infested (-) by *Ityogonimus* spp. and in the digeneans. SE, Standard Error.

		Host (+)	Host (-)	Host (+) vs (-)	
		Mean ± SE	Mean ± SE	Mann-Whitney	
		Median (min-max)	Median (min-max)	U	P
Hg	Kidney	249.6 ±77	218.6 ±52		
		111 (16.3-927.4)	141 (17.5-861.6)	150	0.455
	Liver	356.2 ±94.01	435.6 ±203.1		
		230 (17.7-974.3)	138 (22.3-4499)	147	0.418
	Parasite	20891.7 ±5766			
		14745 (365.7-73956)			
Pb	Kidney	324.1 ±58.5	337.7 ±46.1		
		245.3 (115-824)	278.4 (75.5-1010)	144	0.381
	Liver	149.3 ±31.8	141.0 ±12.6		
		93.0 (51-458)	133.5 (54.4-324)	117	0.120
	Parasite	12374.6 ±3648			
		7149 (1375-45038)			
Cd	Kidney	4247.6 ±769	4516.8 ±725		
		4031 (292-10183)	4128 (517-14944)	152	0.481
	Liver	3149.5 ±389	3703.5 ±344		
		3167[a] (587-5642)	3845 (664-6927)	122	0.156
	Parasite	4260.4 ±443			
		4602[a] (1339-7155)			

[a] Cd concentration is significantly higher in the parasite than in liver of infected hosts (U=56; P=0.0278)

Table 2 Mercury, lead and cadmium bioaccumulation factors (BF = [Parasite] / [Host (+)]) in renal and hepatic tissues of *T. occidentalis* infested by *Ityogonimus* spp.. ns, not significant.

			Kruskal- Wallis		Dunn's test		
	BF Kidney	BF Liver	H	P	Kidney *vs* Liver Adj-P	Parasite *vs* Kidney Adj-P	Parasite *vs* Liver Adj-P
Hg	83.7	58.6	24.6	<0.0001	ns	<0.0001	0.0002
Pb	38.2	82.9	31.2	<0.0001	ns	0.0012	<0.0001
Cd	≈1	1.3	3.18	0.2038	ns	ns	ns


[Hg] ng.g-1 ww
30000
20000
10000
1000
0
kidney (+)
kidney (-)
liver (+)
liver (-)
parasite
[Pb] ng.g-1 ww
20000
10000
500
0
kidney (+)
kidney (-)
liver (+)
liver (-)
parasite
[Cd] ng.g-1 ww
6000
4000
2000
0
kidney (+)
kidney (-)
liver (+)
liver (-)
parasite