VERSITA

Cadmium and lead concentrations in *Moniliformis moniliformis* (Acanthocephala) and *Rodentolepis microstoma* (Cestoda), and in their definitive hosts, *Rattus rattus* and *Mus domesticus* in El Hierro (Canary Archipelago, Spain)

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Abstract

Information on parasites of vertebrates living in terrestrial ecosystems as monitoring tools for heavy metal environmental pollution is scarce. The present study evaluates the potential suitability of the models *Rattus rattus*/*Moniliformis moniliformis* and *Mus domesticus*/*Rodentolepis microstoma* as promising bioindicator systems for cadmium and lead pollutions under natural conditions. The highest level of cadmium was found in one specimen of *M. moniliformis* (335.2 ng g^{-1} wet weight) and the average concentration of Cd in the acanthocephalan was significantly higher than values found in *R. rattus* liver and kidney tissues. The maximum concentration of lead occurred in one specimen of *R. microstoma* (567.4 ng g^{-1} wet weight) and the average concentration of Pb in the cestode was significantly higher than values found in *M. domesticus* liver, kidney and muscle tissues. The present results allow proposing both models as promising biomonitoring systems to evaluate environmental cadmium pollution (mainly *R. rattus*/*M. moniliformis*) and lead contamination (especially *M. domesticus*/*R. microstoma*) in terrestrial non-urban habitats.

Keywords

Heavy metals, *Moniliformis moniliformis*, *Rodentolepis microstoma*, biomonitoring system, environmental pollution, Canary Archipelago

Introduction

Heavy metals are widespread in the environment and can be responsible for toxic effects in the biota. The use of rodents to evaluate these effects is limited despite their proven relevancy to predict environmental risk (Alleva *et al*. 2006, Hamers *et al*. 2006, Sánchez-Chardi *et al*. 2007). Using small mammals for biomonitoring is useful for the assessment of environmental quality, especially in areas of high ecological interest such as El Hierro Island (Canary Archipelago).

Apart from the use of small mammals when inferring about the presence of toxic elements in certain habitats, recent studies evidenced that some helminth parasites are able to accumulate much more heavy metals than their hosts in various environments. However, most of the helminth/host systems tested for this purpose (involving fish, birds or mammals) have been examined in freshwater habitats (e.g. Sures 2004, 2008; Vidal-Martínez *et al*. 2010). Therefore, the available information about systems involving rodents and some of their helminth parasites is scarce. In fact, few experimental (Scheef *et al*. 2000; Sures *et al*. 2000a, 2002) and field studies (Sures *et al*. 2003; Torres *et al*. 2004, 2006) have been performed using cestode and acanthocephalan parasites of rodents. This general lack of information motivates the need for further studies on organisms or models that may reflect small-scale differences in heavy metal pollution in terrestrial ecosystems.

In non-synanthropic areas of El Hierro Island, *Rattus rattus* and *Mus domesticus* are the only existing rodent species, which are often infected by *Moniliformis moniliformis* and *Rodentolepis microstoma*, respectively. The aim of the present study is to evaluate the potential suitability of both host-

parasite systems (*R. rattus/M. moniliformis* and *M. domesticus/R. microstoma*) as promising bioindicators for cadmium and lead pollution under natural field conditions. In addition, this study allowed inferring about the degree of environmental quality in this emblematic island of the Canary Archipelago.

Materials and methods

El Hierro (27°44′N, 18°33′W), the smallest island of the Canary Archipelago, Spain, was declared a Biosphere Reserve by UNESCO in 2000. It has a surface of 268 km². Rodents were collected using Sherman traps in several areas of the island during 2008 and 2009. After rodents have been scanned for helminths, 14 *Rattus rattus* parasitised by *M. moniliformis* (collected in the "Lagartario" area) and 10 *Mus domesticus* parasitised by *R. microstoma* (collected in the "Fayal-Brezal" area) were selected for cadmium and lead analysis.

The "Lagartario" capture site (27°46′N, 17°59′W and only 76 meters a.s.l.) is close to the main road in the island connecting Valverde (the capital of the island) and the village of Frontera. The "Fayal-Brezal" capture site (27°44′N, 17°59′W) is located far away of any village in the highest part of the island (1305 meters a.s.l.).

Samples of kidney, liver and muscle of each rodent as well as adult specimens of *M. moniliformis* and gravid *R. microstoma* were taken using stainless-steel instruments. All samples were frozen at –20°C until being processed for metal analysis. Around 50–150 mg (wet weight) of the above mentioned samples were mineralized in Teflon vessels using 2 ml HNO_3 (Merck, Suprapur) and 1 ml H_2O_2 (Panreac) and left overnight in an oven at 90°C. The whole process was standardized and validated in the (detection limits and accuracy of results) as described in detail in Torres *et al.* (2004). All concentrations were presented as ng g⁻¹ wet weight. Bioaccumulation factors (BF) in helminths were calculated as proposed by Sures *et al.* (1999).

Results

Mean cadmium and lead concentrations in tissues of analysed rodents and their helminths are shown in Table I. The detection limits (mean blank value plus three standard deviations of the mean blank) for both elements were lower than 1 ng ml⁻¹ and accuracy values were greater than 95%.

The highest level of cadmium was found in one specimen of *M. moniliformis* (335.2 ng g⁻¹ wet weight). The maximum concentration of lead occurred in one specimen of *R. microstoma* (567.4 ng g⁻¹ wet weight). Similarly, the highest average concentration of cadmium was found in *M. moniliformis* (95.6 ng g⁻¹), whereas the highest average concentration of lead was found in *R. microstoma* (241.3 ng g⁻¹).

There were significant differences in Cd average concentrations among *R. rattus* tissues and also between hosts' tissues and the acanthocephalan *M. moniliformis* (Kruskal-Wallis, H = 39.77, P<0.001). In fact, the cadmium average concentration is lower in *R. rattus* muscle when comparing to both liver and kidney tissue (Dunn's test, P<0.05 and P<0.001, respectively) and its average concentration in the acanthocephalan is higher than in *R. rattus* liver and muscle tissue (Dunn's test, P<0.01 and P<0.001, respectively). With respect to *M. domesticus*, there were also significant differences in cadmium average concentrations among the analysed tissues when comparing host and *R. microstoma* (Kruskal-Wallis test, H = 27.04, P<0.001). Both *R. microstoma* and kidney tissue presented higher average cadmium concentrations than muscle tissue (Dunn's test, both P<0.001). When comparing the analysed helminths, the average cadmium concentrations in *M. moniliformis* (parasitising *R. rattus*) was higher than in *R. microstoma* (parasitising *M. domesticus*) (Mann-Whitney test, U = 30.00, P = 0.02).

With respect to lead, there were significant differences in average concentrations among *R. rattus* tissues and also when comparing host tissues to *M. moniliformis* (Kruskal-Wallis, H = 42.78, P<0.001). In fact, considering the hosts' tissues, the lead average concentration is higher in kidneys when comparing to both liver and muscle tissue (Dunn's test, P<0.01

Table I. Concentrations of cadmium and lead in tissues of *R. rattus* and *M. domesticus* and in *M. moniliformis* and *R. microstoma* (ng g⁻¹ wet weight; mean ± SE). BF = bioaccumulation factors

		R. rattus	BF	*M. domesticus*	BF
Cd	Kidney	77.8 ± 23.9	1.2	41.5 ± 10.1	1.2
	Liver	14.7 ± 3.0	6.5	12.5 ± 0.9	3.9
	Muscle	1.1 ± 0.2	86.9	0.8 ± 0.2	60.6
	M. moniliformis	95.6 ± 21.9			
	R. microstoma			48.5 ± 14.6	
Pb	Kidney	87.4 ± 14.7	1.6	9.2 ± 0.9	26.2
	Liver	22.5 ± 2.2	6.3	13.3 ± 2.0	18.1
	Muscle	12.6 ± 1.8	11.2	8.6 ± 0.6	28.1
	M. moniliformis	141.6 ± 27.7			
	R. microstoma			241.3 ± 51.6	

and P<0.001, respectively). Also, the lead average concentration in *M. moniliformis* is higher than in the liver and muscle tissue of *R. rattus* (Dunn's test, P<0.001 and P<0.001, respectively). The most interesting results refer to the significant differences detected among the lead average concentrations in *M. domesticus* tissues and *R. microstoma* (Kruskal-Wallis, H = 24.51, P<0.0001). In fact, the lead average concentration in *R. microstoma* was higher than in *M. domesticus* liver, kidney and muscle tissue (Dunn's test, P<0.05, P<0.001 and P<0.001, respectively). When comparing the average lead concentrations detected in hosts' tissues, kidney and liver presented higher values in *R. rattus* than in *M. domesticus* (Mann-Whitney test, U = 00.00, P<0.001 and U = 25.00, P = 0.0092, respectively).

The highest bioaccumulation factor (BF) was obtained for cadmium (Table I) considering that the average concentration of Cd in *M. moniliformis* was 86.9-times higher than that in *R. rattus* muscle. The average concentration of Cd in *R. microstoma* was also 60.6-times higher than that in *M. domesticus* muscle. The average concentration of lead in *M. moniliformis* was 11.2-times higher when compared to muscle of *R. rattus*. On the other hand, the average lead concentration in *R. microstoma* was 18 to 28-times higher than in tissues of *M. domesticus* (Table I).

Discussion

Most studies aiming at assessing environmental quality have focused on areas, which were at least expected to be polluted such as floodplains, landfills, dumping sites, mine areas, or where accidents have occurred (Grimalt *et al*. 1999, Hamers *et al*. 2006, Pereira *et al*. 2006, Torres *et al*. 2006, Sánchez-Chardi *et al*. 2007). Contrarily, insufficient attention is paid to areas of high ecological importance that may also be subject to several types of lower or indirect anthropogenic chemical stress. The environmental quality of protected areas is often high because they are usually subjected to low disturbance. However, it should be taken into account that long-term pollutant activities may disturb some ecosystems making them less suitable for wildlife.

An earlier study using the model *Apodemus sylvaticus*/*Gallegoides arfaai* (Torres *et al*. 2004) under natural field conditions in NE continental Spain revealed much higher Cd concentrations in kidneys (644.9 ng g^{-1} wet weight) than those found in either of the hosts evaluated in El Hierro. Another earlier study using the model *A. sylvaticus*/*Skrjabinotaenia lobata* (Torres *et al*. 2006) in the urban dumping site of Garraf (located only 32 km away from Barcelona city) reported Pb levels only slightly higher (144.8 and 15.1 ng g^{-1} w.w. in kidney and muscle, respectively) than those evidenced in El Hierro but the reported Cd concentrations were again much higher (959.2 and 9.5 ng g^{-1} w.w. in the same tissues) than those detected in the present survey (Table I).

Other studies on small mammals from contaminated sites including *A. sylvaticus* from a landfill in NE Spain (Sánchez-Chardi *et al*. 2007) and *R. rattus* and *M. spretus* from an abandoned mine area in SE Portugal (Pereira *et al*. 2006) revealed higher amounts of Pb and Cd than levels found in the evaluated rodents collected in El Hierro. Therefore, it is evident that contamination levels in El Hierro are much lower than those reported in other areas of the Iberian Peninsula (Spain and Portugal). However, when comparing the areas included in the present study, there were significantly higher Pb concentrations in liver and kidney of *R. rattus* collected in "El Lagartario" than in *M. domesticus* collected in "Fayal-Brezal". Apart from the possible effect of interspecific variability (which cannot be confirmed considering the available data) the observed differences should be a result of anthropogenic contamination in "El Lagartario" area where human pressure is higher, including the presence of motor vehicles. These results confirm that the environmental quality of zones that have partial or complete protection status is often high. However, long-term pollutant activities might disturb some ecosystems that require a systematic control using suitable species or helminthological models as biomonitors of pollution by heavy metals.

The study on *A. sylvaticus*/*G. arfaai* (Torres *et al*. 2004) constituted the first evaluation of Cd bioaccumulation using a terrestrial host-cestode system. However, the authors concluded that this system was not efficient in monitoring environmental Cd pollutions. Since then, other terrestrial vertebrate/cestode models have been evaluated such as *Oryctolagus cuniculus*/*Mosgovoyia ctenoides* (Eira *et al*. 2005), *Skrjabinotaenia lobata*/*Apodemus sylvaticus* (Torres *et al*. 2006) and *Raillietina micracantha*/*Columba livia* (Torres *et al*. 2010). However, they were all found to be unsuccessful as promising bioindicator systems of environmental Cd pollution, much like the model *M. domesticus*/*R. microstoma* evaluated in the present study.

With respect to acanthocephalan parasites of mammals, few terrestrial models for the evaluation of Cd bioaccumulation are available. A study involving specimens of *Macracanthorhynchus hirudinaceus* infecting pigs revealed 32-times and 5-times more Cd in the acanthocephalan than in their hosts' liver and kidney, respectively (Sures *et al*. 2000a). Also, the system including *M. moniliformis* and experimentally infected rats had already been demonstrated as a highly sensitive bioindicator in terrestrial and urban ecosystems (Scheef *et al*. 2000). The same system was evaluated in the present study under natural conditions and bioaccumulation factors with respect to liver and muscle seem to corroborate its usefulness for the evaluation of Cd, although values obtained by Scheef *et al*. (2000) in their experimental study were much more significant.

As for the evaluation of Pb bioaccumulation using acanthocephalans parasites of mammals in terrestrial habitats, two systems involving *Macracanthorhynchus hirudinaceus* and *Moniliformis moniliformis* have been evaluated (Sures *et al*.

2000a, b). In our study, the bioaccumulation factors obtained for *R. rattus*/*M. moniliformis* are in line with those obtained experimentally by Sures *et al.* (2000b), thus supporting the suitability of this system as a useful and promising tool in environmental monitoring.

Also considering the terrestrial compartment, the system *Rattus norvegicus*/*Hymenolepis diminuta* was the first host/cestode system to be evaluated for its possible capacity of Pb accumulation both under experimental conditions (Sures *et al.* 2002) and in the field (Sures *et al.* 2003). The latter was performed in the city of Cairo (Egypt) and the evaluated host-parasite model was confirmed as a promising bioindicator system for Pb in urban ecosystems (Sures *et al.* 2003). Other subsequent field studies included cestode parasites of rodents (*Apodemus sylvaticus*; Torres *et al.* 2004, 2006), lagomorphs (*Oryctolagus cuniculus*; Eira *et al.* 2005) and terrestrial urban birds (*Columba livia*; Torres *et al.* 2010). The BFs obtained in the present study seem to confirm that cestodes with a relatively large tegumental surface in respect to their weight should reach high bioaccumulation factors and therefore they could be theoretically considered good biomonitoring tools as postulated by Torres *et al.* (2006). Also, cestodes efficiently collect bile salts, which have formed organometallic complexes with metal ions, becoming available in the intestine through the hepatic intestinal cycle (Sures and Siddall 1999). It is possible that this capacity for collecting bile salts is enhanced in *R. microstoma* because it is usually found in the liver (inside the bile ducts) and duodenum. In fact the BFs reported in our study for this cestode are quite high (Table I) considering the scarcely polluted sampling area.

Considering the presently available data, it can be concluded that the widely distributed rodents *R. rattus* and *M. domesticus* are suitable biomonitoring species of environmental pollution by heavy metals. This has been confirmed by the lower contamination expected in El Hierro in comparison to several unprotected areas in the Iberian Peninsula. However, even though El Hierro is a very small island, the anthropogenic pollution was emphasised in the Lagartario area, which presented Pb contamination levels similar to continental Spanish areas. In addition, the present data also allow proposing both models as promising biomonitoring systems to evaluate environmental Cd pollution (*R. rattus*/*M. moniliformis*) and Pb pollution (mainly *M. domesticus*/*R. microstoma*) in terrestrial non-urban habitats. In fact, due to its hepatic and duodenal sites of infection and its small size, *R. microstoma* can be considered a good indicator for environmental Pb pollution in non-urban terrestrial habitats.

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