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10. Heavy metal accumulation by intestinal helminths of vertebrates

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Abstract. The relevancy of parasites as potential indicators of environmental quality has been increasing over the last years, mostly due to the variety of ways in which they respond to anthropogenic pollution. The use of fish parasites as bioindicators of heavy metal pollution in aquatic ecosystems has been widely studied. However, little information concerning terrestrial habitats is presently available. In fact, in the last two decades several studies have been performed worldwide in different habitats and/or conditions (theoretically both in polluted and unpolluted terrestrial ecosystems, but mainly in aquatic ecosystems) in order to investigate heavy metal pollution using parasitological models. Different groups of vertebrates (mainly fish, mammals and birds) and several parasitological models have been tested involving acanthocephalans mostly, but also cestodes and nematodes. It is not the aim of this chapter to do a complete revision of the available data concerning this subject. Instead, we emphasize some general aspects and compile a mini-review of the work performed in this

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field by our research group. The results obtained until now allow confirming several parasitic models as promising bioindicator systems to evaluate environmental cadmium and mainly lead pollution in terrestrial non-urban habitats, as it was already demonstrated for aquatic ecosystems. The present knowledge also allows confirming that parasites can reveal environmental impact. Environmental parasitology is an interdisciplinary field, which needs simultaneous expertise from toxicology, environmental chemistry and parasitology. Furthermore, environmental parasitology should be taken into account in order to increase the efficiency of environmental monitoring programs.

1. Introduction

Over the last decades parasites have been attracting attention as potential indicators of environmental quality due to the variety of ways in which they respond to anthropogenic pollution, particularly in aquatic ecosystems. In fact, several revision papers have been available for a long time now [1-6]. A pioneer study in this field addressed the issue of parasites as indicators of water quality as a whole, including the use of helminth transmission in marine pollution studies. Among other aspects, the study pointed out that: i) levels of infection of certain parasites could be used as early warning indicators of deteriorating water quality, ii) laboratory experiments should be undertaken to investigate the response of parasite transmission stages to selected pollutants, and iii) guidelines for the selection of the most appropriate host-parasite systems were needed [1]. Just a few years later, the first review on the use of fish parasites as bioindicators of heavy metals in aquatic ecosystems [2] presented some conclusions and perspectives on parasites as accumulation indicators. Some problems or limitations of this kind of studies were also evidenced, such as: i) helminth infestations may affect host sensitivity to heavy metals, ii) the vitality of fish aqueously exposed to cadmium was markedly reduced when they were infested with larval cestodes, and iii) adult parasites may have their own detoxification mechanisms or take up metals, which is in contrast to the extreme metal sensitivity of certain free-living parasite stages like cercariae. It was also pointed out that in order to evaluate the relationship between environmental exposure and acanthocephalan metal bioconcentration and to validate the role of parasites in environmental biomonitoring more laboratory studies on experimentally infected fish are needed. These studies should allow determining the bioconcentration factors, defined as the ratios of the metal concentration in the parasites to those in the host tissues ($C_{[parasite]} / C_{[host\ tissue]}$) [7].

Until recently little information was available on the simultaneous effects of parasites and pollutants on the physiological homeostasis of organisms.

However, today it is possible to assert that: i) parasites influence the metabolism of pollutants in infected hosts, ii) parasites interact with contaminants in synergistic or antagonistic ways, and iii) parasites induce physiological reactions in hosts that are thought to be pollutant-related. In addition, there is a close interaction between the effects of pollutants and parasites, which seems to be mediated at least partly by the endocrine system, which itself is closely related to the immune system of some hosts [8]. In fact, since it is well accepted that parasitism and pollution affect the physiological homeostasis of aquatic hosts [8], it seems reasonable to speculate that the same should occur in terrestrial hosts. The effects of pollution on parasitism are variable, i.e. pollution may increase parasitism if the pollutant mainly affects the host rather than the parasite (immunotoxic chemicals) or it may exercise a negative effect over certain parasites, which are more susceptible to the particular pollutant than their hosts. The interaction between parasites and pollution can be even more complex. In fact, some parasites may even have a "positive" influence on their hosts when exposed to environmental pollution or, on the contrary, they may have synergistic effects becoming more harmful to the host or modeling gonad development of the host [8]. It has been postulated that where hosts face a polluted environment parasitism might be advantageous. This hypothesis deserves further investigation, as it is unclear if the "negative" effects of parasites may be outweighed by the positive impact of reduced pollutant levels [5]. Therefore, it would be important to study this effect under realistic environmental conditions.

Concerning the main goal of this mini-review, it must be said that there are several works on the uptake and accumulation of metals by acanthocephalans of fish most of which have been summarized elsewhere [3, 4]. According to that data the most conspicuous metal accumulation was found in *Pomporhynchus laevis* parasitizing the chub, *Leuciscus cephalus*, which reduce the metal levels in fish host tissues [9]. This fact motivated some experimental works that evaluated the kinetics and processes of metal uptake by fish tissues and their parasites [10-12]. In fact, a model explaining metal uptake by *P. laevis* and the associated reduction of lead in host tissues was developed [10]. In this work the central element of the hypothesis is that acanthocephalans take up bile-bound lead in the small intestine. The liver expels lead ions by binding them to steroids in the bile and these complexes pass down the bile duct into the intestine. In uninfected fish, the intestinal wall reabsorbs bile-bound lead, which then runs through the hepatic intestinal cycle. This cycle can be interrupted by parasites by taking up bile-bound lead thus reducing the available amount of lead that could be reabsorbed by the fish host. As a consequence, infection reduces the amount of lead available for accumulation in fish tissues. This fact has been partially analyzed in the terrestrial model *Rodentolepis microstoma* / *Mus domesticus* in El Hierro Island (Canary Archipelago, Spain) considering that this cestode inhabits within the duodenum near the bile ducts of this small mammal [13]. This uptake has also been described for other elements and even for important physiological cations [7,14,15]. For example, it was seen that the levels of several essential elements in the liver of *Perca fluviatilis* were negatively correlated with the size of the infrapopulation of *Acanthocephalus lucii* [14]. It appears that acanthocephalans take up these essential elements as a by-product when taking up bile salts with extreme efficiency, as most intestinal parasites are unable to synthesize their own steroids and fatty acids [16,17].

Acanthocephalans seem to meet most of the criteria commonly accepted for ideal bioindicators, even though their individual age cannot be determined. However, it seems unnecessary to specify their exact age, which is known to range from 50-140 days; in addition as metal uptake occurs more rapidly in the acanthocephalan than in host muscle tissue this ratio could provide information on the duration of environmental exposure and a higher ratio must be expected after a short-term exposure than in longer exposure periods [3, 18-20]. The accumulation capacity of some acanthocephalans such as *Acanthocephalus lucii* from natural infected perch, *Perca fluviatilis* was compared to other established free-living organisms such as the zebra mussel *Dreissena polymorpha* from the same site. The following conclusions were obtained: i) the zebra mussel allows to discriminate between gradients of pollution most likely due to its immobility as a consequence of being attached to the substratum by byssal threads and ii) the bioconcentration values of Cd and Pb in *A. lucii* (despite the high variability of metal burden values) were several times greater than those in the zebra mussel. These observations suggest that acanthocephalans are even more useful as environmental indicators for assessing metal pollution in aquatic habitats than other established indicator organisms [21, 22]. In this context we should answer and contextualize the question why do we need new accumulation indicators especially if we are dealing with endoparasites of vertebrates? Among other aspects, it can be said that the use of parasites supposes a contribution to the exploration of remote areas, by investigating whether or not a specific metal is present in a given habitat at bioavailable concentrations [15]. Another interesting possibility is to study the biological availability of noble metals such as the platinum group elements (PGE: palladium, platinum and rhodium) emitted with exhaust fumes from automotive catalytic converters [23]. Around a decade ago it was evident that most of the studies performed in this field were made in aquatic habitats and only a few involved terrestrial hosts emphasizing that it would be worthy intensifying the research in terrestrial ecosystems [3]. Information using terrestrial vertebrates such as rodents is still quite limited even though they are thought to be useful in predicting environmental risk and in assessing environmental quality [24-26]. There is even less information about models involving small mammals and their helminth parasites, which can be summarized in only some experimental works [27-29] and in a reduced number of field studies [13, 30-32] involving cestodes and acanthocephalans as bioindicator models of heavy metal pollution (mainly Pb, Cd and Hg). Under natural conditions, there is a very limited amount of research dealing with the way parasitism and pollution can interact with each other in terrestrial organisms. Nowadays this subject continues to be more studied in the aquatic environment despite the increasing awareness that parasitism should be investigated in the light of the respective environmental conditions. In fact, since pollution can favour or decrease parasitism depending on an uncountable number of interacting variables, environmental parasitology is an interdisciplinary field which needs simultaneous expertise from toxicology, environmental chemistry and parasitology [1, 5, 33].

2. Materials and methods

Even though such studies have used a wide range of sample types and methodologies, there is a common denominator. Samples may include host tissues (liver, kidney, muscle, gonads, bone, feathers, etc.) as well as parasites (mainly larval and adult stages of several groups of helminths, either whole individuals or portions) that must be taken using stainless-steel or other non-contaminating instruments.

The analytical methodology varies according to different studies. Our particular work is performed at the “Centres Científics i Tecnològics de la Universitat de Barcelona” (CCiTUB), which holds the ISO 9001:2008 certifications. At the proper facilities, samples are weighed (if possible between 100-200 mg wet weight) and then digested in Teflon vessels with suprapure HNO_3 (2 mL) and H_2O_2 (1 mL) in an oven at 90ºC and left overnight. All material used in the digestion process is thoroughly acid-rinsed. After digestion, samples are diluted with Milli-Q water and then analyzed for trace elements by inductively coupled plasma-mass spectrometry (ICP-MS; Perkin-Elmer Elan 6000).

The whole process must be standardized and validated by use of certified standards and blanks. In our surveys two standards are mainly used (DORM-2 and DOLT-3; National Research Council, Canada). The accuracy of all results is higher than 90% and very close to 100% for As, Hg, Pb and Cd.

Detection limits are very low for each element and are calculated as the mean blank value plus three standard deviations of this mean blank.

All concentrations are usually presented as ppb (ng g^{-1}) or ppm (μg g^{-1}) wet weight. Bioaccumulation factors (BFs) in helminths are calculated as the ratios of the metal concentration in the parasite to those in the host tissues ($C_{[parasite]}$ / $C_{[host\ tissue]}$) [7].

3. Studies performed

In the last two decades several studies have been performed worldwide in different habitats and/or conditions (theoretically both polluted and unpolluted terrestrial and mainly aquatic ecosystems, including freshwater, estuarine and marine environments) in order to investigate heavy metal pollution using parasitological models. Different host-parasite models using several groups of vertebrates (mainly fish, mammals and birds) and parasites have been assessed. These models often included acanthocephalans, but also cestodes and nematodes were evaluated. Considering the wide diversity within this type of research, it is possible to categorize studies according to more homogeneous groups. We do not intend this chapter to be a complete revision of the available data on this subject. In fact, it emphasizes some general aspects and reviews the work performed by our research group.

3.1. Aquatic ecosystems

Toxic element pollution has been more extensively investigated in aquatic environments than in terrestrial habitats. Fish and birds inhabiting aquatic ecosystems have been largely used as organisms for monitoring environmental pollution. Marine ecosystems are continuously threatened by contaminants, including heavy metals, which are frequent products of both anthropogenic activities and natural processes. The biomagnification of some metals such as mercury along the food chain is a major concern in marine environments increasing the potential for human exposure [34-36]. The use of bioindicators allows evaluating the risk of exposure, acting as early warning systems for environmental deterioration but firstly the selection of representative species is required [37]. With respect to the marine compartment, seabirds have been intensively used because of their high trophic position and bioaccumulation capacities together with their ability to process certain inorganic elements [38]. In this context, we have studied the concentration of some toxic elements in two seabird species using fresh carcasses obtained from beached bird surveys along the central coast of Portugal: the Atlantic gannet, *Morus bassanus* (Sulidae) and the razorbill

Alca torda (Alcidae) [39, 40]. In addition we are studying the usefulness of the model *Tetrabothrius* sp. / *M. bassanus* as a bioindicator of marine toxic element pollution. When assessing potentially toxic elements in *M. bassanus* it was evidenced that: i) mercury was roughly above the minimum level for adverse effects in birds, ii) there was a higher accumulation of Se and Cd in kidney, of Pb in feathers, and of Mn in liver, and iii) the age of the analyzed birds (juveniles, sub-adults and adults) seems to affect the accumulation of Cd, Co, Hg, Mn, Se and Zn [39]. Preliminary data concerning the model *Tetrabothrius* sp. / *M. bassanus* seem to indicate that it could be proposed as a good bioindicator system to evaluate environmental Cd and Pb pollution in marine environments (*personal data*). In the similar study involving *A. torda* again it was possible to evidence a possible contamination risk by mercury and also by chromium [40]. A higher accumulation of Se and Cd was detected in kidney, of Zn and Pb in feathers, and of As and Mn in liver of *A. torda*. Razorbill's age was also found to affect the accumulation of Cd, Cr, Cu, Hg, and Mn, with juveniles presenting higher levels of Cu and Mn than older individuals [40].

In the polluted estuarine habitat of the Ria de Aveiro (Portugal) we analyzed trace element concentrations in *Proteocephalus macrocephalus* (Cestoda) and *Anguillicola crassus* (Nematoda) in comparison to their fish host, the European eel, *Anguilla anguilla* [41]. Our results confirmed that the consumption of eels collected in this study area represents no risk for humans, because the values of toxic elements quantified in the edible portion of the analyzed eels were below the maximum limits allowed by EU regulations. Consequently, the consumption of these fish by humans should be safe after removing all viscera, which at times presented higher concentrations than those in the edible portion. Nonetheless, since eels are prey to birds and carnivores, eels might represent a real contamination risk for wildlife. The survey also allowed concluding that the model *P. macrocephalus* / *A. anguilla* is a promising bioindicator system to evaluate environmental Cr, Ni, Pb and Zn exposure in aquatic areas where both species are present.

3.2. Terrestrial ecosystems

As mentioned earlier, most studies dealing with environmental terrestrial conditions, using or not parasitic models, have been designed under experimental conditions [27-29] or focused either on theoretically polluted areas such as floodplains, landfills, dumping sites, mine areas and polluted cities, or on areas where major ecological accidents have occurred [e.g. 25, 26, 30, 32]. Contrarily, insufficient attention is devoted to areas not included in the latter circumstances, where environmental quality is often high because they are usually subjected to low disturbances [13, 15]. However, it should be taken into account that long-term pollutant activities may disturb the more pristine ecosystems.

In this sense, we performed an environmental study in an area of high ecological interest, the El Hierro Island in the Canary Archipelago, declared a reserve of the biosphere by the UNESCO in 2000. Later we compared the obtained insular data (El Hierro Island) on Cd and Pb pollution using the models *Rattus rattus* / *Moniliformis moniliformis* and *Mus domesticus* / *Rodentolepis microstoma* with those previously reported in NE Spain using the model *Apodemus sylvaticus* / *Gallegoides arfaai* also obtained under natural field conditions. We verified that, although lead values were similar in both studies, cadmium tissue levels in the continental area were much higher than those found in El Hierro [13, 31]. In the urban dumping site of Garraf (located only 32 km away from Barcelona, Spain) concentrations of Cd and Pb were also recently tested using another parasitic model (*Apodemus sylvaticus* / *Skrjabinotaenia lobata*) [32]. The reported levels of lead were only slightly higher than those evidenced in El Hierro, but again, cadmium concentrations were much higher than those registered in El Hierro [13, 32]. In general, it could be stated that pollution in El Hierro is lower than in the several areas analyzed in the Iberian Peninsula, corroborating that the environmental quality of zones that have partial or complete protection status is often high. However, as pointed above, long-term pollutant activities (such as tourism-related activities and traffic) might disturb remote or protected ecosystems that require a systematic control using suitable species or helminthological models as bioindicators of pollution by heavy metals.

The model *Apodemus sylvaticus* / *Gallegoides arfaai* was the first terrestrial host-cestode system evaluated for its potential capacity for accumulating cadmium. However, it was found that this model could not be considered an effective system for monitoring environmental cadmium pollution [31]. The same was found for another rodent / cestode model (*A. sylvaticus* / *Skrjabinotaenia lobata*), for a lagomorph / cestode model (*Oryctolagus cuniculus* / *Mosgovoyia ctenoides*), and for a columbiform bird / cestode model (*Columba livia* / *Raillietina micracantha*) [32, 42, 43]. More recently, the same was found for the model *M. domesticus* / *R. microstoma* [13].

Precedents of terrestrial models involving acanthocephalan parasites of vertebrates to study Cd bioaccumulation are scarce. The large acanthocephalan pig's parasite *Macracanthorhynchus hirudinaceus* was considered an important cadmium bioaccumulator on the basis of the study performed in Bolivia under naturally conditions [44]. The acanthocephalan *M. moniliformis* was tested under laboratory conditions on infested *Rattus* *norvegicus* and it was concluded that *M. moniliformis* might be used as a highly sensitive bioindicator of cadmium pollution in terrestrial and urban ecosystems [27]. This presumption has recently been corroborated in the above mentioned field study reported from El Hierro although it is necessary to note that natural conditions are much more heterogeneous than experimental premises [13].

As indicated for cadmium, again a reduced number of terrestrial systems involving acanthocephalans parasites of vertebrates were evaluated in respect to their potential for lead bioaccumulation. The species *M. hirudinaceus* was able to bioaccumulate lead at least under the above mentioned conditions in which the study was performed in Bolivia and it was pointed out that *M. hirudinaceus* might be used as an important lead bioaccumulator in terrestrial biotopes especially as it is a very abundant parasite of domestic and wild pigs throughout the world [44]. The model *R. norvegicus* / *M. moniliformis* was tested under experimental conditions and it was considered a useful and promising bioindicator system for lead in urban ecosystems in temperate regions [28]. Despite the variability of natural conditions in contrast to experimental controlled variables, the bioaccumulation factors reported from El Hierro show little difference from those obtained experimentally, and confirm the suitability of this system as a useful and promising tool in environmental monitoring [13, 28].

The first terrestrial model involving a cestode parasite of rodents studied for the possible capacity of lead accumulation was *Rattus norvegicus* / *Hymenolepis diminuta*, probably because the host is widely distributed and easily accessible, meeting some of the criteria set for a good bioindicator [18], either under experimental conditions or in the field [29, 30]. The survey performed in the city of El Cairo (Egypt) allowed considering this model as a promising bioindicator for lead in urban ecosystems [30]. In the same study from El Cairo, the bioaccumulation capacity of the larval stage of *Taenia taeniaeformis* located in cysts in liver tissue of the same rats parasitized by *H. diminuta* was also tested. It was observed that the level of lead in *T. taeniaeformis* was similar to those of the different evaluated tissues. This observation allowed postulating that the cestodes' ability to take up lead is related with the parasite's microhabitat within the host and/or its developmental stage [30]. Furthermore, it was pointed out that heavy metals are not distributed evenly in the cestodes being concentrated especially in the gravid proglottids of the strobila. Thus it was suggested that those parts of strobilae, which are similar in size and have gravid proglottids should be preferred when assessing heavy metals in cestodes [30].

Other studies have also been performed in terrestrial habitats from Spain and Portugal to evaluate the relationship between bioaccumulation in cestodes of vertebrates and environmental lead exposure. Similar models were used involving rodents, but also lagomorphs and even urban birds (*A. sylvaticus* / *G. arfaai* and *S. lobata*, *O. cuniculus* / *M. ctenoides* and *C. livia* / *R. micracantha*) [13, 31, 32, 42, 43]. The need to use the above mentioned portions of cestodes is easy to fulfill when trying to test entire cestodes smaller than *H. diminuta* such as *R. microstoma*, *G. arfaai* and *S. lobata*.

Table 1 Mean lead bioaccumulation factors [7] calculated for diverse cestode parasitic models in respect to several terrestrial vertebrate tissues.

Model	Parasite Family	Liver	Kidney	Muscle	Ref.
A. sylvaticus *G. arfaai*	Anoplocephalidae	20	6	24	[31]
A. sylvaticus *S. lobata*	Catenotaeniidae	33 – 53	6 – 8	52 -81	[32]
R. rattus *H. diminuta*	Hymenolepidiidae	29 – 87	6 – 11		[30]
M. domesticus *R. microstoma*	Hymenolepidiidae	18	26	28	[13]
O. cuniculus *M. ctenoides*	Anoplocephalidae	1.5	2		[42]
C. livia *R. micracantha*	Davaneidae	10	15	80	[43]

Table 1 presents the lead BFs for models involving cestodes belonging to several families and their vertebrate hosts in terrestrial ecosystems. The *A. sylvaticus* / *G. arfaai* model was proposed as an alternative to evaluate environmental lead exposure, especially outside urban areas where the wood mouse may be readily available [31]. Similarly, the model *A. sylvaticus* / *S. lobata* was tested and proven to be a useful tool for biomonitoring lead pollution in terrestrial habitats [32]. In addition, the BFs reported in the model *M. domesticus* / *R. microstoma* are in agreement with the hypothesis that cestodes with a relatively large tegumental surface in respect to its weight should reach high bioaccumulation factors and therefore they could be considered potentially good bioindicators [13, 43]. It is well-known that cestodes inside the intestine are very efficient taking up bile salts produced in the liver through the hepatic intestinal cycle [10]. This capacity should be increased in the case of *R. microstoma* due to its hepatic (inside the bile ducts) and duodenal location. In fact the BFs reported for this cestode are quite high (see table 1) particularly if we consider that the sample was obtained from

El Hierro Island, an area with very low lead pollution [13]. With respect to models involving parasites of terrestrial birds, only a few have been assessed as bioindicators of environmental pollution. The model *Columba livia* / *Raillietina micracantha* from the city of Santa Cruz de Tenerife (Canary Islands, Spain) was proposed as another promising bioindicator to evaluate environmental toxic element exposure, particularly Pb and Mn, in areas where pollution levels are still relatively low and where both common species are present [43]. The concentration of some toxic elements was also studied in a cestode parasite of lagomorphs, concretely in the model *Oryctolagus cuniculus* / *Mosgovoyia ctenoides* collected from Portugal. Unlike the above mentioned models, the obtained results from *M. ctenoides* and wild-rabbits disproved this model as a promising bioindicator system [42]. The contrasting results obtained in the different models might be due to the large size of *M. ctenoides* in comparison to the other evaluated cestodes and/or due to differences in the tegumental absorption processes between cestode families.

4. Conclusions

The information available to date confirms that several parasitic models are promising bioindicator systems to evaluate environmental cadmium and mainly lead pollution in terrestrial non-urban habitats as it has been widely demonstrated in aquatic environments. In addition, the present knowledge gives a positive answer to the question on whether or not parasites can really reveal environmental impact. In the future, some recommendations for the design of monitoring studies should be taken into question in order to increase the efficiency of environmental monitoring programs.

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