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Trace Element Concentrations in *Raillietina micracantha* in Comparison to Its Definitive Host, the Feral Pigeon *Columba livia* in Santa Cruz de Tenerife (Canary Archipelago, Spain)

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Abstract The use of systems involving bird parasites as bioindicators of environmental pollution has been scarcely studied in comparison to other models involving fish and rodent parasites, which have been demonstrated as particularly adequate due to their bioaccumulation capacities. The present study evaluated the accumulation of nine trace elements in the cestode *Raillietina micracantha* and in its host *Columba livia* collected from the densely populated city of Santa Cruz de Tenerife (Canary Islands, Spain). Samples (kidney, liver, pectoral muscle, feathers, and *R. micracantha*) of 27 infected *C. livia* were selected for trace element analysis by inductively coupled plasma–mass spectrometry. Element levels in pigeon tissues revealed some degree of pollution in Santa Cruz de Tenerife, particularly by Pb and Zn. Pb and Mn mean concentrations were higher in *R. micracantha* than in the pigeon's soft tissues, with subsequent high bioaccumulation factors for Pb (kidney = 15.38, liver = 10.38, muscle = 79.83) and Mn (kidney = 6.81, liver = 7.52, muscle = 19.89, feathers = 6.11), among others. The negative relations detected for As concentrations between liver and *R. micracantha* emphasize a possible role of the cestode in As detoxification in host tissues. Considering the obtained bioaccumulation factors, the system *R. micracantha*/*C. livia* is proposed as another promising bioindicator system 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.

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Large quantities of pollutants have continuously been introduced into cities as a consequence of anthropogenic activities such as urbanization, traffic, and industrial processes. In urban environments, the motor vehicle is one of the major sources of pollution, mostly due to exhaust emissions and deterioration of tires, engines, and brakes, among others (Rayson 1990). In fact, in a metropolitan area in Greece, exhaust emissions presently represent the major release source of Cd and Mn (Ewen et al. in press). Furthermore, some of the modifications meant to reduce the amount of pollution from motor vehicles (unleaded petrol, catalytic converters, etc.) became new sources of different trace elements such as Platinum Group Elements from catalytic converters (Ward et al. 2004).

Assessing pollutants in different ecosystem components is an important task in order to identify possible risks (Gragnaniello et al. 2001). With respect to trace elements, biomonitoring studies might be critical to reflect the potential environmental pollution status and consequences (e.g., Burger and Gochfeld 2000; Loranger et al. 1994). In fact, information about pollutant bioavailability should be more reliable when organisms, rather then their abiotic environment, are evaluated (Phillips 1977).

Among possible biomonitoring species, birds have been widely used to assess environmental contamination, and in urban environments particularly, the usefulness of the feral pigeon (*Columba livia*) as a bioindicator of pollution has been assessed over the last decades (Klein et al. 2008; Nam et al. 2004b; Schilderman et al. 1997). These metropolitan sedentary birds forage on the ground and present abundant, stable populations, mostly associated with human activities. Their atmospheric exposure in the urban environment is similar to that of the human population and, consequently, pigeons have been extensively used to monitor heavy metal pollution (Hutton and Goodman 1980; Nam and Lee 2006; Schilderman et al. 1997).

However, relatively recent studies have reported that some helminth parasites are able to accumulate much more heavy metals than their hosts in both aquatic and terrestrial environments (see review by Sures 2004). With respect to urban habitats, data on the use of parasites as potential bioindicators of heavy metal pollution are still very scarce and refer almost exclusively to cestode/mammal models (Sures et al. 2003; Torres et al. 2006). Although models using aquatic birds (Baruš et al. 2000; Tenora et al. 2002) or raptors (Baruš et al. 2000) have also been proposed, there are no data concerning urban birds.

Columba livia is very abundant in the forests, cities, and villages of Tenerife Island (Canary Archipelago, Spain). In the city of Santa Cruz de Tenerife, the cestode *Raillietina micracantha* (Fuhrmann 1909) is one of the pigeon's most prevalent helminths (Foronda et al. 2004). Therefore, considering that cestode/pigeon systems have never been evaluated, the main goal of the present study was to assess the accumulation of trace elements in the cestode *R. micracantha* and in *C. livia* in the city of Santa Cruz de Tenerife (Canary Islands, Spain), thus evaluating the model *R. micracantha*/*C. livia* as another promising bioindicator system.

Materials and Methods

The Canary Archipelago is located between 13°23′–18° 8′ W and 27°37′–29°24′ N in Macaronesia. The city Santa Cruz is located in the oriental part of Tenerife Island, which is the largest one of the archipelago. The city includes an area of 150 km^2 comprising one-third (225,000 people) of the Tenerife population.

After obtaining the appropriate permits from the Canary government, the Santa Cruz de Tenerife municipal services captured several pigeons during 2007. Forty individuals were frozen for later analysis in the laboratory. After thawing, pigeons were dissected and samples of the kidney, liver, pectoral muscle, and primary feathers were collected from all individuals. These samples were stored in glass vials and deep-frozen until later processing for trace element analysis. All digestive tracts were removed and scanned for helminths using a stereomicroscope with the help of stainless-steel instruments and Milli-Q water. Several individuals of *R. micracantha* from all 27 pigeons parasitized by this cestode (67.5% of prevalence) were frozen for trace element analysis.

First, feathers were triple rinsed in Milli-Q water alternated with acetone, to remove loosely adherent external contamination, and air-dried overnight. Then all samples (kidney, liver, pectoral muscle, feathers, and *R. micracantha*) collected from the infected *C. livia* were processed for trace element analysis. Samples were weighed (±100 mg wet weight) and digested in Teflon vessels with HNO_3 (2 mL) and H_2O_2 (1 mL) (Merck; Suprapure), at 90°C in an oven and left overnight. All material used in the digestion process was thoroughly acid-rinsed. After digestion, samples were diluted with Milli-Q water and then analyzed for trace elements by inductively coupled plasma–mass spectrometry (ICP-MS; Perkin-Elmer Elan 6000). The analytical procedure was checked using standard reference material Dogfish (*Squalus acanthias*) liver (DOLT-3) and muscle (DORM-2; National Research Council, Canada). Several analytical blanks were also prepared and analyzed along with samples in order to determine the detection limits.

A normal distribution of all data was obtained after $\log(x + 1)$ transformation. Differences between element concentrations among the analyzed tissues were detected by ANOVA followed by Tukey's test. Linear regression analysis was used to detect relationships between toxic element levels in pigeons and parasites. Statistical analysis was performed using the Statview 4.5 software package. For all tests, a significance level of $p < 0.05$ was applied. The bioaccumulation factors (BFs) were determined as the ratio of the element concentration in the parasites to that in different host tissues ($BF = C_{[parasite]}/C_{[host\ tissue]}$) (Sures et al. 1999).

Results

Element Distribution in Host Tissues and Parasites

The detection limits (mean blank value plus three standard deviations of the mean blank) for each element were lower than 1 ng/mL, except for Zn (6.1 ng/mL[1]). All accuracy values were greater than 90%.

The mean element concentrations detected in all tissues collected from *C. livia* and in *R. micracantha* are presented in Table 1. All element levels were found to vary according to each analyzed tissue (ANOVA, all $p < 0.0001$). Considering the birds' tissues, feathers presented the highest mean concentrations of Cr, Cu, Pb, Zn (all $p < 0.001$), and Hg (liver and kidney, $p < 0.001$ and muscle ($p < 0.05$). Feathers also presented higher As and

Table 1 Trace element concentrations in tissues of *C. livia* and in *R. micracantha* (μg/g wet weight) from the Canary Islands

	Liver		Kidney		Muscle		Feathers		R. micracantha	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
As	0.0594	0.0202–0.186	0.1892	0.055–0.405	0.0675	0.0319–0.1726	0.2736	0.0884–0.6786	0.1308	0.0144–0.587
Cd	0.1096	0.0088–0.3769	0.6776	0.007–2.598	0.0075	0.0003–0.0436	0.0231	0.0046–0.0627	0.0128	0.0033–0.0342
Cr	0.5206	0.3242–1.042	0.5134	0.2745–0.8625	0.6094	0.4522–0.8433	2.661	1.689–10.5	0.3632	0.2005–0.9804
Cu	3.407	2.069–6.184	2.841	0.9692–7.898	4.023	1.75–5.18	9.607	6.747–14.24	0.6017	0.3271–2.774
Hg	0.0075	0.0002–0.0253	0.0164	0.009–0.0316	0.0184	0.0059–0.03	0.0870	0.0068–0.2347	0.0016	0.0006–0.0054
Mn	1.528	0.5933–2.696	2.693	0.5001–6.368	0.5149	0.2896–0.8198	2.712	0.393–5.699	11.72	1.545–66.36
Pb	0.2907	0.0166–1.66	0.287	0.021–1.724	0.1108	0.0101–0.6272	2.696	0.2824–6.0002	1.732	0.0665–9.208
Se	0.4874	0.2251–0.6989	0.8146	0.2099–1.426	0.3383	0.2006–0.5319	0.8088	0.3185–2.479	0.2132	0.0926–0.3645
Zn	40.91	20.46–96.63	25.02	15.54–69.75	12.22	8.286–31.11	144.9	72.32–267.6	49.18	25.73–106.8

Se mean concentrations than liver and muscle (both $p < 0.001$). As expected, the highest mean Cd concentration was detected in the kidney ($p < 0.001$, for all tissues). In comparison to the other soft tissues, the kidney also presented the highest mean concentrations of As (liver, $p < 0.001$; muscle, $p < 0.01$) and Se (both $p < 0.001$). Considering soft tissues only, muscle presented a significantly higher Cu mean concentration than kidney ($p < 0.001$) and slightly higher (not significant) Cr and Hg mean concentrations.

With respect to trace element concentrations in *R. micracantha* individuals, higher Mn mean concentrations were found in comparison to values detected in the pigeon's soft tissues and feathers (all $p < 0.0001$). Additionally, higher mean Pb concentrations were found in the cestode in comparison to the pigeon's soft tissues (all $p < 0.0001$) and a higher Zn mean concentration was also found in comparison to the pigeon's kidney and muscle (both $p < 0.0001$).

Relations Between Element Concentrations in Pigeon Tissues and *R. micracantha*

Many positive relations were obtained between elements' concentrations in the evaluated pigeon tissues; therefore, only the very significant relations ($p \leq 0.0001$, $R^2 \geq 0.50$) are presented (Table 2). The highest number of very significant relations was detected in kidneys. In fact, the concentration of Mn significantly increased along with increasing concentrations of six other elements (As, Cd, Cr, Cu, Pb, Se). Furthermore, in kidney tissue, the concentration of Se significantly increased along with increasing concentrations of five other elements (Mn, As, Cr, Cu, Pb) and significantly decreased with an increasing concentration of Hg (Table 3). There is also a significant relation involving Cd concentration in feathers (Cd-Pb) and three positive relations involving Zn concentrations in *R. micracantha* (Zn-Cr, Zn-Cu, Zn-Se).

Table 2 Positive linear regressions among element levels in *C. livia* and its cestode *R. micracantha*

		F	R^2
Kidney	[Mn]*[Cu]	56.034	0.69
	[Mn]*[Pb]	23.787	0.51
	[Mn]*[Cr]	24.763	0.52
	[Mn]*[As]	56.652	0.69
	[Mn]*[Cd]	25.036	0.52
	[Mn]*[Se]	65.837	0.73
	[Cu]*[Pb]	23.994	0.50
	[Cu]*[As]	26.000	0.73
	[Cu]*[Cd]	29.885	0.57
	[Cu]*[Se]	25.330	0.50
	[Pb]*[As]	26.461	0.54
	[Pb]*[Cd]	25.329	0.52
	[Pb]*[Se]	29.790	0.56
	[Cr]*[As]	22.904	0.50
	[Cr]*[Se]	23.896	0.51
	[As]*[Se]	53.582	0.68
Feathers	[Pb]*[Cd]	28.409	0.53
Cestode	[Zn]*[Cu]	22.751	0.50
	[Zn]*[Cr]	34.102	0.63
	[Zn]*[Se]	23.427	0.57

Negative relations were detected for As concentrations between the liver and kidney and between the liver and *R. micracantha,* indicating that an increase in As concentrations in the kidney or cestode is accompanied by a decrease in As liver concentrations and vice versa (Table 3).

Bioaccumulation Factors

Considering the ratio between element concentrations in *R. micracantha* to those of host tissues, high BFs were detected (Table 4). With respect to *C. livia* soft tissues, the

Table 3 Negative linear regressions among element levels and tissues of *C. livia* and its cestode *R. micracantha*

		F	R^2
Kidney	[Hg]*[Se]	8.070	0.50
[As]	Kidney*liver	24.758	0.51
[As]	Cestode*liver	32.848	0.69

highest BFs were obtained for Pb (average BFs: kidney = 15.38, liver = 10.38, muscle = 79.83). It was also possible to obtain relatively high BFs for Mn with respect to all soft tissues and with respect to feathers (average BFs: kidney = 6.81, liver = 7.52, muscle = 19.89, feathers = 6.11). On average, the concentration of Cd in *R. micracantha* was 7.90 times higher than that in *C. livia* muscle. With respect to the kidney and muscle, BF values were also obtained for Zn (2.12 and 4.55, respectively), whereas with respect to the liver, BF values were obtained for As (3.48).

Table 4 Mean, standard error (in parentheses), and range of accumulation factors $[C]_{parasite}/[C]_{host\ tissue}$ for some elements detected in *R. micracantha* in relation to *C. livia* tissues

	Kidney	Liver	Muscle	Feathers
As		3.48		
		(1.18)		
		0.18–25.12		
Cd			7.90	
			(2.67)	
			0.14–57.25	
Mn	6.81	7.52	19.89	6.11
	(1.13)	(1.24)	(2.58)	(1.22)
	0.33–19.21	1.02–30.31	1.88–64.29	0.50–29.42
Pb	15.38	10.38	79.83	
	(4.68)	(3.22)	(14.23)	
	0.46–114.18	0.88–72.78	4.11–219.87	
Zn	2.12		4.55	
	(0.19)		(0.40)	
	0.95–5.96		0.99–11.05	

Note: Ratios corresponding to less than a twofold increase are not presented

Discussion

Trace Element Concentrations and Tissue Distribution in *C. livia*

Previous studies using the feral pigeon as a bioindicator of pollution in urban areas have mainly focused on the evaluation of Pb, but some data on Cd, Mn, and Zn are also available (Hutton and Goodman 1980; Loranger et al. 1994; Nam et al. 2004b; Schilderman et al. 1997). Nevertheless, data on tissue concentration of other trace elements in pigeons are scarce (see Adout et al. 2007). The present study reports for the first time the levels of nine trace elements in pigeon soft tissues and feathers from an urban environment.

In Santa Cruz de Tenerife, it was possible to detect that pigeon feathers presented the highest mean concentrations of Cr, Cu, Hg, Pb, and Zn in comparison to soft tissues. Feather concentration can generally be ascribed to both exogenous contamination and to mobilization from internal trace element metabolization. For example, whereas Pb contamination in feathers is known to be both external and internal, Cu and Zn contamination is predominantly internal (Ek et al. 2004). In the present study, feathers were washed before analysis, and although contamination might not have been entirely removed, element concentrations should mostly be regarded as endogenous, resulting, for example, from the sequestration of circulating elements into feathers during molt.

In comparison to a recent study using pigeon feathers to assess environmental trace elements in several areas located in an arid environment (Negev desert) in Israel (Adout et al. 2007), pigeon feathers from Tenerife revealed a much lower amount of Pb, Cd, and Mn. However, Hg (0.09 µg/g wet weight.) and Zn (144.9 µg/g wet weight) feather concentrations in the present study were higher than those reported by Adout et al. (2007) for an urban area (0.04 and 131 µg/g wet weight, respectively) and similar to concentrations reported for an industrial area (0.09 and 146 µg/g wet weight, respectively).

In the past, the use of *C. livia* to monitor Pb pollution resulted from the use of alkyl lead as an antiknocking agent in automobile gasoline. More recently, despite the use of lead-free gasoline, a study on blood of urban pigeons from The Netherlands revealed that high-traffic emissions are still one of the major Pb source (Schilderman et al. 1997). In the present study, the Pb concentration in pigeon liver (0.29 µg/g wet weight) was lower than values presented in Korea (Nam et al. 2004a) for rural, urban, or industrial areas (respectively 1.57, 2.09, and 1.44–2.02 µg/g wet weight). Furthermore, the Pb concentration in pigeon liver from Santa Cruz de Tenerife (present study) was also lower than the liver concentration from pigeons collected by Schilderman et al. (1997) in a high-traffic area in Amsterdam (1.21 µg/g wet weight). However, the liver Pb concentration in the present study was higher than liver concentrations from pigeons collected in a medium traffic area (0.18 µg/g wet weight) and in two other urban low-traffic density areas (0.13 and 0.16 µg/g wet weight) located in The Netherlands (Schilderman et al. 1997).

The highest mean Cd concentration was detected in the kidney (0.68 µg/g wet weight), which was much lower than values obtained for pigeon kidney in Amsterdam (2.73 and 2.51 μg/g wet weight), although it was very similar to values obtained for pigeons from the lower-traffic areas reported in the same study by Schilderman et al. (1997). In fact, the liver/kidney Cd concentration ratio (0.16) obtained in our study indicates a chronic, low-level exposure situation (Scheuhammer 1987).

With respect to Zn values in pigeon liver (40.91 μg/g wet weight, in the present study), the concentrations reported by Schilderman et al. (1997) are relatively lower (30.2, 29.4, and 29.0 μg/g wet weight with respect to decreasing traffic density areas in The Netherlands). The total load of pollutants in pigeons is known to be strongly related to the ingestion of food and soils contaminated by deposited surrounding pollution (Beyer et al. 1994). Therefore, the relatively high mean Zn content found in soil samples from the Canary Islands (Fernández-Falcón et al. 1994) might contribute to these results.

Relations Between Element Concentrations in Pigeon Tissues and in Parasites

The vast number of positive significant relationships among concentrations of trace elements in bird tissues, detected both in the present and in previous studies (e.g., Kim et al. 1998; Mendes et al. 2008) suggests common uptake and storage pathways or similar regulation and detoxification processes. It was possible to detect the occurrence of considerable coaccumulation of several elements in the kidneys, possibly emphasizing the kidneys' storage functional role. The high number of relations involving Mn and Se in kidney tissue might be indicative of these elements' importance in the above-mentioned metabolic processes. The negative relation between Se and Hg concentrations illustrates well the previously described importance of Se in trace element detoxification, particularly Hg (Ikemoto et al. 2004; Thompson 1996).

The very significant relations involving the concentrations of both As and Se (As–Se–Pb, As–Se–Cr, As–Se–Cu, As–Se–Mn) in kidney tissue contribute to the already suggested possible role of Se in As storage/detoxification processes in birds (Ribeiro et al. 2009). Apart from binding to Se, the simultaneous binding of several metals to metallothioneins (MTs) is another important trace element detoxification mechanism, which might explain parallel metal accumulation (Kojadinovic et al. 2007).

Relationships involving the toxic elements Pb and Cd with the essential elements Cu and Mn were also detected in pigeon kidney. With respect to Cu and Mn, these are regulated metabolically, and their concentrations in internal tissues fluctuate according to requirements during molt and breeding and also according to the increment of MTs, considering their high affinity for these metal-binding proteins (Stewart et al. 1994; Walsh 1990). Again, the simultaneous binding of Pb and Cd to MTs according to the available number of binding sites should explain the detected relations among metals. The significant relation involving Cd concentration in feathers (Cd-Pb), whether endogenous or exogenous, seems to be a good indication of traffic-related emissions as an important source of pollution.

With respect to relations involving trace element concentrations in *R. micracantha,* three positive relations involving Zn were detected (Zn–Cr, Zn–Cu, Zn–Se). These might result from common storage/uptake mechanisms, such as metal induction and binding to MTs with subsequent allocation to the cestode. The negative relations detected for As concentrations between the liver and kidney and between the liver and *R. micracantha* emphasize a possible role of the cestode in lowering As contamination in host tissues. It is possible that As detoxification includes As removal from circulation in the liver (by binding to MTs and possibly Se, as mentioned earlier), followed by storage in kidney tissue and, in this case, in the cestode.

Bioaccumulation Factors

Among the BFs (Sures et al. 1999) obtained in the present study, the BFs registered for Mn, Pb, and Zn are noteworthy. With respect to Pb and Cd, Baruš et al. (2000) reported maximum BF values for the liver (10.07 and 10.57, respectively) and muscle (5.95 and 12.3, respectively) in other helminth/bird models. However, in the present study on *R. micracantha*/*C. livia*, it was possible to detect much higher maximum BF values for Pb in the liver (72.78) and muscle (219.87) and for Cd in muscle (57.25).

The results obtained indicate that not only does the system *R. micracantha*/*C. livia* appear to be a suitable bioindicator for Pb, but it also indicates that Pb pollution might have been underestimated in the past. In Santa Cruz de Tenerife, there is 10 times less Pb in pigeon liver than in the evaluated cestode, 15 times less Pb in pigeon kidney, and almost 80 times less Pb in pigeon muscle. The BF values detected for Mn constitute the most surprising results. Notice the lowest average BF indicating a sixfold increase in *R. micracantha* (in comparison to feathers) and the highest average BF indicating an almost 20-fold increase (in comparison to muscle). The analysis of *R. micracantha* revealed a high availability of potentially exogenous Mn, which could not have been detected otherwise.

Although more field and experimental essays are necessary to evaluate the relationship between bioaccumulation in cestode parasites of birds and environmental trace element availability, we presently propose the system *R. micracantha*/*C. livia* as another promising bioindicator system to evaluate environmental As, Cd, Mn, and Pb, with a particular suitability for Mn and Pb exposure in urban areas where both species are present. Although two cestode/rodent systems have already been proposed for urban environments (Sures et al. 2003; Torres et al. 2006), the system *R. micracantha*/*C. livia* is the first model using cestode parasites of urban-dwelling birds.

Finally, although the present study evaluated trace element concentrations in pigeons infected by *R. micracantha*, further studies should allow comparing the present results with element concentrations in tissues collected from pigeons not infected with the cestode. In fact, several studies on aquatic environments reporting lower metal contents in fish hosting helminth parasites in comparison to fish without parasites (Eira et al. 2009; Malek et al. 2007; Sures and Siddall 1999; Turčková et al. 2002) indicate that possible infections in organisms used for ecotoxicological evaluations should be taken into consideration in the future. Furthermore, laboratory results obtained from uninfected hosts might not be reproducible in natural environments, where wild noninfected hosts are uncommon (Klar and Sures 2004).

In conclusion, the present study revealed some degree of pollution, particularly by Mn, Pb, and Zn, in Santa Cruz de Tenerife. However, the relatively high element levels in pigeons might not presently represent a potential exposure risk to humans because pigeon contamination results from both inhalation and ingestion, an exposure route unavailable to humans. Nonetheless, the present results support the usefulness of helminth parasites as sensitive early-warning bioindicators in environments in which pollution levels are still relatively low.

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