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Use of the fish endoparasite *Ligula intestinalis* (L., 1758) in an intermediate cyprinid host (*Rastreneobola argentea*) for biomonitoring heavy metal contamination in Lake Victoria, Kenya

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Abstract

Use of some fish parasites as bioindicators of heavy metal pollution has been demonstrated as a promising approach because of their capacity to bioconcentrate such elements. This study evaluated the effects of a cestode parasite, *Ligula intestinalis*, on the accumulation of lead (Pb), cadmium (Cd), chromium (Cr) and copper (Cu) in the cyprinid fish, *Rastreneobola argentea*, in Lake Victoria, Kenya. This *L. intestinalis/R. argentea* model also was assessed as a bioindicator system for heavy metal contamination in the lake. Samples of 125 fish, 63 parasites, water and sediments were collected at four sites in the Kenya portion of the Lake Victoria basin characterized by variable heavy metal concentrations, for ICP-OES element analysis. The concentration of all four heavy metals in the fish and parasite samples exhibited site-specific variations relative to the metal concentrations in the water. The Pb, Cr and Cd concentrations in the *L. intestinalis* were higher than in the fish samples by a factor 11, 18 and 14 respectively, whereas the Cu concentration in *L. intestinalis* was increased by a factor of 2.5, relative to the Cu concentration in fish. The Pb, Cd and Cr concentrations in the parasite body increased, relative to their concentrations in fish samples, suggesting in the bioaccumulation of these metals by the parasite. The Cu concentration in the fish parasite decreased, relative to increased Cu levels in the fish. This finding was interpreted as being a competition for these elements between the parasite and its fish host. Moreover, the increased Cd and Cr levels in the fish were significantly influenced by the increased abundance of the parasites in the abdominal cavity of the fish samples. Based on the results of this study, the *L. intestinalis/R. argentea* system is proposed as a promising bioindicator model for evaluating environmental Pb, Cd and Cr concentrations where these species occur.

Key words

biomonitoring, endoparasites, heavy metals, Lake Victoria, *Ligula intestinalis, Rastreneobola argentea*.

INTRODUCTION

Pollution from elevated levels of heavy metals is a very important environmental problem in many developed and developing countries (Zoller 1994; Huang *et al.* 2007; Sánchez-Chardi & Nadal 2007; Hang *et al.* 2009; Sánchez-Chardi & López-Fuster 2009; Bose-O'Reilly *et al.* 2010; Zheng *et al.* 2010). Lake Victoria, which is the second largest freshwater lake in the world and the largest lake in the tropics, continues to receive increasing loads of heavy metals, including lead, cadmium, chromium and copper (Wandiga 1981; Wandiga *et al.* 1983; Onyari & Wandiga 1989; Makundi 2001; Jason *et al.* 2002; Kishe & Machiwa 2003; Mwamburi 2003; Birungi *et al.* 2007), attributed to various human activities within its drainage basin. Inadequate environmental legislation and/or enforcement contribute to this problem. At the same time, Lake Victoria still represents an essential source of

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nutrition for millions of in-lake organisms (Simonit & Perrings 2005). The cyprinid fish, *Rastrineobola argentea*, is one of three productive fish species in the lake, being the main protein source for several lakeside communities (Wanink 1999). The populations of *R. argentea* in Lake Victoria, however, exhibit a high degree of infestation with the tapeworm, *Ligula intestinalis* (Cowx *et al.* 2008). *Ligula intestinalis* is a Pseudophyllidean cestode of the family Diphyllobothriidae. In its plerocercoid stage, this tapeworm infests a range of freshwater fish species, particularly members of the Cyprinidae, as its second intermediate host, exhibiting a widespread distribution (Dubinina 1980; Hoole & Arme 1988; Pierce *et al.* 2005).

The association between parasites and the fish they infect has suggested the possibility of developing a sentinel host–parasite association as an indicator of metal pollution in the aquatic environment (Sures *et al.* 1999; Sures 2001; Sures & Siddall 2001, 2003; Thielen *et al.* 2004; Malek *et al.* 2007; Eira *et al.* 2009; Jankovská *et al.* 2009). In fact, the use of some fish parasites as bioindicators of heavy metal pollution has been demonstrated to be feasible because of their capacity to bioconcentrate these pollutants (Sures *et al.* 1999; Sures 2001, 2007; Schuldermann *et al.* 2003; Sures & Siddall 2003; Rymar *et al.* 2008). Most acanthocephalans and nematodes are excellent bioaccumulators of heavy metals, regardless of the environment in which they reside. Thus, an acanthocephalan-fish model sentinel assemblage has been recommended as an effective tool for biomonitoring metal concentrations in a wide range of aquatic environments (Sures *et al.* 1994, 1999; Sures 2001, 2003; Sures & Siddall 2001, 2003; Thielen *et al.* 2004).

Field studies focusing on heavy metal accumulation in cestodes have highlighted a large variability in their specific metal accumulation capacity, relative to their hosts and to their environment (Tenora *et al.* 2000; Baruš *et al.* 2001; Tekin-Özan & Kir 2005; Jirsa *et al.* 2008; Tekin-Özan & Barlas 2008; Eira *et al.* 2009). Cestodes currently affect a large number of fish species, and can survive in a wide range of varying environments (Jirsa *et al.* 2008; Tekin-Özan & Barlas 2008). Further studies on systematically different parasites from different microhabitats, as well as from different environments, are required to evaluate the cestode–fish host association as a potential biomonitoring tool for heavy metal contamination.

Accordingly, the goal of this study was to evaluate the ability of a cestode (*L. intestinalis*) in a cyprinid fish host (*R. argentea*) to bioaccumulate heavy metals. The *L. intestinalis/R. argentea* was also assessed as a bioindicator system for heavy metal contamination in Lake Victoria, where the species is found. This host–parasite assemblage from different sites in the lake was analysed to characterize spatial changes in the ability of the cestode–fish host association in Lake Victoria to accumulate heavy metals.

MATERIALS AND METHODS

Study area and sampling sites

Lake Victoria is generally shallow (mean depth 40 m), being located in a catchments of ≈184 000 km^2. The lake is located astride the equator between 2.5°S and 1.5°N, and 32° and 35°E, with its basin shared by the three riparian states of Kenya, Tanzania and Uganda. The lake is fed by a number of large rivers originating in Kenya (Nzoia, Gucha-Migori, Sondu-Miriu, Mara, Yala, Nyando). Its single outlet is the Nile River. The sampling sites in this study were selected on the basis of anthropogenic activity profiles along the coastal zones of Lake Victoria (Fig. 1). Sampling site 1 (S1) is located at Kisumu, which has a population of about 1.1 million. Kisumu is a centre of urban development, the source of various industrial discharges. The lake also receives drainage from intense agricultural activities in this region. Sampling site 2 (S2) is located on Kendu-Bay, which is characterized as a rural agricultural area with virtually no fertilizer inputs. Sampling site 3 (S3) is located at Karungu, and receives drainage from small gold mines. Sampling site 4 (S4) is located at Port Victoria, a rural area that receives inflows from the Nzoia River. The Nzoia River receives effluents from two sugar factories and a paper mill factory situated 100–150 km upstream from Lake Victoria.

Fish and endoparasite collection

The fish host used in this study was a cyprinid, *R. argentea* (Pellegrin, 1904). Fish samples (n = 125) were obtained during five sampling efforts between June and August 2005 at sites S1, S2, S3 and S4, with the fish being caught with gillnets of mesh size 0.2″. The sampling data and composition of the fish catch (i.e. total number of fish caught, fish sampled, sex ratios, size ranges, etc.) are summarized in Table 1. All materials used in this study were initially washed in ultra pure water to minimize contamination. The caught fish were sacrificed, using an overdose of tricaine methane sulphate (MS-222). They were then weighed (to the nearest 0.1 g), measured (folk length in mm) and dissected. The fish dissection was done with stainless steel instruments pre-cleaned in 1% EDTA solution and double-distilled water. For all the caught fish determined to contain parasites (n = 63), stainless steel instruments were used to remove the parasites (n = 63) from the abdominal cavity.

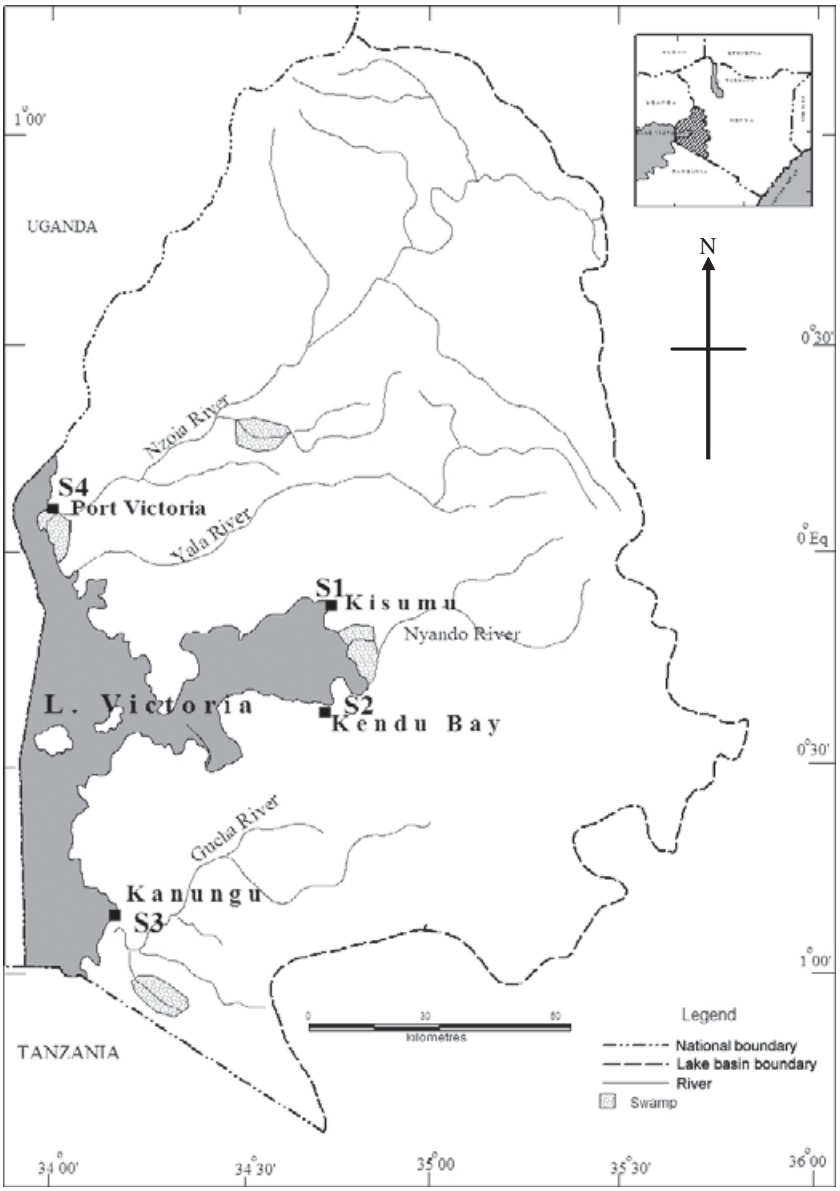

Fig. 1. Map of Lake Victoria basin (Kenya), identifying sampling sites.

Table 1. Characteristics of fish and parasite samples collected from four Lake Victoria sampling sites

	Sampling site			
Fish catch data	S1	S2	S3	S4
Fish catch date	June–July 2005	June–August 2005	June–August 2005	June–August 2005
Number of fish	28	32	31	34
Sex ratio (M:F)	13:15	17:15	16:15	15:19
Mean length (mm)	36.0 ± 1.2	35.4 ± 1.7	37.1 ± 1.3	38.4 ± 2.2
Mean weight (g)	0.49 ± 0.22	0.54 ± 0.64	0.61 ± 0.43	0.55 ± 0.32
Prevalence of *Ligula intestinalis* (%)	23.2	17.2	7.1	23.2
Mean weight of *Ligula intestinalis* (g)	0.22 ± 0.14	0.11 ± 0.05	0.19 ± 0.02	0.13 ± 0.07
Mean abundance in *Rastreneobola argentea*	1.95 ± 0.15	2.31 ± 0.22	2.17 ± 0.02	1.07 ± 0.07

The parasites were then counted, weighed and stored in glass vials. They were then transferred to Teflon vials and freeze-dried (−80°C), awaiting subsequent heavy metal analysis. The parasitized and unparasitized fish were also freeze-dried. All the samples were bagged and frozen at −4°C until processed in the Netherlands.

Sampling and analysis of water

The river water samples were obtained with the Grab Technique Method, using 0.5 L, metal-free Van Dorn bottles. All the water samples were collected at 0.5-m depth in the lake. The water samples were then transferred to half-litre polythene bottles pre-soaked in nitric and sulphuric acid solutions of 1:1 volume ratio, washed in 2 L of tap water and rinsed three times in distilled water dried prior to the field work. The collected water samples were acidified to pH = 2 with concentrated nitric acid (APHA 1998). The samples were then placed into an ice box and transported to the laboratory for chemical analyses.

Sediment samples

A total of 36 sediment samples were collected from each of the four sites. The sediments were collected with Ekman Grab Samplers. A polypropylene spatula was used to transfer the sediment subsamples to acid-rinsed polypropylene bottles. The samples were placed in an icebox and transported to the laboratory for chemical analyses.

Heavy metal analyses

All samples were analysed at the Physical Geography Laboratory of the University of Amsterdam in the Netherlands. All frozen fish, parasite and sediment samples were thawed, crushed and homogenized, using a Pulver Settle 5′ planetary mill (Fritsch GmbH, Idar-Oberstein, Germany) for 5 min at 400 rpm. The whole fish samples (≈0.5 g) were weighed for digestion. The weighing instrument calibrated the weight to 0.5000 g for fish samples weighing less than, or more than, 0.5000 g. As all the parasite samples weighed <0.5 g, the actual weight of the individual parasites were determined and calibrated to 0.5000 g during the final heavy metal analyses. In a similar manner, 0.5000 g of sediment samples was weighed for digestion. About 0.5 mL of the water samples was transferred via pipette to a high-pressure quartz vessel. Digestion of all the samples were performed in a solution of 4 mL concentrated nitric acid and 1 mL concentrated hydrochloric acid, in a closed higher-pressure quartz vessel, using a micro-wave digester (Canton Paarä GmbH, GAZ, Austria). The samples were digested using an optimized microwave method at a temperature 200°C and pressure of 75 atmospheres. After digestion, the samples were diluted with 50 mL of nanopure water, and transferred into cleaned 50 mL polystyrene bottle. The heavy metals were analysed with a Perkin Elmer DV 4300 Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The quality of the analytical process for the fish also was controlled by analysis of the NIST-CE278 certified standard reference material for fish tissues. Analytical blanks were used to determine the instrument detection limits. The measured values deviated <10% from the certified values (Table 2). The heavy metal concentrations in the fish, parasite and sediment samples were expressed as mg kg^{-1} dry weight, whereas the heavy metal concentration in the water samples was expressed as mg L^{-1}.

Statistical analyses

The *W*-test developed by Shapiro and Wilz (Gilbert 1987) was used to evaluate the normal/log-normal distribution of the data. A normality test of the data indicated that only a few datasets conformed to a non-parametric distribution. To achieve the criterion of data normality before statistical procedures were performed, all the non-parametric data were log-transformed, using the equation: $x' = \log(x + 1)$ (Zar 1996). Differences in the metal concentrations in the biota, water and sediment samples between sites were analysed, using one-way ANOVA. Whenever the null hypothesis was rejected, a multiple comparison test (Tukey's HSD test) was used to localize the differences. The relationships between the heavy metal concentrations were based on a set of regression analyses between data from the host fish and its parasites. As it was unknown whether or not population Y is zero when X is zero, the regression analysis option of 'No model intercept' was not utilized for any dataset (Zar 1996). The heavy metal bioaccumulation

Table 2. Detection limits (ng mL^{-1}) and heavy metal concentrations (μ g^{-1}) in standard reference material NIST-CE278, as determined by inductively coupled plasma mass spectrometry (ICP-OES)

Heavy metal	Detection limit	Standard	ICP-OES value means (±SD)	Certified value mean (±SD)	Accuracy
Lead (Pb)	0.06	NIST-CE278	0.31	0.32	96.88
Cadmium (Cd)	<0.01	NIST-CE278	17.94	19.38	92.57
Chromium (Cr)	0.98	NIST-CE278	31.25	34.70	90.06
Copper (Cu)	<0.01	NIST-CE278	1.90	2.04	93.14

factor was determined according to the method of Sures *et al.* (1999), being expressed as the ratio of the heavy metal concentration in the parasites to that in the whole fish samples ($C_{parasite}/C_{host\ tissue}$).

RESULTS

The first step in this study was to determine the concentrations of four heavy metals, including lead (Pb), cadmium (Cd), chromium (Cr) and copper (Cu) in the water and sediment samples from the four sampling sites (Table 3). The concentrations of Pb, Cd, Cr and Cu in the water samples were significantly different between the sampling sites ($P < 0.05$). The water samples from sampling site 1, which is located near an urban area, contained significantly higher Pb, Cd and Cu concentrations than the other sampling sites. In contrast, the Cr concentration was elevated for sampling site 3, compared with the other sites. For the sediment samples, those from sampling site 1 exhibited higher concentrations of Pb and Cd, whereas Cr was found to be elevated in the sediment from sampling site 3 than for the other sites.

The metal concentration in the fish and their intermediate parasites, *L. intestinalis*, were then compared among the sampling sites (Fig. 2). The concentrations in all the fish samples exhibited significant spatial variations. The specific metal contents in the fish parasites ($P < 0.05$) also exhibited significant spatial variations. There were no discernable differences between male and female fish, for either the parasitized and non-parasitized samples ($P > 0.05$) for any of the specific metals. Accord-

Table 3. Heavy metal concentrations in water (mg L^{-1}) and sediment (mg kg^{-1}) samples for four Lake Victoria sampling sites in Lake Victoria

	Sampling site							
	S1	S2	S3	S4	S1	S2	S3	S4
Heavy metal	Water				Sediment			
Lead (Pb)	0.99 ± 0.07^{c}	0.32 ± 0.05^{b}	0.31 ± 0.03^{b}	0.26 ± 0.02^{a}	3.64 ± 0.21^{c}	2.42 ± 0.18^{b}	2.55 ± 0.24^{b}	1.87 ± 0.17^{a}
Cadmium (Cd)	0.06 ± 0.009^{c}	0.02 ± 0.004^{a}	0.02 ± 0.005^{a}	0.03 ± 0.003^{b}	0.12 ± 0.05^{c}	0.09 ± 0.04^{b}	0.06 ± 0.054^{a}	0.05 ± 0.03^{a}
Chromium (Cr)	0.39 ± 0.04^{b}	0.23 ± 0.04^{a}	0.79 ± 0.02^{c}	0.36 ± 0.03^{b}	0.91 ± 0.08^{c}	0.62 ± 0.06^{b}	1.12 ± 0.11^{d}	0.42 ± 0.05^{a}
Copper (Cu)	0.94 ± 0.03^{c}	0.69 ± 0.04^{a}	0.71 ± 0.05^{a}	0.82 ± 0.05^{b}	3.44 ± 0.19	3.31 ± 0.23	3.31 ± 0.35	3.34 ± 0.54

Mean values with the same superscript letters are not significantly different ($P > 0.05$) in water and sediment samples across the rows.
SE, standard error, calculated from the ANOVA mean-square of error.

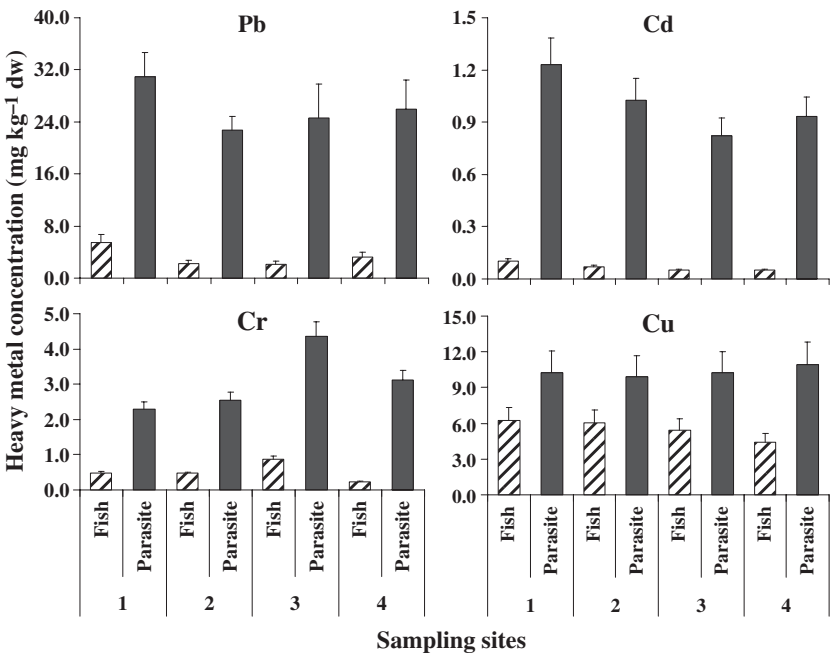

Fig. 2. Mean metal concentrations in fish and fish parasites (mg kg^{-1} dw ± SEM) from the four sampling sites in Lake Victoria (dw, dry weight of sample; SEM, standard error of mean; Pb, lead; Cd, cadmium; Cr, chromium; Cu, copper).

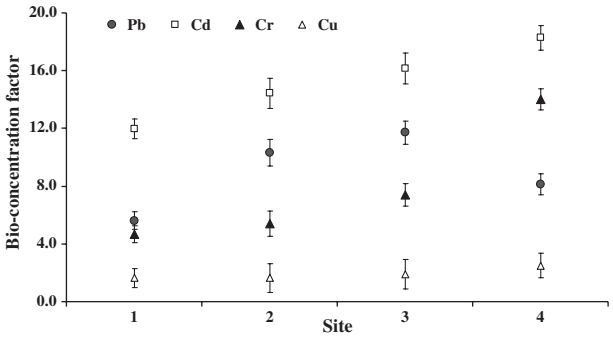

Fig. 3. Mean (±SEM) of bioaccumulation factors ([C]parasite/[C]host sample) for heavy metals analysed in *Ligula intestinalis*, in relation to *Rastreneobola argentea* samples (Pb, lead; Cd, cadmium; Cr, chromium; Cu, copper).

ingly, these data were pooled for the purpose of analyses. The Cr concentration displayed more prominent site-specific differences in the fish and fish parasites than did the other measured heavy metals, with sampling site 3 exhibiting the highest Cr concentration. The fish parasite samples contained systematically higher concentrations of all four heavy metals for all the sampling sites than did their fish host.

Considering the trace element levels detected in *R. argentea* and respective host, it was possible to determine that all four heavy metals were present at higher levels in the cestode than in *R. argentea* (Fig. 3). The Cd bioaccumulation factor (12–18) was the highest of all the analysed metals, being consistently higher for sampling site 4, and lowest for sampling site 1. The Pb concentration in the cestode was consistently higher (bioaccumulation factor = 5–11) in the parasites than in the fish, with the highest bioccumulation factor being observed for sampling site 3 and the lowest for sampling site 1. The accumulation of Cr ranged from 4 to 6 times higher in the cestode than in the fish for sampling sites 1, 2 and 3, but increased to higher values of 12 for sampling site 4. The Cu bioaccumulation factor (1.5–2.5) was the lowest among all the heavy metals analysed, displaying a consistent similarity for all four sampling sites.

Subsequent regression analyses served to highlight relationships between the concentration of some heavy metals in the parasite and in some of the hosts (Fig. 4). In fact, it was found that the Pb, Cd and Cr concentration in *L. intestinalis* increased as a linear function of the metal concentrations in *R. argentea*, albeit the increased Pb and Cr concentrations in the cestode parasite, relative to the fish, were more predictable than the Cd changes. It was also possible to determine that the increased Cu concentration in the parasite was associated with a linear reduction of the Cu concentration in the fish.

The changes in the heavy metal body burden of the *R. argentea*, relative to the changes in number of parasites counted in the fish abdominal cavity, was also modelled (Fig. 5). The results demonstrated no discernable

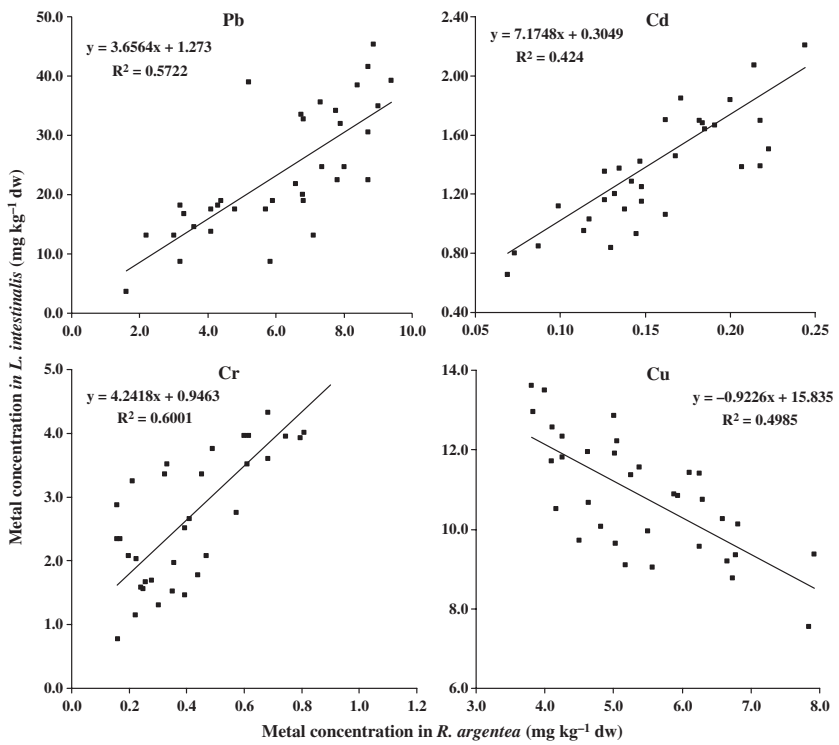

Fig. 4. Regression models illustrating relationships between heavy metal concentrations (mg kg^{-1} dw) in *Ligula intestinalis* and in *Rastreneobola argentea* (dw, dry weight of sample; Pb, lead; Cd, cadmium; Cr, chromium; Cu, copper).

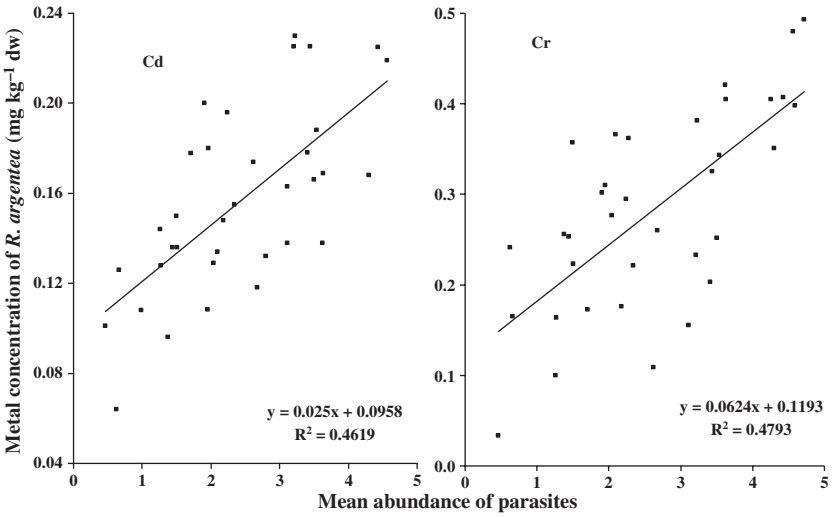

Fig. 5. Regression analyses illustrating relationships between heavy metal concentration (mg kg^{-1} dw) in *Ligula intestinalis* and mean intensity of *Ligula intestinalis* (dw, dry weight of sample; Cd, cadmium; Cr, chromium).

changes in the Pb and Cu concentrations in the fish samples, as the abundance of the parasite changed (data not shown). Nevertheless, the increased Cd and Cr concentrations were influenced by the increased parasite abundance in the fish abdominal cavity.

DISCUSSION

The concentration of the four analysed heavy metals in the water and sediment samples from Lake Victoria displayed significant spatial distribution, suggesting similar patterns of heavy metal enrichment of water and sediments within the lake. A similar observation was previously reported by others (see Mwamburi 2003). It is evident that sites located in areas influenced by anthropogenic activities along the lakeshore exhibited different heavy metal concentrations. Although there has been a progressive increase in the heavy metal concentrations in the lake over the past 20 years (Wandiga 1981; Wandiga *et al.* 1983; Ochieng 1987; Onyari & Wandiga 1989; Mwamburi & Oloo 1996/1997; Kishe & Machiwa 2003), the concentration of the four heavy metals examined in this study are still low, compared with heavy metal concentrations observed in water systems in industrialized countries of Europe and America (Schuldermann *et al.* 2003; Thielen *et al.* 2004; Sánchez-Chardi & Nadal 2007; Tekin-Özan & Barlas 2008; Eira *et al.* 2009).

The general pattern of heavy metal accumulation in this study exhibited the following progression: fish parasite > fish > sediment > water. As a result of the low heavy metal concentrations in the lake water, sediment and fish, it appears that the parasite *L. intestinalis* was better at accumulating heavy metals to a higher concentration than observed in their environmental media or in their respective intermediate host fish, *R. argentea*. The bioaccumulation factors of Pb, Cd and Cr in *L. intestinalis* were higher by up to factors of 11, 18 and 14 respectively, when the heavy metals' concentrations in the cestode parasite and the fish hosts were compared. The bioaccumulation ability of *L. intestinalis* for Pb, Cd and Cr in the present study compares well with metal bioaccumulation ability of *Caryophllaaus laticeps* in nase, *Chondrostoma* (Jirsa *et al.* 2008). The bioaccumulation ability of *L. intestinalis* is higher in *R. argentea*, however, compared with tench (*Tinca tinca*) from Lakes Kovada (Tekin-Özan & Kir 2005) and Beysher (Tekin-Özan & Barlas 2008), both in Turkey. The bioaccumulation of Cd by *L. intestinalis* in *R. argentea* is lower than for another cestode (*Monobothrium wageneri*) and *Bothriocephalus scorpii* in *Tinca tinca* (Sures *et al.* 1997). The higher accumulation of heavy metals by *L. intestinalis* in a cyprinid (*R. argentea*) is not reflected in the metal accumulation ability of *B. scorpii* and *Gallegoidae surfacai* in other cyprinids (Sures *et al.* 1997), or in rodents (*Apodemus sylvaticus*) (Sures 2004). Malek *et al.* (2007) hypothesized that, because cestodes cannot synthesize their own cholesterols and fatty acids, they efficiently obtain them from their host's intestinal lumen, thereby reducing the absorptive capacity of the heavy metals in the fish body cavity with the presence of parasites. Considering that *L. intestinalis* has a very large surface area-to-volume ratio (Cowx *et al.* 2008) in the host, they can easily take up organo–metallic complexes, which have been released from the host's bile duct to the small intestines. Consequently, these metals can accumulate in the body of the parasites, thereby rendering the parasites as sinks for these metals. It is also possible that fish have developed effective mechanism by which many heavy metals are

transported, stored and excreted (Bijvelds *et al.* 1998). Such cellular mechanism possibilities, however, have not yet been reported for cestodes. Thus, the higher bioaccumulation of metals in the cestode could be reflected by this physiological mechanism.

The cestode parasites exhibited a consistent site-specific bioaccumulation pattern in this study. The highest bioaccumulation factor of the parasite was exhibited for sampling site 2. This finding might indicate a coupling of geological metals from the environment that could increase the metal burden in fish. To this end, alluvial gold mining activities by local community members were observed within the proximity of sampling site 2. This variability, however, which might reflect the mobility of the fish host, can obscure differences that might otherwise be detected between sampling sites.

With the exception of a higher bioaccumulation capacity of an acanthocephalan (*Acanthocephala lucii*) in zebra mussel (*Dreissena polymorpha*) (Sures 2003), the high Cu bioaccumulation factor for the cestode fish parasite in this study, has not previously been reported. As Cu is an essential element, the high Cu bioaccumulation capacity by a cestode parasite, and the negative correlation between the Cu concentrations in fish and parasites, might reflect elemental competition between the fish and parasite. Both parasite and fish hosts have been shown to compete for several elements, including Ca, Cu, Fe, Zn and Sr (Sures 2002). There is limited information on inter-elemental relationships in fish–parasite associations, however, because of a paucity of data from studies dealing with simultaneous analyses of different elements in fish, and even information on metal kinetics and metal metabolism in fish–parasite associations. It is probable, therefore, that competition for elements between hosts and parasites for essential elements could lead to increased absorption of other essential heavy metals, including Cu. The Cu concentration might be regulated by the fish, as well as by the parasite, albeit the physiologically required concentrations are actually higher for the parasite. This higher Cu accumulation in the parasite, therefore, cannot be considered as the bioaccumulation of environmental pollutants.

It was also observed that fish with higher abundances of endoparasites exhibited higher CD and Cr concentrations (Fig. 5). This finding, however, cannot be interpreted as meaning that fish heavily infested with parasites necessarily contain higher metal concentrations from the parasites. Rather, it could be associated with disruption of fish physiological functions by the parasites that impair the ability of the fish to regulate metal contents in their tissues. Moreover, infested fish already weakened by the parasite infection might be more vulnerable to other suboptimal or adverse environmental conditions, such as food shortage or water pollution. Fish endoparasites, mainly *L. intestinalis*, have been reported to affect normal physiological functions (Cowx *et al.* 2008), some of which would render their host prone to metal bioaccumulation from the aquatic environment. The parasitized fish samples in this study were observed to be heavier than the unparasitized fish samples, contrary to the observations of Sures (2003) for acanthocelephalans heavily infested by parasites. This scenario might not be related to either the metal content or kinetics in the fish, but rather by hormonal disturbances through endocrine disruption (Jobling & Tyler 2003). The latter have been widely reported for roach (*Rutilus rutilus*) and godgeons (*Gobio gobio*) (Van Aerle *et al.* 2001). Furthermore, *L. intestinalis* has been reported to interfere with pituitary–gonodal axis of its host, thereby delimiting reproduction (William *et al.* 1998; Cowx *et al.* 2008). Thus, the energy budget associated with reproduction can be substituted for feeding and, together with heavy metal uptake, could induce gigantism of the fish.

In conclusion, this study illustrates that the *L. intestinalis* in *R. argentea* bioaccumulate heavy metals in variable quantities in its fish host. The heavy metals Pb, Cd and Cr were bioaccumulated by factors up to 11, 18 and 14 respectively, and Cu by a factor of 2.5 in the fish endoparasites. Whereas Cu was demonstrated to be the subject of element competition between the fish and the parasite, the heavy metals Pb, Cd and Cr displayed a partitioning in the fish hosts, with parasites having higher concentrations of these metals. This finding suggests that the *L. intestinalis* in its *R. argentea* host are a promising biomonitor for assessing exposure to these heavy metals.

Kennedy (1997) has outlined the disadvantages of using fish parasites for this purpose, including the possibility of the parasite being directly or indirectly influenced by the host metabolic factors or immune system, by parasite overdispersion and aggregation, by inadequate knowledge of their physiological responses to metal pollution, and, in this study, to site-specific variations in metal accumulation. In regard to the bioaccumulation factor, it appears that *L. intestinalis* is a sensitive indicator and early warning sign for Pb, Cd and Cr pollution in water systems. As this parasite is easy to identify, even in different hosts (Olson *et al.* 2000; Tekin-Özan & Kir 2005; Tekin-Özan & Barlas 2008), because of its high abundance and high prevalence, it should be considered as a biomonitor model and early warning sign for localized Pb, Cd and Cr pollution in waterbodies.

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