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Use of cestodes as indicator of heavy-metal pollution

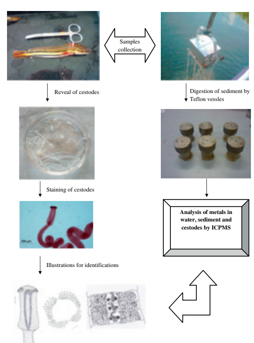
Tran Thi Yen Nhi [a,*], Noor Azhar Mohd Shazili [b], Faizah Shaharom-Harrison [a]

[a] Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia
[b] Vice Chancellor, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Malaysia

HIGHLIGHTS

- We measured metal concentration in cestodes, fish tissues, sediment and water.
- We used an inductively-coupled plasma mass-spectrometry (ICP-MS) equipment.
- Cestodes from fish host accumulated some heavy metals higher than the water.
- Cestodes accumulated some heavy metals higher than fish tissues but lower than sediment.
- Cestodes have potential as a bioindicator of heavy-metal pollution.

GRAPHICAL ABSTRACT



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ABSTRACT

Thirty snakehead fish, *Channa micropeltes* (Cuvier, 1831) were collected at Lake Kenyir, Malaysia. Muscle, liver, intestine and kidney tissues were removed from each fish and the intestine was opened to reveal cestodes. In order to assess the concentration of heavy metal in the environment, samples of water in the surface layer and sediment were also collected. Tissues were digested and the concentrations of manganese (Mn), zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) were analysed by using inductively-coupled plasma mass-spectrometry (ICP-MS) equipment. The results demonstrated that the cestode *Senga parva* (Fernando and Furtado, 1964) from fish hosts accumulated some heavy metals to a greater extent than the water and some fish tissues, but less than the sediment. In three (Pb, Zn and Mn) of the five elements measured, cestodes accumulated the highest metal concentrations, and in remaining two (Cu and Cd), the second highest metal accumulation was recorded in the cestodes when compared to host tissues. Therefore, the present study indicated that *Senga parva* accumulated metals and might have potential as a bioindicator of heavy-metal pollution.

1. Introduction

Parasite species are widely considered as indicators of local pollution. The parasite specificity to their host or their strict environmental requirements, such as water temperature and salinity have been used to separate stock and tag populations of fish or species of commercial value. Environmental changes can make parasite populations increase or decrease. Wastewater or industrial pollutants may lead to an increase in fish parasites due to a decrease in immunological defence and a lower resistance of fish to infections (Sasal et al., 2007).

Parasites are a major part of the aquatic environment and represent a significant proportion of the aquatic biomass. The relationship between environmental pollution and parasitism in aquatic organisms and the potential role of endoparasites as water-quality indicators have received increasing attention during the past two decades (Sures, 2003, 2004). Polluted conditions can make parasites tend to increase in numbers since they are sensitive to

* Corresponding author. Fax: +60 9 6683390.
E-mail addresses: kimhue12588@yahoo.com.vn (T.T. Yen Nhi), faizah@umt.edu.my (F. Shaharom-Harrison).

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environmental changes (Sures, 2004). In the aquatic environment, there are several types of pollutants such as domestic sewage, pesticides, heavy metals, paper effluents, petroleum and acid rain which are known to affect aquatic animals (Law, 1993). Because of their great diversity in terms of number of species and their number of life-history strategies, there is an increasing interest in using parasites as biological or ecological indicators of their fish host life conditions (Sindermann, 1979; Williams et al., 1992; Marcogliese and Cone, 1997; Marcogliese, 2005).

Certain parasites, particularly intestinal acanthocephalans and cestodes of fish, can accumulate heavy metals at concentrations that are orders of magnitude higher than those in the host tissues or the environment (Sures et al., 1999, 2001; Sures, 2003, 2004; Turcekova and Hanzelova, 1997; Jirsa et al., 2008; Jankovská et al., 2011, 2012). However, currently, little is known about the accumulation of toxins within parasites in Malaysia. The present study was conducted in Lake Kenyir, Malaysia. The lake is a unique tourist destination because of beautiful scenario of tropical forest and is widely known for its valuable flora and fauna species. The aim of this study is to evaluate the metal accumulation ability of the cestodes compared to their host as a possible sentinel in monitoring increasing heavy-metal pollution in a lacustrine environment. Incidentally, the potential suitability of cestodes as sentinel organisms for metal pollution under natural environmental conditions is determined.

2. Materials and methods

2.1. Fish tissues and worms collection

Fish were collected at Lake Kenyir. Upon capture, the fish were killed by pithing on the head before measurement of the total length, standard length and weight. Based on the findings of previous studies on the high bioaccumulation in certain tissues, the liver, muscle, intestine and kidney were selected and collected with wooden or plastic forceps. These tissues were dissected out and placed in clean bottles with lids. They were kept frozen at −20 °C until metal analysis was done.

The intestine was removed from each fish and placed in a plastic Petri dish with saline solution. The intestine was cut open to reveal the cestodes. The worms were carefully removed by using the same instruments and placed in pre-cleaned bottles with lids for each fish. Thereafter the cestodes were stored at −20 C until further processing.

2.2. Water collection

The water samples were collected in the surface layer and stored in polyethylene bottles. For total metals, 1 mL pure nitric acid was added to 500 mL unfiltered water samples. For dissolved metals, water samples were taken back to the laboratory, filtered through 0.45 μm membrane filter and stored at 4 °C after addition of 1 ml pure nitric acid.

2.3. Sediment collection

Before sampling, the equipment was cleaned by bathing it in nitric acid for a week at room temperature followed by rinsing with deionised water in order to remove metal contamination. The soil sediment at the bottom of the lake was taken by using an Ekman-Grab at different stations. The samples were placed in plastic bags and frozen.

2.4. Digestion of fish tissues and cestodes

Approximately, 0.05 g each of tissues sample was accurately weighed and placed into a Teflon vessel. Then, 2 mL HNO_3 and 1 mL H_2O_2 were added to the Teflon vessel which was encased in its jacket and heated at 100 °C for 5 h and 35 °C for 8 h. The digested samples were placed in 10 mL polypropylene test tubes followed by addition of deionised water to a volume of 10 mL. Then 1 mL sample was transferred into another test tube followed by addition of deionised water to a volume of 10 mL. After that, samples were analysed by using an inductively-coupled plasma mass spectrometry (ICP-MS). Concentrations of cadmium (Cd), manganese (Mn), zinc (Zinc), copper (Cu) and lead (Pb) were recorded. In order to determine the accuracy of the extraction procedure, a standard reference material (SRM: DORM-3, NRCC) was digested using the same protocol. The recovery range for heavy metals must be between 90% and 100%.

2.5. Digestion of sediment

Sediment was sieved through a series of sieves 4 mm to <63 μm. Samples (0.05 g) were digested as described for tissues and tapeworms but with HCl, HNO_3 and HF acids in ratio of 3:3:2 and followed by heating at 100 °C for 5 h. To determine the accuracy of the extraction procedure, a standard reference material (SRM: BCR-667 Estuarine sediment, IRMM) was digested using the same protocol. The recovery range for heavy metals must be between 90% and 100%.

2.6. Scanning ICP-MS

The standard solutions for ICP-MS (20, 40, 60, 80, 100 and 120 ppb) were prepared. These solutions were diluted in 50 mL milli-Q water. Then, samples were injected in ICP-MS.

2.7. Statistical analysis

Element concentrations in fish tissues, parasites and sediment of the lake were determined as mg/kg (dry weight) while water levels were determined as μg/L. One-way ANOVA was used to evaluate the significant difference in the concentration of metals in different studied fish tissues and cestodes. A probability at a level of 0.05 or less was considered significant. All statistical calculations were performed with SPSS 16.0 for windows.

3. Results

3.1. Metal concentration in water

The variation of total (T) and dissolved (D) metals concentrations in the lake water are presented in Table 1. For total metal, average contents were found in the following order:

Table 1
Total metal (T) and dissolved metal (D) concentrations in the lake water.

Metal		Sampling site		
		1	2	3
Pb	T (μg/L)	13.20 ± 1.59^{a}	5.89 ± 1.57^{b}	5.24 ± 1.03^{bc}
	D (μg/L)	0.82 ± 0.36^{ab}	2.68 ± 1.14^{b}	0.50 ± 0.77^{ac}
Cu	T (μg/L)	53.10 ± 3.48^{a}	3.86 ± 1.01^{b}	3.55 ± 1.09^{bc}
	D (μg/L)	2.47 ± 0.99^{a}	3.12 ± 0.37^{a}	2.73 ± 0.63^{a}
Zn	T (μg/L)	51.00 ± 4.00^{a}	31.60 ± 1.69^{b}	14.00 ± 0.80^{c}
	D (μg/L)	12.20 ± 0.79^{ab}	14.3 ± 1.40^{b}	6.27 ± 0.64^{ac}
Mn	T (μg/L)	67.40 ± 1.70^{a}	5.16 ± 0.49^{b}	5.13 ± 0.30^{ab}
	D (μg/L)	1.25 ± 0.67^{a}	1.29 ± 0.42^{a}	1.30 ± 0.34^{a}
Cd	T (μg/L)	3.90 ± 1.10^{a}	0.25 ± 0.11^{b}	0.07 ± 0.02^{bc}
	D (μg/L)	0.13 ± 0.11^{a}	0.09 ± 0.09^{a}	0.02 ± 0.24^{a}

Letters a, b and c show differences among sites. Data shown with different letters are statistically different at $P < 0.05$ level.

Table 2
Total metal concentrations in sediment.

Rep	Site	Concentration of heavy metal (mg/kg dry weight)					Total metal
		Pb	Cu	Zn	Mn	Cd	
1	1	1.93	4.21	11.12	53.10	0.99	71.35
	2	4.40	3.58	8.82	48.69	1.15	66.66
	3	4.11	3.08	6.97	115.04	0.94	130.14
2	1	1.98	5.43	12.08	55.20	1.04	75.73
	2	5.34	4.09	7.97	53.34	1.05	71.79
	3	4.25	4.13	7.03	118.06	0.87	134.34
3	1	2.37	4.34	12.03	67.83	0.85	87.42
	2	3.93	3.67	8.01	66.54	1.01	83.16
	3	4.34	3.02	5.55	115.57	0.96	129.44
Mean ± SD	1	2.09 ± 0.24^{a}	4.66 ± 0.67^{a}	11.74 ± 0.54^{a}	58.71 ± 7.97^{a}	0.96 ± 0.09^{a}	78.17 ± 8.31^{a}
	2	4.56 ± 0.72^{b}	3.78 ± 0.27^{ab}	8.27 ± 0.48^{b}	56.19 ± 9.26^{ab}	1.07 ± 0.07^{a}	73.87 ± 8.44^{b}
	3	4.23 ± 0.12^{bc}	3.41 ± 0.62^{bc}	6.52 ± 0.84^{bc}	116.22 ± 1.6^{c}	0.92 ± 0.04^{a}	131.3 ± 2.65^{c}

Letters a, b and c show differences among sites. Data shown with different letters are statistically different at $P < 0.05$ level.

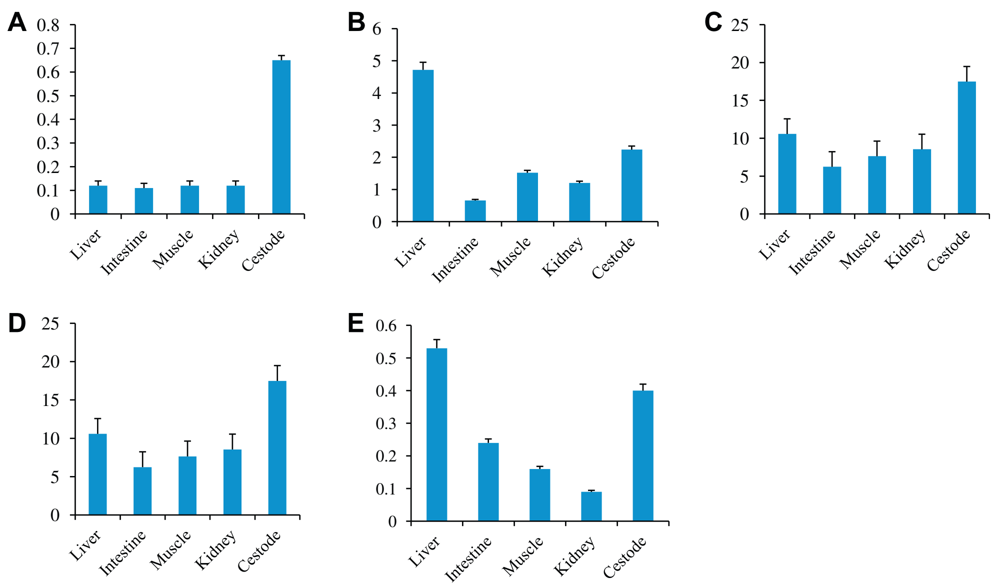

Fig. 1. Concentrations of metals in fish tissues and cestodes ((A) concentration of Pb, (B) concentration of Cu, (C) concentration of Zn, (D) concentration of Mn and (E) concentration of Cd).

Table 3
Comparison of heavy-metals concentrations in lake water with USEPA (1986) and Interim National Water Quality Standards for Malaysia (INWQS).

Metals	This study (mg/L)	USEPA (1986) (mg/L)	Classes (INWQS) (mg/L)				
			I	II	III	IV	V
Cd	<0.001	0.01	–	0.01	0.001	0.01	–
Cu	0.003	1.0	–	1	–	0.2	–
Pb	0.001	0.05	–	0.05	0.01	5	–
Mn	0.001	0.05	–	0.1	0.2	0.2	–
Zn	0.011	1.0	–	5	0.4	2	–

Zn > Mn > Cu > Pb > Cd. This order was different for dissolved metal being Zn > Cu > Pb > Mn > Cd.

3.2. Metal concentration in sediment

The total metal concentrations and average values for each sampling site found in sediments in this study are as shown in Table 2. Metal contents were arranged from higher to lower mean content in this area as: Mn > Zn > Cu > Pb > Cd.

3.3. Metal concentration in fish tissues and cestodes

Cestode in this study is closed to *Senga parva* reported by (Fernando and Furtado, 1964) with small, long and creamish in colour, rectangular scolex with two rows of semicircular hooks on apical disc. The heavy-metal content in different organs and cestodes of *Channa micropeltes* are shown in Fig 1. The highest content of Pb, Zn and Mn were found in cestodes, while Cu and Cd were highest in the liver of the fish.

Generally, cestodes accumulated the highest concentrations of three (Pb, Zn and Mn) out of the five elements studied and accumulated Cu and Cd at the second highest concentration. The liver had the highest accumulation of two elements (Cu and Cd).

4. Discussion

The aquatic environment with its water-quality is considered the main factor controlling the state of health and disease in both cultured and wild fish. Pollution of the aquatic environment by inorganic and organic chemicals is a serious threat to the survival of aquatic organisms including fish (Samir and Ibrahim, 2008). With regards to Malaysian interim water-quality standard (Ahmad et al., 2009) and permissible limits recommended by USEPA (1986), Lake Kenyir still has good water quality (Table 3).

Metals enter the aquatic environment from a variety of sources, including those naturally occurring through biogeochemical cycles and those added through anthropogenic sources, such as industrial and domestic effluents, urban pollution, stormwater runoff, landfill leachate and atmospheric sources (Forstner and Wittman, 1981). By comparing the accumulation of heavy metals in water and sediments in the present study, it may be concluded that the heavy metals accumulated more in sediments than water, since the sediments acted as reservoir for all contaminants and dead organic matter descending from the ecosystem above. Similar findings were reported by other authors (Hamed, 1998; Nguyen et al. 2005). Sediments have a high storage capacity for pollutants. Within any part of the hydrological cycle, far less than 1% of these are actually dissolved in the water; more than 99% are stored in the sediments (Salomons and Stigliani, 1995).

Aquatic organisms tend to integrate all the stresses placed on the aquatic system and reflect combined effects over an extended period of time. In addition, aquatic organisms are recognized as bio-accumulators of pesticides and heavy metals and consequently can be used to monitor pesticides and metal pollution. Thus, fish are considered to be a suitable component of the aquatic system to monitor because they integrate the effect of detrimental environmental change as consumers that are relatively high in the aquatic food chain. Furthermore, fish as indicator organisms for biological monitoring have a high public profile and consequently are used extensively for conservation status and bioaccumulation studies (Hellawell, 1986).

In the present study, cestodes accumulated the highest metal concentrations. The second highest metal concentrations were found in the liver of the host, while the lowest were detected in the intestine of the host. Cestodes accumulated the highest metal concentration in three (Pb, Zn and Mn) of the elements studied. The second highest concentration of metals in cestodes was that of Cu and Cd. In many instances, metal concentrations were substantially higher than in the host tissue, for example concentrations of Mn in cestodes were 4.7, 11.2, 15.4 and 5.5 times higher than liver, muscle, kidney and intestine of their hosts.

Tenora et al. (2000) found that Pb, Cr and Cd concentrations in *Ligula intestinalis* plerocercoids were 15.6 and 2.6 times higher than in fish muscle. Similarly, Tekin-Özan and Kir (2005) reported that the iron level in *L. intestinalis* plerocercoid was 37.4 times higher than that of the muscle, was 2.4 times higher than that of the liver and 5.6 times higher than that in the gill. Retief et al. (2009) investigated cestode *Bothriocephalus acheilognathi* from the largemouth yellowfish and found that they had potential as bio-indicators, because in 8 out of the 23 elements measured cestodes had accumulated the highest metal concentrations. Results from the present study corroborated that of European researchers (Galli et al., 1998; Sures et al., 1999; Sures, 2003, 2005) on metals accumulated in endoparasites. The accumulation of metals in the liver could be due to the tendency of the elements to react with the oxygen carboxylate, aminogroup, nitrogen and sulphur of the mercapto group in the metal lothionein protein, whose concentration was highest in the liver (Yousuf et al., 2000). However, muscle tended to accumulate lower metals as it is not an active tissue for heavy-metal accumulation (Karadede et al., 2004).

Heavy metals such as copper, zinc and iron are essential for fish metabolism while some others such as mercury, cadmium and lead have no known role in biological systems. For the normal metabolism of fish, the essential metals must be taken up from water, food or sediment. However, nonessential ones are also taken up by fish in a similar manner and accumulate in their tissues (Mustafa and Atli 2002). The difference in metal concentrations of the tissues could be as a result of their capacity to induce metal-binding proteins such as metallothioneins. The present data showed that metal concentrations in the liver and kidney were highest in the fish studied. It is well known that a large amount of metallothionein induction occurs in the liver of fish (Canli and Furness, 1993). In fish tissues, it is clear that Zn showed the highest concentration in all tissues. In muscle tissue, Zn level was followed by Cu, while Pb showed the lowest concentration. In intestine, Zn concentration was followed by Mn and Cu, with Pb was the lowest one. Similarly, Zn and Cu showed the highest level in liver and kidney, while Pb and Cd showed the lowest levels. This could be explained by the fact that Zn and Cu are essential elements for living organisms and have an important role in different physiological processes.

Alterations in metal uptake and accumulation in organisms due to parasites are a comparatively new but very important field in terms of ecotoxicological research. Following the first evidence of reduced metal uptake in infected fish compared to uninfected ones (Sures et al., 1999), there is now an increasing number of papers describing alterations of chemical uptake in intermediate hosts (Evans et al., 2001) as well as in final hosts due to parasitism (Sures, 2003). A reduced chemical uptake in organisms used for ecotoxicological studies, for example as accumulation indicators, may erroneously indicate low levels of pollution. Therefore, biomonitoring programmes should take into account the influence of parasite infections on the levels of pollutants in sentinels.

The trend for all of the elements in fish tissues, cestode, water and sediment in the present study was obtained as the following: sediment > cestodes > fish tissues > water. Statistical analysis indicated that the means were significantly different between sediment, water and cestode ($p < 0.05$).

5. Conclusion

Cestodes *Senga parva* from fish hosts *Channa micropeltis* accumulated some heavy metals higher than in the water and fish tissues but lower than in sediment. Cestodes accumulated the highest concentration of Zn while three elements (Pd, Cd and Mn) were found with the second highest level in cestodes when compared to sediment, water and fish tissues. This study supports the idea that cestodes are very useful for determining the level of heavy-metal pollution in an aquatic system.

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