

USE OF THE FRESHWATER CRAB *TRICHODACTYLUS FLUVIATILIS* TO BIOMONITORING AL AND MN CONTAMINATION IN RIVER WATER

USO DO CARANGUEJO DE ÁGUA DOCE *TRICHODACTYLUS FLUVIATILIS* COMO BIOMONITOR DA CONTAMINAÇÃO DE AL E MN EM AMBIENTES FLUVIAIS

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ABSTRACT

Determinations of Al and Mn concentrations in the tissues (gills, hepatopancreas and muscle) of the freshwater crab *Trichodactylus fluviatilis* and water samples, both collected from sites on tributaries of the Corumbataí River (São Paulo, Brazil) were performed. The Bioaccumulation Factor (BAF), calculated for different sites with respect to the water concentration, ranged from 173-555 for Al and from 636 - 921 for Mn. Dissolved concentrations of Al and Mn in water samples (collected in different sites) were related to the accumulation of these metals in crabs, suggesting that *T. fluviatilis* is good biomonitor for Al and Mn pollution in aquatic ecosystem.

Keywords: Bioaccumulation. Bioindicator. *Trichodactylus fluviatilis*. Metals. Freshwater

RESUMO

Foram determinadas as concentrações de Al e Mn nos tecidos (brânquias, hepatopâncreas e músculos) do caranguejo de água doce *Trichodactylus fluviatilis* e em amostras de água, ambos coletados em afluentes do Rio Corumbataí (São Paulo, Brasil). O Fator de Bioacumulação (BAF), calculado para os diferentes sítios de coletas em relação às respectivas concentrações nas amostras de água, encontrou-se entre 173 e 555 para Al e entre 636 e 921 para Mn. As concentrações de Al e Mn dissolvidos nas amostras de água (coletados nos diferentes sítios) foram relacionadas à acumulação desses metais nos caranguejos, sugerindo que *T. fluviatilis* é um bom biomonitor da poluição de Al e Mn em ecossistemas aquáticos.

Palavras chaves: Bioacumulação. Bioindicador. *Trichodactylus fluviatilis*. Metais. Água doce

1. INTRODUCTION

Aluminum is a non-essential metal that can be toxic to plant and aquatic animals (AZEVEDO and CHASIN, 2003). Manganese is an important micronutrient that is responsible for the hardness of the shell of crustaceans (SANDERS et al., 1998) and it acts as activator of enzyme system of animals. Though, manganese is an essential metal, it is also toxic in aquatic environment at upper limit availability. The effect of trace metals (essential or non-essential) present in an aquatic system basically depends on bioavailability (AZEVEDO and CHASIN, 2003).

The bioavailability is affected by the species of the metals and depends on several factors, such as pH, salinity, temperature, amount of dissolved carbon. Generally, the main fraction of Mn found in river waters from South America is bound in suspended particle (AZEVEDO and CHASIN, 2003; ZAGATTO and BERTOLETTI, 2006). Thus, this metal normally is removed from water column by sedimentation before toxic levels can be attained. However, the dissolved fraction of Mn may be increased by the solubilization of sediment-bound manganese by a shift to reducing conditions. Aluminum is soluble at low pH values, so suggesting considerable bioavailability only for acid freshwater. However, it is recently recognized that aqueous Al can be toxic to freshwater fish and invertebrates (WARD; MCCROHAN and WHITE, 2006) even for neutral pHs. For example, in the range of 250 – 500 μL^{-1} , Al affects the filtering activity of the bivalve *Anodonta cygnea* (KÁDÁR et al., 2001).

Bioaccumulation can be used to evaluate the contaminant-specific bioavailability since it consists in the net retention of a substance by an organism over the time. This approach has focusing extensively the use of bivalves and, also, some marine crustaceans as indicator species to assess loads of metals in the environment (McPHERSON and BROWN, 2001).

In the case of crustaceans, the metals are taken from the surrounding environment through the gills or through the intestinal wall with the ingestion of metals absorbed in the food. These characteristics provide an integration of both sediment and water derived pollutant stress.

Some species of marine crabs successfully have been proposed as biomonitors of metals and metalloids pollutions (McPHERSON and BROWN, 2001; TÜRKMEN et al., 2006). Two freshwater crab species (*Potamonautes warreni* and *Potamonautes perlatus*) from South Africa also have been evaluated as biomonitors of trace element contamination in fluvial waters and the main findings of these studies are summarized in Table 1. Conclusively, *Potamonautes warreni* has been considered as a good indicator of Fe and Mn pollutions.

The principal aims of this work were to evaluate the level of Al and Mn concentration in the freshwater river crab *Trichodactylus fluviatilis* and its potentiality of bioindication of Al and Mn pollution in river water. Basic questions that normally are associated with the use of a crab as a biomonitor (the relationship to the level of dissolved contaminant in the river water, effects of the sex, tissue and size on the metals accumulation) were also investigated.

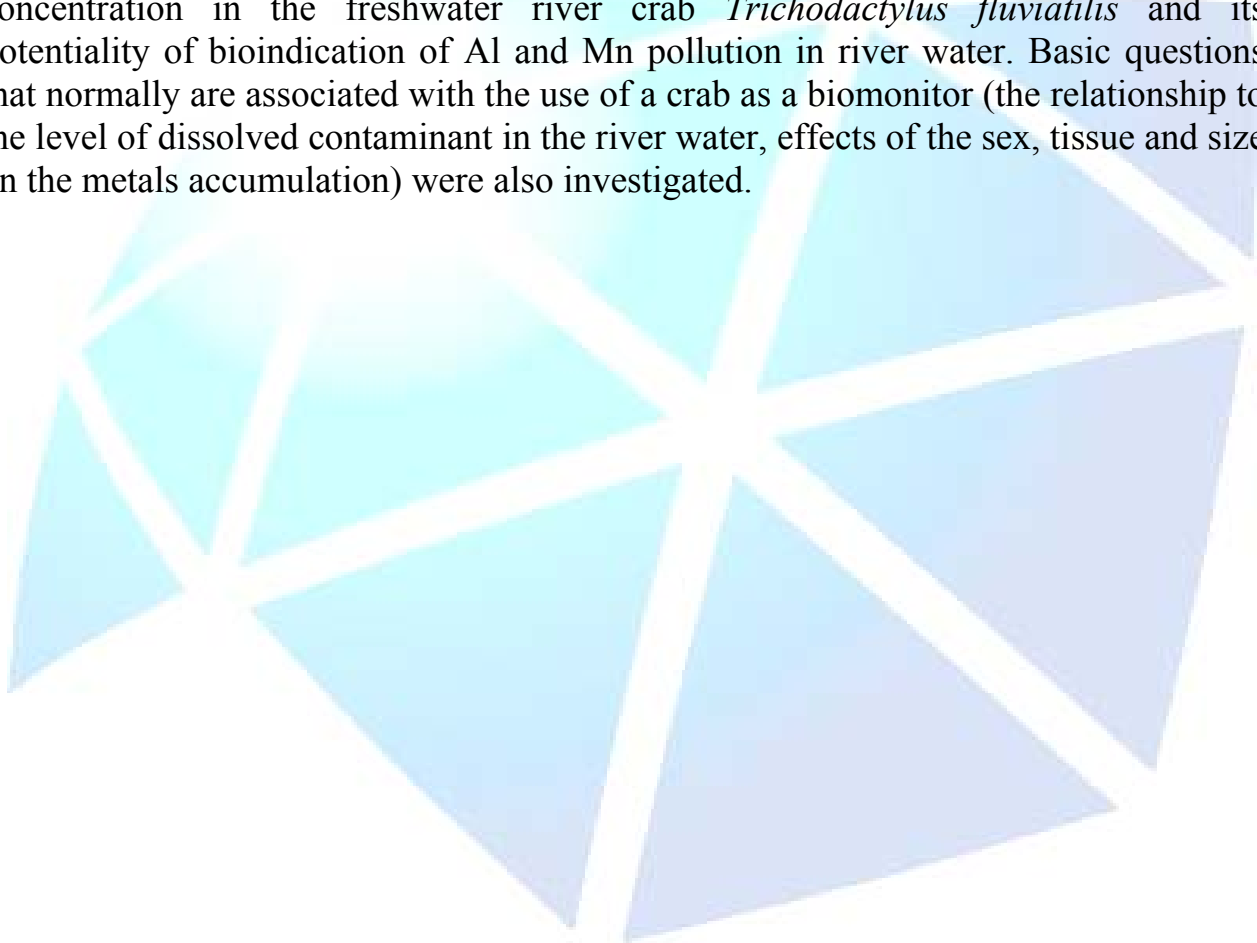


Table 1 - Summary of studies reporting the use of freshwater river crabs as potential biomonitor

Species	Factor examined	Metals	Main finding(s)	Reference
<i>Potamonautes perlatus</i>	Tissue, size, site, seasonality	Cu	– Body loads don't reflect the level of the metal in water and sediment. – Useful to evaluate the bioavailability of Cu	SNYMAN; REINECKE and NEL, 2002
<i>Potamonautes perlatus</i>	Tissue, size, site, seasonality	Cd, Pb	– Body loads don't reflects the level of the metal in the environment – Useful to evaluate the bioavailability of Cd and Pb	REINECKE; SNYMAN and NEL, 2003
<i>Potamonautes warreni</i>	Tissue, size, site, seasonality	Cu	– Probably able to regulate the metal concentration in its tissues and thus is not a suitable indicator of the metal in the aquatic environment	STEENKAMP; DU PREEZ and SCHOONBEE, 1994a
<i>Potamonautes warreni</i>	Tissue, size, site, seasonality, sex	Mn	– Mn concentration in the tissue reflects the degree of Mn contamination of the surrounding aquatic environment	STEENKAMP et al., 1994b
<i>Potamonautes warreni</i>	Size, site, seasonality, sex	Cd, Zn	– May be useful as a indicator for Zn contamination – Cannot be determined as a bioaccumulative indicator for Cd.	SANDERS; DU PREEZ and VAN VUREN, 1998
<i>Potamonautes warreni</i>	Tissue	Cd, Cu, Zn	– Increase in the concentrations of Cd in the gills and digestive gland highlight the potential of <i>P. Warreni</i> as a biomonitor species.	SANDERS; DU PREEZ and VAN VUREN., 1999
<i>Potamonautes warreni</i>	Tissues, sites, size	Cd, Zn	– Sediment and water metal concentrations were not representative of metal accumulation in the crab tissues.	THAWLEY; MORRIS and VOSLOO, 2004
<i>Potamonautes warreni</i>	Size, site, sex, seasonality	Fe, Mn	– May be useful as a biomonitoring protocols as they provide information on the levels of contamination of the elements in different sites	SCHUWERACK; LEVIS and JONES, 2001

2. MATERIALS AND METHODS

Twenty-eight *T. fluviatilis* specimens were collected during January-may/2006 from Cabeça River (S 22° 24' 34.5"; WO 47° 39' 27.2"; n = 7), Passa Cinco River (S 22° 25' 07.8"; WO 47° 42' 48,1"; n = 5) and Claro stream (S 22° 41' 35.3"; WO 47°

32' 26.1"; n= 16). Samples of water were collected (three time at each site) during January/February/May/2006.

Passa Cinco River (on west margin) and Claro stream (on east margin) are tributaries of Corumbataí River (Figure 1). The Cabeça River is the main affluent of the Passa Cinco River. The Corumbataí River basin is a populated area (CONCEIÇÃO and BONOTTO, 2004). Sources of contamination in this Basin have been attributed to domestic and industrial wastes and to agricultural processes related to sugar cane crops (CONCEIÇÃO and BONOTTO, 2004). The Claro stream site is situated in a protected area (Floresta Estadual Edmundo Navarro de Andrade). Discharge of domestic waste from the municipality of Rio Claro is the main potential source of pollution in this site (FERREIRA and PETRERE, 2007). The Passa Cinco and Cabeça sites are located in an area with similar anthropogenic influences: agricultural activity, mainly the intensive use of the soil to sugar cane cultivation. Thus, the crabs collected in Passa Cinco and Cabeça rivers were grouped and this area, potentially polluted by agricultural process, was considered as site 2 (n = 12). The crabs collected in Claro stream site (n = 16) were associated to a potentially polluted area by domestic waste (site 1).

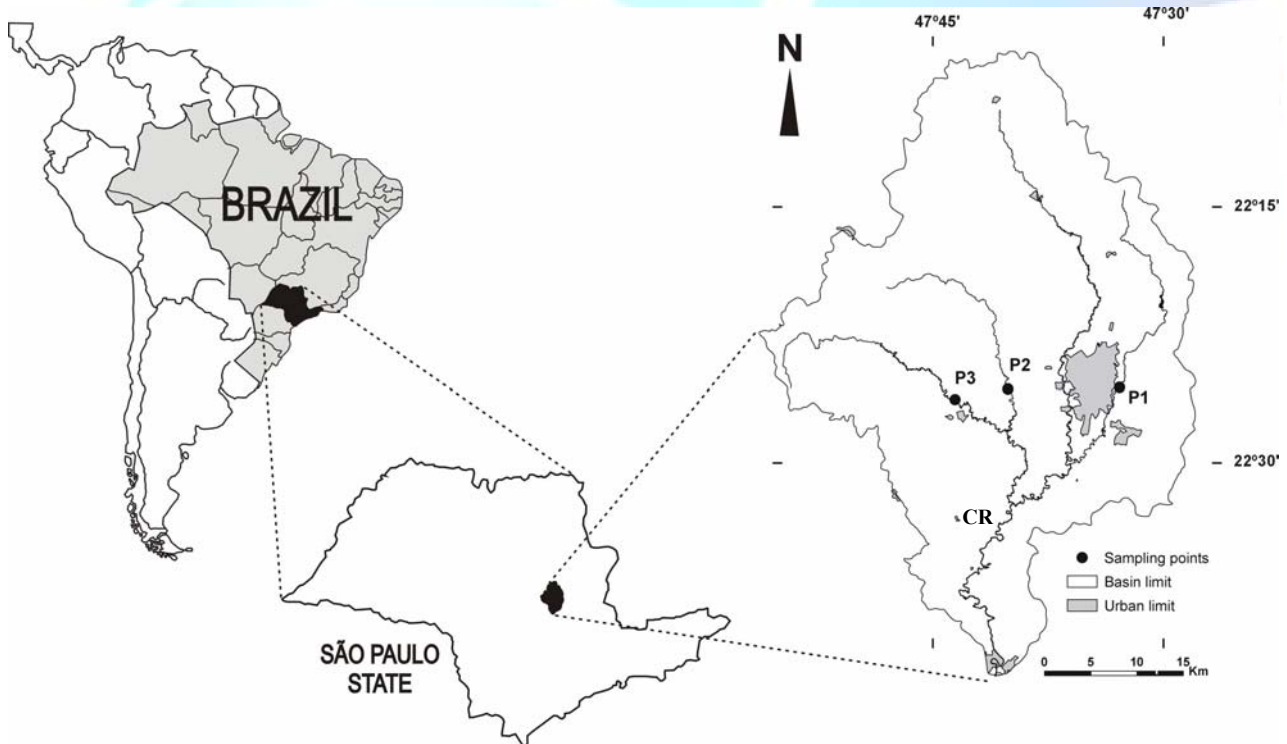


Figure 1. Sampling points in the Corumbataí River (CR) Basin: Claro stream (P1) Cabeça River (P2), Passa Cinco River (P3).

The water samples were filtered (0.45 µm), acidified at pH 2.0-2.5 with HNO₃ and stored at 4 °C. The sampled crabs were transported to the laboratory, where they were euthanased by chilling at -10 °C. The animals were thawed, rinsed three times with deionised water, sexed and measured (carapace length) prior to the dissection. Gill, hepatopancreas and muscle tissues were removed from each individual, weighed and stored in 2 ml-Eppendorf tubes at -10 °C until analysis. Wet tissues samples (0.15 - 0.64 g of gill, 0.07 - 0.87 g of hepatopancreas and 0.2 – 1.33 g of muscle) were transferred to digestion tubes and 2.5 mL of concentrated nitric acid (*pro analisi* grade, Merck, Germany) were added. The tubes were allowed to stand overnight and heated in a digestion block at 160 °C for about 2 hours. The mixture was allowed to cool and then 0.2 mL of concentrated perchloric acid (*pro analisi* grade, Merck, Germany) was added. Finally, the tubes were heated at 210 °C for about 2.5 hours and, after cooling, the solutions were transferred to 25 mL volumetric flasks. An inductively coupled plasma optical emission spectrometer (GBC model Integra XL, Australia) was used for metal determinations in water samples and biological digests. Multielement standard stock solution containing 1000 mg L⁻¹ of Al and Mn (Specsol MICPG2, Brazil) was used.

All glassware and plasticware used during the samples collection, preparation and analysis processes were soaked in a 20% acid nitric solution for 8 hours, followed by rinsing with deionised water. Deionised water (18.2 MΩ cm) produced in a Milli-Q system (Millipore, USA) was used.

Standard reference materials (SRM) of river water (SRM 1640, certified by the National Institute of Standards and Technology) and the biological tissues Fish Homogenate (SRM MA-A-2 / TM, International Atomic Energy Agency) and Copepod Homogenate (SRM MA-A-1 / TM, International Atomic Energy Agency) were used to check the accuracy of the analytical procedure.

We have not available biological SRM with certified values for Al. Thus, recoveries tests were undertaken to evaluate the accuracy of Al determination in this type of samples. Concentration and recoveries of Al and Mn in SRM are presented in Table 2. Satisfactory analyte recoveries were obtained for all analyzed samples.

Table 2 - Concentrations (mean ± confidence limit) and recoveries of Al and Mn from Certified Reference Material (CRM)

CRM	Certified, µg kg ⁻¹		Spike, ng	Found ^a µg kg ⁻¹		Recovery, %	
	Al	Mn		Al	Mn	Al	Mn
River water SRM 1640 (NIST)	52.0 ± 1.5	121.5 ± 1.1	-	60 ± 8.6	123 ± 8.6	115 ± 4	101 ± 2
Fish SRM MA-A-2/TM (IAEA)	-	810 ± 40	6250	< LD ^c	747 ± 1	79 ± 13 ^b	92 ± 6
Copepod SRM MA-A-1/ TM (IAEA)	-	2900 ± 200	6250	14 ± 1	2452 ± 147	98 ± 26 ^b	85 ± 6

a. Without spike

b. Based on spike recovery

c. Limit of Detection

The concentration of Al and Mn in the water were compared using *t* tests. Analysis of variance (ANOVA) was used to evaluate differences on metal concentrations between the factors sex, site and tissues. Tukey test was used to compare the mean values of metal concentration on tissues of crabs. Product-moment correlation coefficients were used to test for significance relationship between metal concentration and the size of crabs (carapace length).

3. RESULTS AND DISCUSSION

Table 3 shows the mean values of Al and Mn concentrations (dissolved fraction) found in river water sampled from sites 1 and 2 and maximum level values for the quality of the freshwater (Brazilian Directive Conama Resolution No. 357/2005; BRASIL, 2006) destined for human consumption (level 1 and 3) and for the protection of aquatic life (level 1). The Mn level was not significantly different in samples from sites 1 and 2 ($t = - 0.059$, $P = 0.953$) and lower than the values defined by Conama (Brazilian National Council for the Environment). The mean concentration of Al in samples from site 2 (Table 3) was higher than the maximum values established by Conama and significantly higher than the mean value of samples from site 1 ($t = - 1.86$, $P = 0.089$).

Table 3 - Aluminum and manganese dissolved concentration in river water from sites 1 and 2

	Found ^a , $\mu\text{g L}^{-1}$		Guide level ^b , $\mu\text{g L}^{-1}$			
	Al	Mn	Al		Mn	
			Level 01	Level 03	Level 01	Level 03
Site 1	184.6 ± 104.4	59.5 ± 42.3	100	200	100	500
Site 2	311.4 ± 116.4	61.4 ± 53.7	100	200	100	500

a. Mean ± sd

b. Recommended guidance level and maximum values allowed by Conama Resolution (Brazilian National Council for the Environment)

The concentrations of Al and Mn in freshwater depends on interaction of several factors, for example: weathering of rocks in the drainage basin; chemical reactions between water and soil or sediments; pollution action in the basin, such as contamination due to inadequate use of fertilizer and inadequate agricultural practice. Despite fertilizer are potential source of Mn pollution, and site 2 are characterized by intensive agricultural activity, the results of water analysis indicates no man-made contamination of this element. On other hand, high level of Al was found in river water, mainly for site 2. These values can be attributed as a consequence of the higher level of aluminum in the studied area (16,92% for rocks from site 2 and 13,88% for site 1, CONCEIÇÃO and BONOTTO, 2004) and the higher erosion rates that

normally occur in cultivated lands, mainly for site 2.

Concentrations of Al in the gills, hepatopancreas and muscle of *T. fluviatilis* from site 1 and 2 were not significantly correlated with its carapace length ($P > 0.05$, Table 4). Manganese concentrations in above-mentioned tissues of *T. fluviatilis* from site 1 and 2 also indicate no significant correlations with carapace length ($P > 0.05$, Table 4).

Table 4 - Correlation (P value) between metal concentration and carapace length (cc) of *Trichodactylus fluviatilis* from site 1 and 2.

	Site 1		Site 2	
	Al	Mn	Al	Mn
Gills vs. cc	0.74	0.86	0.95	0.90
Hepatopancreas vs. cc	0.25	0.60	0.06	0.46
Muscle vs. cc	0.94	0.60	0.62	0.12

The influence of animal size on metal levels in some crabs has been previously reported. Positive correlations for essential (Zn) and non-essential (Hg) metals were reported for the marine species *Pseudocarcinus gigas* (TUROCZY et al., 2001). This related-size trend was attributed to physiological exchange for Zn and due to accumulation of the metal for Hg. For others marine species from unpolluted area, negative correlation between carapace length and Cd, Co, Cu, Mn and Zn concentrations were reported (BJERREGAARD and DEPLEGGE, 2002). In particular for Mn, this relationship was explained as consequence of trend in the decrease of Mn concentration in the body due to accumulation of the metal in the exoskeleton of older crabs (STEENKAMP et al., 1994b; BJERREGAARD and DEPLEGGE, 2002).

For the freshwater river crab *Potamonautes perlatus*, negative correlation between carapace length and concentrations of non-essential metals (Pb e Cd) were reported (REINECKE; SNYMAN and NEL, 2003). For other freshwater river crab (*Potamonautes warreni*) collected in an unpolluted area, it was also reported a negative correlation between the concentration of the essential metals Fe and Mn as a consequence of differences on metabolism, diet and superficial area of old and young individuals (SANDERS; DU PREEZ and VAN VUREN, 1998). This size-related trend was masked for *P. warreni* collected from polluted area (with elevated concentration of Mn) as a consequence of the accumulation of Mn by old and young crabs (SANDERS; DU PREEZ and VAN VUREN, 1998). For comparison, in the studied area we have found dissolved Mn concentrations of about $60 \mu\text{g L}^{-1}$ (Table 3) while Mn concentrations in river water (dissolved fraction) from unpolluted and polluted sites reported by Sanders; Du Preez and Van Vuren, (1998) were 32 ± 38 and $111 \pm 69 \mu\text{g L}^{-1}$, respectively. Thus, based on results from this study (no size-related tendency) and assuming the behavior of *P. warreni* as a model, it can be

concluded that Al and Mn were accumulated in *T. fluviatilis* collected from site 1 and 2.

In addition of crab size, other factors that determine metal concentrations in *T. fluviatilis* may include: sex, tissue type and site. The concentration of Al in *T. fluviatilis* did not differ significantly for tissue and sex (ANOVA, $P < 0.05$). A value on the order of 5 mg kg^{-1} (wet basis) has been found for muscle tissue (claw) of *Carcinus maenus* (SKONBERG and PERKINS, 2002), while values of 1.23 - 10.65 and 2.12 - 17.56 mg kg^{-1} (dry basis) were reported for *Callinectes sapidus* collect in relatively unpolluted and polluted sites, respectively (TURKMEN et al., 2006). These values are lower, when compared with the mean concentration of Al found in the muscle of *T. fluviatilis* (11.96 mg.kg^{-1} , wet basis). The baseline of Al level in the hepatopancreas previously reported by McPherson and Brown (2001) for the crab *Portunus pelagicus* ($0.71 \pm 0.45 \text{ mg kg}^{-1}$, wet basis) was also lower than the level of Al found in the *T. fluviatilis* ($138.7 \pm 349.5 \text{ mg kg}^{-1}$, wet basis).

ANOVA indicates that tissue type is a significant factor for the concentration of Mn, but shows no significant difference between sexes ($P < 0.05$). The same pattern (tissue dependence and no sex dependence) has been reported for the marine crab *Tachypleus tridentatus* (KANNAN; YASUNAGA and IWATA, 1995) and for the freshwater crab *P. warreni* (STEENKAMP et al., 1994b). Mean concentrations of Mn in gills, hepatopancreas and muscle of *T. fluviatilis* are presented in Table 5. The highest Mn concentration was found in the hepatopancreas. It is a common result the Mn concentrations to be higher in hepatopancreas (TUROCZY et al., 2001), possibly due to the detoxification function of this organ.

Table 5 - Comparison of Mn concentrations (mg kg^{-1} , wet basis) in tissues of *Trichodactylus fluviatilis* using Tukey test.

Tissue	Mean concentration	group
gills	39.88	A
hepatopancreas	86.18	B
muscle	11.58	B

Table 6 shows Bioaccumulation factors (BAFs) and values of Al and Mn concentrations in *T. fluviatilis* from site 1 and 2. The BAFs, a value that is used to quantify the bioaccumulation of environmental pollutants in aquatic and terrestrial biota, was calculated with respected to the water dissolved concentrations:

$$\text{BAF} = C_{\text{crab } i} / C_{\text{water } i};$$

where, $C_{\text{crab } i}$ and $C_{\text{water } i}$ are the mean concentrations of the metal in crabs and waters collected from site i , respectively (KANNAN; YASUNAGA and IWATA, 1995).

Table 6 - Bioaccumulation factor (BAF) and mean concentration of Al and Mn (mg kg^{-1} , wet basis) in *Trichodactylus fluviatilis* from sites 1 and 2.

	Site 1		Site 2	
	BAF	Mean concentration	BAF	Mean concentration
Al	173.8	32.08	555.5	173.0
Mn	636.6	37.86	921.2	56.56

For both sites, the BAFs of Mn were 2-4 times higher than those of Al. The Al concentration in the crabs from site 2 was significantly higher than the crabs from site 1 (Table 6). On other hand, there was not significant difference on Mn concentration in *T. fluviatilis* from site 1 and 2. This difference clearly reflects the differences of Al and Mn concentrations soluble in river water samples from site 1 and 2 (Table 3).

The present work reports the first information on the concentration of Al and Mn in the freshwater crab *T. fluviatilis*. The results of this study suggest including the analysis of *T. fluviatilis* in monitoring programs of freshwater. Particularly in areas that are subjected to high levels of Al and Mn, important information on concentration of these metals encountered in different sites are provided.

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