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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

CHEMICAL ANALYSIS TROUGH "ENERGY-DISPERSIVE SPECTROSCOPY (EDS)" OF DIGENEA METACERCARIAE FOUND INFESTING SPECIMENS OF *HOPLOSTERNUM LITORALLE* (HANCOCK, 1828) (SILURIFORMES: CALLICHTHYDAE), CAPTURED IN MANAUS POLLUTED IGARAPES

CARACTERIZACIÓN QUÍMICA EN TREMATODOS DIGENÉTICOS PARASITOS DE HOLPOSTERNUM LITORALLE (HANCOCK, 1828) (SILURIFORMES: CALLICHTHYDAE) USANDO LA ESPECTOSCOPIA DE ENERGÍA DISPERSIVA (EDS) CAPTURADOS EN IGARAPES CONTAMINADOS DE MANAUS

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Abstract

We examined 82 *Hoplosternum littorale* captured in two Manaus polluted igarapes, namely, São Raimundo and Educandos, in October and November 2010. Fish specimen's standard length and weight showed to be 14.87±71 cm and 116.53±24.01 g, respectively. We carried out the chemical analyses of tissues with the aid of an Energy-Dispersive Spectroscopy (EDS) system in conjunction with a Scanning Electron Microscope (SEM). We determined the punctual qualitative and semi-quantitative chemical composition of the metacercariae of *Clinostomum marginatum*, *Tylodelphis destructor* and of the pro-genetic metacercaria *Herpetodiplostomum caimanicola,* by using the characteristic X-ray emission. Microanalyses by EDS determined the presence of carbon (C); oxygen (O); iron (Fe); zinc (Zn); aluminum (Al); siliceous (Si); phosphorous (P); sulfur (S); potassium (K); calcium (Ca); chlorine (Cl); copper (Cu); lead (Pb); manganese (Mn); platinum (Pt); tungsten (W) and cobalt (Co). The detected heavy metals Cu, Pb and W indicated that igarapes is heavily polluted, and that Digenea metacercariae are highly tolerant to the accumulation of these elements.

Keywords: Amazon - chemical elements - fishes - Platyhelminthes - pollution.

Resumen

Fueron examinados 82 *Hoplosternum littorale* capturados en dos arroyos contaminados en la ciudad de Manaus: São Raimundo y Educandos, entre octubre a noviembre del 2010. La longitud y peso promedio de los peces analizados fueron de 114,87±71cm y 116,53±24,01g, respectivamente. Los análisis químicos de los tejidos fueron realizados con ayuda de un sistema de Espectroscopía por Dispersión de Energía (EDS) en conjunto con un Microscopio Electrónico de Barrido (MEB). Fue determinada la composición química puntual cualitativa y semi-cuantitativa en larvas de *Clinostomum marginatum*, *Tylodelphis destructor* y de la metacercaria progenética de *Herpetodiplostomum caimanicola*, utilizando la emisión de rayos X característicos. Los microanálisis por EDS determinaron la presencia de carbono (C); oxígeno (O): hierro (Fe); Zinc (Zn); aluminio (Al); silicio (Si); fósforo (P); azufre (S); potasio (K); calcio (Ca); cloro (Cl); cobre (Cu); plomo (Pb); manganeso (Mn); platino (Pt); tungsteno (W) y cobalto (Co). Los metales pesados detectados Cu, Pb y W son indicadores de polución en los arroyos, y las metacercarias de Digenea son altamente tolerantes a estos elementos de acumulación.

Palabras clave: Amazonía - elementos químicos - peces - Platyhelminthes - polución.

INTRODUCTION

Rising population density and heavy anthropic activity surrounding urban water bodies, has affected the water quality along with its biodiversity. Changes in trophic structure and elimination of spawning and recruiting sites, strongly affect the aquatic fauna, thus diminishing diversity and consequently affecting the ecosystem as a whole (Silva, 1995).

Biological monitoring is a water quality evaluating method, which takes into account the biological community responses to the extant environmental conditions (Whitfield & Elliott, 2002; Goulart & Callisto, 2003). Bio-indicators serve as indicators of the level of contamination, and the changes in the number and distribution of individuals and species, may point out environmental deterioration (Karydis & Tsirtsis, 1996; Harrison & Whitfield, 2004; Buron *et al*., 2009).

There have been studies carried out on a large variety of organisms to evaluate their ability to serve as bio-indicators, bio-monitors or biological indicators, as well as on their use as markers pointing out any kind of pollution occurring on that aquatic environment. Fish parasite species hold abilities, which enable them to be bio-indicators and markers (Gunkel, 1994; Sures, 2004, 2008; Buron *et al*., 2009).

Fish parasite species have shown to be good indicators of pollutant accumulation, because they provide valuable information on the environment's chemical status, both by pointing out the presence or lack of pollutants, and the ability to concentrate them in their tissues (Sures *et al*., 2000; Buron *et al*., 2009).

The utilization of fish parasites as pollutant concentrators is justified by their much greater ability than that of hosts or environment themselves to bio-accumulate or bio-concentrate heavy metals (Sures & Siddall, 1999; Sures, 2003). That bio-accumulative characteristic of the parasite is not the result of a slow accumulation process, but rather a relatively quick adhesion to a leveled status (Sures *et al*., 2000).

Currently, several techniques, such as Energy-Dispersive Spectroscopy (EDS), detect chemical elements in fish parasites. EDS analysis: electrons interacting with the sample's atoms generate signals from these interactions, which provide information on the sample's properties. When the beam hits the samples their atoms are exited, as they return to their fundamental status, they emit photons with the atom's characteristic energies, (characteristic X-rays). Photons identified in terms of their energy and counted by the X-rays detector, located within the vacuum chamber (Heckmann *et al*., 2007).

The joint system's *hardware* and *software*, stores information generating the spectrum relative to the number of counts according to kev energy (kilo electron volt), identifying the chemical elements present in the sample (Heckmann *et al*., 2007). Compared with the analysis by wave-length dispersion, the EDS allows detecting all chemical elements present in a sample during a single run, instead of only one element per run (Whallon *et al*., 1989; Johnson, 1993). Unlike atomic absorption spectrophotometry, it is a practically non-destructive method for chemical composition microanalysis, and minute-sized samples can be analyzed (Vaughn, 1989; Johnson, 1993).

The present work studies the chemical composition of Digenea metacercariae trematodes parasites of *H. littorale* the only fish species that was captured in the studied environment. It analyzes heavy metal accumulation in pollution-exposed parasites.

MATERIAL AND METHODS

We performed necropsies on 82 *H. littorale* specimens captured from October to November 2010. Fish averaged 14.87±71 cm and 97.49±24.01g. They had been captured in polluted streams within the city of Manaus, in the State of Amazonas Figure (1). These streams were, São Raimundo (3º 09' 47"S and 59º 54' 29" W) and Educandos (3º 04' 16"S and 59º 55' 62" W). The collected Digenea metacercariae were fixed in cold AFA (alcohol, formaldehyde, acetic acid) solution with and without compression.

Digenea metacercariae qualitative and semi-quantitative composition determination was done by using the Energy-Dispersive Spectroscopy (EDS) system in conjunction with a FEI Quanta 200 model Scanning Electron Microscope (SEM). The characteristic X-rays emission identifying elements chemically present in Digenea metacercariae trematodes. Microanalyses were done in the Electronic Microscopy and Microanalyses Laboratory at "Universidade Estadual de Londrina (UEL)".

EDS microanalyses: Twenty (20) fixed parasites were washed three times in 0.1M cacodylate buffer for 10 min; dehydrated in an ascending series of ethyl alcohol; dried to a critical point in a BAL-TEC CPD 030 model drier, and mounted in metal cylinders (*stub*). Afterwards, they were covered with carbon film in a BAL-TEC SCD 050 model "Sputter coater" metalizer.

The spectra were obtained with the aid of an INCA X-ray secondary electron detector system (155 eV resolution) using the INCA-Analyzer software for identifying the specific peaks for the elements of interest, mainly the heavy metals present in the samples, since the main purpose of EDS ananylis is to to detect all known chemical elements present within a sample during a predefined time, unlike any special element per run as other techniques do. Therefore, our quantitative results were based on each element's peak height in the generated spectrum, and 300 s was how long it took for each specimen to be analyzed. The EDS system was very efficient it detected minute amounts of 17 chemical elements present in the tissues from a sample comprised by in the three metacercariae species: *Clinostomum marginatum* (Rudolphi, 1819), *Herpetodiplostomum caimanicola* (Dollfus, 1935) and of *Tylodelphis destructor* (Szidat & Nani 1951).

Taxonomical identification is in accordance with Travassos *et al*. (1969) and Kohn *et al*. (1995). Vouchers specimens' C. *marginatum* (INPA 639), *H. caimanicola* (INPA 640) and of *T. destructor* (INPA 641) were deposited in the collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

RESULTS

The parasites were morphologically identified as metacercariae of *C. marginatum, H. caimanicola* (progenetic metacercaria) and *T. destructor*. There was no detected chemical element difference for different parasite species analyzed by DES in the two collecting sites.

EDS microanalyses recorded the presence of 16 chemical elements in Digenea metacercariae captured in igarapé São Raimundo: carbon (C); oxygen (O); iron (Fe), zinc (Zn), aluminum (Al), siliceous (Si); phosphorous (P); sulfur (S); potassium (K); calcium (Ca); chlorine (Cl); copper (Cu); lead (Pb); manganese (Mn); platinum (Pt) and tungsten (W) (figure 2). In addition to these 16 chemical elements, cobalt (Co) was also present in Digenea metacercariae captured in igarapé Educandos (figure 3).

EDS chemical elements semi-quantitative distribution shows percentile differences between elements in the studied sites. Igarapé Educandos presents a higher contribution of heavy metals, such as, Cu, Pb, Pt and Zn, as compared to igarapé São Raimundo (figure 4).

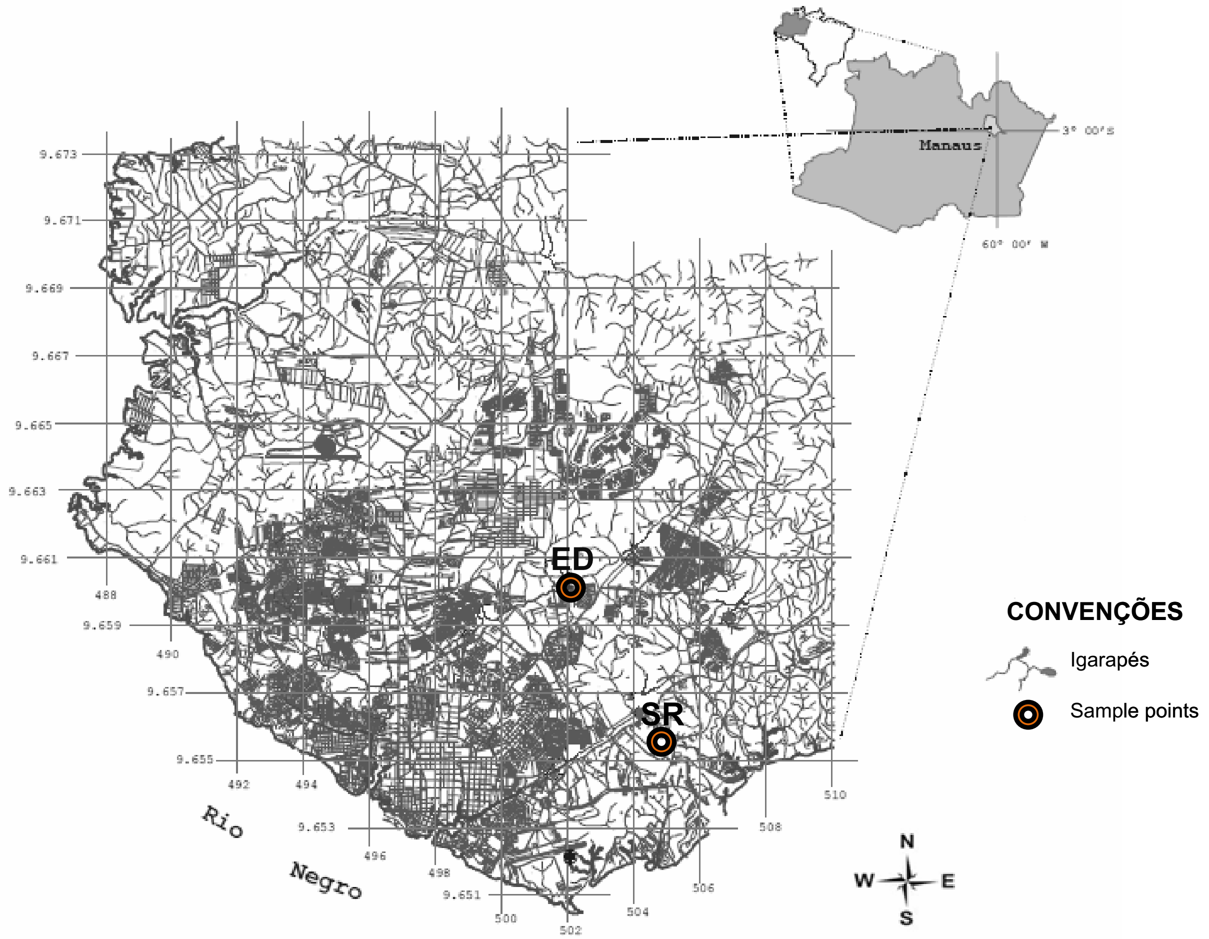

Figure1. Manaus city map showing the collecting sites. Igarapé São Raimundo (SR) and igarapé Educandos (ED).

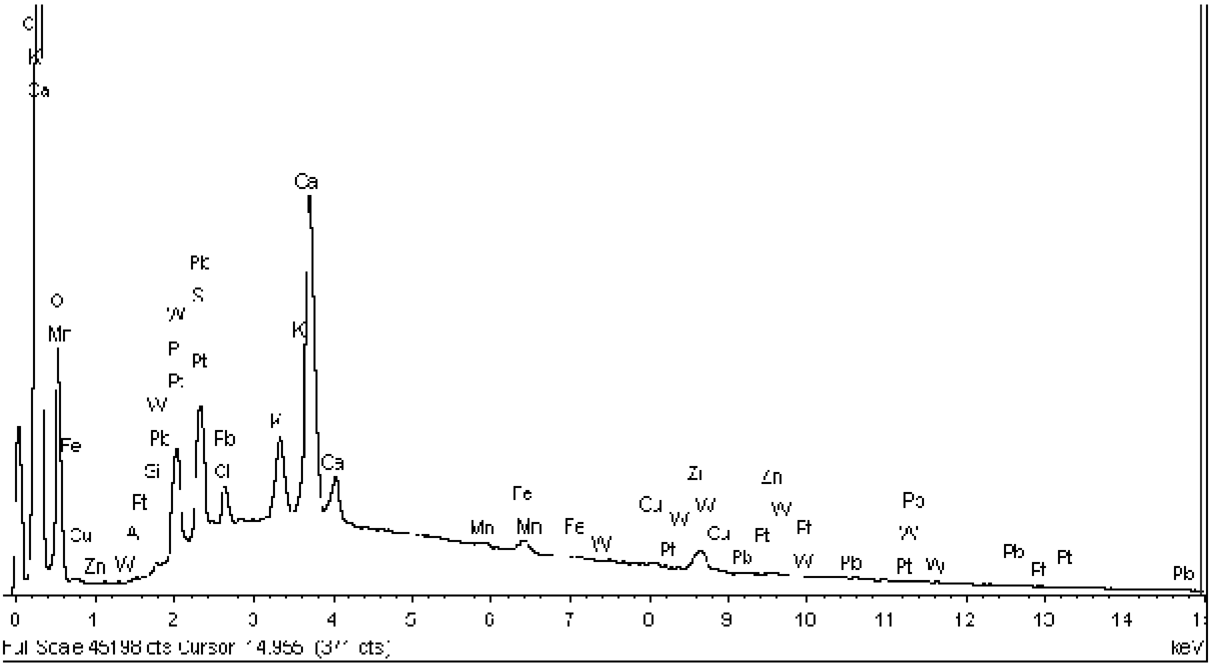

Figure 2. EDS - generated spectrum for *Hoplosternum littorale* metacercariae from igarapé São Raimundo.

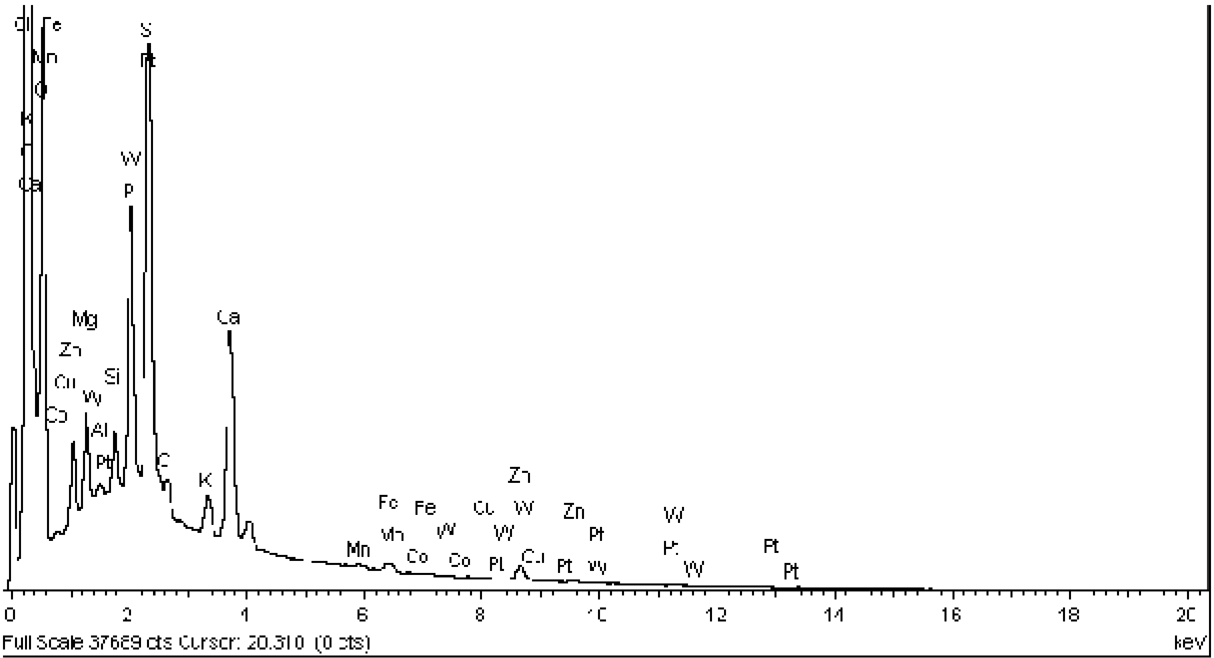

Figure 3. EDS - generated spectrum for *Hoplosternum littorale* metacercariae from igarapé Educandos.

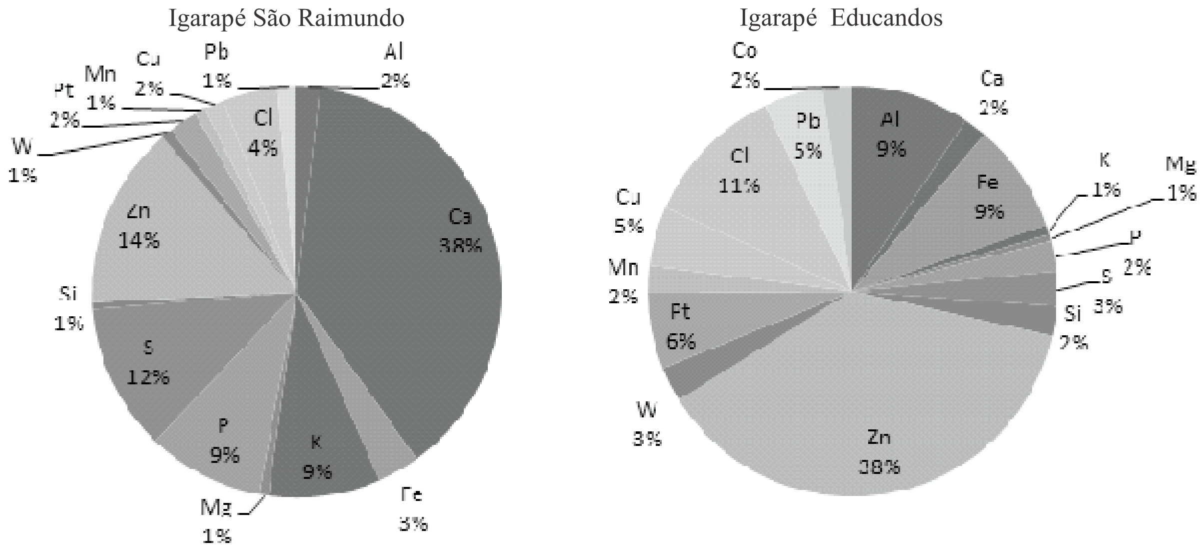

Figure 4. EDS found the chemical element semi-quantitative distribution in *Hoplosternum littorale* Digenea metacercariae captured in igarapés São Raimundo and Educandos. Yet, Carbon (C) and oxygen (O) were overlooked because of being common in the analyzed metacercariae tissues.

DISCUSSION

The energy-dispersive spectroscopy system (EDS) is a valuable tool in studying and characterizing microscopic materials. It is much employed in petrography characterization and petrological studies in geosciences. It can determine the chemical constituents of living organism, i.e., heavy metals present in fish parasite tissues (Heckmann *et al*., 2007).

The coupled EDS- SEM system determined the chemical constitution of proboscis and hooks of five Acanthocephala species: *Neoechinorhynchus qatarensis* Amin, Saoud & Alkuwari, 2002; *Acanthocephalus dirus* (Van Cleave 1931); *Neoechinorhynchus idahoensis* Amin and Heckmann 1992; *Echinorhynchus salmonis* (Müller 1784) and *Pseudoacanthocephalus* sp. High sulfur levels were found (Heckmann *et al*., 2007).

In this work by using the same procedure we detected 16 chemical elements in Digenea metacercariae from igarapé São Raimundo: carbon (C); oxygen (O); iron (Fe), zinc (Zn), aluminum (Al), siliceous (Si); phosphorus (P); sulfur (S); potassium (K); calcium (Ca); chlorine (Cl); copper (Cu); lead (Pb); manganese (Mn); platinum (Pt) and tungsten (W). In addition to these 16, cobalt (Co) was also present in Digenea metacercaria from igarapé Educandos.

The EDS system was very efficient; it detected minute amounts of 17 chemical elements present in the tissues from a sample comprised by three metacercariae species: C. *marginatum, H. caimanicola* and *T. destructor*. It determined the spatial distribution of elements chemical in every sample analyzed. It generated X-ray composition maps showing its practicality for analyses of anthropogenic-impacted environments.

Heavy metal contamination, mainly by: zinc (Zn); cobalt (Co); nickel (Ni); copper (Cu); iron (Fe) and lead (Pb) threatens the whole world. It can contaminate soil, water and accumulate in tissues of living organisms (Loumbourdis, 1997). Determination of low concentrations of heavy metals present in the samples requires the use of sufficiently susceptible and versatile techniques. The present work shows the EDS system technique to be very efficient in analyses determinations.

The EDS system was utilized in species of Acanthocephala *N. qatarensis*, *A. dirus*, *N. idahoensis*, *E. salmonis* and *Pseudoacanthocephalus* sp. Following 60 s of exposition we detected the presence of: sodium (Na); aluminum (Al); magnesium (Mg); siliceous (Si); potassium (K); sulfur (S); calcium (Ca) and phosphorous (P) (Heckmann *et al*., 2007).

In this study, we increased, this exposition time in each Digenea metacercariae sampled point, to 300 seconds. This resulted in the detection of: lead (Pb); tungsten (W); copper (Cu) and platinum (Pt). Therefore we recommend the fish parasites samples exposition time in EDS to be higher than 60 seconds, so as to run no risk of not detecting low-concentrated elements present in the sample.

Manaus city igarapés ranked as being environments presenting diversified characteristics, ranging from pristine to degraded, there occurring polysaprobic environments presenting heavy pollution (Lopes *et al*., 2008).

The biological diversity differs in igarapés São Raimundo and Educandos. São Raimundo presented higher macro-invertebrate diversity than that of Educandos. The latter is subjected to the inflow from chemical effluents contaminated with copper (Cu); nickel (Ni) and zinc (Zn) originating from the Manaus Industrial District (Cleto-Filho, 1998).

Igarapé Educandos presents cleared banks and secondary vegetation along some of its stretches. Its upper course gets the sewage from the Manaus Industrial District, receiving the inflow from around 400 chemical; thermoplastic; metallurgic; mechanical and electronic industries, the main polluting agents of which, happen to be heavy metals (Lopes *et al*., 2008).

The present study showed the chemical composition found in Digenea metacercariae trematodes tissues of *C. marginatum, H. caimanicola* and *T. destructor*, parasites of *H. littorale* to be the same on the two igarapés studied. But for cobalt (Co), which only occurred in metacercariae from igarapé Educandos.

Analyses of Digenea metacercariae collected in the two igarapés presented quantitative differences. Samples from igarapé Educandos presented higher heavy metal concentration values. This is due to the continuous inflow of industrial pollutants into this igarapé.

Chemical elements as copper (Cu); iron (Fe); manganese (Mn); cobalt (Co); selenium (Se); einsteinium (Es); iodine (I) and chrome (Cr) are considered to be essential to the metabolism of living organisms. Cadmium (Cd), mercury (Hg), lead (Pb), nickel (Ni), aluminum (Al), platinum (Pt), tungsten (W) and Argonne (Ar) exert no known biological function and are usually toxic to a large number of organisms (Roesijadi & Robinson, 1993; Heath, 1995).

Igarapés contaminated through anthropic action present a reduction on dissolved oxygen contents, increase of pH and show high concentrations of heavy metals (Zn, Co, Ni, Cu, Fe, and Pb). There occur at high fecal and total coliform rates, much above those acceptable for human consumption or recreation (Silva, 1995; Melo *et al*., 2005; Santana & Barroncas, 2007).

Digenea metacercariae trematodes, parasites of *H. littorale* from igarapés São Raimundo and Educandos contained lead (Pb), platinum (Pt), aluminum (Al) and tungsten (W) in their tissues. All this contamination came about through the inflow of toxic effluents originating from the Manaus Industrial District factories sewage. That contamination determined the death of living organisms inhabiting the water bodies within these ecosystems.

Pollutants cause a significant impact on the host-parasite system in aquatic environments. By assessing this relationship one concludes the fish parasite fauna, mainly digenetic trematode species, to be important parameters in monitoring pollutant-contaminated water quality (Sures, 2008; Vidal-Martinez *et al*., 2009; Khalil *et al*., 2009).

The present work demonstrated the EDS system to be very efficient. It detected 17 chemical elements in tissues of Digenea metacercariae trematodes, parasites of *H. littorale* including heavy metals with no known biological function. It showed to be a valuable tool as well as an efficacious and fast method in assessing anthropically contaminated water bodies' actual water quality.

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