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RESEARCH ARTICLE

The effects of heavy metals on the incidence of morphological deformities in Chironomidae (Diptera)

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ABSTRACT. Streams in urban areas are strongly impacted by the input of organic matter and metals, for instance copper (Cu) and zinc (Zn). These metals are essential for the aquatic biota, but when absorbed in excess they are toxic. In Chironomidae larvae, the deleterious effects of heavy metals can be ascertained by analyzing the morphological deformities of the larval mentum, a structure of the oral cavity. In this study, we evaluated I) the bioavailability of Cu and Zn in urban stream sediments and II) the relationship between Cu and Zn concentrations and the incidence of deformities in the mentum of *Chironomus* larvae. Chironomid flies were collected from four locations in two streams at an urban area in southern Brazil. They were identified and the incidence of deformities in the mentum was quantified. Sediment samples were collected at the same locations where larvae were collected, to quantify the bioavailable fractions of Cu and Zn. The concentrations of Cu in the sediment were similar between the collection sites. However, Zn concentrations varied among sites, being greater in the stretch directly influenced by the input of the organic waste. In total, 2,895 Chironomid larvae were collected. The incidence of deformities in the mentum was above 30% and was correlated with the concentrations of Cu (r = 0.68) and Zn (r = 0.87). This correlation indicates that the municipal waste that is thrown into the city's streams has influenced the occurrence of deformities.

KEY WORDS. Biomonitoring, copper, environmental quality, mentum, zinc.

INTRODUCTION

Aquatic environments are among the most threatened natural resources of the world, since they are directly affected by what happens in their surroundings (Machado et al. 2015). Among the many substances that contaminate rivers and lakes, heavy metals are critical. They are not biodegradable and can have long-term toxic effects (Mahlangeni et al. 2016). Heavy metals in aquatic environments can originate from natural sources (e.g. weathering of rocks) or anthropogenic contamination (e.g. urban, industrial or agricultural waste) (Maldonado and Wendling 2009). Among the waste generated by human activities, urban effluents may vary in their chemical composition, with a high load of organic matter, nutrients and metals (Baird 2002).

Metals are amongst the most common pollutants of aquatics ecosystems (Ali et al. 2013). When released into water bodies, can form ions. These ions either remain as suspended particles, or they settle in limnic sediments (Tuna et al. 2007), which act as a sink for heavy metals and other pollutants (Sharley et al. 2008). After heavy rains, these metals are re-suspended in the water, contaminating the food chain, and adversely affecting the water column and water communities (Pitt 1995, Linnik and Zubenko 2000, Simpson et al. 2005).

Copper (Cu) and zinc (Zn) are considered essential to aquatic organisms (Torres et al. 2008). However, they can become toxic when absorbed (e.g. food intake) and/or adsorbed (e.g. contact with contaminated water) in excess (Tüzen 2009). According to Magalhães et al. (2015), feeding is the main source of exposure to these metals. Large amounts of metal can cause physiological or morphological effects (Al-Shami et al. 2010, Planelló et al. 2013).

The main morphological effects caused by metals in Diptera larvae are deformities in the mentum, a structure in the oral cavity of these immatures (Brinkhurst et al. 1968, Di Veroli eta al. 2012, Sensolo et al. 2012). Larvae of *Chironomus* Meigen, 1804 (Diptera: Chironomidae) are very prone to heavy metal contamination for two reasons. First, they feed from detritus (Armitage 1995); second, they remain in direct contact with the limnic sediment (Odume et al. 2012, Corbi and Froehlich 2010).

The cause of deformity in some Chironomidae is still complex and not fully understood. Some studies on the effect of heavy metals on the incidence of deformities in *Chironomus* have been conducted. In Malaysia, Al-Shami et al. (2010) investigated the influence of agricultural, industrial and anthropogenic stressors on the incidence of deformities in Chironomidae. Di Veroli et al. (2014) documented the morphological deformities in *Chironomus* in a lake contaminated with heavy metals, including Cu and Zn. Žunić et al. (2015) stated that *Chironomus* larvae exposed to high concentrations of Cu and Pb showed a higher rate of deformities. Some studies have reported that the percentages of changes in the oral cavity may range from zero to 8%, even when minimally impacted streams were analyzed (Warwick 1988, Nazarova et al. 2004, Ochieng et al. 2008).

In regions where urbanization is intense, water bodies are subject to human disturbances that change the quality of the water (Milesi et al. 2008). In this study, we evaluated I) the bioavailability of Cu and Zn in urban streams and II) the relationship between the concentration of these metals with the incidence of deformities in the mentum of *Chironomus* larvae. The following hypotheses were tested: I) the sites with the greatest influence of municipal waste will have higher bioavailable concentrations of Cu and Zn in the sediment and hence, II) in places with higher bioavailable concentrations of Cu and Zn there will be higher incidence of deformities in the mentum of *Chironomus* larvae.

MATERIAL AND METHODS

This study was conducted in streams located at the basin of the Tigre River in Erechim, southern Brazil (27°29’6” to 27°47’10”S, 52°21’33” to 52°08’43”W, Fig. 1). The local climate is classified as Cfb under Köppen’s classification, subtropical temperate. The annual average temperature is 17.6 °C and rainfall is well distributed throughout the year, with an average rainfall of 1912.3 mm (Alvares et al. 2013). The vegetation is characterized by a mixture of seasonal evergreen Araucaria forest and semi-deciduous forest (Oliveira-Filho et al. 2015). Urban use comprises 86% of the total area of the drainage area where the studied streams are situated (Budke et al. 2012).

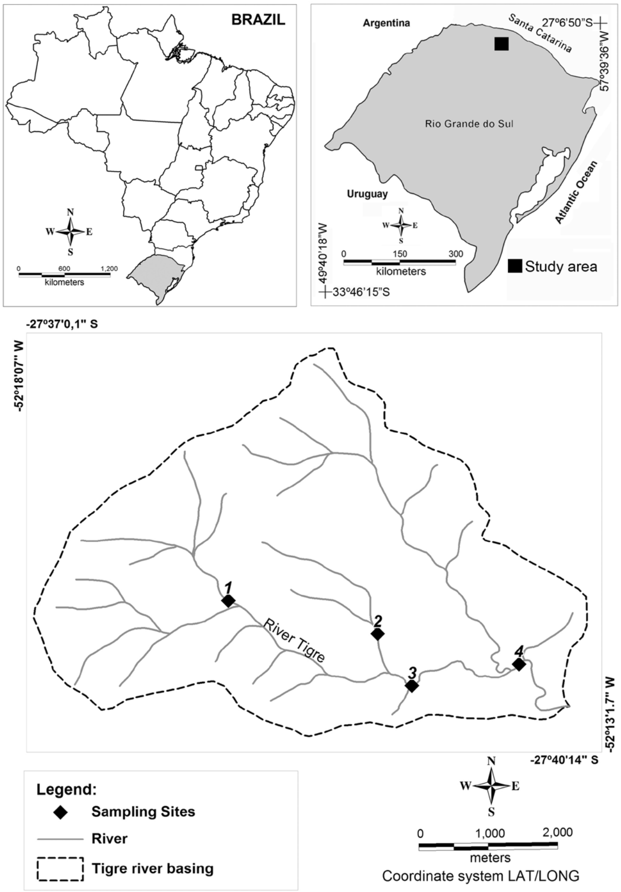


Figure 1. Geographic location of the collection sites by the Tigre River, Erechim, RS, Brazil.

The collection sites were located within the boundaries of the city and were characterized as follows: the first site (P1) has riparian vegetation on both sides, extending to about 20 to 30 m. The bed is composed of stones, leaves and sand, with consistent water flow, facilitating oxygenation. The second site (P2) is located upstream of a housing complex. The waste generated by the houses is released directly into the riverbed. There is about 5 to 10 m of riparian vegetation on both banks, where the substrate is rocky. The third site (P3) is located about 2 km from P2 and its substrate is composed of rocks. The vegetation of the margin is low and there is a small community over the stretch. The fourth site (P4) is located about 4 km from P3. It has about 2 m of riparian vegetation on both banks, and a rocky substrate. On one of the banks there is a concrete factory. Every site at the river is shallow (some stretches reach 1 m, but most are <1 m deep).

Organisms were collected over four stretches of the streams (Fig. 1), between August and September 2015, using a Surber sampler (two sub-samples/stretch, mesh: 250 µm, area: 0.09 m^2) and fixed in the field with 80% ethanol. For identification of larvae, semi-permanent slides with Hoyer solution (Trivinho-Strixino and Strixino 1995) were made for observation of the organisms in optical microscopy with 1000x magnification. Identification to genus level was done using key of the Trivinho-Strixino (2011).

The incidence of deformities in the mentum of *Chironomus* larvae for each sample collected was analyzed. The larvae that had deformities of the mentum were separated and photographed to better visualize abrasions, additions or deletions in their teeth. We considered any change in the normal pattern of the mentum, such as wear, addition, fusion and absence of teeth (Sanseverino and Nessimian 2008) as deformities. The identified bodies were listed and deposited in the Benthic Invertebrate Collection of MuRAU (Regional Museum of the Alto Uruguay) URI Campus de Erechim.

To characterize the quality of the water at the stretches studied, some physical-chemical characteristics of the water and sediment were measured. Throughout the four sites, some abiotic variables were also measured. The following variables were quantified: water temperature, turbidity, electrical conductivity, total dissolved solids, pH and dissolved oxygen with a multiparameter analyser (Horiba® U50). Water samples for the concentration analysis of total organic carbon and total nitrogen in total organic carbon fixer with a nitrogen detector TOC-VCSH (Shimadzu) were collected. The methods for the analysis of these parameters are described in APHA (1998).

In each stretch, we collected sediment samples with a corer sampler (70 mm diameter) at a depth of 5 to 10 cm. In the laboratory, we dried the samples in an oven (60 °C/48 h) and sifted them (62 µm mesh) to separate the grain size fraction intended for the extraction of metals. The potentially bioavailable fraction of Cu and Zn in the sediment was extracted from 0.5 g of sediment and 10 mL of HCl 0.1 mol L^{-1} for 24 h at room temperature (20 ± 2 °C). Subsequently, the samples were filtered in 25 mL volumetric flask and the total volume was measured with HNO_3 1 mol L^{-1}. The metals were quantified by atomic absorption spectrophotometry on an atomic absorption spectrophotometer (Varian AA55).

To verify the incidence of deformities in organisms, we calculated the percentage of larvae with a deformed mentum. To verify the differences between the abiotic variables along the sampling sections, we used a Repeated Measures Analysis of Variance (RM-ANOVA) followed by a posteriori Tukey test ($p < 0.05$). To evaluate the relationship between Cu and Zn concentration in the sediment and the incidence of deformities in the larvae, we used Pearson's linear correlation analysis. The deformity percentage values and the concentrations of the obtained metals were transformed into log (x+1) to avoid distortions caused by outliers and homogenization of the variances. The analyses were conducted using the BioEstat 5.3 program (Ayres et al. 2007).

RESULTS

The average water temperature was 15.7 ± 2.6 °C. The pH was slightly acidic (5.9 ± 1.1) and the water was well-oxygenated (10.4 ± 2.3 mg L^{-1}). The electrical conductivity ($F_{(3,11)} = 40.6$, $p < 0.001$), turbidity ($F_{(3,11)} = 23.1$, $p < 0.001$), total dissolved solids ($F_{(3,11)} = 41.2$, $p < 0.001$) and total nitrogen ($F_{(3,11)} = 297.1$, $p < 0.001$) differed among the collection sites. The electrical conductivity, total dissolved solids and total nitrogen presented higher values in the downstream sections (P3 and P4), while turbidity was higher only in P3 (Table 1). The pH and percentage of organic matter in the sediment differed between the sites ($F_{(3,11)} = 23.9$, $p < 0.001$; $F_{(3,11)} = 19.8$, $p < 0.001$, respectively) (Table 1). The pH was higher in P1 (8.8 ± 0.1), while the organic matter (61.1 ± 10.2) was higher in P3.

The bioavailable concentrations of Cu in the sediment were similar between the collection sites ($F_{(3,11)} = 1.9$, $p = 0.17$). On the other hand, the bioavailable concentrations of Zn varied between sites ($F_{(3,11)} = 45.4$, $p < 0.001$), with P2 being the site with the highest concentration (115 ± 6.9 mg kg^{-1}, Fig. 2).

Table 1. Mean and standard deviation of abiotic variables in four stretches of the River Tigre, Erechim, Rio Grande do Sul.

	P1	P2	P3	P4
Water temp. (°C)	16.8 ± 3.5^{a}	16.3 ± 2.7^{a}	15.9 ± 2.7^{a}	15.4 ± 3.2^{a}
DO (mg L^{-1}) nnn	11.5 ± 1.8^{a}	8.8 ± 2.9^{a}	10.6 ± 2.6^{a}	10.4 ± 2.6^{a}
pH water	6.4 ± 0.5^{a}	5.5 ± 1.8^{a}	5.4 ± 1.5^{a}	6.3 ± 0.1^{a}
Turbidity (UNT)	6.9 ± 4.8^{b}	3.4 ± 1.8^{b}	17.2 ± 4.2^{a}	2.9 ± 0.5^{b}
EC (mS cm^{-1})	0.07 ± 0.01^{b}	0.16 ± 0.02^{a}	0.19 ± 0.04^{a}	0.23 ± 0.02^{a}
TDS (mg L^{-1})	0.04 ± 0.01^{b}	0.10 ± 0.01^{a}	0.12 ± 0.03^{a}	0.15 ± 0.01^{a}
TOC (mg L^{-1})	17.3 ± 1.4^{a}	18.5 ± 13.6^{a}	19.1 ± 12.6^{a}	20.1 ± 1.9^{a}
TN (mg L^{-1})	2.9 ± 0.2^{a}	7.9 ± 0.4^{b}	9.6 ± 0.8^{c}	10.1 ± 0.3^{c}
pH sediment	8.807 ± 0.11^{a}	8.607 ± 0.08^{b}	$8.417 \pm 0.07^{a},^{b,c}$	$7.97 \pm 035^{b,c}$
OM (%)	44.91 ± 2.27^{a}	32.65 ± 11.14^{a}	61.09 ± 10.21^{b}	28.35 ± 2.43^{c}

DO: dissolved oxygen; EC: electrical conductivity; TDS: Total Dissolved Solids; TOC: Total Organic Carbon; TN: Total Nitrogen. pH and OM: Organic Matter measured in the sediment. Means followed by different letters in the lines have significant difference by the Tukey test ($p < 0.05$).

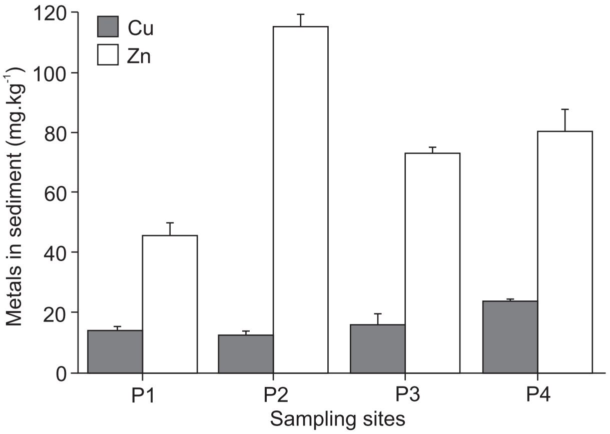
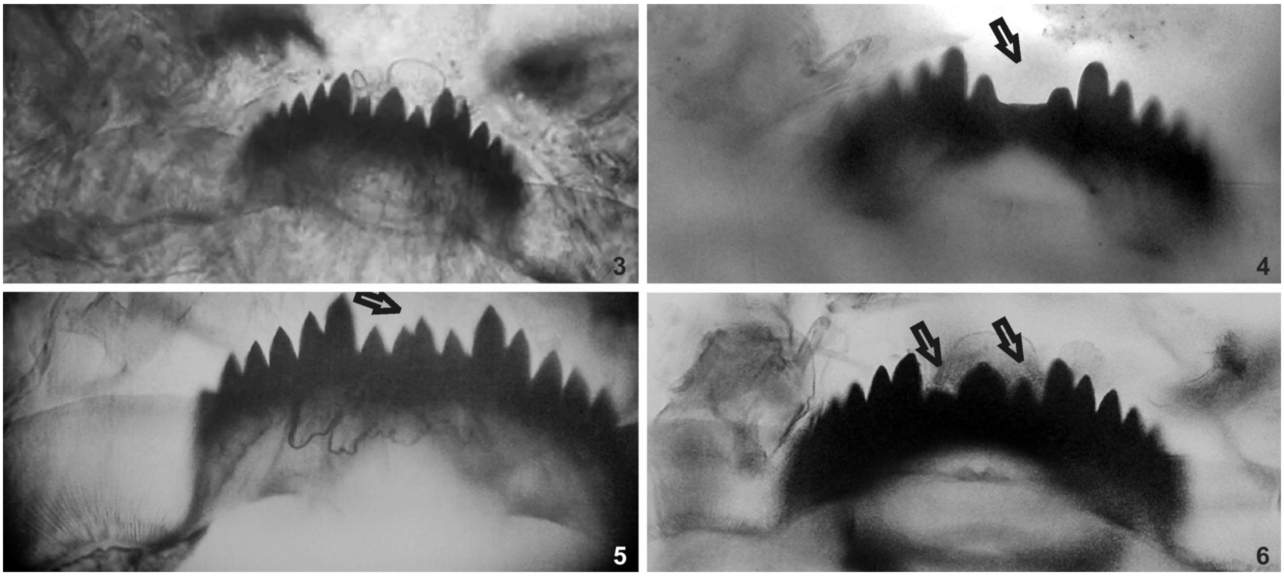
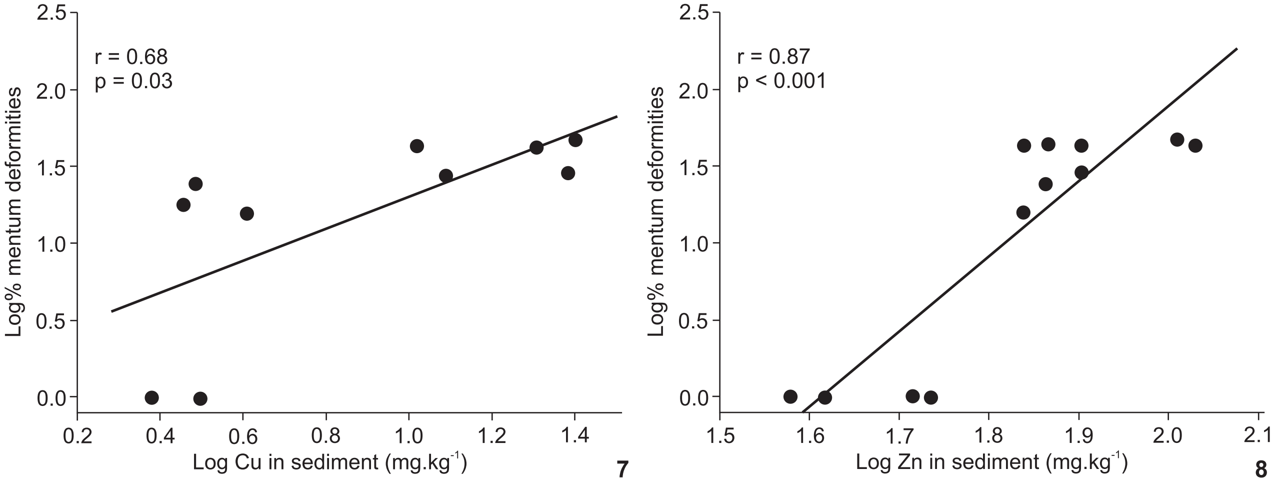


Figure 2. Zinc concentration (mean ± standard error) and copper at the collection sites of Tigre River, Erechim, RS, Brazil.



Figures 3–6. Deformities in the mentum of *Chironomus* (oral cavity): (3) standard mentum; (4) deformed mentum (deletion of teeth); (5) deformed mentum (addition of teeth); (6) deformed mentum (tooth wear).



Figures 7–8. Pearson's linear correlation between Copper (7) and zinc (8) concentrations and the incidence of deformities in the mentum of *Chironomus* (oral cavity) *Chironomus*.

In total, 2895 *Chironomus* larvae were sampled. Site P3 had a greater count of organisms (62.5% of the total, 1808 organisms) followed by site P4 (32.1%, 930 organisms). Of the total number of larvae identified, 881 (30.4%) had deformities of the mentum (Figs 3–6). The majority of deformities observed were by attrition and deletion of the mentum.

Sites P3 and P4 were the sites with the highest percentage of larvae with deformity of the mentum (31.8% and 31.7%, respectively). The concentrations of Cu and Zn were positively correlated with the incidence of such deformities in *Chironomus* larvae. The relationship between deformities and Zn concentrations was greater than that of Cu concentrations ($r = 0.87$, $p < 0.001$; $r = 0.68$, $p = 0.03$, respectively) (Figs 7–8).

DISCUSSION

The electrical conductivity, turbidity and total dissolved solids varied among the collection sites. Urban effluents are significant sources of chlorides in the water surface, which influence the variation in electrical conductivity (Santos 2010, Hepp et al. 2013). Similarly, as solids dissolved in water indicate the presence of salts, a correlation between dissolved solids and electrical conductivity is expected (Porto et al. 1991). Moreover, in places with large suspended matter, colloids and dissolved and particulate organic matter (which can be generated by urban environments), the water tends to be significantly turbid (Medeiros et al. 2015). The variation of these variables indicates a contamination gradient of the water body (spring–mouth).

P1 is situated in a small vegetation fragment, which ensures that the aquatic environment is somewhat protected (Hepp et al. 2010, Miserendino et al. 2011). In addition, its drainage area does not have any source of organic matter of anthropogenic origin. Riparian vegetation does not always guarantee water quality if there is a source of contamination at the site. The great problem of urban water bodies lies in the fact that they receive domestic waste *in natura* (Hepp et al. 2013). In this case, the domestic effluents are dumped directly into the river under study, since in P2 there is an upstream housing complex, which explains the high concentrations of Zn.

High concentrations of organic matter are fundamental for metals to bind, which is of great relevance for their transfer into biological systems (Campbell et al. 1988). In our study, we observed that increased concentrations of organic matter were positively associated with Cu concentrations.

Although the concentration of Zn did not increase in the nascent-mouth direction of the stream, the sites with the greatest influence of urban waste (P2 and P3) presented the highest concentrations of Zn, corroborating our first hypothesis.

The incidence of deformities in the mentum if *Chironomus* was positively correlated with the concentrations of Cu and Zn, thus corroborating our second hypothesis.

Chemical compounds can accumulate in the sediment and are the main cause of morphological alterations in the mentum of *Chironomus* larvae (Macdonald and Taylor 2006), making these immatures suitable indicators of water quality (Janssens and Gerhardt 2003, Ochieng et al. 2008).

Some studies have reported that the oral cavity of *Chironomus* larvae may present changes in up to 8% of the individuals even in minimally impacted streams (Warwick 1988, Ochieng et al. 2008). This is considered normal and is a consequence of the natural wear and tear caused by the dietary habits of these larvae. In this study, we observed more than 30% of the larvae with deformities in the mentum (wear, deletion and extra teeth). This high percentage of deformed larvae (four times more than expected from natural wear) is indicative of the effect of chemical stressors dissolved in the water. According to Wiederholm (1984) and Odume et al. (2012), in heavily polluted environments, 30–40% of Chironomidae larvae present deformities in their mouth parts. Martinez et al. (2002) found that 3.8% to 10.3% of *Chironomus* larvae presented morphological deformities associated with sediments impacted with high levels of Cd, Cu, Pb and Zn. Biasi and Restello (2010) observed 12.2% of *Chironomus* larvae with deformities in the mentum.

The high incidence of deformities in larvae collected at sites P3 and P4 is directly correlated with the high concentrations of Cu and Zn. Organisms exposed to a stressor (e.g. metals) may have their adaptive responses suppressed to the extent that it decreases their chances of survival (Karouna-Renier and Zehr 2003). High levels of incidence of deformities at site P3 and site P4 may be directly related to environmental stressors, such as the high concentrations of metals in the sediment.

In conclusion, our study indicates that the presence of Cu and Zn from the deposition of waste from urban environments causes deterioration in the quality of water and the sediment of streams, thus increasing the incidence of deformities in Chironomidae. Although we did not observe variations in the concentrations of Cu between the sections, the high incidence of deformities may be caused by this metal in the presence of certain components. As the relationship between Zn concentrations and the incidence of deformities was higher, we are led to believe that the association of Cu and Zn enhances the negative effects of the release of organic residues on aquatic organisms. Another important conclusion of our study is related to the observed morphological effects. Most studies on the use of bioindicators of water quality have indicated that environmental degradation affects aquatic populations and communities. In this study, we showed that the morphological and physiological structure of organisms is impacted, resulting in even greater damage to aquatic communities. Therefore, the use of biological assessment approaches, such as studies of morphological variations in the presence of pollutants, are excellent tools for assessing the health of aquatic environments, serving as important information for water resource management.

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