

The relationship between sediment metal concentration and Odonata (Insecta) larvae assemblage structure in Cerrado streams

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ABSTRACT

The relationship between sediment metal concentration and Odonata (Insecta) larvae assemblage structure in Cerrado streams

Metals can be incorporated into stream sediment affecting benthic invertebrate assemblages in different ways. Odonata larvae have variable tolerances to metals; sublethal levels accumulated in larval tissue can indirectly influence assemblage structure in environments with differences in types and concentrations of metals in the sediment. This research evaluated the relationship between Odonata larvae assemblages and sediment metal content in Cerrado streams. We evaluated genus composition, abundance, richness, Shannon-Wiener (H') diversity index and Pielou's evenness index (J') of the assemblages from 12 streams. Cluster analysis was used to identify groups of streams according to sediment concentrations of Cu (copper), Zn (zinc), Ni (nickel), Fe (iron) and Mn (manganese). Canonical Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA) were performed to determine how metals influence Odonata assemblage metrics. Cluster analysis revealed three distinct groups of streams according to metal concentration in the sediment. RDA showed a negative relation between Pielou evenness (J') and the concentration of Ni, Cu, Zn and Mn, while abundance, genus richness and Shannon-Wiener diversity were positively related with Fe. CCA indicated that some taxa showed an opposite relation with metal concentration, but others were more abundant in streams subjected to high metal concentrations. Although the increase in iron concentration in streams can lead to an increase in the abundance of Odonata larvae, high concentrations of copper, zinc and manganese can lead to a reduction in taxon evenness.

Key words: benthic communities, macroinvertebrates, pollution, aquatic insects

RESUMO

A relação entre a concentração de metais no sedimento e a estrutura de assembleias de larvas de Odonata (Insecta) em córregos de Cerrado

Os metais podem ser incorporados ao sedimento dos riachos afetando as assembleias de invertebrados bentônicos de diferentes maneiras. As larvas de Odonata têm tolerâncias variáveis aos metais e os níveis subletais acumulados no tecido larval

podem influenciar indiretamente a estrutura das assembleias em ambientes com diferentes tipos e concentrações de metais nos sedimentos. Esta pesquisa avaliou a relação entre as assembleias de larvas de Odonata e a concentração de metais no sedimento de riachos do Cerrado. Avaliamos a composição de gêneros, abundância, riqueza, índice de diversidade de Shannon-Wiener (H') e índice de equidade de Pielou (J') das assembleias de 12 riachos. A análise de agrupamento foi utilizada para identificar grupos de riachos de acordo com as concentrações de Cu (cobre), Zn (zinco), Ni (níquel), Fe (ferro) e Mn (manganês) nos sedimentos. Análise de Redundância Canônica (RDA) e Análise de Correspondência Canônica (CCA) foram utilizadas para determinar como os metais influenciam as métricas das assembleias de Odonata. A análise de agrupamento revelou três grupos distintos de riachos, de acordo com a concentração de metais no sedimento. A RDA mostrou uma relação negativa entre a equidade de Pielou (J') e a concentração de Ni, Cu, Zn e Mn, enquanto a abundância, a riqueza de gênero e a diversidade de Shannon-Wiener apresentaram relação positiva com o Fe. A CCA indicou que alguns táxons apresentaram uma relação oposta à concentração de metais, mas outros foram mais abundantes em riachos sujeitos a altas concentrações de metais. Embora a maior concentração de ferro em riachos possa levar a um aumento na abundância de larvas de Odonata, altas concentrações de cobre, zinco e manganês podem levar a uma redução na equidade dos táxons.

Palavras chave: comunidades bentônicas, macroinvertebrados, poluição, insetos aquáticos

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INTRODUCTION

Metals are considered one of the most common contaminants in water bodies and may have anthropogenic origins such as disposal of industrial effluents and agricultural activities (Corbi *et al.*, 2006). Metals can be incorporated into the sediment and cause persistent and long-term impacts including reduction of water quality, bioassimilation and bioaccumulation by aquatic organisms. Consequently, metals can be incorporated into the food chain (Lindegarth & Underwood, 2002), resulting in potential long-term implications to animal and human health (Mertz, 1986, Ip *et al.*, 2007).

Studies conducted in different metal-contaminated aquatic systems have reported the presence of the metals in sediment and negative effects in the benthic fauna (Murphy & Davy-Bowker, 2005, Dural *et al.*, 2006, Solà & Pratt, 2006, Corbi *et al.*, 2011, Zhang *et al.*, 2016). Toxic effects of metals can occur at the individual level, for example, increasing mortality of sensitive species and changing other vital processes such as growth and reproduction (Casper, 1994, Amisah & Cowx, 2000, Chanu *et al.*, 2017). Similarly, the exposure of insect larvae to the sediment of streams located in agricultural areas reduced the number of DNA bands, causing a loss of genetic diversity (Colombo-Corbi *et al.*, 2017).

Benthic macroinvertebrate fauna are essential organisms in lentic and lotic trophic webs, influ-

encing energy flow and nutrient cycling (Nicacio & Juen, 2015, Nascimento *et al.*, 2018). These organisms can reflect environmental changes and are the most widely used organisms in freshwater biomonitoring of human impact providing reliable information of aquatic ecosystems integrity (Bonada *et al.*, 2006, Ceneniva-Bastos *et al.*, 2017, Corbi & Trivinho-Strixino, 2017).

In watercourses polluted by metals, species richness and diversity of benthic macroinvertebrate communities can be reduced by direct and indirect impacts of contaminants (Smolders *et al.*, 2003, Carter *et al.*, 2017). Oligochaetes, chironomids, and Hydropsychidae caddisflies, for example, are relatively tolerant to metals, whereas some genera of Ephemeroptera such as *Rhithrogena* and *Cinygmula* are considered extremely sensitive to metals (Winner *et al.*, 1980, Clements *et al.*, 2000).

Odonata larvae comprise a diverse group in aquatic macroinvertebrate communities with species associated with differences in environmental conditions (Kalkman *et al.*, 2008). They are influenced by the variation in water bodies such as from riparian deforestation, pH changes, and metal contamination (Hilton, 1985). The close association of Odonata larvae with sediments in streams (Corbi & Trivinho-Strixino, 2008), and their metal accumulation capacity (Guimarães *et al.*, 2019) might result in the transference of metals to higher trophic levels, including fishes and

terrestrial animals (Clements, 1991, Wayland & Crosley, 2006, Tollett et al., 2009). Since Odonata larvae are predators, they could accumulate more metals than the other aquatic insects they prey upon (Corbi et al., 2008).

Few studies evaluating metal concentration influences on Odonata larvae indicate variable tolerance to metals with little direct effect on mortality (Rockwood et al., 1991, Tenessen, 1993, Tollett et al., 2009). However, sublethal levels accumulated in larval tissue are proposed to influence predator-avoidance, development, or appetite, resulting in assemblage structure variation in environments with different kinds and concentration levels of metals in sediment (Tollett et al., 2009).

The Brazilian Cerrado is considered a hotspot since it presents high biodiversity but also strong environmental impacts (Myers et al., 2000). The aquatic environments in the domain of the Cerrado biome have high levels of macroinvertebrate

richness including dragonflies and damselflies (De Marco Jr. & Vianna, 2005). The conversion of natural areas of Cerrado into agricultural fields results in the use of fertilizers, containing different concentrations of metals such as lead, nickel, chromium, cadmium, aluminum and zinc depending on different kinds of agriculture cultivation. This situation, in addition to the deforestation of riparian vegetation, favors the contamination of aquatic sediments and benthic macroinvertebrate fauna, including Odonata juveniles (Corbi et al., 2010, 2013, 2018, Guimarães et al., 2019).

In this context, this study evaluated the relationship between Odonata larvae assemblages and metal concentrations in the sediment of Cerrado streams, located in Central Brazil. We expected to find higher assemblage structure metric values in streams with lower levels of metal concentration, considering the potential negative effects of metals in Odonata larvae.

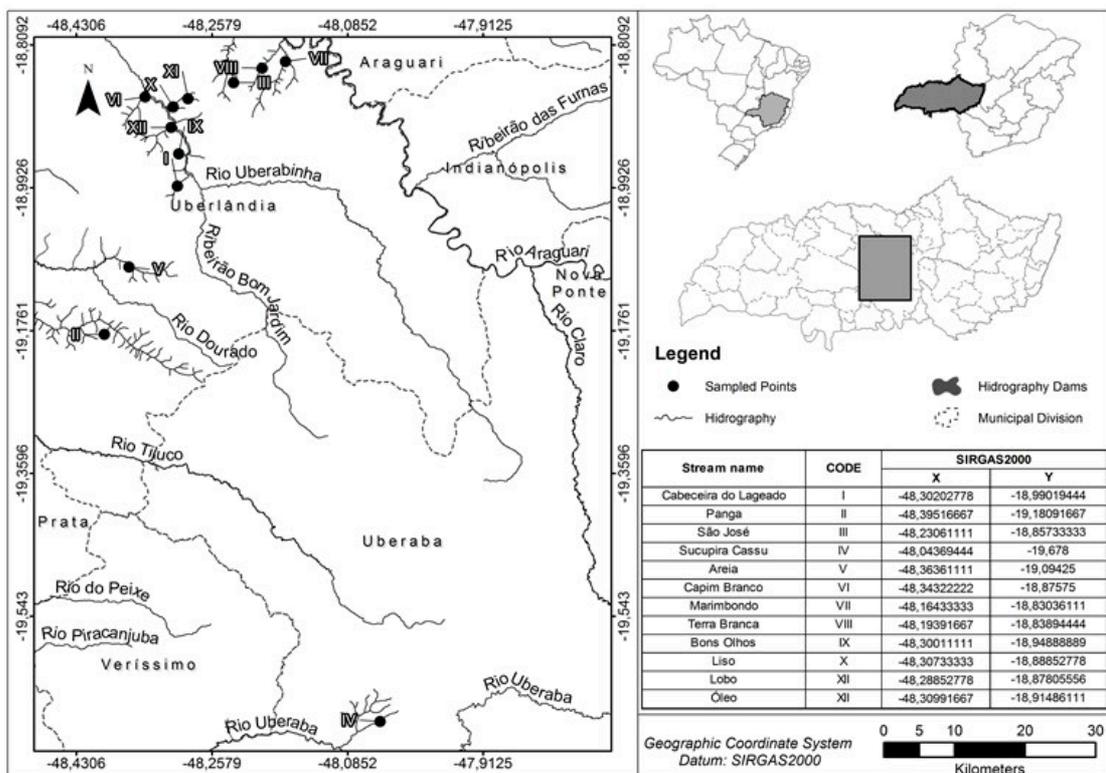


Figure 1. Location of the study area, in the State of Minas Gerais, Brazil. *Localização da área de estudo, no estado de Minas Gerais, Brasil.*

MATERIALS AND METHODS

Study area

The study was conducted in 12 low order streams in the Triângulo Mineiro region, Minas Gerais state, Central Brazil (Fig. 1). The climate in the region is tropical and according to the Köppen climate classification, is type Aw, megathermic, with summer rains between October and March and winter from April to September (Gomes *et al.*, 1982).

The Triângulo Mineiro region was marked by extensive cattle ranching until the end of the 1960s. In the late 70s, soybean cultivation was introduced. The flat topography and the ease of access to limestone deposits in the area were factors that favored agricultural expansion and with it, the intense use of pesticides. Tax incentives also stimulated pine and eucalyptus plantation (Schneider, 1996). More recently, large areas of the region have been dedicated to sugarcane cultivation. This agricultural development leads to an increase in contamination of regional aquatic ecosystems (Brito & dos Reis, 2012).

All the streams are remarkably similar in physical structure. They have low water velocity (< 1 m/s), shallow depth (< 1 m) and have predominantly sand (40 %) and fine gravel (~20 %) substrates. The streams were chosen with the aim of sampling the variability of land use and environmental conditions of the region. Streams I, II and III are located in environmental protection areas; stream IV has preserved riparian vegetation predominantly surrounded by sugarcane cultivation. Streams V, VI, VII and VIII are predominantly covered by pasture areas dedicated to livestock, all of them with an altered riparian vegetation, except for stream VI which does not have natural vegetation. Streams IX, X, XI and XII have altered riparian areas and are located in urbanized regions.

Sample collection and processing

Sampling in each stream was conducted in October/November 2010 and February/March 2011. Samples of both periods were joined for analysis.

The Odonata larvae assemblage structure was

studied selecting one hundred-meter sections in each of the 12 streams. Each 100 m section was divided into 20 segments of five meters, from which five segments were randomly sampled. In each sampling segment, we collected Odonata larvae using a “D” net (0.25 mm mesh size), screening different types of microhabitats for six minutes. Each biological sample was represented by all the larvae collected during the 30 minute periods for each stream.

The specimens were put in plastic bags and preserved in 10 % formalin. In the laboratory, the larvae were identified to genus, using dichotomous keys (Carvalho & Calil, 2000, Costa *et al.* 2004, Mugnai *et al.* 2010). Assemblage metrics (abundance, genus richness, Shannon-Wiener diversity index (H') and Pielou's evenness (J')) were calculated for each stream.

Surface sediment subsamples (~5 cm deep) were collected in each segment of the 100 m section with a plastic spoon and they were put together in a plastic bag for each stream. The spoons and plastic bags were metal-free. They were transported to the laboratory, frozen at -20 °C and subsequently lyophilized for Cu, Fe, Mn, Ni and Zn extraction. In beakers (100 mL), we added to each 3.0 g subsamples of the sediment 10 ml of nitric acid (HNO₃), then heated it on a hotplate at approximately 90 °C for two hours. These samples were then cooled at room temperature, filtered using filter paper and transferred to volumetric flasks. The solutions were analyzed for metals in a Pye Unicam flame atomic absorption spectrophotometer. Digestion and detection were undertaken in triplicate (Pourang, 1996). Blanks and standards were run with each batch of samples.

The results of Cu, Zn and Ni concentrations were compared with guide values available, those adopted by the Companhia de Tecnologia de Saneamento Ambiental (CETESB) and by the project QualiSed (Mozeto *et al.*, 2006).

Data Analysis

Cluster analysis (UPGMA method, Euclidean distance coefficient) was used to classify streams according to the metal concentrations (previously standardized due to differences in scale) in sedi-

Table 1. Taxonomic composition (family and genera) and abundance of Odonata larvae in the sampling sites. *Composição taxonômica (famílias e gêneros) e abundância das larvas de Odonata nos locais de amostragem.*

TAXONOMIC GROUP	STREAM											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Aeshnidae												
<i>Castoraeschna</i> Calvert, 1952	-	-	3	-	-	-	-	-	-	-	-	-
Calopterygidae												
<i>Mnesarete</i> Cowley, 1934	1	1	2	1	2	1	-	-	-	-	-	6
Coenagrionidae												
<i>Argia</i> Rambur, 1842	-	-	-	-	-	3	-	-	-	-	-	-
<i>Oxyagrion</i> Selys, 1876	-	-	-	-	-	-	-	-	-	1	-	3
Unidentified (NI)	-	-	-	1	-	-	-	1	-	7	2	2
Cordulidae												
<i>Aeschnosoma</i> Selys, 1870	2	-	-	-	-	-	-	-	-	-	-	-
<i>Neocordulia</i> Selys, 1882	1	2	1	1	-	-	-	-	-	-	-	-
Unidentified (NI)	-	1	-	-	-	-	-	-	-	-	-	-
Gomphidae												
<i>Gomphoides</i> Selys 1850	-	-	-	-	1	-	-	-	-	-	-	-
<i>Phyllocycla</i> Calvert, 1948	-	-	-	-	1	2	1	-	-	-	-	-
<i>Phyllogomphoides</i> Belle, 1970	-	-	3	1	1	-	-	-	-	-	-	-
<i>Zonophora</i> Selys, 1854	-	-	4	1	-	-	-	-	-	-	-	-
<i>Progomphus</i> Selys, 1854	-	-	1	-	4	1	5	3	-	-	-	-
Libellulidae												
<i>Anatya</i> Kirby, 1889	-	-	-	-	-	-	-	-	-	-	2	-
<i>Dythemis</i> Hagen, 1861	-	-	-	-	-	-	-	-	1	1	-	-
<i>Elasmothemis</i> Westfall, 1988	-	-	-	-	-	-	1	-	-	1	-	4
<i>Erythrodiplax</i> Brauer, 1868	-	-	-	-	-	-	-	-	-	6	-	-
<i>Orthemis</i> Hagen, 1861	-	-	-	-	-	-	-	-	-	6	-	-
<i>Perithemis</i> Hagen, 1861	-	-	1	-	-	-	-	1	-	-	-	1
<i>Tramea</i> Hagen, 1861	-	-	-	-	-	-	-	-	-	1	-	-
Unidentified (NI)	-	-	-	-	-	-	-	-	1	-	-	-
Total abundance	4	4	15	5	9	7	7	5	2	23	4	16

ments. Significant difference among groups was tested using Permutational Multivariate Analysis of Variance (PERMANOVA). Canonical Redundancy Analysis (RDA) was used to correlate environmental variables, represented by standardized metal concentrations and biological metrics (richness, Shannon index diversity, evenness and total abundance) of Odonata assemblages. Canonical Correspondence Analysis (CCA) was used to describe and compare the distribution and abundance of Odonata taxa among streams with different metal concentrations. The choice of whether a linear or a unimodal response model is more appropriate was based on the gradient length of the axes (0.633 for diversity metrics and 7.609 for taxa abundance data). Significance of the RDA and CCA models, axis, and terms (metal variables) were tested through Monte Carlo permutation (Ter Braak & Šmilauer, 2002). All mul-

tivariate analyses were conducted in R software, version 4.0.2 (2020-06-22) (R Core Team 2020) using the vegan package (Oksanen et al., 2013). Both RDA and CCA were used only for descriptive purposes, so no attempt was made to select explanatory variables.

RESULTS

We recorded a total of 101 Odonata larvae, within 18 genera (Table 1). Abundance of taxa varied from 2 to 23 in the streams (Fig. 2a). Specimens of Gomphidae were the most abundant, corresponding to 28.7 % of Odonata larvae. Genus richness varied from two to seven taxa (Fig. 2b). The Shannon-Wiener (H') diversity varied from 0.7 to 1.8 (Fig. 2c) and the evenness (J') varied from 0.7 to 1.0 (Fig. 2d).

In stream sediments, the concentrations of

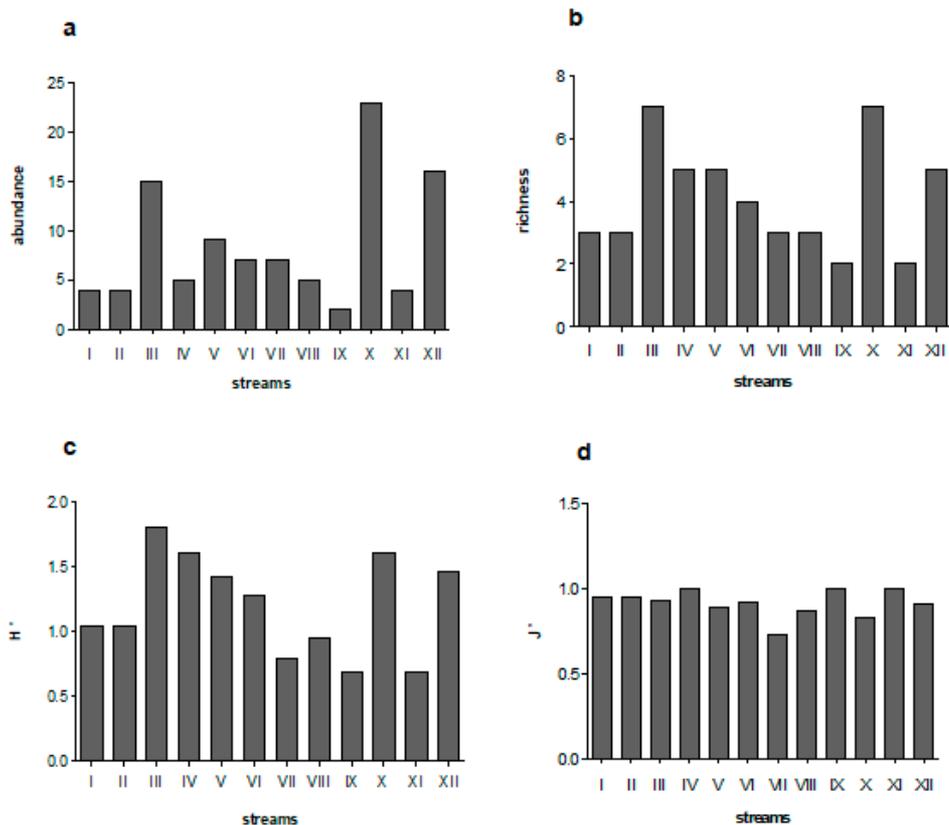


Figure 2. (a-d). Values of the biological parameters of the Odonata larvae assemblages. a) abundance b) richness c) Shannon-Wiener (H') diversity index d) Pielou's evenness index (J'). *Valores dos parâmetros biológicos das assembleias de larvas de Odonata. a) abundância b) riqueza c) índice de diversidade de Shannon-Wiener (H') d) índice de equitabilidade de Pielou (J').*

Cu varied from 0 mg/kg to 114.96 mg/kg (Fig. 3a); Zn concentrations varied from 2.61 mg/kg to 99.68 mg/kg (Fig. 3b); Ni concentrations varied from 0 mg/kg to 59.81 mg/kg (Fig. 3c); Fe varied from 284.08 mg/kg to 10 774.30 mg/kg (Fig. 3d) and Mn concentrations varied from 7.57 mg/kg to 279.73 mg/kg in the streams analyzed (Fig. 3e).

Cluster analysis revealed three groups of streams that differed statistically from each other (PERMANOVA, $F_{1,10} = 26.59$; $p = 0.022$), according to the metal concentration in the sediment (Fig. 4). The single stream (VII) from group 1 showed high concentrations of Cu, Zn, Ni, and Mn. Group II was characterized by lower concentrations of Cu, Zn, Ni, Fe, and Mn, joining the three streams from protected areas (I, II, and III), stream V from a pasture area, and three streams

from urban areas (X, XI, and XII). Group 3 included the stream from a sugarcane area (IV), two streams from pasture areas (VI and VIII) and one stream from an urban area (X), all of them with higher concentrations of Fe.

Comparing the Cu, Zn and Ni concentrations with the guide values (Mozeto *et al.*, 2006), streams IV and VII showed concentrations above PEL (probable effect level) for Ni. Streams VII, VIII and X showed concentrations above TEL (threshold effect level) for Cu. Stream VIII showed concentrations above TEL for Ni (Table 2). High concentrations of iron were recorded mainly in streams VI, VIII and X.

The first two axes of the RDA explained 73 % of the variance, with 49.25 % on the first axis, which was negatively correlated to Fe concentra-

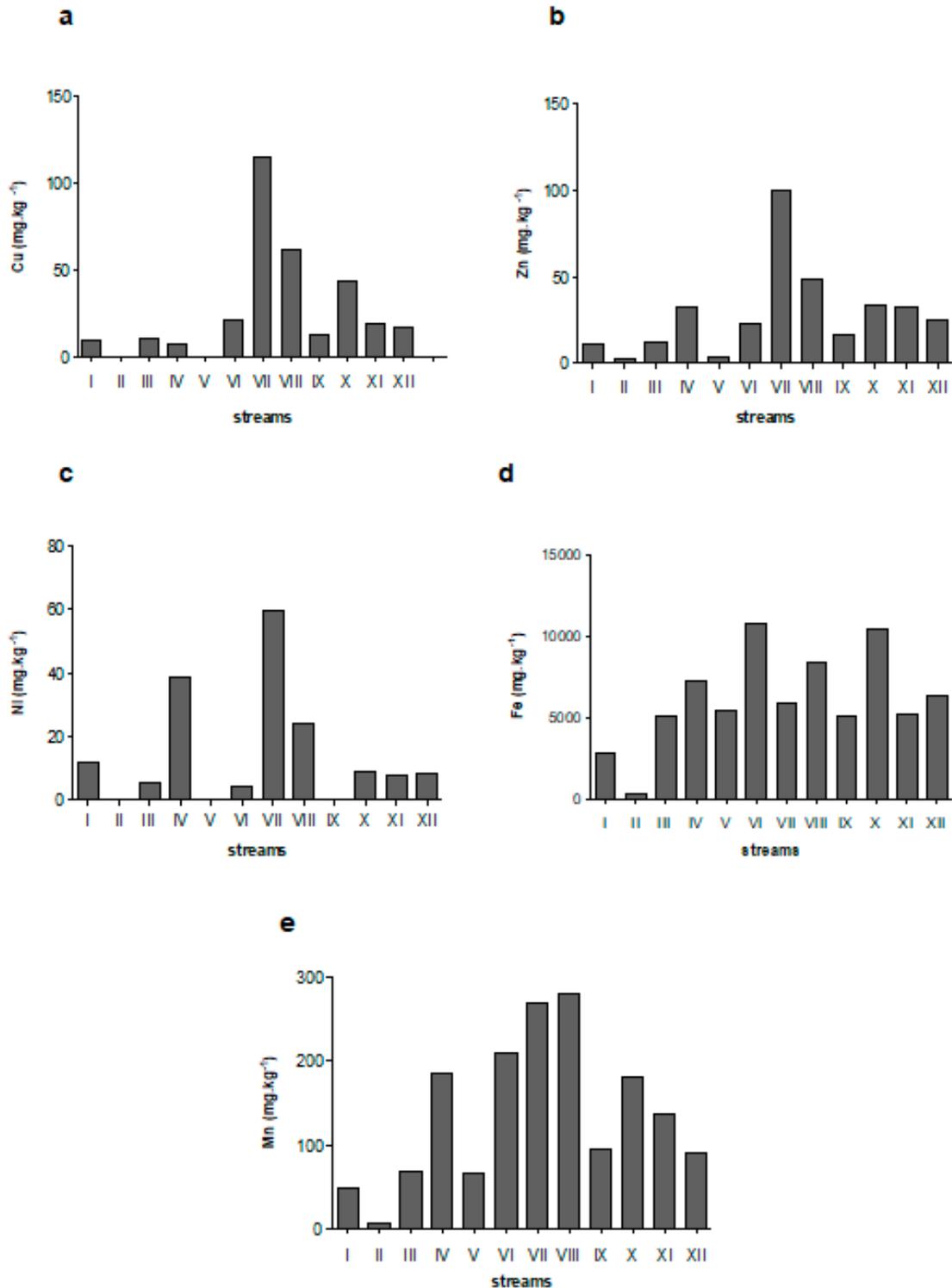


Figure 3. (a-e). Mean metal concentrations in the sediment of 12 streams (mg/kg). a) Copper (Cu) b) Zinc (Zn), c) Nickel (Ni) d) Iron (Fe), e) Manganese (Mn). (a-e). *Concentrações médias de metais no sedimento dos 12 córregos (mg/kg). a) Cobre (Cu) b) Zinco (Zn), c) Níquel (Ni) d) Ferro (Fe), e) Manganês (Mn).*

tion (biplot scores = -0.5090), and 23.69 % in the second axis, negatively correlated to Cu (-0.932), Zn (-0.802), Mn (-0.553), and Ni (-0.540) concentrations (Fig. 5). Abundance, richness, and diversity increased with the concentration of Fe.

However, evenness decreased with the concentrations of Ni, Cu, Zn and Mn (Fig. 5). The RDA model ($F_{5,6} = 3.9158; p = 0.014$) and the first axis ($F_{1,7} = 14.6957; p = 0.020$) were statistically significant. Metals significantly correlated to di-

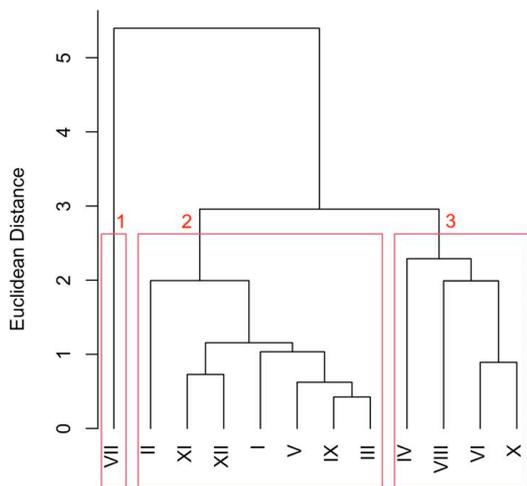


Figure 4. Dendrogram resulting from cluster analysis of streams based on metal concentrations (UPGMA method, Euclidean coefficient, cophenetic correlation = 0.9006). Group 1: stream with the highest metal concentrations; group 2: streams with low metal concentrations; group 3: streams with intermediate metal concentrations. *Dendrograma resultante da análise de agrupamento dos córregos baseada nas concentrações de metais (método UPGMA, coeficiente Euclidean, correlação copenética = 0.9006). Grupo 1: córrego com as maiores concentrações de metais; grupo 2: córregos com concentrações baixas de metais; grupo 3: córregos com concentrações intermediárias de metais.*

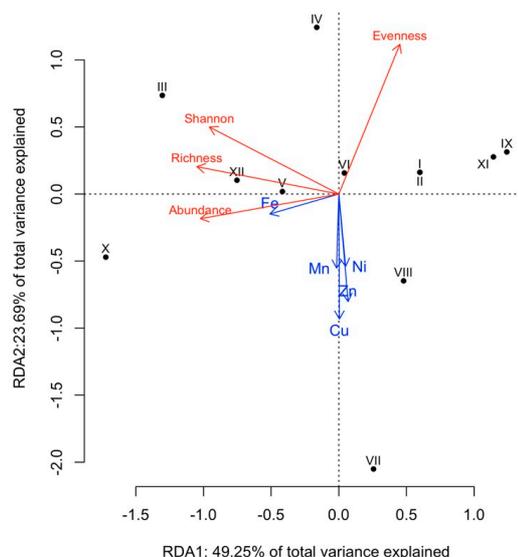


Figure 5. Ordination diagram of streams according to Canonical Redundancy Analysis (RDA), considering the concentrations of metals and biological parameters (richness, abundance, H' and J'). *Diagrama de ordenação dos córregos de acordo com a Análise de Redundância Canônica (RDA), considerando as concentrações de metais e os parâmetros biológicos (riqueza, abundância, H' e J'). Legend: H' – Shannon-Wiener Diversity Index; J' – Pielou's evenness index; Fe – iron; Cu - copper; Mn – manganese; Ni – nickel.*

Table 2. Comparison between Cu, Zn and Ni concentrations and the guide values (Mozeto *et al.*, 2006). *Comparação entre as concentrações de Cu, Zn e Ni e os valores guia (Mozeto *et al.*, 2006).*

Metals	Guide Values		Stream											
	TEL*	PEL**	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Cu	35.70	197.00	9.95	nd	13.94	7.45	nd	21.75	<u>114.96</u>	<u>62.40</u>	12.62	<u>43.63</u>	19.54	16.94
Zn	123.10	315.00	10.94	2.61	17.60	32.49	3.62	22.64	99.68	48.92	16.27	33.94	32.79	25.35
Ni	18.00	35.90	11.61	nd	3.32	38.86	nd	4.45	59.81	<u>23.81</u>	nd	8.76	7.95	8.45

* TEL - Threshold Effect Level; ** PEL - Probable Effect Level, nd: not detected. Underline: transgress TEL; Bold: transgress PEL

versity metrics of the Odonata assemblage were Cu ($F_{1,6} = 5.2895$; $p = 0.012$), Fe ($F_{1,6} = 5.0389$; $p = 0.013$) and Mn ($F_{1,6} = 7.0278$; $p = 0.004$).

Metal concentrations explained 38.15 % of the total inertia (4.819) in the Odonata larvae assemblage. The first axis of the CCA explained 14.78 % of the total variance and was negatively correlated with Fe (-0.871), Mn (-0.5777), Cu (-0.4018) and Zn (-0.3491), separating, on the positive side, streams with consistently lower concentrations of metals in protected areas (I, II, and III), and on the negative side, streams from urban areas (IX, X, XI, and XII). Taxa less tolerant to high metal concentrations and found only in the streams from protected areas were *Aeschnosoma*, unidentified Corduliidae (NI), *Neocordulia*, and *Castoraeschna*. In contrast, *Progomphus*, and *Phyllocycla* were more abundant in streams from pasture areas and *Anatya*, *Dythemis*, *Erythrodiplax*, *Orthemis*, *Tramea*, and unidentified Coenagrionidae (NI) were found only in streams from urban areas.

DISCUSSION

In general, metals are essential to the biota. Organisms require trace quantities of some metals, such as cobalt, copper, manganese, zinc and iron to complete vital functions in the body (Mertz, 1986, Melo et al., 2012, Corbi et al., 2018). However, some metals have reference values that indicate toxicity if exceeded. Other metals like mercury, lead and cadmium are considered toxic, have no function in organisms and their presence and bioaccumulation can cause diseases, particularly in mammalian species at the highest level of the food chain (Mertz, 1986).

Low values of metals were detected in sediment of all streams located in preserved areas (I, II, II). Corbi et al. (2008, 2018) also recorded low levels of metals in areas with preserved vegetation. However, streams located in urban, pasture and sugarcane areas showed considerable variation in metal concentration. This situation could be related to different causes, including differences in riparian forest integrity and intensity of metal contamination in adjacent areas. Riparian vegetation is very important for absorption of toxic products which come from the neighboring areas

(Dudgeon, 1989, Primavesi et al., 2002, Angelotti-Netto et al., 2004, Corbi et al., 2018).

The Cluster Analysis was consistent with guide values for almost all the streams that showed high metal concentrations (X, VIII, IV, VII), as they showed values above TEL and/or PEL for Cu, Zn and Ni. Although these metals can be part of the metabolism of most organisms, in high concentrations they can cause impacts to ecosystems, such as biodiversity loss by toxicity. Kiffney and Clements (1993), for example, observed significant changes in invertebrate communities exposed to critical concentrations of Cu and Zn. Abel (1989) included the metals Cu, Zn and Ni on a list of toxicity to aquatic organisms.

Despite the higher iron concentrations recorded in streams VI, VIII and X, this metal showed

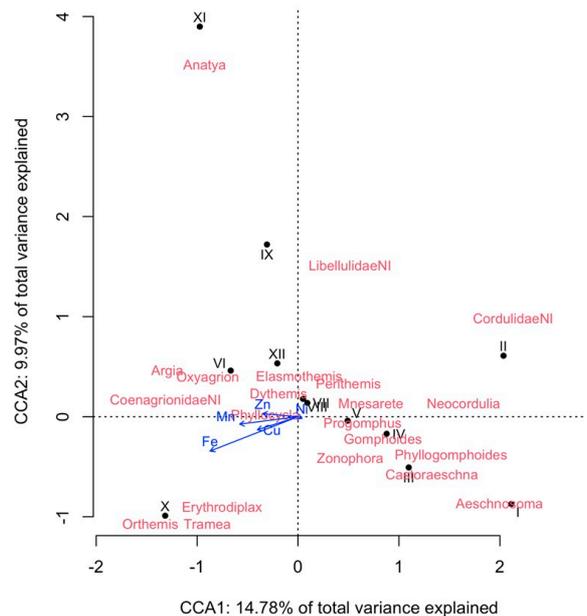


Figure 6. Ordination diagram of the first two axes of the Canonical Correspondence Analysis (CCA), considering the concentrations of metals and the Odonata taxa studied. Legend: Fe – iron; Cu - copper; Mn – manganese; Ni – nickel. Metals providing strong influence on Odonata larval assemblage composition have longer arrows (Fe and Mn). *Diagrama de ordenação dos dois primeiros eixos da Análise de Correspondência Canônica (CCA), considerando as concentrações de metais e os táxons de Odonata estudados. Legenda: Fe – ferro; Cu - cobre; Mn – manganês; Ni – níquel. Metais exercendo forte influência na composição da assembleia de larvas de Odonata têm setas mais longas (Fe e Mn).*

high concentrations at almost all sampling points. Much of the Cerrado biome is covered by ferruginous lateritic crusts. These crusts in combination with quartzitic material forms sandy clay soils poor in nutrients and with high levels of iron oxides (Reatto & Martins, 2005). This pattern was also observed by Corbi *et al.* (2006) and Corbi *et al.* (2018), wherein the iron appeared in high concentrations in all streams, related to the type of soil.

Although streams V, VI, VII and VIII were predominantly covered by pasture areas they showed different concentrations of metals. This is probably related to the variable level of integrity of riparian vegetation and the presence of cultures. Stream V had an altered riparian forest around the sampling section surrounded by pastures, which could explain the low concentrations of most metals similar to those found in protected areas. Stream VIII had as altered riparian forest and a silviculture (*Eucalyptus* sp.) section along the sampling stretch. The sampling section of stream VI was located in a pasture area without riparian forest, a situation similar to stream VII. However, this last stream had a large area of culture upstream from the sampling section that could explain the high levels of metals detected. As already mentioned, the removal of the riparian vegetation favors the input of toxic substances in aquatic ecosystems coming from the neighboring areas. In cultivated areas, the intense use of fertilizers and pesticides can significantly modify benthic communities of small streams (Corbi & Trivinho-Strixino, 2008) and can cause contamination of sediment and benthic invertebrates (Corbi *et al.*, 2008; Guimarães *et al.*, 2019).

The RDA analysis indicated that among analyzed metrics, evenness was more negatively influenced by the concentrations of metals, particularly with Mn, Cu and Zn. Abundance, richness and diversity are positively related with high levels of Fe in the sediment. Overall, these results do not corroborate the hypothesis of high biological metric values in streams with low sediment metal concentrations suggesting that traditional community metrics are not good indicators of sediment contamination by metals using Odonata assemblages, at least at the concentration levels recorded in the present study. Masson *et al.*

(2010), while investigating relationships between benthic macroinvertebrate communities and abiotic parameters, found that granulometry, dissolved organic carbon, nitrogen, phosphorus and sulfur concentrations in the sediment explained more clearly the changes in the abundance and richness of communities than did the concentration of metals in the sediment, which had little influence on these changes.

The CCA results showed Odonata genera had different responses to metal concentration. Many genera of Odonata showed low abundance in streams with high metal concentrations. Specifically, we could observe an opposite distribution of the taxa *Aeschnosoma*, *Neocordulia*, *Castoraeschna*, *Mnesarete*, *Zonophora*, *Gomphoides*, *Phyllogomphoides* and Corduliidae NI (unidentified) in relation to high concentration of metals in the sediment. However, the CCA also indicated that *Erythrodiplax*, *Orthemis*, *Tramea*, *Dythemis*, *Phyllocycla*, *Argia*, *Perithemis*, *Anatya*, and Coenagrionidae NI (unidentified) may be more tolerant to the presence of metals, as they occurred in streams with high concentrations of Zn, Mn, Cu, Fe and Ni. For ecological data, the percentage of explained variance is usually low; often ~10 %. This is not of concern, as it is an inherent feature of data with a strong presence/absence component, as is our data on the Odonata larvae assemblage.

Variations in tolerance of different genera of Odonata to metals and other environmental parameters could be responsible for the distribution patterns recorded (Nasirian & Irvine, 2017). Ferreira-Peruquetti and Fonseca-Gessner (2003) and Corbi *et al.* (2010), found, for example, that the genera *Erythrodiplax*, *Orthemis* and *Tramea* show a preference for impacted habitats.

Fletcher *et al.* (2017) emphasize the taxa-specific nature of trace element accumulation and provide evidence of differences in accumulation of some trace elements among dragonflies that differ in body form and utilize different microhabitats within the reaches of a stream. They found that high trace element concentrations appeared to accumulate in larvae closer to contaminant sources, and factors such as body form and habitat use appeared more influential on trace element accumulation than phylogeny for several elements (Ni, Ba, Sr, V, Be, Cd, and Cr).

Studies conducted in tropical regions also recorded metal accumulation in Odonata larvae in areas with sediment metal contamination adjacent to cultures. Corbi et al. (2008, 2011) found that dragonflies showed high concentrations of metals in streams located on impacted areas by sugarcane cultures but in general, metal concentrations in larvae were lower than in sediments. However, a different pattern was detected by Guimarães et al. (2019), with bioaccumulation factors (BAF) higher than 1 for some metals and sampled streams in different trophic groups of benthic invertebrates, including Odonata larvae.

A study conducted by Lesch and Bouwman (2018) using adult male dragonflies as indicators of environmental metallic elements, recorded high concentrations of As, and Pb in dragonflies collected near mines when compared to other sites. Although they did not evaluate the origin of metals in the adult Odonata, the contamination of larvae could be one source of these metals. This situation would be worrying because terrestrial predators of adult Odonata, such as falcons (Clarke et al., 1996), could also be contaminated.

In streams with agricultural and livestock activities, we recorded the highest metal concentrations, indicating that these streams are strongly influenced by the use of products with metals in their compositions, such as fertilizers. The results indicated that some genera of Odonata larvae are associated to preserved streams, indicating a positive relationship to habitat quality, but others occurred predominantly in streams with high metal concentrations. The hypothesis of high biological metric values in streams with low sediment metal concentrations was not corroborated. The probable metal tolerance variability of Odonata larvae genera makes traditional assemblage structure metrics for this insect group not effective indicators of habitat quality for metal sediment contamination, at least at the concentration levels recorded in the present study.

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