Bioindicators of water quality as a tool of environmental impact assessment in the Rio Toledo watershed/Brazil

Jéssica Manfrin. Author, M.Sc,[1] Daniel Schwantes. Author, Dr.,[2] Affonso Celso Gonçalves Jr. Author. Dr,[3] Andréia da Paz Shiller. Author, M.Sc,[4] and Valdemir Aleixo. Author, Dr.[5]

[1]Post Graduate Program in Agronomy, State University of Western Paraná (UNIOESTE), Marechal Cândido Rondon, State of Paraná, Brazil, Pernambuco Street, nº 1777; e-mail: jessicamanfrinn@gmail.com
[2]Educational College of Medianeira, Department of Agronomy (UDC), Medianeira, State of Paraná, Brazil, Rio Branco Street, nº 1820; e-mail: daniel_schwantes@hotmail.com
[3] Center of Agrarian Sciences, State University of Western Paraná (UNIOESTE), Marechal Cândido Rondon, State of Paraná, Brazil, Pernambuco Street, nº 1777; e-mail: affonso133@hotmail.com
[4] Center of Agrarian Sciences, State University of Western Paraná (UNIOESTE), Marechal Cândido Rondon, State of Paraná, Brazil, Pernambuco Street, nº 1777; e-mail: andreia.schiller@hotmail.com
5 Department of Environmental Engineering, Pontifical Catholic University of Paraná (PUCPR), Toledo, State of Paraná, Brazil, Union Avenue, nº 500; valdemir.aleixo@pucpr.br

ABSTRACT

This study aimed to identify and quantify environmental impacts under the Toledo River watershed by means of biological monitoring. Six samplings were carried out at seven points. The macroinvertebrates were sorted and identified according to order and taxonomic family. Them were analyzed from the biological metrics, biological index, and submitted to statistical analysis. The total concentrations of metals was obtained in the macroinvertebrates. The quality of the Toledo River is negatively impacted by the activities in the watershed since the concentrations of toxic metals in the benthic macroinvertebrates were high, indicating its bioaccumulation. Low rates of richness, organisms susceptible to contamination, and diversity of families is observed, and a high rate of dominance of the Chironomidae species, indicating a highly polluted environment.

INTRODUCTION

The introduction of metals into the aquatic environment can be responsible for innumerous problems to aquatic biota, and considering the interdependencies between these organisms in this environment, the modification can cause high rates of ecological imbalances (Rocha and Azevedo 2015).

Metals such as cadmium (Cd), lead (Pb) and chromium (Cr), can be highly toxic to most diverse living beings. The Cd, for example, even if introduced at low levels, can quickly accumulate in living tissues, mainly in microorganisms and mollusks and in these organisms remain (Siegel 2002; International Programme on Chemical Safety 2012; Martins et al. 2014).

Living beings need small amounts of certain metals to perform their vital functions, such as cupper (Cu), manganese (Mn), and zinc (Zn), but at excessive levels, these metals can be extremely toxic and cause damage. Other metals, such as Cd and Pb, do not play any role in organisms and their insertion may generate accumulation (Ferreira et al. 2010).

One of the ways of assessing the levels of toxicological contamination in a specific water resource is the use of bioindicators. They respond to changes caused to the environment through the disappearance or decline of organisms or community, or by the abundant growth and increase of the reproductive capacity (Siegel 2002). Organisms that are commonly used and internationally accepted as biological indicators are benthic macroinvertebrates. These organisms have ideal characteristics such as, low mobility and long life cycles; thus reflecting impacting conditions for a long time period and presenting the real conditions of the place and they have taxonomic identification and low cost of realization (Rosenberg and Resh 1993). Therefore, they are good indicators of changes in aquatic ecosystems, including sources of diffuse pollution (Toledo and Nicolella 2002).

The macroinvertebrates are responsible for the mixing of the superficial part of the sediment and for fragmenting the vegetation that ends up depositing in the bed of the river. This fact release nutrients and other compounds to the water and enables the aeration of the sediments (Cummins et al. 1989; Callisto and Esteves 1995). In this sense, Pimenta et al. (2016) suggest that there is a possibility of correlation between contamination and bioindicators resulting in the identification of impacts and analysis of water quality in urban and rural environments.

This study aimed to evaluate the biological quality of Toledo River, located in Toledo city west of the State of Paraná – Brazil, by means of the evaluation, identification and establishment of indexes using benthic macroinvertebrates. Also, to evaluate the occurrence of bioaccumulation of metals in these organisms and their possible insertion into the food chain.

MATERIAL AND METHODS

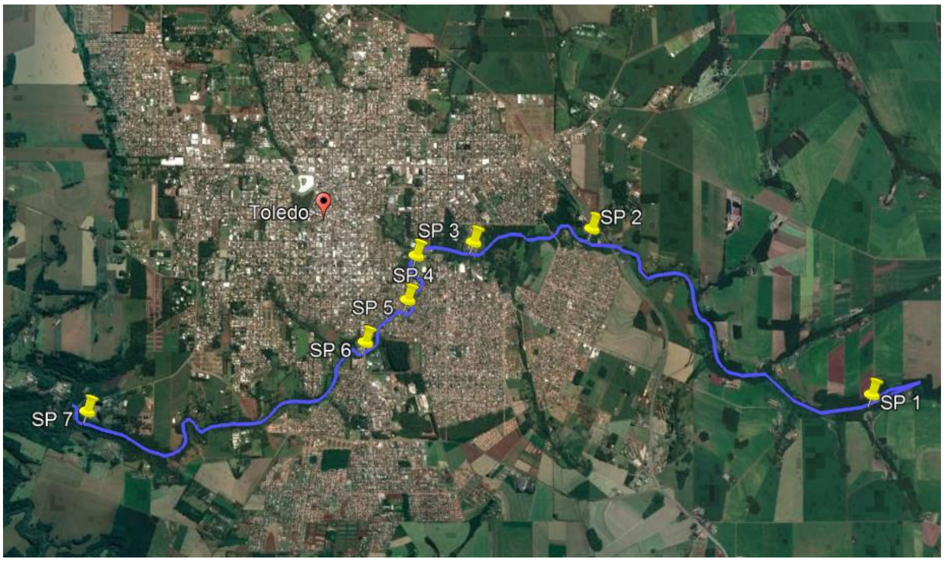
The city of Toledo is nationally known for the intensity of agroindustrial activities, with emphasis on the production of pigs, poultry, milk production, and agricultural production. In addition, the industrial area is rising and exhibit a considerable diversity of size and branches of production.

For the sampling seven sample points were chosen, being them: SP 1 (24°45'02.43"S, 53°39'51.86"W), SP 2 (24°43'50.39"S, 53°42'12.55"W), SP

3 (24°43'56.18"S, 53°43'13.14"W), SP 4 (24°43'59.52"S, 53°43'44.77"W), SP 5 (24°44'27.58"S, 53°44’3.677"W), SP 6 (24°44'40.92"S, 53°44'12.89"W) and SP 7 (24°45'11.75"S, 53° 46'36.69"W), exhibited in Figure 1.

All sampling points were determined from an on-site visit, considering the environmental assessment of the sites and the anthropic activities developed around the water body (Table 1). Six samples were taken in a period of 12 months, with an interval between 60 days.

Figure 1. Location of sampling points at Toledo River in the city of Toledo/PR/Brazil



Source: Google Earth, 2016.

Table 1.1 Characterization of the macroinvertebrates and sediment sample points at Toledo River, in the city of Toledo/PR/Brazil

Sample points	SP 1	SP 2	SP 3	SP 4	SP 5	SP 6	SP 7
Rural area	X						X
Urban area		X	X	X	X	X	
Preserved riparian forest	X						X
Degradeted riparian forest		X	X	X	X	X	
Agricultural activities	X						
Industrial activities					X	X	
Urban solid waste		X		X	X	X	X
Wastewater disposal					X	X	
Fish-farming			X				

Benthic macroinvertebrates samplings were performed using a hand net collector with a 500µm mesh for scanning at the seven sampling points and the kick-sampling methodology was applied (Cetesb 2011). The collected material was stored in properly identified plastic bags. The samples were washed with running water under a sieve of 85 mm.

After washing, the samples were screened based on the visual separation of the benthic macroinvertebrates from the vegetable debris as well as from the inorganic particles present in the sample (Kuhlmann 2012). The triage occurred in two ways, with the naked eye using a white tray with saline solution and superior illumination and with the aid of a magnifying glass (stereoscopic microscope). The first screening format was used to separate the coarser material followed by a more rigorous inspection in search of smaller sized individuals under the magnifying glass.

In the samples with high concentration of coarse inorganic material such as gravel and sand or with many clods of clay, supersaturated saline solution was used; which consisted of the addition of 500 g of cooking salt in 1 liter of water, homogenized and then gradually added to the sample (Kuhlmann 2012). After the separation of the material, with the use of the magnifying glass, the samples were analyzed in small portions homogeneously distributed in petri dishes.

The macrofauna was identified using a magnifying glass (stereoscopic microscope), based on the visualization of external morphological characters, such as the oral apparatus, structure and shape of the organism (Kuhlmann 2012). The identification of the organisms occurred through the classification in order and family using the identification methodologies of Pérez (1988), Bouchard (2004), Merrit et al. (2008), and Mugnai et al. (2010).

The points with high population densities were submitted to the technique of sub-sampling proposed by Kuhlmann (2012). In this study, high-density taxa were stipulated in a number equal to or greater than 300 organisms. For the calculation of the final densities of the rate, we considered the densities obtained in the chosen quadrant and this value multiplied by four, which guarantees the continuity of the quantitative character of the performed analysis.

Biological data were analyzed using biological metrics of richness, abundance, dominance, Shannon diversity and equitability, using Past software 2.15 and percentage (%) analyzes of Chironomidae and Ephemeroptera/Tricoptera through Excel 2010 software. Statistical analyzes were carried out using the principal component analysis (PCA) techniques for the grouping of biological data and analysis of their distribution and canonical correspondence analysis (CCA) for the evaluation of some parameters and their relation to the community of benthic macroinvertebrates. The Non Metric Multidimensional Scaling (NMDS) was also performed. Statistical analyzes of PCA, CCA and NMDS were performed using the PCORD 5.31 software. All the biological data were also compared to Biological Monitoring Working Party' (BMWP') index; which is used in the state of Paraná/Brazil to classify the water resources.

The determination of the metals Cu, Zn, Fe, Mn, Cd, Pb and Cr in sediments and benthic macroinvertebrates was performed by means of atomic absorption spectrometry of flame atomic absorption spectrometry (Welz and Sperling 2008), although, before the determination both samples (sediments and macroinvertebrates) were submitted to nitroperchloric digestion (AOAC 2005).

RESULTS AND DISCUSSION

The samples taken in the Toledo River were carried out in the months of September, October and December/2015 and March, May and June/2016, totaling six sampling of biological material and with 7,797 macroinvertebrates. Of these, 7,751 macroinvertebrates were identified according to order and taxonomic family, and 246 according to the taxonomic class.

Nine orders and 24 families (Table 2), as well as four classes were identified (Table 3). The most frequent order was Diptera, representing 92.52% of all sampled organisms, and the Chironomidae family was the most frequent (83.01%), followed by the Ceratopogonidae family (8.79%). The Chironomidae family, the most representative, is characterized by its adaptation to highly impacted environments and consequently these organisms have physiological mechanisms to survive in environments with low dissolved oxygen (DO). In this way, they usually exhibit a high number of individuals when evaluated an urban river; which is the case of Toledo River (Hepp and Restello 2007; Sá et al. 2010).

Table 2. Benthic macroinvertebrates sampled at the Toledo River - Toledo/PR/Brazil classified in order and taxonomic family with the values of attribution of the BMWP' index and their classification by trophic group (T.G.)

Toledo River					
Order	**Family**	**Abundance**	**%**	**BMWP'**	**T.G.**
Coleoptera	Elmidae	13	0.17	6	CC
	Hydrophilidae	5	0.06	3	P
Crustacea	Aeglidae	13	0.17	5	F
Diptera	Ceratopogonidae	685	8.79	4	P
	Chironomidae	6472	83.01	2	CF
	Dolichopododidae	26	0.33	4	P
	Empididae	3	0.04	4	P
	Muscidae	5	0.06	-	P
	Psychodidae	2	0.03	4	CC
	Simuliidae	9	0.12	5	CF
	Tipulidae	11	0.14	5	P
Ephemeroptera	Baetidae	9	0.12	5	CC
	Leptophlebiidae	5	0.06	10	CC
	Oligoneuridae	2	0.03	6	CF
Gordioidea	Chordodidae	48	0.62	-	S
Hemiptera	Hebridae	1	0.01	-	P
	Naucoridae	1	0.01	3	P
Megaloptera	Corydalidae	5	0.06	4	P
Odonata	Coenagrionidae	27	0.35	6	P
	Gomphidae	31	0.4	8	P
	Libellulidae	21	0.27	8	P
Trichoptera	Hydropsychidae	25	0.32	5	CF
	Glossosomatidae	101	1.3	8	S
	Hydrobiosidae	31	0.4	7	CF
Total		**7551**	**96.84**	**112**	

Note: T.G. = trophic group, P = predator, F = filtrator, CC = collector/colletor, CF = collector/filtrator and S = scraper.

Table 3. Benthic macroinvertebrates sampled at the Toledo River - Toledo/PR/Brazil classified according to the class and the values of the BMWP '

Toledo River			
Class	**Abundance**	**%**	**BMWP'**
Bivalvia	65	0.83	6
Gastropoda	13	0.17	-
Hirudinea	167	2.14	-
Oligochaeta	1	0.01	1
Total	**246**	**3.16**	**7**

According to Silveira (2004) the way organisms feed and their own feeding is directly related to the possible impacts suffered by water bodies. In this aspect, the Chironomidae family is the most observed family in this study, belonging to the filtration functional group, which is responsible by filtering the materials and water. Callisto et al. (2001) also observed that the most abundant order was Diptera, with the family Chironomidae, followed by Ceratopogonidae, representing more than 80% of the total sampled in streams with urban characteristics.

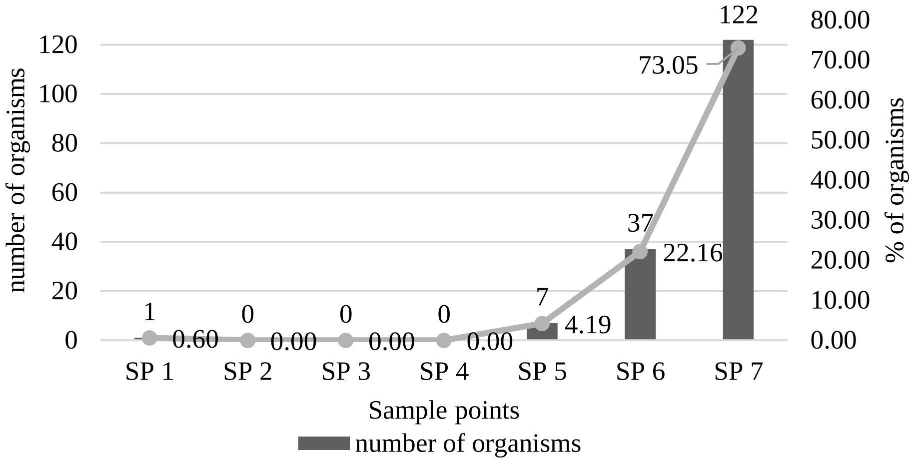
The second most abundant order was Trichoptera, exhibiting 2.02%. Galdean, Callisto and Barbosa (2001) also observed that the second most common order was Trichoptera, which, according to the authors, can be attributed to the capacity of organisms of this order to inhabit different places and to eat different forms of food; which is directly related to the water body characteristics. In this sense, the Toledo River, located mainly in urban areas and because it receives high organic loads (Table 1), exhibit high nutrient rates, which assists in the development of organisms with characteristics of collectors/filters.

The highest taxonomic class was Hirudinea (2.14%). The organisms of this class were found mostly at points SP 6 and SP 7, as shown in Figure 2.

In a study by Colpo, Brasil and Camargo (2009), Hirudinea class was found in sites with low DO rates, consequently these areas are intensely urbanized and receive industrial and domestic effluents. In this way, the Hirudinea class is characterized by to inhabit impacted. Also, according to the continuous Rio theory established by Vannote et al. (1980), the pollutant loads as well as the impacts suffered by the water body tend to be increasing in the nascent direction to the mouth. All these characteristics were predominantly observed in the sample point sites such as SP 6 and SP 7.

The points SP 6 and SP 7, besides having their own characteristics of impacts such as urbanization and industrial activities in their vicinity, also features of cumulative effect of the Toledo River. Because before the watercourse reach these points it receives all the other influences suffered by the points prior to these, which justifies and intensifies the results found in these two points (Strieder et al. 2006).

Figure 2. Number of the organisms from Hirudinea class identified at the seven sample points at Toledo River – Toledo/PR/Brazil



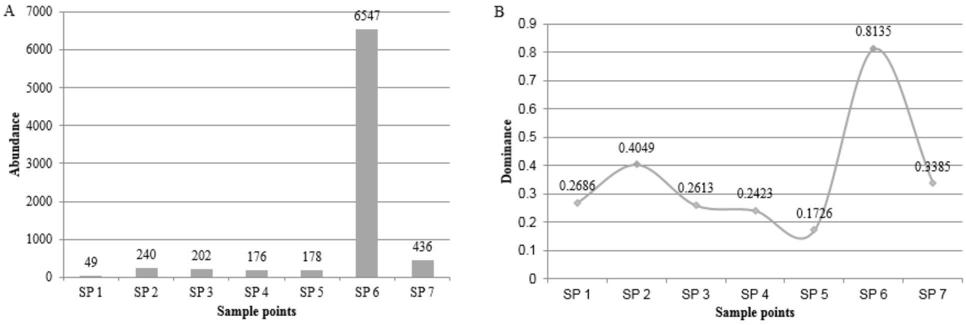
When the richness of individuals is observed, it can be stated that the wealth found in SP 1 (rural) (16.00) is higher than the remaining points sampled in urban environment (13.83). The point sampled in the rural area (SP 1) has well conserved margins and no urban interference; on the contrary, the points characterized as urban receive numerous organic loads during the course of the water body, have poor conservation margins, and as cited before, most direct contact with urbanization. Likewise, Hepp and Restello (2007), obtained higher values of richness in points near the source of the water body.

As shown in Figure 3A, there was a greater abundance of individuals at SP 6. According to Hepp and Restello (2007), in areas with very intense urban characteristics, such as SP 6 and SP 7, there is an abundance of organisms, however, low levels of richness are evident. In this sense, sites with high pollution rates tend to exhibit low richness and high density of individuals belonging to more tolerant groups. In this way, the SP 6 is located after a domestic sewage treatment plant and a sewage treatment plant of a large food industry, which possibly alters and favors the development conditions of certain organisms, such as the presence of tolerant organisms such as those of the order Diptera, family of Chironomidae and Ceratopogonidae and those of the class Hirudinea.

The dominance shows that SP 6 also exhibit greater dominance when compared to the other sampling points (Figure 3B).

Koning et al. (2008) observed that sites with higher urban influences showed alterations in the diversity and richness of aquatic organisms, as can be seen in points SP 6 and SP 7. When SP 6 and SP 7 are compared, SP 6 exhibit higher abundance (Figure 3A) and dominance (Figure 3B). This occurs possibly due to the characterization of the SP 6, while the point SP 7 receiving all the cumulative load of the river and located after the point SP 6, shown characteristics of self-purification.

Figure 3. A – Abundance of the benthic macroinvertebrates sampled at the Toledo River – Toledo/PR/Brazil in the seven sampling points; B – Dominance of the benthic macroinvertebrates sampled at the Toledo River – Toledo/PR/Brazil in seven sampling points



The diversity of Shannon_H exhibited greater diversity in SP 5 (2.284) and lower diversity in SP 6 (0.354), Figure 4A. In addition, it is possible to verify that there was not a high variation between the other points and then it is possible to consider that the number of individuals is evenly distributed among the different species in SP 1, SP 2, SP 3, SP 4, and SP 7. In a study conducted by Milesi et al. (2008), the authors observed that the diversity of Shannon_H varied between 0.022 and 3.266, and in the same way as in this study, the point with the lowest diversity was also the one with the greatest abundance and dominance of organisms, being represented by the Chironomidae family.

Equitability exhibited values ranging from 0.3226 (SP 6) to 0.7756 (SP 5) (Figure 4B). Colpo, Brasil and Camargo (2009) observed values of equitability between 0.1 and 0.6 (values considered low by the authors) reflecting the poor quality of the water body. According to Hepp and Restello (2007) the higher the values found for this parameter, the better the quality of the water. Thus, it is considered that SP 6 is the sampling point with the most compromised quality.

The percentage of Chironomidae (Table 4) demonstrates the representativeness of this family in relation to the total number of individuals in the sample per sampling point. It is observed that, in all sampled points family Chironomidae is found, and when considering the data from Table 1, Chironomidae family represents 83.01% of all sampled macroinvertebrates, totaling 6,472 individuals of the 7,797 macroinvertebrates. Mormul et al. (2009) found a high frequency of Chironomidae reaching 98% of the total sampled. The results found in the Toledo River for the SP 6 confirm the result found for dominance, low diversity and equitability.

The analysis of % ET is important because according to Biasi et al. (2010) these organisms are extremely sensitive to environmental interference, in this way, they are considered good indicators of water quality, and it exhibited 2.04% of abundance in the Toledo River. Barbola et al. (2011), in a study carried out to evaluate the water quality of the Pitangui River, also in the State of Paraná, found 3.4% ET, a value considered low by the author.

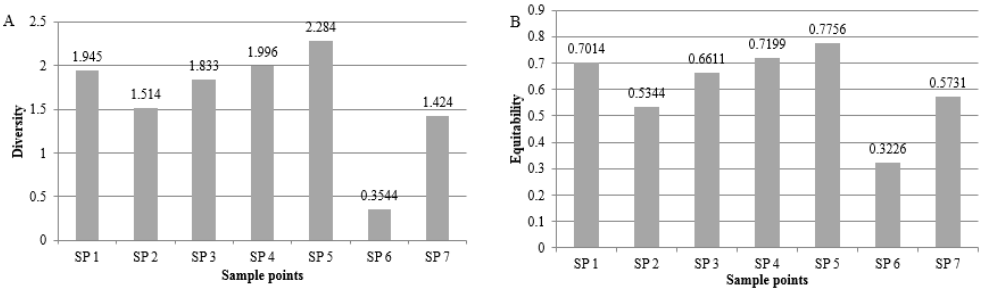
Thus, if it is compared the value found in this study, it can be stated that the Toledo River presents a low % ET, suggesting a poor water body quality.

Table 4. Percentage of Chironomidae Family sampled at Toledo River – Toledo/PR/Brazil per sampling point

Sampling point	% of Chironomidae	% of other families
SP 1	2.20	97.80
SP 2	61.80	38.20
SP 3	60.30	39.70
SP 4	52.60	47.40
SP 5	43.00	57.00
SP 6	90.20	9.80
SP 7	71.90	28.10

Note: SP 1: First sample point; SP 2: Second sample point; SP 3: Third sample point; SP 4: Fourth sample point; SP 5: Fifth sample point; SP 6: Sixth sample point; SP 7: Seventh sample point.

Figure 4. A – Diversity of Shannon_H of benthic macroinvertebrates sample at Toledo River – Toledo/PR/Brazil; B – Equitability of benthic macroinvertebrates sampled at Toledo River – Toledo/PR/Brazil;



When the values of the BMWP' index for water quality classification (Table 5) were analyzed, it can be observed that the Toledo River is in class III, presenting acceptable quality, with a total of 119 points.

Table 5. Values to water classification according to the BMWP' index

Class	Value	Classification of quality
I	>150	Great
II	121-150	Good
III	101-120	Acceptable
IV	60-100	Doubtful
V	36-60	Polluted
VI	16-35	Very polluted
VII	<16	Heavily polluted

Source: Adapted of Paraná (2015). Note: BMWP': Biological monitoring working party.

According to Paisley, Trigg and Walley (2014) the BMWP' index is based on the total of the values attributed to each group (order or class) of benthic macroinvertebrates, and it considers only the presence of a certain group, not the number of organisms in this group. This index is currently used by environmental agencies of the State of Paraná to evaluate water bodies; however, since it is a qualitative and non-quantitative index, it requires great attention, since it may not shows the real quality of a particular water body.

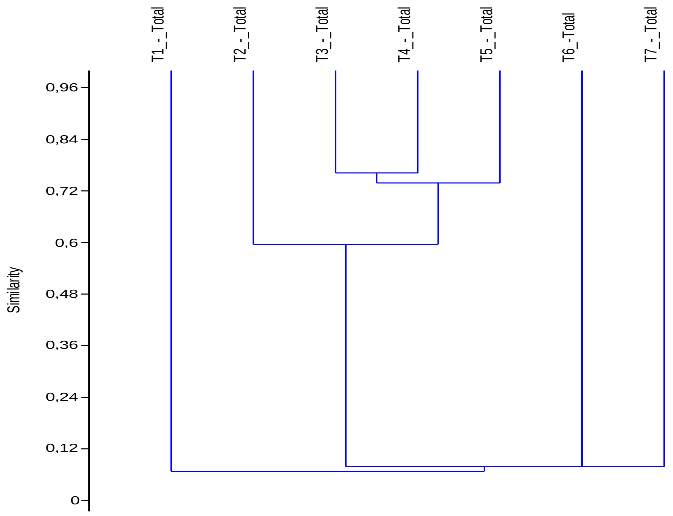
When comparing the points of the Toledo River under the NMDS analysis; which represents a proximity analysis between organisms with greater similarity between them, it is observed that points SP 2, SP 3, SP 4, SP 5 and SP 7 are more similar between them and points SP 1 and SP 6 show high differences between them.

These results possibly occur because SP 1 is located in an agricultural region with no urban interference, and SP 6 is located in an urban environment and receives all the cumulative effect of high nutrient loads, especially from point sources of pollution.

In a study by Strohschoen et al. (2009), authors showed that organisms tend to approach according to their habitat, regardless of the external factors that influenced them, however, in this study, the opposite was evidenced. In the Toledo River, although some families of organisms are sampled in several points, they are ordered according to the external interferences, independently of their trophic characteristics; which suggests the importance that the anthropogenic activities to the environment of the basin.

The analysis of hierarchical groupings through the similarity dendogram (Figure 5) confirmed the NMDS analysis data suggesting that the SP 4 and SP 5 are more similar to each other, and the SP 1 point exhibit a lower degree of similarity, possibly by the point characteristics (rural point). Another fact to be considered from the analysis of the dendogram is the similarity between points SP 6 and SP 7, justifying the cumulative effect condition, and the continuous river theory.

Figure 5. Dendogram of similarity among the sampling points in the watershed of Toledo River located in the city of Toledo/PR/Brazil by the similarity of Bray Curtis



Note: T1 represents SP 1; T2 represents SP 2; T3 represents SP 3; T4 represents SP 4; T5 represents SP 5; T6 represents SP 6; T7 represents SP 7.

The results obtained for the metals in the tissues of the benthic macroinvertebrates are exhibited in Table 6. Some samples did not reach the minimum weight to carry out the tests, in this way, only the results of the samples that had a weight equal to or greater than 0.1g were performed.

Table 6. Concentration average of metals in the benthic macroinvertebrates sampled in the sediments of Toledo River during the sampling period

Sampling point	Cd	Cr	Pb	Cu	Fe	Mn	Zn
	mg kg^{-1}						
SP 2	1.516	≤LQ	31.268	4.359	815.138	26.341	6.633
SP 3	3.031	≤LQ	203.790	3.038	1803.389	80.058	13.105
SP 4	2.856	≤LQ	156.181	2.185	1116.186	35.483	6.513
SP 5	3.699	≤LQ	240.438	5.277	1497.759	117.207	19.201
SP 6	1.449	≤LQ	68.261	12.579	3832.303	135.870	33.220
SP 7	0.549	≤LQ	31.394	2.976	1595.467	21.798	24.201

Note: LQ (limit of quantification) in mg kg^{-1}: Cd = 0.005; Cr = 0.01; Pb = 0.01; Cu = 0.005; Fe = 0.01; Mn = 0.01; Zn = 0.005.

When the values of metals found in benthic organisms are evaluated, it is possible to compare them with the results found in the sediment (Table 7), since it has a bioavailable fraction of these elements that can be incorporated by aquatic organisms, characterizing the bioaccumulation.

Table 7. Total concentration of metals in the sediments sampled at Toledo River during the sampling period

Sampling point	Cd	Cr	Pb	Cu	Fe	Mn	Zn
	mg kg^{-1}						
SP 2	≤0.005	≤0.01	1925.000	175.000	89652.700	430.000	170.000
SP 3	15.000	≤0.01	1123.000	140.000	71144.114	665.714	152.857
SP 4	5.000	≤0.01	1848.333	128.750	82806.825	913.750	186.250
SP 5	36.250	≤0.01	1783.750	102.500	68862.017	427.500	167.500
SP 6	27.500	≤0.01	2390.000	118.333	83138.667	446.667	218.333
SP 7	31.667	≤0.01	1727.500	185.000	83730.400	507.500	195.000

Note: LQ (limit of quantification) in mg kg^{-1}: Cd = 0.005; Cr = 0.01; Pb = 0.01; Cu = 0.005; Fe = 0.01; Mn = 0.01; Zn = 0.005.

In order to demonstrate the occurrence of bioaccumulation in the tissues of the benthic macroinvertebrates sampled in the Toledo River, the Pearson correlation was performed considering the toxic metals in the tissues of the benthic macroinvertebrates and the metals in sediments. The results showed that the SP 6 had moderate, medium and high correlation for some metals (Cd = 0.54, Cu = 0.78, Mn = 0.99 and Zn = 0.98). On the other hand, the SP 7 exhibited similar results to those found for Cd and Mn.

At points SP 6 and SP 7 the correlation was high, and in this sampling points the highest percentages found were for Diptera and Chironomidae family. These organisms are classified as filtering agents, and they develop and perform their activities in the sediments of water bodies (Silveira 2004; Silveira et al. 2006). The performed ecological functions of these organisms and the large sampled amount at SP 6 and SP 7 explains the high bioaccumulation of metals and nutrients.

Fe and Pb were the elements with the highest concentrations in the tissues of the benthic macroinvertebrates, reaching averages of 1929.88 mg kg^{-1} for Fe and 163.96 mg kg^{-1} for Pb. The toxic metal Pb it is not characterized as being part of the soil of the primary chemical composition of soils and river sediments in the region, suggesting that the metal is being introduced into the environment and incorporated by biota.

According to Nacke et al. (2013), Pb is a contaminant usually found in fertilizers and pesticides, widely used in agricultural areas, which significantly characterizes the region where the Toledo River is inserted, justifying these high levels.

Zn ranged from 5.688 to 59.061 mg kg^{-1}, considered to be high when compared to other studies, such as that carried out by Chiba, Passerini and Tundisi (2011), which cite high results for this element, indicate risks to the ecosystem due to its high toxicity.

The only element that did not exhibited concentrations in the living tissues and in the sediments was Cr. For all other evaluated metals (Cd, Fe, Mn, Pb and Zn), all results were higher than the quantification limits, exhibiting higher values than those found in water samples.

50% of the Cd evaluated concentrations in macroinvertebrates are higher than the sediment concentration. Similarly, some concentrations of Cu, Fe, Mn and Zn, in sediments were higher than those from benthic macroinvertebrates, being preferentially accumulated in the solid fraction of the riverbed sediment.

According to Góes-Silva (2014), there is an intrinsic relationship between water, sediment and macroinvertebrates, because even if the water or sediment contain low concentrations of metals, these will be filtered by benthic macroinvertebrates occurring the bioaccumulation.

The canonical correspondence analysis showed that the points SP 6 and SP 2 received major influences by the metals concentration. The point SP 6 showed higher relations with the elements Mn, Cu and Zn, while SP 2 was more related to the elements Cd and Pb. In study conducted by Jesus et al. (2004), the authors identified highest presence of Cu, Mn and Zn at the sampling points which received the greatest contribution of domestic and industrial liquid effluents. In this sense, SP 6 had a direct relationship with these elements and is characterized by the receipt of industrial and domestic effluents, besides receiving the entire urban load received by all the route of the Toledo River.

The SP 2 point is located at the beginning of the urban area of the municipality of Toledo and at the end of direct contact with the agricultural area. Given this characterization, it is probable that the relationship with the metals Cd and Pb originates from the contamination by pesticides and fertilizers in the agricultural area. According to Gonçalves Jr. et al. (2014), regions that are subject to intensive cultivation for long periods, tend to exhibit higher levels of toxic metals, mainly by the use of soil and fertilizer correctives that have considerable doses of Cd and Pb in their compositions. Some macroinvertebrates, due to their functional group, tend to exhibit a higher level of bioaccumulation, such as the organisms of the filtering group, among them the Bivalves and those of the order Diptera, of the family Chironomidae (Bergonci and Thome 2005). In this research, the family with the highest representability is Chironomidae and Bivalvia. Repula et al. (2012), cite that there should be great concern when it comes to Cd, Cr and

Pb, because in addition to being toxic, they are bioaccumulated by aquatic flora and fauna, translocating at different trophic levels.

CONCLUSION

In view of the toxicological analyzes carried out in the Toledo River, it is possible to verify that there is concentration of toxic metals in the tissues of benthic macroinvertebrates. At point SP 2, for example, the average levels of Cd exceed the average sediment concentrations, which indicates the bioaccumulation of metals by these organisms.

From the biological results obtained through biological and statistical metrics, the activities carried out around its course, such as agricultural pollution sources and urban influences; allow a process of cumulative effect on its environmental quality, considerably influencing the Toledo River.

From the perspective of the BMWP' index, the Toledo River exhibit itself as acceptable, which suggests the need to update the tools of evaluation and classification of water resources in the state of Paraná, considering that the other results show that the quality of the Rio Toledo is terrible.

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