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Forensic Evaluation of Metals (Cr, Cu, Pb, Zn), Isotopes ($\delta^{13}C$ and $\delta^{15}N$), and C:N Ratios in Freshwater Sediment

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A forensic approach was used to evaluate sediments from Portão Stream, including analysis of metals, carbon (C) and nitrogen (N) stable isotopes, and C:N ratios. Samples collected at various points located along the stream were tested in order to investigate a possible illegal leachate input. The studied stream is heavily impacted by sewage and industrial discharges from two cities along its course. Among the metals analyzed, chromium (Cr) was noticeably the main pollutant, showing the highest levels, above regulatory limits, downstream from some potential sources of effluents enriched with this metal. Isotope analyses revealed a general trend of depletion in the heavier isotope along the stream for C and N. The exception was one point near a hazardous waste landfill, where relatively more enriched $\delta^{13}C$ and $\delta^{15}N$ values were found. The isotope and metal analysis results indicated that this site was affected by a particular source, demonstrating the combination of these parameters could be used for the discrimination of sources in a heavily polluted stream. Nevertheless, further investigations are necessary to provide a comprehensive evaluation of the biogeochemical processes involved in the incorporation of leachate in sediments to use this analysis as evidence for the illegal leachate discharge.

Keywords: sediment, metals, C and N isotopes, C:N ratio

Introduction

The degradation of environmental quality associated with industrial activities is a matter of great concern in the definition of public policies on human and environmental health, especially in densely industrialized regions as the Sinos River Watershed, located in southern Brazil. The cities of Estância Velha and Portão, located in the lower reaches of this watershed, are sources of environmental impact on watercourses, more specifically at Portão Stream, whose headwaters are located within the boundaries of the city of Estância Velha. This watercourse runs through the urban area of Estância Velha and subsequently passes through the urban area of Portão, receiving discharges of domestic sewage and industrial effluents from both cities before the end of its course on the left bank of the Sinos River,

20 km downstream. The contributions received along the passage through both cities pollute its waters at such levels that threaten the aquatic biota diversity and determine the use of the stream only for the dilution of effluents from anthropogenic activities. The water pollution reaches such high levels that it has an impact on the water quality of the Sinos River. This contributes to the occurrence of episodes of fish kills, as the emblematic event occurred in October 2006, with the death of 86 tons of fish reported by the Municipal Environmental Agency of Sapucaia do Sul, RS, Brazil, responsible for the cleanup of the decomposing fish mass. In this episode of massive fish deaths, the competent Prosecutor brought criminal charges against several facilities that have been identified as having released effluents contrary to the State Environmental Agency regulations, the **Environmental Protection Foundation (Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler [FEPAM], 2005).** Among these being a landfill of hazardous industrial waste. The manager of this landfill was sentenced with one of the largest penalties for defendants in environmental crimes in the country, stipulated in 30 years of reclusion.

Although the aforementioned massive fish deaths episode has probably been the most extreme, since 2002 other fish deaths

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were recorded by the State Environmental Agency, the FEPAM that has systematically monitored the water quality of the Sinos River since then. The report issued by FEPAM (2005) demonstrates that the segment of the Sinos River more susceptible to occurrence of fish deaths was actually the surroundings of the mouth of the Arroio Portão. A more recent report of metal analyses, which included cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn), indicates that Cr, Cu, and Pb were most frequently found above the levels set out in the regulation at the sampling point located near the mouth of the Arroio Portão (FEPAM, 2011). Monitoring of water and sediment quality of Arroio Portão is held by the environmental agencies of the two cities in segments belonging to their respective geographical limits. However, to distribute the responsibilities for the stream pollution it is necessary to evaluate the concentrations of metals in sediments, since this compartment concentrates metals in the aquatic environment (Axtmann and Luoma, 1991). Salomons and Förstner (1984) pointed out that the sediments are particularly useful in the identification, monitoring and control of pollution sources. Several studies have reported high concentrations of metal in freshwater sediments caused by industrial sources (Ramamoorthy and Rust, 1978; Rule, 1986; Singh et al., 1997) as well as domestic sewage (Rubin, 1976; Ntekim et al., 1993).

The development of isotope geochemistry has led to significant advances in the determination of pollution sources on contaminated sites (Philp, 2006) providing information for the discrimination of pollution from domestic sewage and industrial discharge through the study of ammonia N isotope ratios (δ^{15}N-NH$_4$) (Lindau et al., 1989). The determination of stable isotope ratios is used for resolution of cases involving environmental contamination by various sources in other countries, mainly in the United States, as described by Mancini et al. (2008).

The leachate produced in solid waste landfills is a source of groundwater and surface water contamination (Baker, 2005) and it has been reported that small amounts of leachate could pollute large volumes of groundwater (Bakare et al., 2000; Baderna et al., 2011; Kjeldsen et al., 2002). The consequence of the isotope fractionation processes that occur in the decomposition of organic waste is a distinctive isotopic signature of C from landfill leachate that can be used to identify leachate contamination in groundwater (Baedecker & Back, 1979). North et al. (2006) have utilized, besides the C isotopes in dissolved inorganic C (δ^{13}C-$_{DIC}$), ammonia N isotope ratios (δ^{15}N-NH$_4$) to detect the presence of leachate in watercourses associated with landfills concluding that isotopic measurements have the potential to be used as a tracer of leachate in surface waters.

In this study, a preliminary criminal environmental forensics exams procedure was performed to investigate a possible environmental crime characterized by the illegal discharge of leachate into a stream. This approach included the collection and evaluation of preexisting data on metal determination in sediments at points distributed along a heavily polluted watercourse and analyses of selected group of metals, C and N isotopes and

C:N ratio, which were discussed taking into account the sources of pollution.

Study Area

The study area encompasses approximately 10 km of Portão Stream, along which seven sampling sites were located (Figure 1). One of these sites (Point 4) was located downstream some leachate outfalls of a landfill that was criminally charged for releasing untreated leachate directly into Portão Stream. The untreated effluent contained both particulate and dissolved phase contaminants.

Portão Stream is a watercourse tributary of the Sinos River's right margin and drains an area located in the western portion of the Sinos River Watershed. The study area is located in the lower part of the watershed of the Sinos River, which is densely populated and industrialized, and the most critical segment in terms of degradation due to contamination by industrial effluents is located in the surroundings of the mouth of the Portão Stream (FEPAM, 2011). The geological substrate of the studied area is predominantly sandstone belonging to the Pirambóia Formation of the São Bento group (Oliveira et al., 2008, Machado and Freitas, 2005). The major mineral constituent is quartz and the predominant grain size in sediments is sand (Robaina et al., 2002).

Materials and Methods

This study approach consisted of an initial evaluation of preexisting data from sediment monitoring analyses conducted by the local environmental agencies to assess possible trends of metal distribution in the sediments. The evidence-gathering procedures included a single sampling campaign due to the goals and resource limitations of a criminal investigation procedure. C and N isotope and C:N ratio analyses were also performed in sediment samples to search for evidence of leachate discharge on the stream which could be defined as an environmental crime by Brazilian legislation in this situation. The goal of the inclusion of a different set of parameters was the addition of redundancy to obtain more reliability on the evidence gathered.

Sampling Sites

Sampling points were recorded referenced to Datum SAD69, in UTM Zone 22J, using a Garmin global positioning system (GPS) receiver unit, Model Etrex Summit HC (Garmin International, Inc., Olathe, KS), with estimated position error of less than 5 meters. Quantum GIS program, Version 1.5.0, Thetys (Quantum GIS Development Team, 2010) was used to the geoprocessing of spatial data and preparation of maps.

Seven sediment-sampling sites were defined along Portão Stream course in accordance with monitoring sites of the respective municipal environmental agencies in order to evaluate the historical levels of metals in those sites. The spatial distribution of the sampling sites is shown in Figure 1. Point 1 is situated
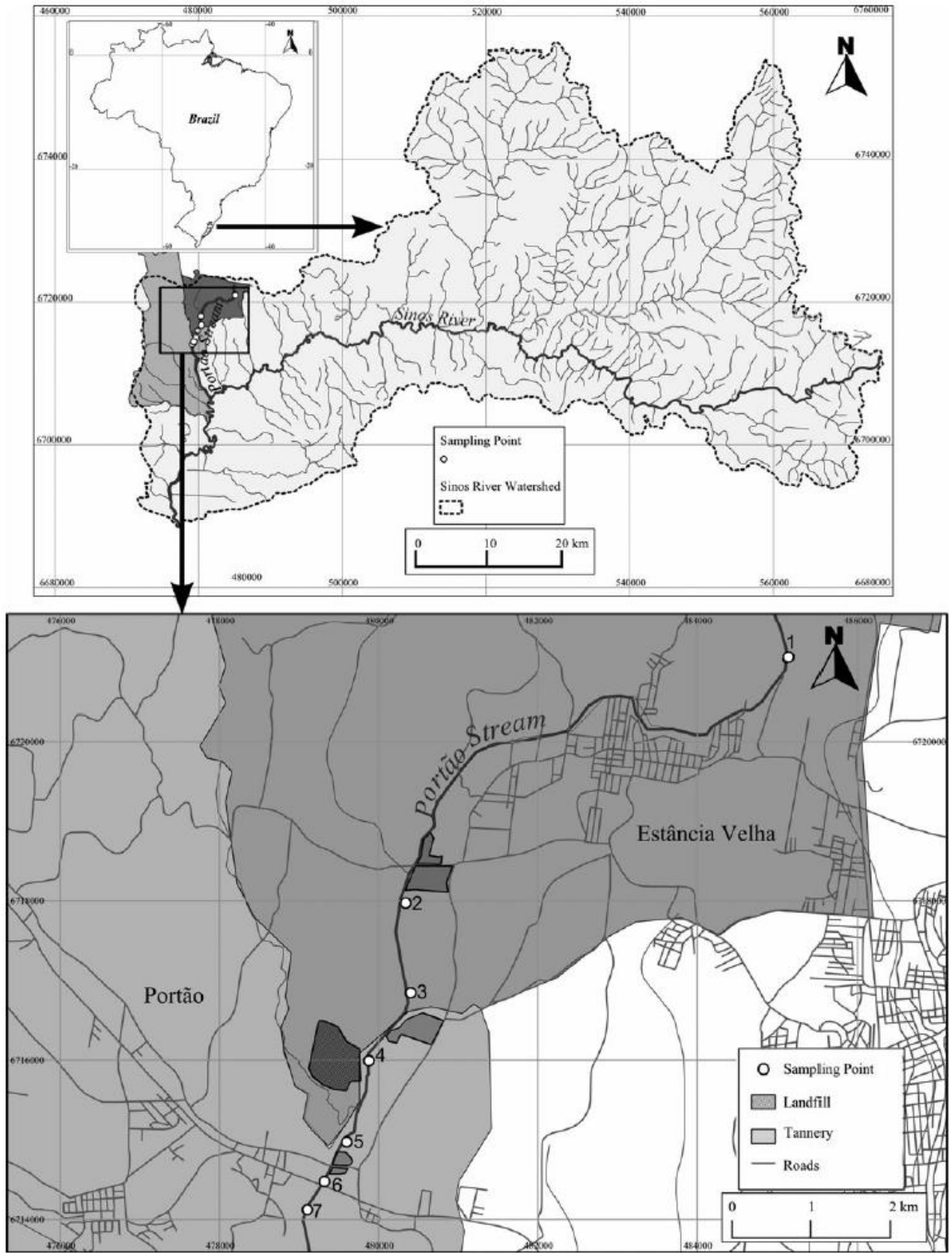
Figure 1. Map of the study area location and spatial distribution of sampling points and major point pollution sources (Datum SAD69, UTM Zone 22J).

close to headwaters of the stream. Nearby this site, there are no significant sources of pollution; therefore, this point was admitted as reference point to background levels. The Points 2 to 7 are distributed downstream of the watercourse segment where there are major sources of pollution.

Sediment Pollution Monitoring Data

Metal analyses on sediment were performed regularly in the pollution monitoring programs of the cities of Estância Velha and Portão. Data obtained from these analyses, were compiled in order to assess metal contamination on the stream in a broader period of time. Cr, Cu, Pb, and Zn analyses data were used in this work. These four elements were selected because they were the most often found in concentrations above the guidelines on the segment of Sinos River impacted by Portão Stream mouth, according to the results of long time monitoring of Sinos River's waters performed by the Environment Protection Agency (FEPAM, 2011). These analyses were carried out by the Laboratory of Leather Technology of SENAI (National Service of Industrial Training). The results evaluated were obtained from superficial sediment samples collected in four sampling campaigns conducted in 2008, two in 2009 and the other two in 2010. Point 7 was included in the 2009 campaign. The sediments were digested with H_2SO_4 and HNO_3 in open system at temperature below 60°C. Quantification of metals was done by flame atomic absorption spectrometry (SM 3111 B 2005), and graphite furnace (SM 3113 B 2005) (APHA, 2005).

Descriptive and Statistical Treatment

Means, maximum values, minimum values, and relative standard deviations of the four heavy metals were calculated based on eight data sets for each site, except for site 7 where there were only four data sets obtained from the environmental quality monitoring of Portão Stream sediments. These results were plotted in a Box-plot chart using the PAST Program, version 2.04 (Hammer et al., 2001). These data were also compared with the values established in the Brazilian legislation, the Resolution Conselho Nacional de Meio Ambiente [CONAMA] No. 344/2004 (CONAMA 2004), and a principal components analysis (PCA) was performed based on a correlation matrix with the mean values, also in the PAST package.

Sampling

Sediment samples were collected using a hollow cylinder of polyvinyl chloride with 100 mm in diameter and 1.2 m in length. This cylinder was immersed vertically in the water and pressed after contact with background material, and then, the lid was inserted on its top in order to keep the sample inside the core. Samples were collected in each of the seven points of sampling in areas of deposition near the bank of the stream. Each sample was stored in an identified plastic bag, sealed and refrigerated until analysis.

Analyses

Prior to analysis, the samples were placed in an oven at ~60°C until the mass was stabilized. After cooling, the samples were sieved with plastic sieve to break clusters and remove debris.

Grain size analyses. Grain size analysis was performed by dry sieving method adapted from Suguio (1973) and Abessa (2005). Approximately 30 g of dry sediment were weighed and transferred to the sieve positioned on top of a series of Went-Worth graded sifters, stacked in descending order of size. The stacked column of sieves was maintained for a period of 15 minutes in an automatic sieve shaker. The mass of sediment retained by each sieve was weighed and the fraction <63 μm was calculated.

Metals. Sediment samples were analyzed for Cr, Cu, Pb, and Zn concentrations in the Laboratory of Chemical and Isotopic Characterization (CQMA)/IPEN/USP. The dry sediment was ground with a mortar and pestle of agate and a 0.5-g portion of the homogenized sample was digested using a microwave-accelerated reaction system, Model MARS 5 (CEM Corporation, Matthews, NC, USA). The acid extraction solution consisted of a mixture containing 9 mL of HNO_3 and 3 mL of HCl sub-boiling, according to recommendations of 3051A USEPA method. This mixture was added to the portion of sediment's sample and/or certified reference material on microwave tubes HP-500 (closed-system vessels HP-500, CEM Corporation; Teflon, PFA fluorocarbon polymer), which were properly sealed and heated in the microwave.

The digestion was performed according to the following parameters: power of 600 W, ramp time of 9 min, temperature of 175°C and hold time of 4.5 min. After cooling, the contents of the tube were transferred to a 50 mL centrifuge vial and completed up to 40 g with water (Milli-Q) with a resistivity of 18 MΩ. cm^{-1} at 25°C. Metal analysis was performed after decanting or centrifuging the residues of the vial. The 3051A method is not intended to perform the total decomposition of the sample, so the concentration of analyte extracted may not reflect the total content of the sample. Metal concentrations were measured using flame atomic absorption spectrometer (FS-FAAS, Varian, model SpectrAA 220-FS, Varian, Australia).

All glassware was cleaned in 10% HNO_3 (w/v) prior to each experiment. Chemicals used for digestion and extraction experiments were analytical reagent grades. The validation of this method was performed by analyzing certificate reference sediments (SRM 2704 Buffalo River Sediment) in three replications.

Carbon and nitrogen. Aliquots of dry sediment samples were sent to the Laboratory of Isotopic Ecology of the CENA/USP, Piracicaba, SP, Brazil where the total C (TC) and N were determined using an NA 1500 CNS Elemental Analyzer Carlo Erba (Thermo Fisher Scientific Inc., Waltham, MA, USA), burning sediment samples in tin capsule with an oxidant (V_2O_5) at 1000°C in a stream of oxygen. The C to N atoms ratio (C:N) can be used to identify the source of organic matter (Cravotta III, 1997; Andrews et al., 1998).

Carbon and nitrogen isotope ratio. The isotopic compositions of total C ($\delta^{13}C$) and N ($\delta^{15}N$) in sediments were determined in samples by on-line automated combustion coupled to mass spectrometer Finnigan MAT Delta-S (Thermo Fisher Scientific Inc.). The $^{13}C/^{12}C$ ratio is reported in relation to the VPDB standard C dioxide from calcium carbonate from the Pee Dee Belemnite formation, by convention, in $\delta^{13}C$ units per mil (‰). The precision of this analysis is 0.3‰. The $^{15}N/^{14}N$ ratio is reported in relation to the atmospheric air in $\delta^{15}N$ units per mil (‰) and the precision of this analysis is 0.5‰.

Descriptive and statistical treatment. The concentrations of metals, fine grain percent (<63 μm), and concentration and isotopic ratios of C and N were compared in a correlation matrix to evaluate the degree to which these variables correlate. The correlation analysis was performed with the data matrix, using the Pearson coefficient at a significance level of $\alpha = 0.05$. This analysis was performed using the software BIOESTAT version 5.0.21 (Ayres et al., 2007). A PCA was performed with metal concentrations, isotopic ratios of C and N and C to N ratio using the PAST package.

Results

Sediment Pollution Monitoring Analyses

Sediment analyses carried out by environmental agencies in the municipalities of Portão and Estância Velha, through which Portão Stream flows, which included Cr, Cu, Pb, and Zn in the period from 2008 to 2010 showed high variability in the concentrations of metals in this environmental compartment among
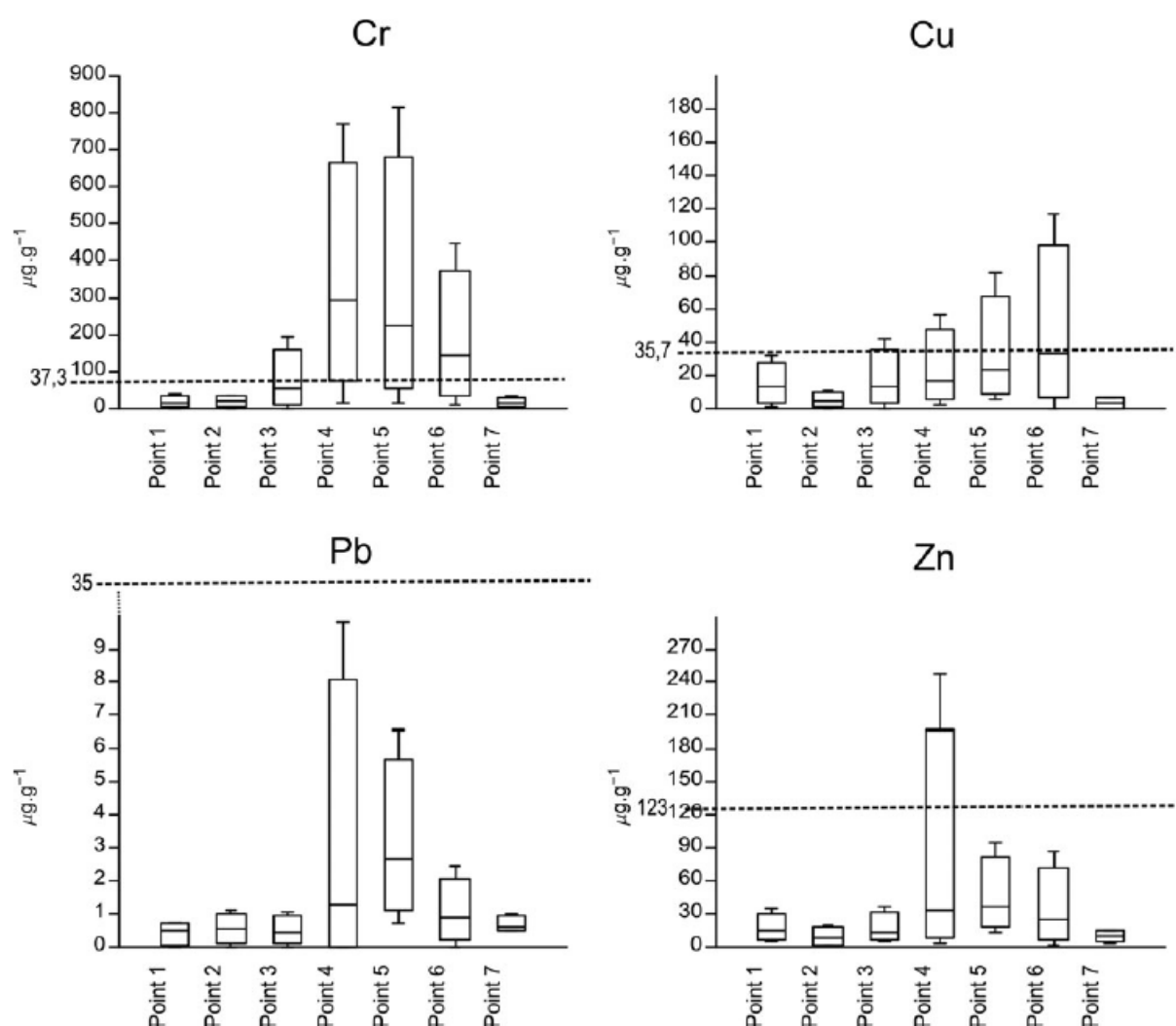

Figure 2. Graph of the concentration ranges and mean values of chromium (Cr), copper (Cu), lead (Pb), and zinc (Zn) in the sampling points. The horizontal dashed lines indicate the limits established by CONAMA Resolution No. 344/04. The limit for lead is 35 μg.g^{-1}; thus, it is not represented, because it is out of the range of the chart since it is far superior to the levels found in the analyses.

sampling sites as well as over time. Also, the results did not reveal any increasing or decreasing trends of metal concentrations in sediments in the time period comprised in this study. The Points 4 to 6 exhibited the highest concentrations of the metals tested (Figure 2), and Point 4 presented the highest mean concentrations for metals Cr, Pb, and Zn. The highest mean concentration of Cu (Cu) was found in Point 6, followed by Point 5. The mean and maximum concentrations of Cu in sediment increased gradually from Point 4 to 6, and, in Point 7, were lower than those found at Point 1, that was the closest to the headwaters.

Concerning potential pollution sources, Points 4, 5, and 6 are within the area of influence of large-scale tanneries and a hazardous waste landfill (Figure 1). Point 4 is the nearest to the landfill drainage systems exits into the stream. Points 2 and 3 are located downstream of the Estância Velha urban area, source of sewage and industrial, including tanneries, effluents discharged to the studied watercourse. Point 2 is located approximately 500 m downstream of two tanneries and Point 3 1.2 km downstream of Point 2. Between Points 2 and 3 there are no potentially polluting activities on the margins of the watercourse.

The concentrations of metals in sediments collected in the sampling points were compared to the limits established by the national environmental council (CONAMA) through its Resolution No. 344/2004 (CONAMA, 2004). This resolution sets limits of contaminants in sediments for dredging purposes of beds of watercourses and provide guidelines for dredged sediment analysis with the basic objective to avoid contamination in the area of disposal. The limits set by this regulation for the metals studied are based on Sediment Quality Guidelines-SQG issued by the Canadian Council of Ministers of the Environment [CCME]). The concentrations of metals in sediments were compared with the limits established by the Level 1, for fresh water, of that Resolution, since this is the only national guideline that refers to sediment contamination. Cr and Cu concentrations were above the regulatory levels in Points 3 to 6, and Zn in Point 4. Comparing the mean values with regulatory boundaries, it is seen that only Cr exceeds this limit in Points 3 to 6 (Figure 2). Cr values surpasses even the limit set for Level 2, for fresh waters of the mentioned resolution, which is of 90 μg.g^{-1} in mean values found for Points 4 to 6 and in maximum values determined in Points 3 to 6.

The PCA, multivariate statistical method, which reduces the number of potentially correlated variables to a smaller number of vectors that can be plotted in two dimensions, is mentioned by Mudge (2009) as a way to investigate sources of environmental contamination. The author states that this technique may be used to demonstrate which chemicals have similar behaviors in a set of samples and, thus, could be associated with a source. PCA was used in this study to distinguish sampling points according to their constituent concentrations. The PCA scatter diagram in Figure 3 shows that Point 4 loaded positively on PC1, followed by Points 5 and 6. As PC1 is the major factor of these data, corresponding to 77% of the total variance, this means that it has the most influence in the data. PC1's variance is explained

mostly by the variables Cr and Zn, therefore these metals are responsible for the overall distribution of the points along the x axis and for the differences between points. Point 4 is located in the site where higher concentrations of Cr and Zn were found in the sediments and it was located downstream a tannery and close to the landfill of hazardous waste that is filled mostly with wet-blue leather solid waste, which has high Cr content. Tariq et al. (2006) also found Cr, Pb and Zn correlated with releases of effluents from tanneries, and a strong association of Cr with other metals, indicating that this shared a common origin with them.

PC2 (20% of the total variance), on the other hand, is explained predominantly by the variable Cu, and behaves oppositely to PC1. In this axis, Point 6 loaded most positively, followed by Point 1. This indicates that Cu sources are probably not associated to Cr and Zn and thus, do not share a common origin with them.

Points 2 and 7 plotted almost overlapping because they exhibited high similarity of the mean levels determined for all metals analyzed. Point 2 is located downstream two tanneries, but the mean levels of contaminants found in sediment samples collected in this site were similar to Point 7 that is farther from pollution sources. This could be explained by efficient effluent treating systems of these plants or by the flow and sediment deposition dynamics of this particular site.

Metal and Grain Size Analyses

The reference materials' analyses are presented in Table 1. The mean recovery of sediment samples of reference material for metals determined during the validation process was 91,6%. Recoveries for Cr were the lowest obtained (58.01%). This is in accordance with the results obtained by Rao and Formoso (2006) who reported similar values on a study of sequential extraction of metals in sediments. This could be explained because the reference material is approved for the total extraction of metals. Cr is known as a refractory element, strongly linked to silicates, which are hard to digest, which confirms the relatively low recovery of this metal. According to the method 3051A, silicates cannot be dissolved and in some cases can isolate target elements analyzed. The validation results obtained in the analysis of the reference material were considered satisfactory for the purposes of this work.

Table 1. Measured values, certified values, and recovery of reference material (Buffalo River Sediment)

Metal	Measured value[1]/ (μg kg-1)	Certified value[2]/ (μg kg-1)	Recovery/ %
Cr	78.32 ± 5.02	135 ± 5	58.01
Cu	95.98 ± 1.75	98.6 ± 5	97.34
Pb	161.19 ± 7.92	161 ± 17	100
Zn	487.90 ± 1.49	438 ± 12	111.39

[1]Mean ± SD
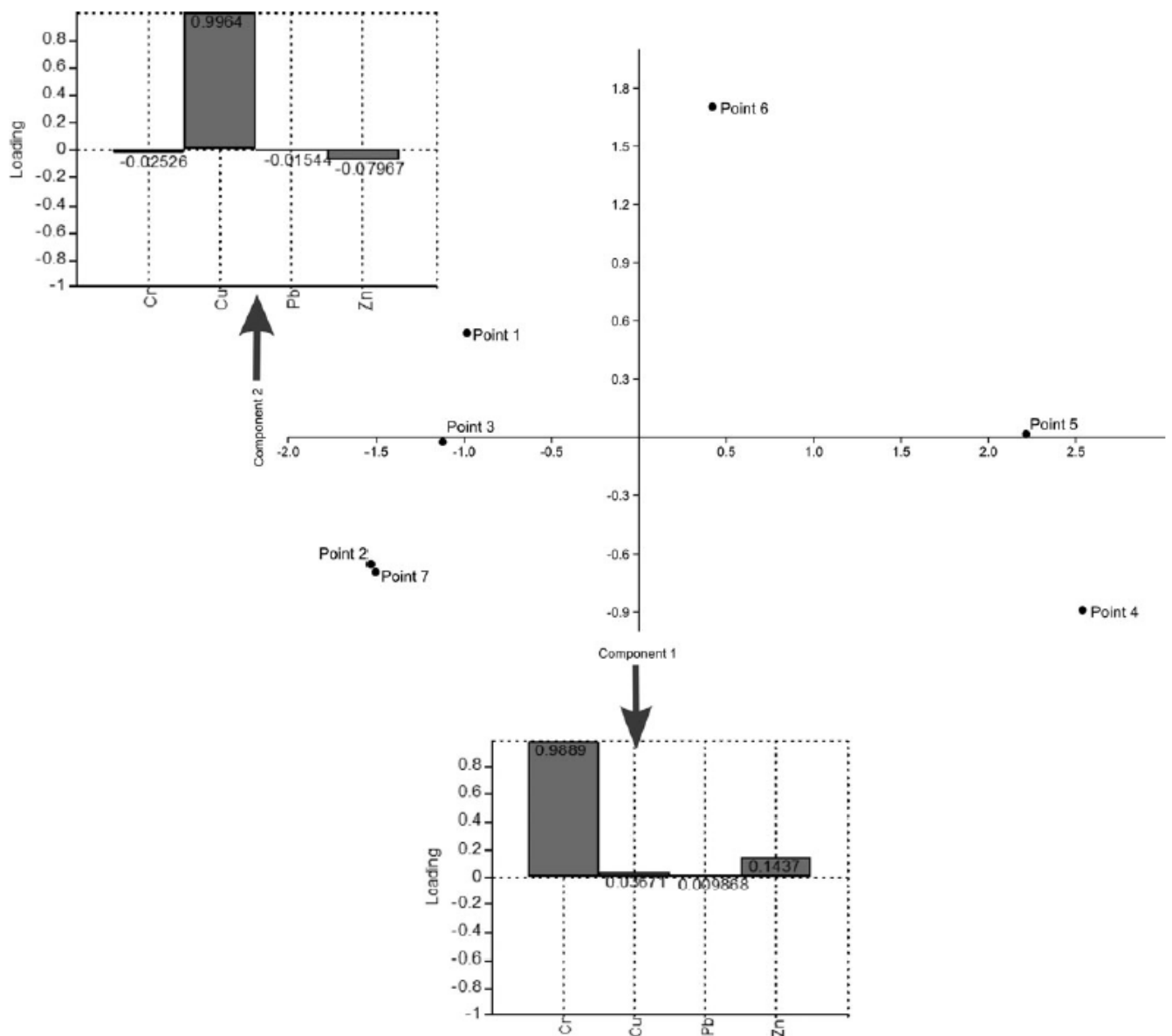[2]Mean ± 95% confidence limit.

Figure 3. Graph obtained from principal component analysis of metal determinations' mean values in the sampling Points 1–7, showing the contribution of variables for each component. PC1 explains 77% of the variance in the data and PC2 explains 20%.

The results obtained in the analysis of Cr, Cu, Pb, and Zn in sediment samples are shown in Table 2. Point 4, in accordance to historical data, showed the highest concentrations for all metals and very high concentrations of Cr. None of the metals analyzed showed an increase in their concentrations along the watercourse. Unlike historical data, relatively low concentrations of metals were found in Point 5, and high Cr and Zn concentrations were determined in Point 2. Since the analytical methodologies employed were not the same, the data sets cannot be compared in a more strict sense. Besides that, the geochemical spatial heterogeneity of stream sediments could lead to a type 2 error, which, in this case, would be to reject the pollution hypothesis, in a single sample analysis.

The results obtained in grain size analysis reveals a high predominance of sand in the grain size composition of sediments throughout the watercourse. The percentage of fines (particles with diameters below 63 μm) ranged from 0.7% in the sample collected in Point 2 up to 12.08% in Point 6. Although the geological substrate explains the predominance of the sand fraction in sediments of the Portão Stream, the low fraction of thins found could also be related to the method used for their estimation. Bordon et al. (2011) and Cesar et al. (2007) described low fraction of fine grain, using a similar method of grain size analysis. In sediments, higher concentrations of metals are found in the grain size fraction below 63 μm (Dalziel et al., 1993). There is a strong correlation between the decrease in grain size

Table 2. Metal concentration, percent fines, isotope ratios of C and N, percentage of C and N, and C/N ratio in the sediment samples collected in Points 1–7 and the geographical location in UTM coordinate system

Coordinate UTM[1]		Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Regulatory limits[2]
	x	485116mE	480327mE	480413mE	479940mE	479603mE	479322mE	479117mE	
	y	6721004mN	6718030mN	6716852mN	6716068mN	6714996mN	6714478mN	6714113mN	
Cr (μg/g)		30.400	437.610	85.850	953.890	61.350	22.460	12.150	37.3
Cu (μg/g)		12.790	10.100	14.680	17.870	10.790	9.290	10.370	35.7
Pb (μg/g)		5.560	1.010	7.500	21.680	7.260	10.230	8.290	35.0
Zn (μg/g)		19.420	43.100	32.740	50.870	26.480	17.310	35.050	123.0
% <63μm		4.010	0.690	3.000	11.900	3.840	12.850	10.600	—
δ^{15}N (‰)		7.060	3.750	4.130	6.050	4.000	3.880	3.320	—
N (%)		0.036	0.015	0.028	0.190	0.039	0.280	0.179	—
δ^{13}C (‰)		−22.150	−23.690	−24.320	−23.430	−24.850	−26.330	−27.920	—
C (%)		0.669	0.112	0.223	1.602	0.368	3.982	4.501	—
C/N		18.800	7.320	7.980	8.430	9.380	14.240	25.200	—

[1]Zone 22, Datum SAD69
[2]CONAMA (2004)

and the increasing concentrations of metals, as well as the presence of sand can serve as a diluent for finer particles rich in metals. According to Horowitz (1991), sediments with larger sand fractions, as seen in this study, may ultimately dilute significant concentrations of metals.

Considering the above mentioned, due to the effects of grain size in metal concentrations in sediment it is important to adjust the concentration of metals to the percentage of fines in order to determine the degree of enrichment derived from pollution and, also, to enable comparisons between sites with different grain size compositions, thus reducing the effects of dilution and the contribution of large particle size. A method proposed by the Department of Resources of Wisconsin (2003) is based on the assumption that the majority of the metals in a sample are associated with the fine fraction (silt + clay). The normalization of metal concentration is made by dividing the mass concentration by percent of fine fraction, expressed as a decimal fraction of mg kg^{-1} of fine metal. However, different metals exhibit variable degrees of association with the fine fraction and, when the fine fraction is below 50% of the total combined fractions, which occurred in all sampling points in this study, this type of standardization might not represent the actual concentration of metals (Horowitz, 1991).

The correlation between metals and fine grain percent can be seen in the correlation matrix (Table 3). The results reveal that only Pb exhibited a positive correlation with the fine sediments content. Significant positive correlations were observed between Cr and Zn in the data set. In tannery effluents, Tariq et al. (2006) found significant positive correlations for Cr, Zn, and Pb, which may indicate that even though these metals share a single origin, their dynamics in the environment can change the relationships that occur in the source itself.

No correlation of Cr concentrations and fine grain content of sediment has been found in this analyses, which is in accordance with other studies (Szalinska et al., 2010; Pawlikowski et al., 2006). Tariq et al. (2006) observed that the concentrations of metals in groundwater and soil samples showed non-Gaussian distributions with high variation around the average, a behavior similar to the findings of this study. In these analyses, the concentrations of most metals in sediments were below regulatory limits (CONAMA, 2004), except for Cr that showed concentrations significantly above these limits, particularly at Point 4.

Concentration and Isotopic Ratios of Carbon and Nitrogen

The results of the analyses of organic C isotope ratio (δ^{13}C ‰) show a gradient of depletion in the heavier isotope from the source to the mouth of the watercourse by observing a variation of −22.2 ‰ to −27.9 ‰ from Point 1 to Point 7. This trend is not maintained in Point 4, which displays a greater enrichment in the heavier isotope in relation to previous and subsequent points in terms of spatial distribution, as can be seen in Figure 4. This enrichment in the heavier isotope noted in Point 4, could be explained by the inputs of a source enriched in δ^{13}C in that region.

As sediments samples collected at Point 4 had higher contents of Cr and Zn, and this site was located downstream a tannery and close to the landfill of hazardous waste the hypothesis is that the observed increase in the δ^{13}C values is caused by these effluents. This suggestion is preliminary, and obviously other
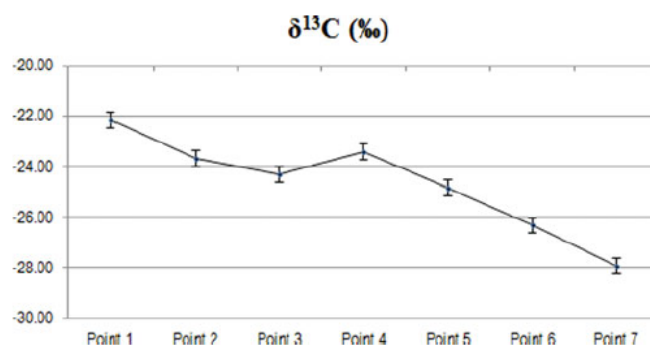
Figure 4. Graph of the δ^{13}C of organic carbon in sediment samples.

Table 3. Correlation matrix of metals, percent fines, isotope ratios of C and N, percent C and N, and C/N ratio in the sediment samples (n = 7)

	Cr μg/g	Cu μg/g	Pb μg/g	Zn μg/g	% < 63μm	δ^{15}N (‰)	N (%)	δ^{13}C (‰)	C (%)	C/N
Cr μg/g	1.000	—	—	—	—	—	—	—	—	—
Cu μg/g	0.681	1.000	—	—	—	—	—	—	—	—
Pb μg/g	0.639	0.707	1.000	—	—	—	—	—	—	—
Zn μg/g	**0.840**	0.546	0.391	1.000	—	—	—	—	—	—
% <63μm	0.187	0.114	**0.737**	0.000	1.000	—	—	—	—	—
δ^{15}N (‰)	0.333	0.606	0.348	−0.033	0.002	1.000	—	—	—	—
N (%)	0.119	−0.059	0.606	−0.074	**0.969**	−0.131	1.000	—	—	—
δ^{13}C (‰)	0.399	0.516	−0.010	0.130	−0.525	**0.774**	−0.578	1.000	—	—
C (%)	−0.215	−0.332	0.286	−0.186	0.842	−0.328	**0.870**	−0.814	1.000	—
C/N	−0.504	−0.351	−0.099	−0.377	0.394	0.009	0.343	−0.503	0.701	1.000

*Bold values indicate significant correlation ($p < 0.05$).

factors should be investigated to corroborate these inferences; however, it is known that large volumes of leachate were thrown into the watercourse in the vicinity of Point 4 by the hazardous waste landfill, which, together with the highest concentration of metallic contaminants also observed at this point, could explain the relatively higher δ^{13}C of total C levels found at this site.

Sediments δ^{13}C values in Points 5, 6, and 7 were compatible with predominant C_3 plant-derived organic matter. C_3 plants present δ^{13}C values from approximately −24‰ to −30‰ with an average value of approximately −27‰, while C_4 plants have an expected range from −10‰ to −16‰ with an average value of approximately −12.5‰ (Clark and Fritz, 1997; Boutton, 1991). The values found in Points 6 and 7 are around the mean values found in freshwater sediments, reported as −26% (Trivelin, 2009; Boutton, 1991). The highest values, i.e. relatively more enriched in the heavy isotope, found in points upstream (Points 1, 2 and 3), especially Point 1, probably were due to the influence of C_4 vegetation, once grassland was the dominant vegetation coverage of the stream margins at this site. Around Points 2 and 3 a conspicuous grassland cover was also seen. Around Point 4 no expressive grassland coverage existed to explain the relatively higher content of the heavy isotope.

The δ^{15}N values of sediment samples showed a general trend similar to that observed with C isotopes, that is, depletion in the heavier isotope from upstream to downstream. This trend, similar to C, was discontinued in Point 4, which showed a relatively h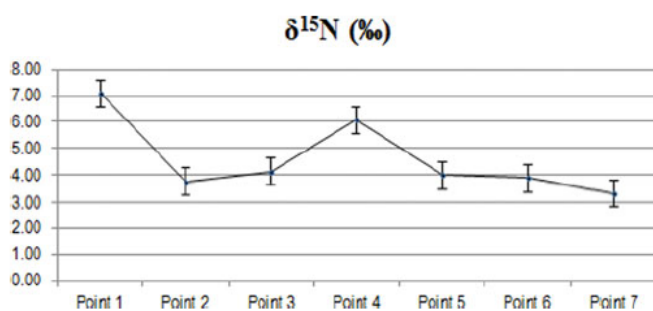igher enrichment in the heavier isotope, as can be seen in Figure 5. Distinct and identifiable signatures of δ^{15}N were determined for fertilizers and sewage, allowing N isotopic ratios to be successfully used in discrimination between these sources of contamination of aquifers, estuaries and oceanic environments (Andrews et al., 1998; Aravena et al., 1993; McClelland et al., 1997; Rogers, 1999; Wassenaar, 1995). Sewage-derived organic matter presents δ^{15}N values in the range of 3 ‰ (North et al., 2004), which is compatible with those found in Points 2, 3, 5, 6, and 7 (Figure 5).

North et al. (2004) identified landfill leachate contamination in waters and their incorporation into plant biomass. The authors report that in the mineralization of organic matter in landfills organic N is converted into ammonia leading to a small change in the N isotope ratios (Kendall, 1998). However, in spite of landfills generally being considered anaerobic environments, the heterogeneous mixture of waste can create rainwater and air pockets, thus oxidant microenvironments. These oxidizing conditions in the landfill can cause two reactions: volatilization and nitrification. The isotopic fractionation caused by the volatilization leaves the remaining N highly enriched in ^{15}N-NH$_4$ (North et al., 2004). The high enrichment in the ^{15}N-NH$_4$ of landfill leachate could explain the relative increase in the heavy isotope observed in Point 4, which lies in the area of influence of the landfill already mentioned.

The C:N ratio is used for identification of sources of organic matter (Dehairs et al., 2000; Bouillon, 2000; Cravotta III, 1997)
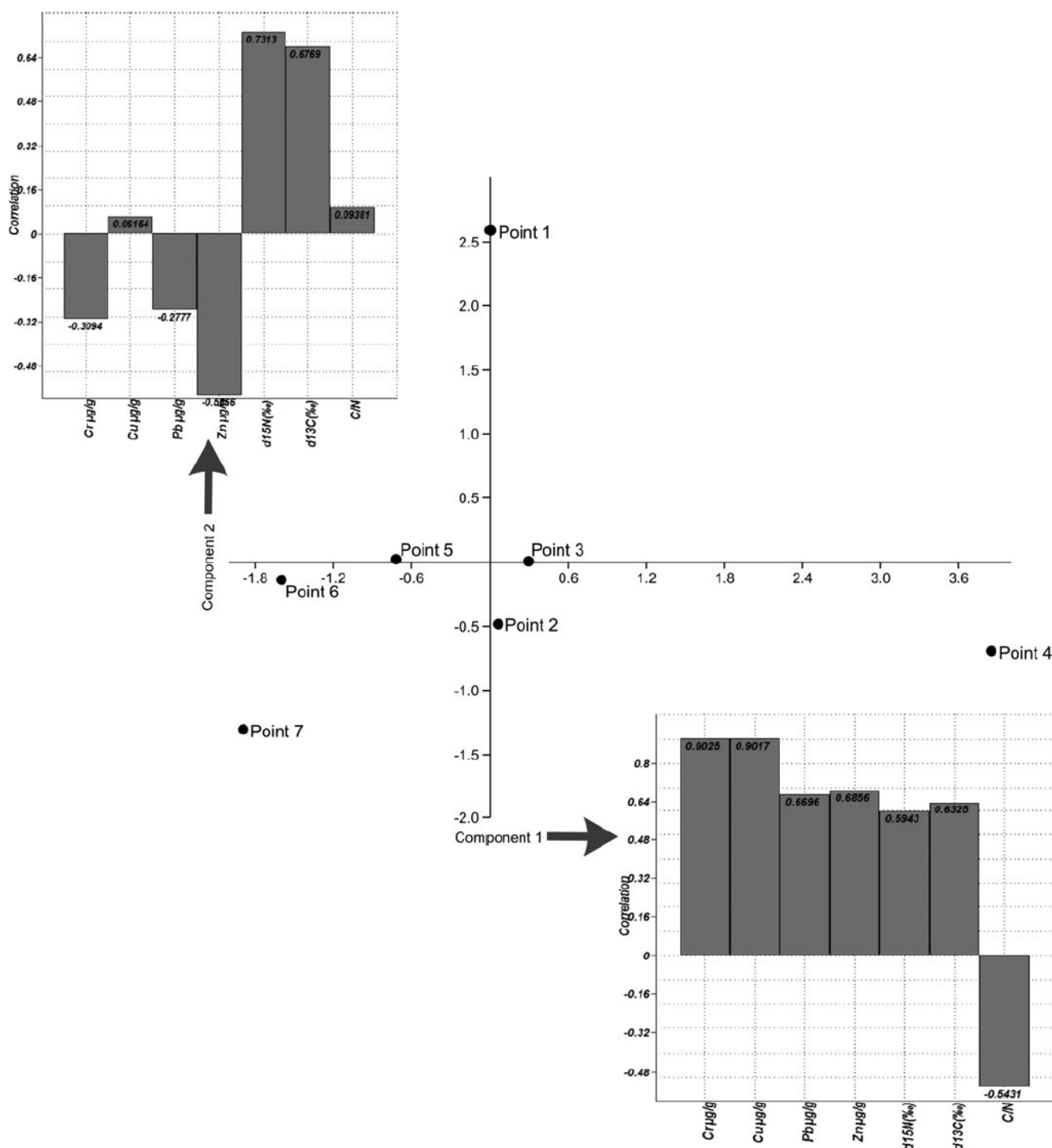

Figure 5. Graph of the δ^{15}N of total nitrogen in sediment samples.
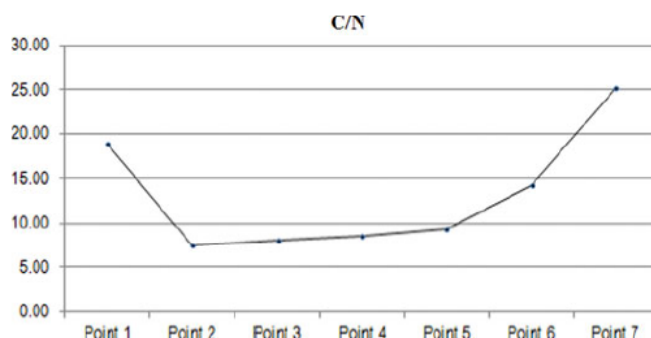
Figure 6. Graph of the C/N ratio in sediments.

Figure 7. Scatter plot obtained from Principal Component Analysis of Cr, Cu., Pb, Zn, $\delta^{13}C$ ‰, $\delta^{15}N$‰, and C/N ratio in the sampling Points 1–7, showing the contribution of variables for each component. PC1 explains 51% of the variance in the data and PC2 explains 22%.

in terrestrial and aquatic environments. Generally, terrestrial plants have C:N ratios higher than 20, while soils have C:N ratios varying from 10 to12, and animals have lower C:N ratios, varying from 3 to 6.

The results of the analyses (Figure 6) showed that the Points 1, 6, and 7 exhibited the highest values of C:N ratio, while Point 2 exhibited the lowest value. The values found for the C:N ratios in sediments collected in Point 1 are compatible

with those reported by Cravotta III (1997) for soils associated with uses that involve the production of cattle manure. In fact, in the immediate vicinity of the damming of the waters near the headwaters, livestock breeding was observed. This finding could also explain the enrichment in ^{15}N found in Point 1, if cattle manure contamination were present, then high levels of ammonia would be expected, and its volatilization could lead to enrichment in the heavier isotope of the remaining N. According to the same author, higher values for C:N ratio, in the range of 29, were found in surface soils of forests, and the lowest were associated with fertilizers (0.59) and waste (4.5).

The results of metal determinations, δ^{13}C, δ^{15}N and C:N ratio analysis in Points 1 to 7 were arranged in a matrix on which was applied PCA with the resulting scatter plot shown in Figure 7. All points were within the boundaries of the 95% confidence limit ellipse.

In this graph, the Points 2, 3, 4, 5, and 6 are related to Component 1, whose variance is mainly explained by metals concentrations, specially Cr and Cu, and N isotope ratios with a contribution of 51% of the variance. The Point 4, that showed high Cr concentration and relatively high δ^{15}N, stood out with the highest positive score of contribution to this component and Point 7 the most negative. Point 1 loaded negatively on PC2 whose variance is mainly explained by δ^{13}C, Cr, and Zn concentrations. Figure 7 demonstrates that sampling Points 1, 4 and 7 are clearly influenced by distinct sources of constituents, while sampling Points 2, 3, 5, and 6, which have values close to zero in both axis, are not clearly distinguishable by the variables studied. These points are located in segments of the stream impacted by industrial effluents, predominantly tanneries, and sewage.

These results are compatible with the spatial distribution of the different pollution sources:

- Point 1, being the closest to headwaters, is not receiving industrial discharges, but is probably impacted by cattle manure and other diffuse sources related to the land use for nearby rural activities;
- Point 4, although located downstream from a tannery, shows a markedly different source based on the variables behavior, which is probably explained by the leachate discharges from the landfill of hazardous waste; and
- Point 7 is the farthest from the pollution sources. Szalinska et al. (2010) have found that distribution of Cr from tannery effluents in Dunajec River sediments mostly reflects the proximity of contamination sources which is in accordance with the observations of this study and could explain the low levels of Cr and other metals in Point 7.

The analyses suggested that a probable leachate discharge from the hazardous waste landfill could be the cause of the highest metal enrichment in the Point 4 sediment. Isotopic analyses showed a distinct trend in this site with enrichment in the heavier isotopes for both C and N that could also be associated with landfill leachate. The monitoring analyses mean values for this site also corroborated these findings, since the highest mean

and maximum metal concentrations were found there. Additionally, Point 4 plotted distinctively from other sampling sites that were located nearby tanneries in the PCA scatter plot (Figure 3 and Figure 7), suggesting that at Point 4 a possible contribution from a different source than the tannery effluents alone.

Conclusions

This study demonstrated that a clear enrichment in metals, especially Cr, is associated with tanneries and a hazardous waste landfill that are located on the margin of the watercourse. Metal concentrations in sediment did not show a tendency to increase downstream; instead, the points located in the closest to the mouth of the stream (Point 7) showed levels closer to the most upstream point (Point 1). The sites that presented higher metal concentrations were those closest to pollution sources, indicating that these contaminants are quickly removed from the water column, depositing in the sediments, since these contaminants are associated with particulate matter. This finding means that, forensic investigation procedures should define sampling point's location within the vicinity of the release of pollutants in order to optimize the campaign.

Chrome stood out as the main pollutant of sediments in the watercourse, showing the highest levels, above the regulatory limits, downstream of some potential sources of this metal-enriched effluent. Zn showed significant positive correlation with Cr, which suggests that these metals share a common origin. Further studies are needed to assess more accurately the grain size composition of sediments and their implications on concentrations of metals along the watercourse, as well as the consistency of the analysis of metals in the fine fraction as a normalization factor for concentrations of metals in sediments with different granulometric composition to be adopted in forensic evaluation that involves sediment contamination.

The results of isotopic and metal analyses suggest the combination of these parameters as potential provider of relevant information for the discrimination of sources in a heavily polluted stream. Although the findings of this study pointed to a possible leachate discharge from the landfill through sediment analyses, which could be characterized as an environmental crime according to Brazilian legislation, further investigations and studies are necessary to define a criminal environmental forensic procedure that could provide evidence for the illegal leachate discharge "beyond a reasonable doubt". These studies should include a comprehensive fingerprinting of the main pollution sources such as the leachate and other effluents discharged to the watercourse as well as the evaluation of the biogeochemical processes involved in the incorporation of leachate in sediments.

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