AN INTRODUCTION TO A MULTIPARAMETER APPROACH TO IMPROVE THE RELIABILITY OF ENVIRONMENTAL CRIME EVIDENCE

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1 INTRODUCTION

Obtaining sound evidence of environmental pollution crimes is usually challenging in environments impacted by multiple sources. This is particularly true when the situation involves running waters systems and the contaminants discharged by the suspect facility must be searched in stream-bed sediments, which are typically heterogeneous in terms of texture and composition. The heterogeneity reflects on the high variability of contaminant content, which implies that a large number of samples must be analyzed in order to reduce the uncertainty of the resulting data to an acceptable level. In environmental crimes investigations, where the State or "People" must provide the scientific proof, resources as laboratory analyses are few, and there are also time restraints, therefore, more effective and fit-for-purpose methodologies must be developed and tested in order to provide the justice operators reliable evidence in a feasible manner.

The present work introduces a case study of leachate discharge from a hazardous waste landfill in a heavily polluted watercourse where the use of a multiparameter approach using metals, stable isotope and organics analysis in stream-bed sediments was performed as an exploratory means provide evidence of this possible crime. A brief review of methods that explore multiple variables to add qualitative consistency for the results obtained is also presented.

Environmental crime is a somewhat new concept in international law. The notion of the environment as being an asset so important for society that it must be protected by the Penal Law is still under construction worldwide, since different approaches are used to its definition and enforcement. According to the European Commission, it "*covers acts that breach environmental legislation and cause significant harm or risk to the environment and human health*" (European Commission [1]). In Brazil, environmental crime definition is no different than the definition for other crimes. It is defined as any act that violates a criminal statute. This is possible because, in 1998, a specific statute that defines the acts subject to criminal litigation known as "Environmental Crime Act" was promulgated. The crimes are divided, in this statute, in five sections: the first are crimes against the fauna; the second, crimes against flora; the third pollution related crimes; the fourth crimes against public property (landscape, architectural, etc.); and the fifth are crimes against the environmental administration, those practiced by subjects within the framework of environmental permits system. This tactic is, in some ways, appropriate, because several criminal conducts need to be defined by other technical legislations and regulations or administrative provisions that can be more easily updated as required, once environmental law is a dynamic law, so new scientific findings, and experience from existing environmental law are used to improve legislation. Other advantage is that criminal conducts are well defined, so the prosecutor does not need to prove a criminal intent or if the conduct was or not a crime, just to prove that there was a crime and who was the responsible for it. Such a system of prosecution implies someone being charged as guilty for the criminal offence, therefore a suspect must be found and investigated in order to sustain a criminal litigation. The burden of proof imposed on the prosecution in the criminal law means that the State must provide the evidence to support the charges and convict the defendant beyond a reasonable doubt. Unlike the U.S. where the environmental crime investigation is usually performed by regulatory agencies, such as USEPA and state agencies, in Brazil it is performed by the Police and the evidence is provided by the Official Forensic Agencies.

The unlawful discharge of effluents in a watercourse, which is the theme of the present work, is one of the pollution related crimes defined in Brazilian Environmental Crime Act. The study case is about hazardous waste landfill whose manager was indicted for discharging untreated leachate in a small watercourse. The discharge occurred systematically, but was noticed after a massive fish die-off that occurred in drought conditions in Sinos River, where the affected stream discharged its waters.

In order to search for evidence of the unlawful leachate discharge from the landfill, which received mostly residues from tanneries and shoemaking factories, in the watercourse, stream-bed sediment samples were collected in the affected watercourse and analysis of metals were performed. These analyses did not provide a sound proof of the leachate discharge since there were others tanneries that discharged its effluents upstream the landfill. Further analyses were needed to distinguish the leachate from the other pollution sources, therefore isotope ratio analyses were performed in the samples.

The isotope analyses were selected because the consequence of the isotope fractionation processes that occur in the decomposition of organic waste is a distinctive isotopic signature of carbon from landfill leachate that can be used to identify leachate contamination in groundwater (Baedecker and Back [2]). North et al [3] have employed, besides the carbon isotopes in Dissolved Inorganic Carbon (δ^{13}C-DIC), ammonia nitrogen 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.

The PAHs are one of the most important classes of anthropogenic persistent organic contaminants. They are relevant in pollution crimes investigation because many of these compounds are listed in group 2A (probable carcinogens) of the International Agency for Research on Cancer (IARC) and benzo(a)pyrene is included in the group 1 meaning that there is sufficient evidence of carcinogenicity in humans for the compound. PAHs are amongst the contaminants found in landfill leachates (Katkevičiūtė *et al* [4]; Zakaria [5]). To add up contaminant information to the previous data set, these compounds were determined in the stream sediments.

1.1 Study area

The watercourse that allegedly received the leachate discharges is Portão Stream. This watercourse runs through the urban area of two small cities, receiving discharges of domestic sewage and industrial effluents before the end of its course on the left bank of the Sinos River, 20 km downstream.

The study area encompasses approximately ten kilometers of Portão Stream, along which seven sampling sites were located (Fig. 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. In this landfill confined about 1,000,000m³ of industrial hazardous waste in the time of the fish die-off. The untreated effluent contained both particulate and dissolved phase contaminants.

The geological substrate of the studied area is predominantly sandstone belonging to the Pirambóia Formation of the São Bento group (Oliveira et al.[6], Machado and Freitas[7]). The major mineral constituent is quartz and the predominant grain size in sediments is sand (Robaina *et al*[8]).

2 MATERIALS AND METHODS

2.1 Sampling sites

Sampling points were recorded referenced to Datum SAD69, in UTM Zone 22J, using a Garmin GPS (Global Positioning System) receiver unit, model Etrex Summit HC, with estimated position error of less than five meters. Quantum GIS program, version 2.0.1, Dufour (Quantum GIS Development Team[9]) was used to the geoprocessing of spatial data and preparation of maps.

Seven sediment sampling sites were defined along Portão Stream course. The spatial distribution of the sampling sites is shown in Fig. 1 (Points 1 to 7). Point 1 is situated close to the headwaters of the stream. Nearby this site, there were no significant sources of pollution; therefore, this point was admitted as reference point for background levels. The points 2 to 7 are distributed downstream of the water course segment where there are major sources of pollution. In these samples, bulk carbon and nitrogen isotope ratios were determined as well as metals Cr, Cu, Pb and Zn were analyzed by Atomic Absorption Spectrometry.

Another sampling campaign was performed and material was collected in nine sampling sites. One of these sampling sites was Point 1 from the previous campaign, Points A to C were located upstream the landfill, Points D to F in the proximities of the landfill and points G and H in another stream located to the south of the landfill that was also expected to be affected by landfill discharges (Figure 2). This stream reaches Portão Stream downstream the main points of leachate discharge of the landfill. Point C is coincident with Point 2 from the previous campaign.

2.3 Metals

Sediment samples were analyzed for chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) concentrations in the Laboratory of Chemical and Isotopic Characterization (CQMA)/IPEN/USP. The dry sediment was acid digested using a Microwave Accelerated Reaction System, Model MARS 5®, according to recommendations of 3051A USEPA method. Metal concentrations were measured using Flame Atomic Absorption Spectrometer (FS-FAAS, Varian, model Spectr-AAS-220-FS).

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.

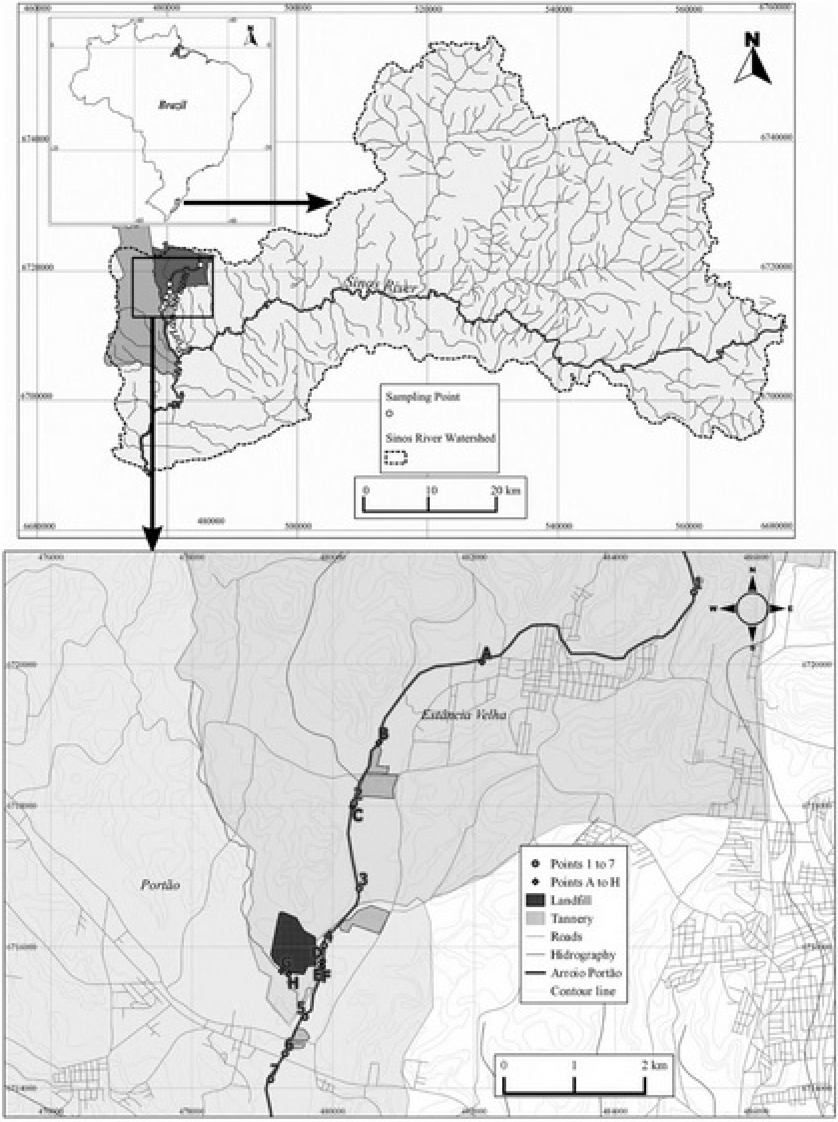

Figure 1 *Study area location depicting the sampling points spatial distribution. Modified from Barbieri et al*[10]

Figure 2. Study area showing the sampling points located nearby the landfill

The samples collected in the second campaign were freeze dried and analyzed by Wavelength-Dispersive X-ray Fluorescence (WD-XRF) at X-ray Fluorescence Laboratory/ CQMA/IPEN/USP. The results obtained were expressed in mean values and standard deviations for a confidence interval of 95%. The detection limit of this method was 50 $\mu g\ g^{-1}$ for the metals Cr, Mn, Zn, Ni, Cu and 0,02% for the elements Si, Al, K, Fe, Mg, S, Na, Ti, P and Ca.

2.4 Carbon and Nitrogen Isotope Ratio

The isotopic compositions of total carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in sediments were determined in samples by on-line automated combustion coupled to mass spectrometer Finnigan MAT Delta-S. The $^{13}C/^{12}C$ ratio is reported in relation to the VPDB standard carbon 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‰.

2.5 Polycyclic aromatic hydrocarbons (16-PAH)

About 500 g of sediments were collected from 0 to 30 cm of depth in a core like device, which was thoroughly rinsed with the stream water and after with distilled water prior to each sampling. The outer layer of the sediment was discarded and the inner part was stored in aluminum foil trays pre-heated to 300°C overnight and freeze stored until analysis. The samples where then freeze dried, a 20g fraction of the homogenized sample was added to a pre-extracted cartridge and extracted in with dichloromethane in an Automated Soxhlet System (SOXTEC) for 4 h using the configurations provided by the equipment manufacturer. The extract was concentrated in a rotary evaporator until approximately 1 mL, passed through an activated copper column, concentrated to 1 mL and fractioned by preparatory liquid chromatography in a silica column to obtain the aromatic fractions following the Jaffé *et al.* [11], method.

The fractions obtained were analyzed using a gas chromatograph Agilent (model 6890) with mass detector (model 5390), splitless injector and a capillary column (30 m X 25 mm X 25 µm) and stationary phase type 5% phenyldimetilpolisiloxane. Injector temperature: 290 °C; detector temperature: 300 °C; column starting temperature 40 °C, isotherm for 1 min, heating rate of 6 °C /min until a final temperature of 290 °C and isotherm of 20 min. The conditions applied were according to the Environmental Protection Agency (EPA), using the technique of selective ion monitoring and electron impact at 70 eV for ionization, based on the methodology EPA 8270C. The results obtained for the 16 priority PAH were qualitative and are shown in terms of presence or absence of the detected compounds.

2.6 Descriptive and statistical treatment

Principal Components Analysis (PCA) was employed as an exploratory tool to distinguish sampling sites based on the variations observed in the metals and carbon and nitrogen isotopic ratios analyses. The results obtained in the first sampling campaign (points 1 to 7) were arranged in correlation matrices in two steps in each sampling campaign data. First, the PCA was employed with metals concentrations and with isotope ratios separately, and after, data from metals concentrations and isotope ratios was assembled in a single matrix and the PCA was performed. The same method was used with the results metal concentrations obtained from WD-XRF analyses in samples collected in the second sampling campaign. The elements Al, Si, K, Fe, Mg, Na, P, Ca, Zr, Rb and Sr were not used in the PCA analysis because it was assumed that some of these elements were mostly associated with the sediments matrix or they were not detected in any of the sampling sites. The statistical analyses were performed using the PAST package, version 3.0 (Hammer [12]) and the graphical outputs obtained were edited in the vector drawing program Inkscape ©, version 0.48.

3 RESULTS AND DISCUSSION

The results obtained in the two sampling campaigns will be presented separately and a discussion of both approaches as well as a brief review of strategies revealed by other authors that could be applied to additional discussion of the criminal environmental forensic methodologies to be developed.

3.1 Metal Concentration (FS-AAS*)*

The results obtained in the analysis of Cr, Cu, Pb and Zn by FS-AAS in sediment samples are shown in Table 1. Point 4, in accordance to historical data from pollution monitoring of the stream, presented in Barbieri *et al* [10], showed the highest concentrations for all metals and very high concentrations of Cr. The metals analyzed did not exhibit increase trend in their concentrations along the watercourse. Relatively high Cr and Zn concentrations were also determined in Point 2. Metal concentrations were relatively higher in the sampling site located downstream the points of leachate discharge from the landfill (Point 4), comparing

Table 1 *Metal concentration obtained by AAS analysis and isotope ratios of C and N in the sediment samples collected in Points 1 to 7.*

Sampling Point	Cr µg g^{-1}	Cu µg g^{-1}	Pb µg g^{-1}	Zn µg g^{-1}	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
1	30.40	12.79	5.56	19.42	7.06	-22.15
2	437.61	10.10	1.01	43.10	3.75	-23.69
3	85.85	14.68	7.50	32.74	4.13	-24.32
4	953.89	17.87	21.68	50.87	6.05	-23.43
5	61.35	10.79	7.26	26.48	4.00	-24.85
6	22.46	9.29	10.23	17.31	3.88	-26.33
7	12.15	10.37	8.29	35.05	3.32	-27.92

to the other samplings sites, so that the PCA analysis allowed to distinguish this Point from the others (Fig. 3). Since there is also other possible sources of these metals upstream this point, the results *per se* could not point to a contamination specific from the suspect source.

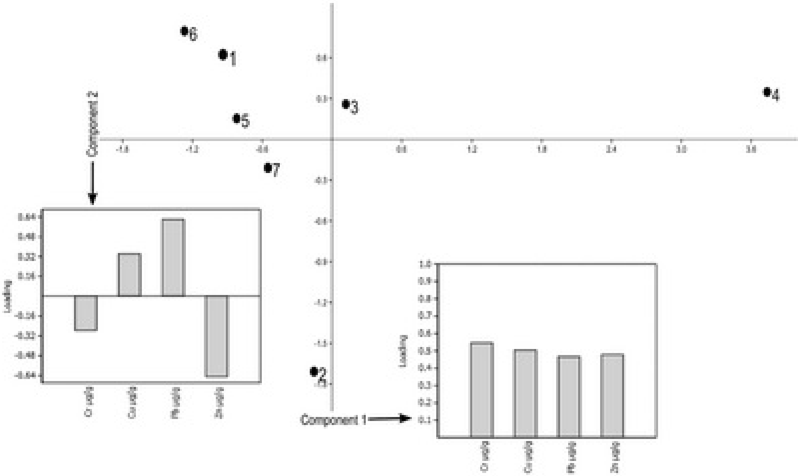

Figure 3 *Scatter plot obtained from Principal Component Analysis of Cr, Cu., Pb and Zn, in the sampling points 1 to 7, showing the contribution of variables for each component. PC1 explains 73% of the variance in the data and PC2 explains 17%.*

3.2 Concentration and isotopic ratios of carbon and nitrogen

As presented in Barbieri *et al* [10], the results of the analyses of organic carbon isotope ratio ($\delta^{13}C$ ‰) 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 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. This enrichment in the heavier isotope noted in Point 4, could be explained by the inputs of a source enriched in $\delta^{13}C$ in that region, such as landfill leachate which $\delta^{13}C$ values can vary from -9.44‰ to 10.6‰, like demonstrated by Mostapa *et al* [13]. In the scatter plot obtained from the PCA analysis of these results (Fig. 4), Point 4 is also distinguished from the others. Point 1, used as reference site regarding industrial and domestic sewage contamination in the stream showed the values most enriched in ^{15}N and the least enriched in ^{13}C. This could explain why it loaded positively in PC1.

The $\delta^{15}N$ values of sediment samples showed a general trend similar to that observed with carbon isotopes, that is to say, depletion in the heavier isotope from upstream to downstream. This trend, similar to carbon, was discontinued in point 4, which showed a relatively higher enrichment in the heavier isotope. Distinct and identifiable signatures of $\delta^{15}N$ were determined for fertilizers and sewage, allowing nitrogen isotopic ratios to be successfully used in discrimination between these sources of contamination of aquifers, estuaries and oceanic environments (Andrews *et al* [14], Aravena *et al* [15], Rogers [16], Wassenaar [17]). Sewage derived organic matter presents $\delta^{15}N$ values in the range of 3 ‰ (North *et al* [3]), which is compatible with those found in Points 2, 3, 5 6 and 7 (Table 1).

In the mineralization of organic matter that takes place in landfills organic nitrogen is converted into ammonia leading to a small change in the nitrogen isotope ratios (Kendall[18]). The volatilization of the ammonia causes an isotopic fractionation, leaving the remaining N highly enriched in ^{15}N-NH_4 (North *et al* [3]), which could explain the values found for the Point 4. The high enrichment in the ^{15}N-NH_4 of landfill leachate could cause the relative increase in the heavy isotope observed in Point 4, which lies in the area of influence of the landfill suspect of leachate discharge.

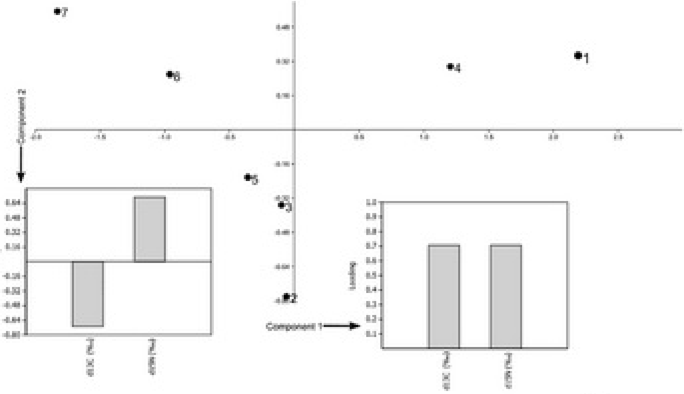

Figure 4 *Scatter plot obtained from Principal Component Analysis of $\delta^{13}C$ ‰ and $\delta^{15}N$‰ in the sampling points 1 to 7, showing the contribution of variables for each component. PC1 explains 89% of the variance in the data and PC2 explains 11%.*

3.3 Metal Concentration (WD-XRF)

The results obtained from WD-XRF analysis on sediments samples are shown in Table 2. In the displayed results, Points A and C show values above the detection limits for the metals Cr, Mn, Zn, Ni and Cu; Points B and F for Cr, Mn and Zn; Point E for Zn, Ni and Cu; Point G, that is located in the Boa Vista stream, for Cr, Ni and Cu. Point H, also situated in Boa Vista stream, did not show any metal concentration above the detection limit of the method. Point 1, which was used as a reference because it was situated upstream from industrial and domestic sewage discharges in the stream, presented the highest concentration of Mn. This could be due to the extensive cattle breeding around the sampling site, as livestock nutritional supplements usually contain Manganese (Nadaska[19]). Point D, although positioned in the segment of the stream likely to be affected by leachate discharges, only exhibited Mn concentrations above the detection limit of the method.

This analytical method was chosen because of the lower effort in sample preparation, comparing to AAS or ICP-MS, and to assess its usefulness in terms of fitness-for purpose as discussed in Ramsey [20], in this kind of situation. The low sensitivity of this method is not expected to be a problem in the greatest part of criminal environmental pollution investigations once most minimum regulatory values for metal contamination in soils, the exception is Ni in Brazilian regulation, are above the detection limit of the method. One limitation of the method used is that it did not include the metals Pb and Cd that have high capacity for accumulation in the environment, disturbing the biosphere equilibrium (Newman and Clements [21]), thus are elements of concern in pollution crime situations. The results of this analysis in the sediment samples were used to obtain the PCA scatter plot presented in Fig. 5. In this graph, Point C loaded positively on PC1 and PC2, since it was the one that presented higher concentrations for most elements while Points 1 and D loaded negatively in this axis. In both sites only Mn was detected. Points D, E and F, located in the area of influence of the landfill discharges showed different behaviors regarding metal contamination, as well as Point G and H, that were located in another watercourse that was also topographically related to the landfill. In the sample collected at Point D only manganese was determined above the detection limit and, in the sample collected at Point H, none of the heavier metals detected was identified. Points A and C, located upstream the landfill, but in industrial effluents polluted area, showed high concentrations of Cr, Mn, Zn, Ni, Cu. Among the points located in the area in the area of influence of the landfill discharges, Point F showed the higher concentrations of Cr and Zn.

The PCA analysis scatter plot displayed in Fig.5 does not discriminate the sampling points according to the inferred pollution sources based on XRF metal determination in sediments, once Point 1 is upstream industrial effluents discharges, points A, B and C are under industrial effluents discharge influence, and points D, E and F, under the landfill effluents discharge, but downstream A, B, and C. Points G and H were also located nearby each other and under the same macro influences in terms of metal contamination deposition.

3.4 Polycyclic aromatic hydrocarbons (16-PAH)

PAHs (Naphthalene, Phenanthrene, Fluoranthene, Pyrene, Chrysene, Benzo [k]fluoranthene, Benzo [a]pyrene, Benzo [g.h.i]perylene, Indeno [1,2,3-cd]pyrene) were detected in Points D, E, F and H as seen in Table 3. The spatial distribution of the sampling sites where these compounds were detected is topographically associated with the landfill. In sampling sites that showed high levels of metal contamination related with industrial effluents, located upstream the landfill, these compounds were not identified. Although

Table 2 *Metal concentration obtained by WD-XRF analysis in the sediment samples collected in Points I and A to H.*

	I	A	B	C	D	E	F	G	H
Si (%)	32±1	39±1	39±1	37±1	37±1	41±1	41±1	43±1	42±1
Al (%)	5,5±0,1	4,6±0,1	5,0±0,1	4,8±1	6,3±0,1	3,9±0,1	3,0±1	2,6±0,1	2,8±0,1
K (%)	0,6±0,1	1,7±0,1	1,5±0,1	1,0±0,1	1,6±0,1	0,8±0,1	0,9±0,1	0,8±0,1	1,0±0,1
Fe (%)	4,4±0,1	1,6±0,1	1,5±0,1	2,1±0,1	1,5±0,1	0,9±0,1	1,0±0,1	0,6±0,1	0,5±0,1
Mg (%)	2,1±0,1	0,23±0,05	0,21±0,05	0,23±0,05	0,42±0,05	0,17±0,05	0,23±0,05	0,10±0,05	0,16±0,05
S (%)	0,28±0,05	0,30±0,05	0,32±0,05	0,64±0,05	1,3±0,1	0,26±0,05	0,29±0,05	0,14±0,05	0,09±0,05
Na (%)	<0,02	0,17±0,05	<0,02	0,14±0,05	<0,02	<0,02	<0,02	<0,02	<0,02
Ti (%)	0,40±0,05	0,23±0,05	0,32±0,05	0,34±0,05	0,23±0,05	0,19±0,05	0,13±0,05	0,09±0,05	0,10±0,05
P (%)	0,07±0,01	0,08±0,01	0,10±0,05	0,35±0,05	0,08±0,01	0,14±0,05	0,17±0,05	0,09±0,01	0,06±0,01
Ca (%)	1,5±0,1	0,36±0,05	0,41±0,05	0,47±0,05	0,37±0,05	0,25±0,05	0,55±0,05	0,19±0,05	0,06±0,01
Zr (µg g-1)	<50	<50	<50	<50	0,007	<50	<50	<50	<50
Rb (µg g-1)	<50	<50	<50	<50	<50	<50	<50	<50	<50
Sr (µg g-1)	<50	<50	<50	<50	<50	<50	<50	<50	<50
Cr (µg g-1)	<50	247±30	242±30	548±50	<50	<50	455±50	270±30	<50
Mn (µg g-1)	1355±100	153±20	160±20	221±20	206±20	<50	154±20	<50	<50
Zn (µg g-1)	<50	530±50	498±50	131±20	<50	530±50	562±50	<50	<50
Ni (µg g-1)	<50	105±10	<50	196±20	<50	54±10	<50	75±10	<50
Cu (µg g-1)	<50	106±10	<50	199±20	<50	55±10	<50	76±10	<50

these compounds were identified in points within the area impacted by the supposed leachate discharge, in Point G, that was located near point H, none of the compounds were detected. In Point D sample, which was collected close to Points E and F, only Perylene, a non-anthropogenic PAH (Ruffino *et al* [22]) was identified.

3.5 Multiparameter approach

The presented results of two sampling campaigns were used to illustrate a criminal environmental forensics procedure where budget and time restrains are frequently, if not always, important issues to be dealt with. This implies that the forensic examiner must make choices and seek alternatives in order to obtain relevant information within this limited framework.

In the case studied, the monitoring of stream pollution was made using metal concentration data which could be valuable for fingerprinting of sources (Yay *et al* [23], Gu *et al* [24]). However, usually a high number of samples are needed for these analyses to be statistically significant. The assemblage of data from additional parameters such as isotope ratio, reported as potential trackers of landfill leachate (North *et al* [3], North *et al* [25]; Mostapa *et al* [13]) and PAH analyses (Gade *et al* [25], Sisinno *et al* [27]), which are also used for

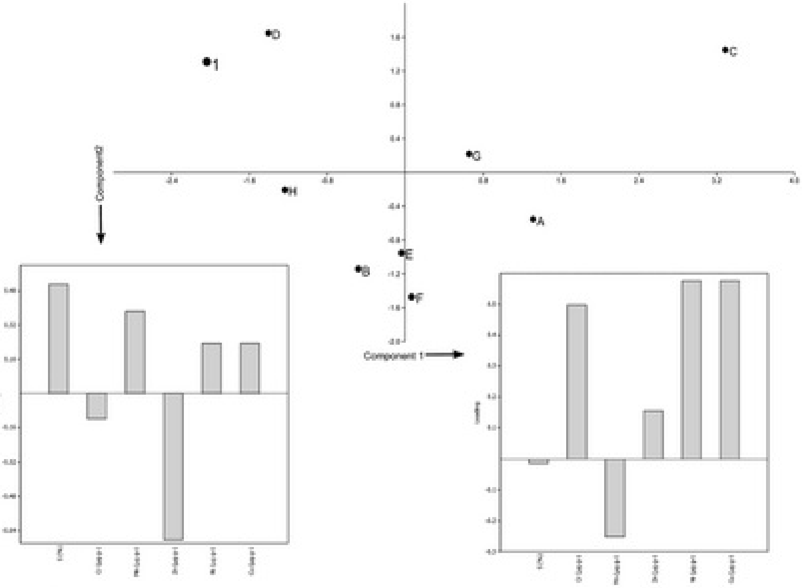

Figure 5 *Scatter plot obtained from Principal Component Analysis of elements (S, Cr, Mn, Zn, Ni, Cu) concentrations analyzed by WD-XRF in the sampling points 1 and A to H, showing the contribution of variables for each component. PC1 explains 44% of the variance in the data and PC2 explains 23%.*

Table 3 *PAH presence*[1] *in the sediment samples collected in Points 1 and A to H.*

	Nap	Phe	Flt	Pyr	Chry	Bkf	Bap	BghiP	Ind
1	-	-	-	-	-	-	-	-	-
A	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-
D	-	-	-	+	-	-	-	-	-
E	-	+	+	+	-	+	-	-	+
F	+	+	+	+	+	+	+	+	+
G	-	-	-	-	-	-	-	-	-
H	+	+	+	+	+	+	+	+	-

[1] + detected and – not detected

Nap: Naphthalene; Phe: Phenanthrene; Flt: Fluoranthene; Pyr: Pyrene; Chry Chrysene; Bkf: Benzo [k]fluoranthene; Bap: Benzo [a]pyrene; BghiP: Benzo [g.h.i]perylene; Ind: Indeno [1,2,3-cd]pyrene;

fingerprinting and source identification, should be evaluated as a potential tool for improving the reliability of pollution evidence and source identification.

The forensic use of a multiparameter approach to identify sources of contaminants could lead to a more accurate identification a suspect source. In this work, a better discrimination of the sampling sites according to the suspect sources is shown in the PCA scatter plot (Fig. 6) where metal concentrations and isotope ratio values for sediments were drawn together for the PCA analysis, using a correlation matrix to standardize the values that are in different scales. In this graph, Point 4, related to the landfill discharges, loaded positively in PC1 and was distinctly separated from other Points. Likewise, Point 1, considered as reference site because it was positioned upstream industrial and domestic sewage releases, loaded positively in PC2, distant from the others. Points 2, 3 and 5, that were under the influence of similar multiple sources, grouped together along the PC1 axis and Points 6 and 7, that were farther from the sources, also grouped. Even though the graph seems to indicate a solid distinction between points regarding the sources, this study was merely exploratory and additional analyses, with larger datasets, are needed to validate this approach as a forensic methodology.

The additional analyses performed in the sediments of the same stream and in another stream that was also topographically related to the landfill in the second sampling campaign, which included PAH and metals determinations, displayed fuzzier data.

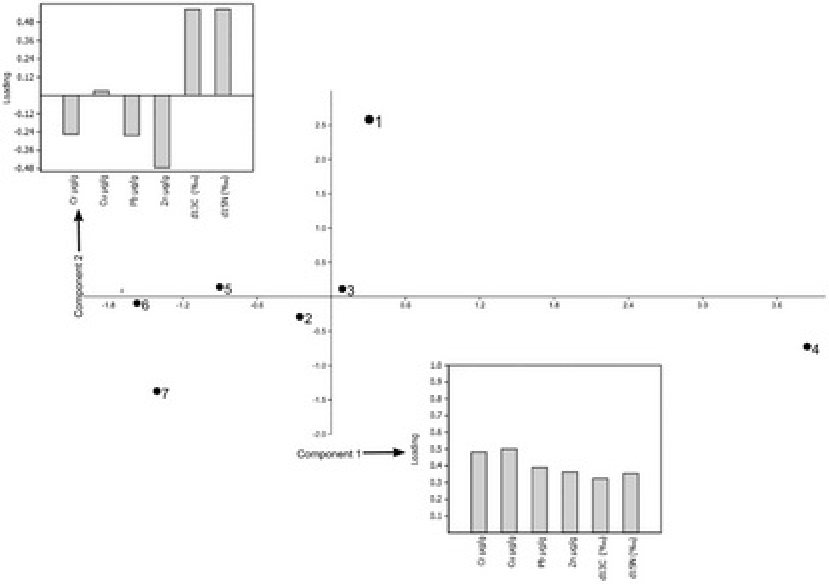

Figure 6 *Scatter plot obtained from Principal Component Analysis of Cr, Cu., Pb, Zn, $\delta^{13}C$ ‰ and $\delta^{15}N$‰ in the sampling points 1 to 7, showing the contribution of variables for each component. PC1 explains 56% of the variance in the data and PC2 explains 25%.*

The PCA scatter plot of the results obtained in the metals analyses by WD-XRF in the samples collected does not discriminate the sampling sites based on the inferred pollution sources. This is probably due to the high detection limit of the method that excludes from the analyses the variations in concentrations below 50 μg g^{-1}, that are loaded in the dataset as "zeros", making this determinative method not suitable for obtaining metals levels to be used in a PCA analysis in this situation.

The qualitative PAH analysis added more information to the investigation because these compounds were detected only in regions supposedly affected by the landfill discharges. Comparing the results of the PAH and metals analyses, in the sampling points it can be seen that Point F is the only one that shows high levels of metals and all range of PAHs that were detected in these analyses. Point E also showed some metals and some PAHs. Points G and H, that were located nearby each other and in a region apparently affected by the same sources, instead , presented uneven results with Point G showing high levels of some metals and no PAH detected and Point H showing some PAHs and no metals.

It is noticeable that there are differences in metals concentrations and PAHs detected even in samples collected in nearby points, such as G and H and D, E and F, for. The heterogeneity of the sediment compartment and small-scale patchiness of this media (Chapman *et al* [28], US Army Corps of Engineers [29], Hadley *et al* [30]) are the probable causes of these apparently inconsistent results.

In criminal environmental situations, it is essential to remember that the absence of evidence is not *necessarily* evidence of absence. The geochemical spatial heterogeneity of stream sediments can easily lead to a type 2 error, which, in this case, would be to reject the pollution hypothesis, with a single or few samples analyses. This could be solved with an extensive sampling plan reaching a sample size *(n)* capable to provide evidence that there is indeed contamination on the suspect site and with fingerprinting methods that allow the identification of the source (the author of the crime) with a reasonable accuracy. The burden of proof is on the side of the State (people) in criminal litigation, therefore the examiner must seek means to provide solid evidence, so that the law officers can conduct the case effectively and the actual offender be properly condemned.

The development of evaluation methodologies to be applied in environmental data that are able to improve the significance of the findings would aid the environmental crimes investigations and verdicts.

One example of these methodologies is the Triad approach described by Chapman *et al* [27] for evaluating and assessing pollution-induced degradation in sediments which is based on three components sediment chemistry, sediment bioassay, and in situ parameters such as benthic community structure. The information provided by each component is unique and complementary and the components are combined to provide comprehensive information that cannot be obtained by a single component analysis. Delconte *et al* [31] used a multi-tracer multi-isotope approach to identify and quantify inputs of NO_3 in groundwater. Recently, Yang *et al* [32] and Xue [33] used a Bayesian model that ran under the open source statistical software R, called stable isotope analysis in R (SIAR) to source apportionment and estimation of proportional contributions of sources of nitrate, respectively. These methods could be useful if explored because they could be applied to Bayesian interpretation methods now commonly applied to evidence in courts (Ehleringer[34]).

4 CONCLUSIONS

In the presented study case, the assemblage of the metals analyses by EAA and stable isotope ratio data in to perform a Principal Components Analysis, provided a better discrimination of sediment sampling points according to pollution sources comparing to the use of the same method in the separate datasets (metals and isotopes) and with the results of metal analyses by XRF. The determination of metals by XRF and the qualitative determination produced fuzzy results requiring additional investigations.

These results were not fit for PCA analysis in this case because of the non-continuous character of the binary data generated by the qualitative PAH analyses and the high number of undetected elements that resulted from the relative high level of detection of the XRF analyses performed.

The apparently inconsistent results of the XRF and PAH analysis was probably due to the high heterogeneity of the sediment and this brings a concern for environmental crimes reports based on analyses in this compartment.

The use of a multiparameter approach, incorporating quantitative PAHs data to the metals and isotope analyses results, should be further explored in situations as the case presented to assess its applicability to improve the reliability of the criminal evidence. This approach could be useful when there are few samples and the environmental forensics experts need to extract the higher amount of information of them. Additionally, other evaluation methodologies as well as methods that can reduce or inform the uncertainties generated by the sediments heterogeneity ought to be developed in order to bring more trustworthy pollution crime evidence to courts. This could aid the environmental pollution crimes to attain the importance they deserve in the Criminal Law system and not be regarded as lesser crimes.

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