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Characterization of a pollution source with isotopic and physicochemical measurements using a forensic metrology approach

Cristina Barazetti Barbieri[a,b], J. E. S. Sarkis[b], and L. F. Rodrigues[c]

[a]Instituto-Geral de Perícias, Porto Alegre, Brazil; [b]Centro de Química e Meio Ambiente, Instituto de Pesquisas Energéticas e Nucleares, Universidade de São Paulo, São Paulo, Brazil; [c]Instituto do Petróleo e dos Recursos Naturais, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil

ABSTRACT

Carbon and nitrogen composition and isotopic ratios (δ^{15}N, δ^{13}C, and δ^{13}C-DIC) as well as physicochemical parameters were measured in hazardous waste leachate connected to an environmental pollution crime. A forensic metrology approach was employed by estimating the measurement uncertainty, including that from the sampling process, using range statistics. The physicochemical parameters demonstrated differences in the leachate from some of the different ponds and this was supported by carbon and nitrogen elemental and isotopic analyses. The isotopic signature of δ^{15}N and δ^{13}C-DIC was highly enriched in the heavy isotope demonstrating that those analyses are a substantial tool for leachate pollution tracking. The uncertainty of the measurements was below or slightly above 20% for most parameters which can be considered fit-for-purpose for a great deal of environmental surveys and was remarkably lower in the isotopic analyses. The uncertainty of measurement including the primary sampling is a relevant information in environmental investigations, especially forensic ones. This knowledge is crucial for a consistent interpretation of environmental samples analyses results, specially from polluted sites and pollution source characterization and tracking, where there is often substantial heterogeneity in environmental samples.

KEYWORDS

isotope ratio; δ^{13}C; δ^{13}C-DIC; δ^{15}N; hazardous waste landfill leachate; pollutant; forensic metrology

Introduction

Landfilling is a waste management method extensively used worldwide, mostly for economic reasons, that causes negative environmental impacts due to the resulting leachate production. The leachate constituents are common sources of surface and groundwater pollution (Han et al., 2016; Xu et al., 2018) and can cause ecosystem damage and human health risks and, thus, environmental pollution crime according to Brazilian legislation. As the deterioration of the facilities' infrastructure may lead to further seepage of leachate (Fleming et al., 1999), those risks increase substantially over time, and 10 times more leakage is expected in the long-term (of over 50 years) (Ya et al., 2018). Therefore, the pollution potential increases with time. Hazardous waste landfills (HWLs) are even more multifaceted and bear additional problems to the environmental monitoring agencies and forensic examiners since they have more complex features and publications on the characterization of the leachate produced from HWLs are scarce, differently from Municipal Solid Waste Landfills (MSWLs) which are more extensively studied (Gautam et al., 2019). As the leachate characteristics are determined by the type of parent waste and, in HWLs the possible types of waste vary according to the predominant industrial waste deposited, it is difficult to assign a general representative signature for HWLs leachate. The leachate produced in a hazardous waste landfill that received mostly tannery (chromium based) wastes was considered the pollution source in an environmental crime that took place in southern Brazil. The release of industrial effluents leading to ammonia and metal toxicity can be the cause of mass fish death pollution in anthropogenically impacted environment (Eddy, 2005; Kangur et al., 2005; Abu-Elala et al., 2016; Raja et al., 2019). Therefore, it is relevant for environmental forensics investigations to determine the source of pollution that led to the catastrophic event to

CONTACT Cristina Barazetti Barbieri cristinabarbieri@alumni.usp.br Instituto-Geral de Perícias, Rua Voluntários da Pátria, 1358 - 3rd floor, 90230-010, Porto Alegre, Brazil.

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guarantee that the administrative and legal measures are directed to the real perpetrator. In order to help improve the robustness of the environmental forensics evidence, $\delta^{13}C$, $\delta^{15}N$ and metals analyses (Barbieri and Sarkis, 2018), as well as ancillary physicochemical parameters measurements, were performed in the leachate from this facility to characterize a distinct fingerprint of this pollution source from similar sources, such as tanneries effluents, that were released in the same aquatic environment.

Stable isotope analysis of C and N can help in the differentiation of pollution sources since the isotopic signatures of both elements in leachate are very distinct from other sources due to the strong enrichment of the reaction substrate in the fractionation that occurs with the carbon isotopes in the methanogenesis and with the nitrogen isotopes in the volatilization of ammonia. North et al. (2004) and Mohammadzadeh et al. (2006) have used $\delta^{13}C$ and $\delta^{15}N$ to identify and study landfill contamination and Engelmann et al. (2018) used the isotopic ratio of the Dissolved Inorganic Carbon ($\delta^{13}C$-DIC), i. e. of the total amount of inorganic carbon species ($CO_2(aq)$, HCO_3^- and CO_3^{-2}) dissolved in the leachate's water, to monitor a landfill area.

A pertinent issue in forensic science is the range of reliability of the data used to support the experts' conclusions. This is the subject of Forensic Metrology (Imwinkelried, 2012; Vosk, 2016), a new area of the forensic sciences which deals with the estimation and expression of the measurements' uncertainties. In environmental forensics, this topic must also be taken into account because in many cases environmental measurements results have to be evaluated within regulatory limits. As the primary sampling component is the major cause for the measurement results' uncertainty in environmental and geochemical investigations (Ramsey, 1998; Barbieri and Sarkis, 2018), its estimation in environmental analysis is key to a proper interpretation and the use of results as forensic evidence. Even in non-regulated environmental parameters used to interpret and/or support the core evidence or as additional evidence of pollution source this range of uncertainty of the measurements is a relevant information in environmental forensics to assess the weight of the data they provide. Therefore, the objectives of this work were,

a. to characterize the hazardous waste landfill leachate, a potential pollutant, by its isotopic signature ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{13}C$-DIC) and ancillary parameters in different sampling sites (leachate collection ponds);
b. to estimate the uncertainty of the measurements, including the sampling component, and report it when presenting the results.

Material and methods

Study area

The Hazardous Waste Landfill where the leachate samples were taken occupies an area of 29.95 ha located at 29° 41′ 1,2" S; 51° 12′ 46,8" circa 5 km from the Estância Velha Town Centre, in the Sinos River Valley Rio Grande do Sul State, Brazil. The landfill started its operation in 1992 and the volume of waste deposited in its more than 10 cells was over 1,000,000 m^3. The disposed waste was mainly constituted by wet-blue leather tanning residues which have high Cr content, reaching percentages of the dry weight. Other types of waste disposed were ashes and sludges from industrial processes including electroplating sludge. The facility was convicted for an environmental crime that occurred in 2006, for illegal discharge of leachate in a stream located nearby its eastern limits, so it was subject to an investigation that revealed malpractices in the infrastructure and operation of the landfill. There were eight leachate ponds (SP1 to SP8) in the facility where replicates of leachate samples were taken (Figure 1). A formal scheme of the leachate treatment system was not available, as the leachate collection infrastructure was built and rebuilt over time. A similar situation occurred with the whole facility infrastructure that included waste disposal cells located on top of older cells and a mixture of older and younger leachate. Nevertheless, the contents and status of the leachate ponds, as informed by the facility's technical manager, follows:

- SP1 – Old leachate storage pond (without recent inputs)
- SP2 – Young leachate pond with daily input, aerated
- SP3 – Old leachate storage pond (without recent inputs)
- SP4 – Chemically treated effluent pond, aerated
- SP5 – Untreated leachate storage pond
- SP6 – Untreated leachate storage pond
- SP7 – Untreated leachate storage pond
- SP8 – Untreated leachate storage pond

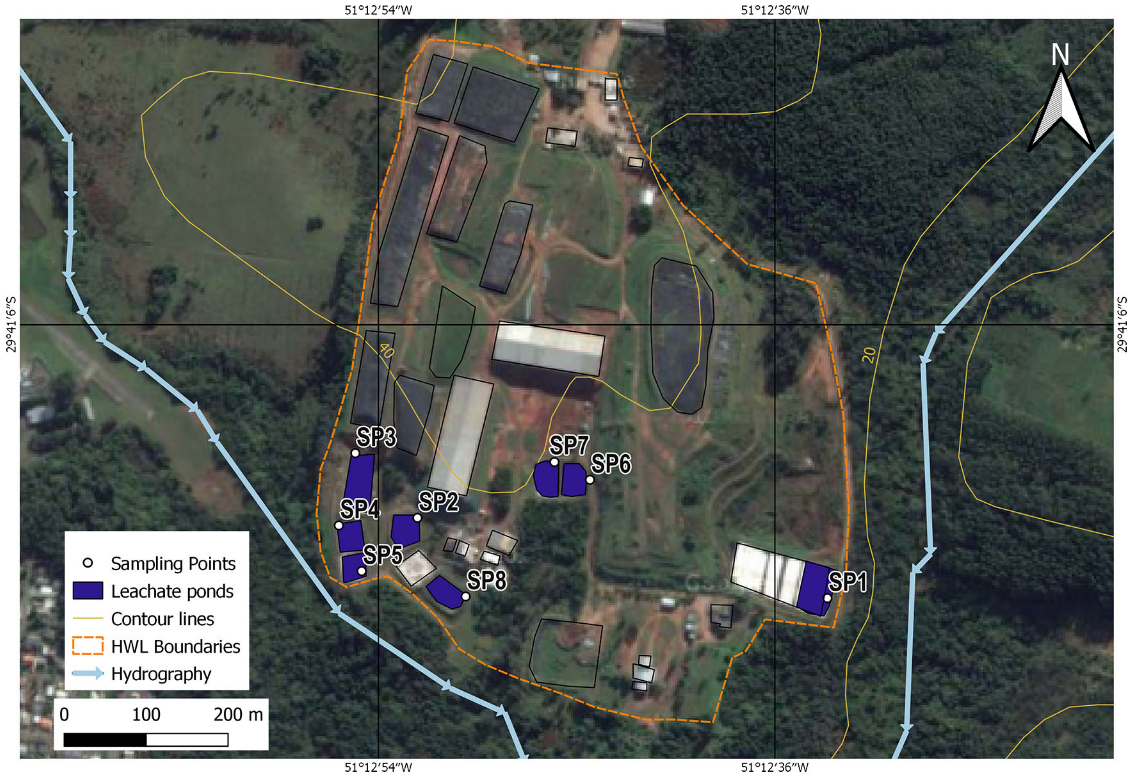

Figure 1. Map of the study area showing the hazardous waste landfill (HWL) location and leachate sampling points.

Sampling, measurements and analyses

In each leachate pond duplicate samples were collected throwing a stainless-steel sampler into the leachate pond about 1 m far from the margin. The sampler was rinsed three times with distilled water before each sample collection. Part of the sampler's contents were poured into an acid washed amber glass bottle and the rest was poured into the multi-parameter device sample container. This procedure was repeated for the duplicate sample. The bottles were refrigerated below 4 °C until analysis. The auxiliary parameters temperature, electric conductivity, pH, oxidation-reduction potential (ORP), and turbidity were measured *in situ* with a multi-parameter water quality meter Horiba, model U-52/10 inserting the probes into the leachate poured into the sample container and waiting for the readings to stabilize. The sample container was rinsed three times with distilled water between samples.

Carbon and nitrogen content and isotopic analyses (δ^{13}C, δ^{15}N and δ^{13}C-DIC) were performed in the leachate samples. The preparation of samples for the total carbon, total nitrogen, δ^{13}C and δ^{15}N analyses involved the evaporation of about 30 mL of each leachate sample until a 2 mL volume was obtained and then dried in a forced ventilation drying oven (Marconi – MA 035, Piracicaba, Brasil) at 50 °C for 48 h. Each sample was placed in a sealed vial and pulverized to $\leq$ 250 µm in a cryogenic mill using liquid nitrogen (-196 °C), avoiding cross contamination. Approximately 150–170 µg of sample, encapsulated in tin, were used for ^{13}C/^{12}C analysis and 1200–1300 µg for ^{15}N/^{14}N. The samples were introduced by the automatic sampler in the elemental analyzer (EA 1108 – CHN – Fisons Instruments, Rodano, Italia) that determined the weight percentages of total carbon (TC) as CO_2 and total nitrogen (TN) as $N_2$and analysed in the isotope ratio mass spectrometer (Delta S – Finnigan MAT, Bremen, Germany).

For the δ^{13}C-DIC analysis, around 10 mL of leachate sample was filtered through a 25 mm PTFE syringe filter, pore size 0.45 μm. The filtered leachate was introduced with a syringe needle into a 30 mL glass flask sealed with a rubber stopper, and crimp-sealed with aluminium cap to which 1 g of phosphorus pentoxide (P_2O_5) and five drops of benzalkonium chloride (Zephiran®) were previously added in order to suppress microbial activity. The procedure for sample gas preparation was performed following Atekwana and Krishnamurthy (1998). The air in the glass bottle was vacuumed through the injected needle whose end continues to the vacuum pump. After vacuuming (<0.06 Torr), the glass bottle was removed from the

Target	Sample A	Sample B	$D_{i1}=\lvert x_{iA}\text{-}x_{iB}\rvert$	$\bar{x}_i = (\frac{x_{iA}+x_{iB}}{2})$	$d_i = \frac{D_i}{\bar{x}_i}$
1	x_{1A}	x_{1B}	$D_1=\lvert x_{1A}\text{-}x_{1B}\rvert$	$\bar{x}_1$	d_1
2	x_{2A}	x_{2B}	$D_2=\lvert x_{2A}\text{-}x_{2B}\rvert$	$\bar{x}_2$	d_2
3	x_{3A}	x_{3B}	$D_3=\lvert x_{3A}\text{-}x_{3B}\rvert$	$\bar{x}_3$	d_3
					$\bar{D}_{measurement} = \frac{\sum d_i}{n}$

Figure 2. Range statistics calculations in single split design investigation for sampling targets (i). To obtain the replicates, two samples (A and B) were collected in each sampling site (target) and each sample was analyzed. The calculation of the Mean Range of Measurement ($\bar{D}_{measurement}$) is demonstrated with three sampling targets. Adapted from Magnusson et al. (2020).

vacuum system. Sample water was injected into the glass by 1 cc plastic syringe on ships. The syringe was removed after 0.5 cc of sample water was automatically injected into the glass bottle. The CO_2 gas inside the glass vial was injected into IRMS by a gastight syringe with stopper.

The $\delta^{13}C\text{-}CO_2$ values were determined using a Thermo Scientific DELTA V Plus isotope ratio monitoring mass spectrometer coupled to a Thermo Fisher Scientific GC gas chromatograph mass spectrometer via a Thermo GC IsoLink and Conflo IV interface (Thermo Fisher Scientific). For analysis, 25 µL of sample was injected in split mode (split 1:20) at 70 °C using gastight syringes (Hamilton). The gas chromatograph was fitted with a CarboxenTM1006 Plot capillary column (30 m and 0.32 mm film thickness) and the following oven temperature program was used: 70 °C initial temperature for 4 min; ramp to 150 °C at 20 $°C.min^{-1}$ and hold for 3 min. Helium was used as the carrier gas. The temperature of the combustion reactor (Ni/CuO/Pt) was 900 °C and the H_2O generated in the combustion interface was removed by the passage of the combustion products through a Nafion tube (fluorinated polymer). It acts as a semi-permeable membrane through which water passes freely while all the other combustion products are retained in the carrier gas stream. Helium was used as carrier gas with a constant flow of 1.5 ml/min.

All $\delta^{13}C$ values are reported relative to a reference CO_2 of known carbon isotopic composition ($\delta^{13}C = -32.82 \pm 0.25‰$) which was introduced directly into the ion source in three pulses at the beginning of each run. The reference gas was calibrated against Vienna Pee Dee Belemnite (VPDB) scale (Coplen, 2011). Each measurement was performed at least in triplicate. The isotopic data are reported using the delta notation with $^{13}C/^{12}C$ variations relative to the international standard Vienna Pee Dee Belemnite (V-PDB):

$$\delta^{13}C‰ = \left(R_{sample}/R_{standard} - 1\right) * 1000$$

where R is the measured $^{13}C/^{12}C$ isotopic ratio in the sample and the standard, respectively, and differences in the ratio between a standard and sample are reported in parts per thousand or per mil (‰).

Statistical analyses

To assess the uncertainties of the measurements, an initial validation procedure was accomplished using relative range statistics with replicate measurements in a single split level design with eight different targets (sampling sites L1 to L8) according to Magnusson et al. (2020). The Range Statistics calculation method was chosen for it is easily implemented using spreadsheet applications. In addition, Leite et al. (2021) found that Range Statistics produced most reliable results under different premises and for the variety of physicochemical parameters evaluated when compared to ANOVA and Robust ANOVA.

The Relative Standard Deviation (RSD), representing the uncertainty of the whole measurement, that includes the process of sampling and analysis of each variable/parameter is obtained using the following equation:

$$RSD = \frac{\overline{D}*100}{1.128}\ \%$$

where the denominator is a factor applied in RSD calculation for replicates (Ramsey et al., 2019) and $\overline{D}$ is the mean range of measurement calculated as shown in Figure 2 for single split design.

The RSD of the measurement corresponds to the combined standard uncertainty, u for the purposes of this study and, according to Magnusson et al. (2020),

Table 1. Physicochemical parameters measurements in leachate in eight sampling points reporting the expanded measurement uncertainty (U) and the relative standard deviation (RSD %).

	Temp (°C)			pH			ORP (mV)			EC (mS/cm)			Turb (NTU)		
Sampling Point ID	Mean value		U	Mean value		U	Mean value		U	Mean value		U	Mean value		U
SP 1	23.45	±	0.87	7.89	±	0.48	−406	±	−101	16.75	±	2.46	257	±	42.79
SP 2	27.10	±	1.00	8.04	±	0.49	−382	±	−95.5	51.75	±	7.59	1000	±	166.8
SP 3	22.15	±	0.82	8.43	±	0.51	−124	±	−31.0	10.85	±	1.59	457	±	76.23
SP 4	24.80	±	0.92	8.02	±	0.49	−401	±	−100	50.10	±	7.35	860	±	143.4
SP 5	25.85	±	0.96	7.10	±	0.43	−308	±	−77.0	33.10	±	4.85	1000	±	166.8
SP 6	21.95	±	0.81	7.52	±	0.46	−343	±	−85.6	17.10	±	2.51	934	±	155.7
SP 7	22.40	±	0.83	8.01	±	0.49	−381	±	−95.1	41.05	±	6.02	1000	±	166.8
SP 8	24.10	±	0.89	7.26	±	0.44	−327	±	−81.8	14.50	±	2.13	1000	±	166.8
RSD (%)	1.85			3.05			12.50			7.33			8.34		

the confidence interval to the report of a sampling uncertainty based directly on one standard deviation ($X = x \pm u$) corresponds to 67%. The authors indicate that the expanded uncertainty, U ($U = 2u$), would increase the confidence interval to 95%, as expected in a normal distribution when two standard deviations (σ) are considered.

Other statistical analyses were performed with the PAST 4.05 software (Hammer et al., 2001).

Results and discussion

Physicochemical parameters

The mean values of the physicochemical parameters' duplicate measurements in leachate samples from the eight sampling sites are shown in Table 1. The mean measurement values are presented with the corresponding Expanded Measurement Uncertainty (U), including the uncertainty from sampling, estimated with the range statistics method (Figure 2). The complete data set of the raw measurements is included in Supplementary Material (SM 1).

The data demonstrate a high variability of these physicochemical parameters among the eight different leachate ponds. The mean temperature range was from 21.95 ± 0.79 (SP6) to 27.10 ± 0.98 °C (SP2) which can be considered high because the ponds were located at the same site, within a radius of around 600 m, exposed to near identical amounts solar radiation, with the exception of pond SP1 that was partially covered, and had approximately similar dimensions. The pH in all ponds was slightly alkaline, ranging from 7.10 ± 0.43 at SP 5 to 8.43 ± 0.51 at SP3. The pH tends to increase over time reaching 8.3 as reported by Lisk (1991). This is in accordance with the high values at SP3, which indeed contained more aged leachate.

The oxidation-reduction potential (ORP) indicate that the leachates were a highly reductive medium, with values ranging from −124 ± 31.0 mV (SP3) to −406 ± 101 mV (SP1). It is important to mention that the ponds SP2 and SP4 were aerated lagoons and still they were anaerobic reducing environments prone to processes such as sulphate reduction and methane fermentation. The electrical conductivity (EC) levels were very high as expected for landfill leachates particularly in its early stages (Ziyang et al., 2009). The lower EC was found in SP3 samples (10.85 ± 1.59 mS/cm) and the higher in SP 2 samples (51.75 ± 7.59 mS/cm). SP1 samples also showed the lowest turbidity (257 ± 42.79 NTU) amongst all the other ponds' samples, followed by SP3 with 457 ± 76.23 NTU. At SP2, SP5, SP7, and SP8 both samples' turbidity reached, or surpassed, the upmost limit of the equipment scale that is 1000 NTU.

The physicochemical parameters measurements' RSD for the leachate, including the uncertainty from the sampling process, ranged from 1.85% for temperature to 12.5% for ORP measurements. The expanded uncertainty was used to obtain a 95% confidence interval; this means that the results were reported, respectively, with a U of 3.7% for temperature and 25% for ORP.

Temperature, pH, electrical conductivity and turbidity showed expanded uncertainties (U) below 20% for a 95% confidence interval. Ramsey (2002) mentions a Fitness-for-Purpose Criterion that has been suggested, according to which the uncertainty of the measurement (including that from the sampling) should not contribute more than 20% of the total variance for the analyte across all of the samples in a particular survey. Regarding that the leachate is a very heterogenous aqueous matrix since it has a high amount of dissolved and suspended solids, which can be inferred by its high Electrical Conductivity and Turbidity, the uncertainty levels found for these parameters can be considered fit for the purpose of improving the understanding of the environmental features for the interpretation of the main data.

The overall physicochemical parameters measurements demonstrate that the leachate from all the

Table 2. Carbon and nitrogen elemental and isotopic measurements in leachate in eight sampling points reporting the estimated uncertainty of the measurement (U) and the Relative Standard Deviation (RSD %).

	% N_2			$\delta^{15}N$			% CO_2			δ^{13}C-bulk			δ^{13}C-DIC		
Sampling Point ID	Mean value		U	Mean value		U	Mean value		U	Mean value		U	Mean value		U
SP 1	0.55	±	0.35	9.19	±	0.85	3.77	±	1.50	−16.72	±	0.48	19.74	±	4.65
SP 2	0.75	±	0.47	19.60	±	1.80	6.37	±	2.53	−16.92	±	0.49	16.15	±	3.80
SP 3	4.45	±	2.80	37.81	±	3.48	13.99	±	5.56	−14.65	±	0.42	21.13	±	4.97
SP 4	2.62	±	1.65	46.16	±	4.25	8.99	±	3.57	−14.48	±	0.42	−2.63	±	0.62
SP 5	3.24	±	2.04	53.20	±	4.89	13.10	±	5.21	−13.53	±	0.39	26.78	±	6.30
SP 6	0.41	±	0.26	20.68	±	1.90	3.57	±	1.42	−15.56	±	0.45	18.95	±	4.46
SP 7	0.48	±	0.30	14.24	±	1.31	5.86	±	2.33	−16.54	±	0.48	20.46	±	4.81
SP 8	0.16	±	0.10	14.69	±	1.35	1.72	±	0.68	−14.67	±	0.42	25.54	±	6.01
RSD (%)	31.42			4.60			19.88			1.44			11.77		

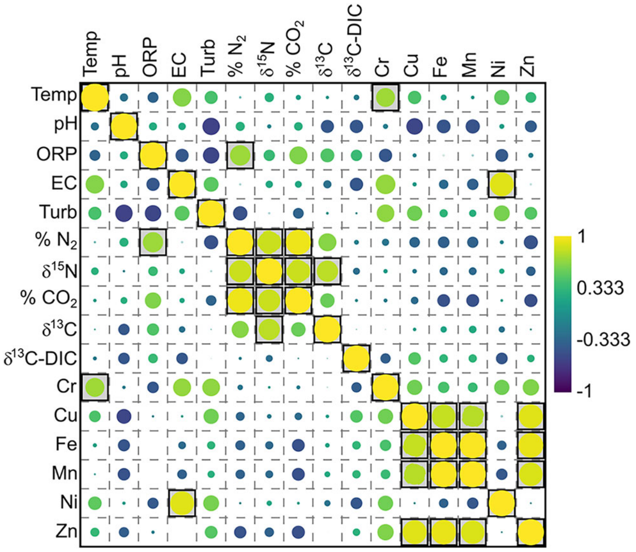

Figure 3. Pearson linear correlation plot of mean values for ancillary parameters, carbon and nitrogen elemental and isotopic measurements and metals with significant values ($p < 0.05$) correlations boxed. Metals' data from Barbieri and Sarkis (2018).

ponds is a potential pollutant for its high contents of dissolved and particulate substances and elements and also that there are substantial differences in its characteristics among the ponds.

Carbon and nitrogen elemental and isotopic measurements

The mean values of the carbon and nitrogen elemental and isotopic duplicate analyses in leachate samples are presented in Table 2. The mean measurement values are presented with the corresponding Expanded Measurement Uncertainty (U), including the uncertainty from sampling, estimated with the range statistics method (Figure 2). The complete data set of the raw measurements is included in Supplementary Material (SM 2).

The total N content of the samples, expressed as N_2 wt % ranged from 0.16 ± 0.10% in SP8 to 4.45 ± 2.80% in SP3. Apart from SP8, the other leachate ponds exhibited very high nitrogen levels if compared to the findings for leachate from Municipal Solid Waste Landfills (MSWL) which are around 0.2 to 0.3% (Chen et al., 2019; Baettker et al., 2020). The lowest C content (1.72 ± 0.68%) was also found in the samples taken from SP8 pond and the highest content (13.99 ± 5.56%), was observed in SP3 demonstrating that the C and N contents are significantly correlated as shown in Figure 3. The positive correlation between C and N contents may be related to the variations in the organic matter content of the leachate ponds.

The C/N ratios (Figure 4) show different proportions of these elements in the sampling points varying from 3.14 at SP3 to 12.09 at SP7. These ratios indicate three different groups concerning the C/N ratios: one formed by SP3, SP4 and SP5 with lower ratios (from 3.14 to 4.09), an intermediate, comprising SP2 and SP6 (8.47 and 8.78, respectively) and one with relatively higher carbon content formed by SP7 and SP8 (12.09 and 10.99, respectively). C/N ratios can reflect the source of organic matter (OM) and the degree of biological degradation of a sample as different sources may show distinct C/N signatures and the ratios are expected to decrease during decomposition (Thornton and McManus, 1994). As a result, leachate from a younger landfill shows a higher C/N ratio (Trabelsi et al., 2000). Andrews et al. (1998) reported C/N ratios of 8.7 for tanning wastes, similar levels were found at SP2 and SP6. In addition, SP6 $\delta^{13}C$ ‰ values also corroborate this predominant source as a mean value of −15.56 ‰ was found in the samples and tanning wastes' $\delta^{13}C$ lay around −15 ‰ (Andrews et al., 1998). As previously stated, the predominant refuse deposited on the landfill is wet-blue tanning wastes, but the findings at SP6 point to a higher contribution of material more recently disposed.

The total carbon content of samples expressed as CO_2 wt % varied from 1.72 ± 0.68% at SP8 to 13.99 ± 5.56% at SP3, similarly to total nitrogen

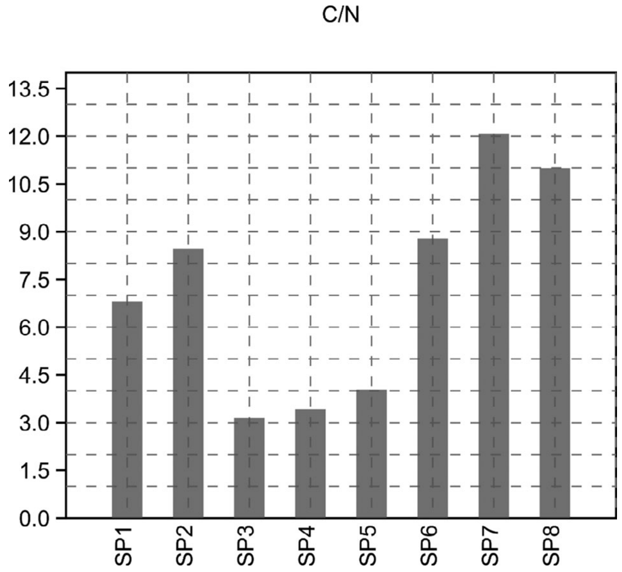

Figure 4. C/N ratios of the leachate in the sampling sites.

content as they are significantly positively correlated, and indicating that SP 8 leachate has relatively lower OM content.

All leachate samples were highly enriched in δ^{15}N with δ^{15}N‰ values ranging from 9.19 ± 0.85 ‰ in SP1 to 53.20 ± 4.89 ‰ in SP5. δ^{15}N showed significant positive correlation with the amount of N in the samples as shown in Figure 3. This may indicate that the majority of the N of the leachate samples, especially those more enriched in the heavy isotope, is ammoniacal nitrogen. Nitrogen in leachate is observed mostly in the form of ammonium, with an extreme case of 8400 mg/L and no matter the source of leachate, the ratio NH_4-N to T-N is high, varying between 0.7 and 0.85, and the concentrations of nitrate (NO_3-N), and nitrite (NO_2-N) are very low (Trabelsi et al., 2000). Source δ^{15}N signatures are altered through kinetic isotope fractionation effects that occur in biogeochemical processes such as ammonification resulting in the enrichment of the precursor substrates with the heavy isotope (Owens, 1987). Oxidizing conditions in the landfill due to the rainfall input, as is the case in the studied landfill since the refuse cells are uncapped, may cause volatilization leaving the remaining NH_{4+} highly enriched in ^{15}N (North et al., 2004). Therefore, δ^{15}N compositions alone may reflect the biogenic alteration that the nitrogenated material went through more than its provenance (Thornton and McManus, 1994). Even though the source fingerprints of the parent material may be missing due to the biogeochemical processes that the refuse goes through in the landfills along time, the leachate is very enriched in ^{15}N which makes it a potential tracer for landfill leachate pollution in surface or groundwater.

The bulk δ^{13}C ‰ values were depleted in the heavy isotope and showed a relatively narrow variation among the sampling sites ranging from −13.53 ± 0.39 ‰ in SP5 to −16.92 ± 0.49 ‰ in SP2. The sampling site SP2 contained a fresher leachate with daily inputs from the leachate collection systems, so the relative higher abundance of the light isotope in these samples may be related to higher contents of degrading organic matter, which typically has a δ^{13}C of −20 to −30 ‰ (Haarstad et al., 2013). Andrews et al. (1998) reported a value of −14.69‰ for the Particulate Organic Matter (POM) δ^{13}Corg‰. The δ^{13}C ‰ values found in this study comprise the organic and inorganic carbon in the dissolved and particulate fractions of the leachate samples. The bulk δ^{13}C ‰ values presented a significant positive correlation with δ^{15}N, which, in turn, was significantly correlated with %N_2 which is probably dominated by NH_4+ species as previously stated. Haarstad and Maehlum (2013) found a correlation between NH_4+ and δ^{13}C.

The inorganic carbon in the dissolved fraction of the leachate samples (δ^{13}C-DIC) is highly enriched in the heavy isotope in the majority of the sampling points (minimum 16.15 ± 3.8‰ in SP2 and maximum 26.78 ± 6.30‰ in SP5) with the exception of SP4, where a value slightly depleted in ^{13}C was found (−2.63 ± 0.62‰). SP4 was a treated effluent pond where lime based additives could have been added to optimize the pH and/or chloride removal. Another possibility is that the relatively low ^{13}C in SP4 was due to input of leachate from more recently disposed refuse that did not undergo the methanogenic phase. Wimmer et al. (2013) report that during the initial aerobic phase of the landfill, δ^{13}C-DIC values are between −20 and −25‰.

The high enrichment in ^{13}C of the leachate δ^{13}C-DIC is reported by Del Rey et al. (2020) that found average δ^{13}C-DIC value of 13.3‰ in leachate from MSW landfill, while Engelmann et al. (2018) found a maximum δ^{13}C-DIC value of 23.9 ‰, still lower than the values found at SP5. Other authors also report positive δ^{13}C-DIC values above 10 ‰ (North et al., 2006; Wimmer et al., 2013).

The RSD for carbon and nitrogen elemental measurements was 19.88 and 31.42%, respectively, and their expanded uncertainties correspondingly 39.76 and 62.84%. Despite the high uncertainty from these measurements the variations between sites were important enough to be observed as demonstrated in Figure 5. The total carbon and nitrogen content

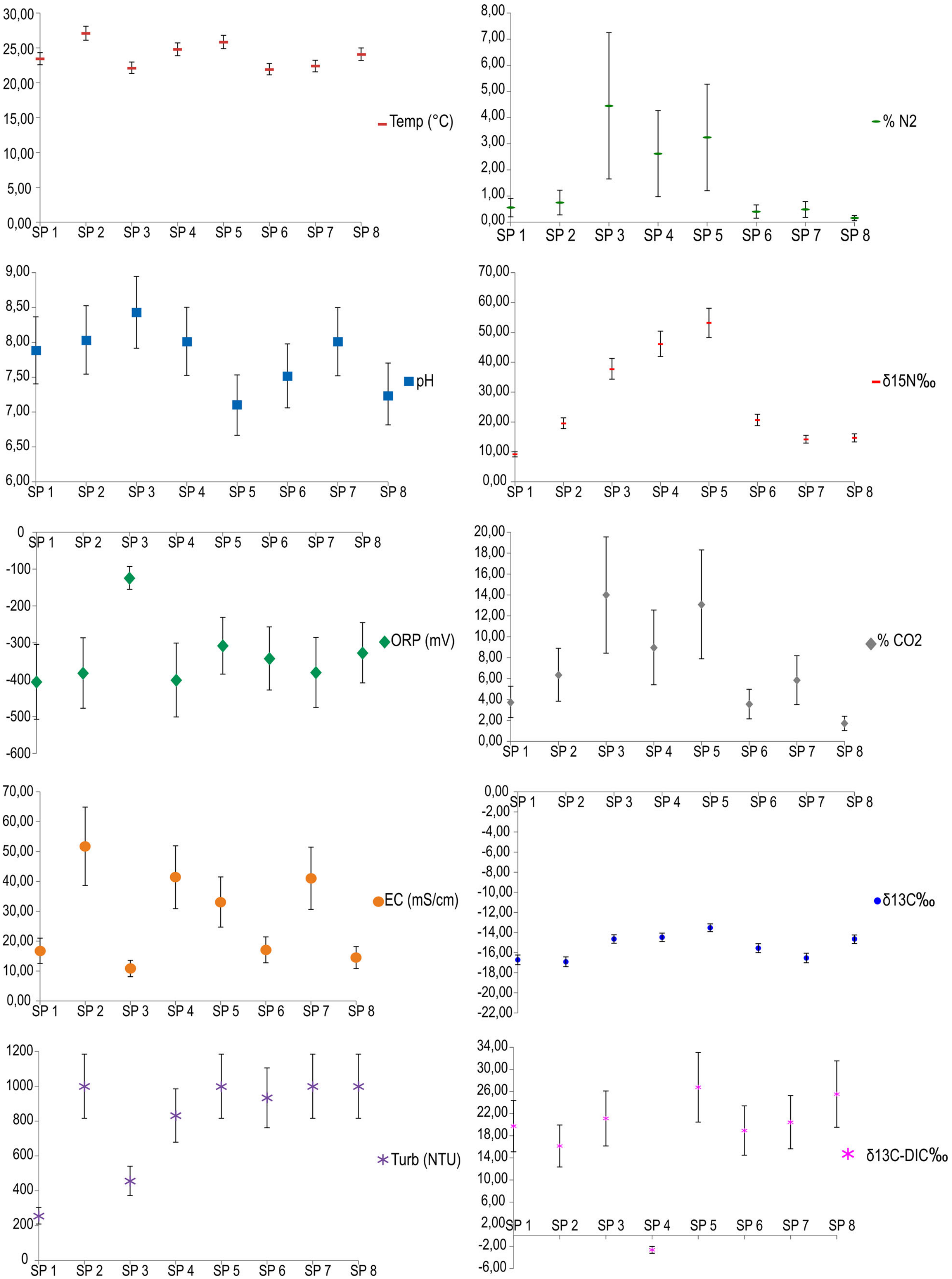

Figure 5. Physicochemical parameters and carbon and nitrogen elemental and isotopic mean values at the sampling points reporting the expanded uncertainty (U) as error bars.

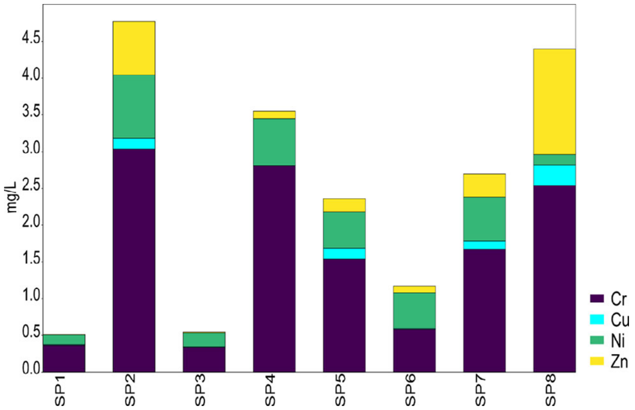

Figure 6. Stacked chart of mean metal concentration in leachate samples from ponds SP1 to SP8. Cr values displayed are 10% of the actual concentration for better visualization (data from Barbieri and Sarkis, 2018).

included the particulate fractions, of which the leachate has very elevated levels, and a heterogeneous distribution in the aqueous matrix that can increase the uncertainty from sampling for these elemental analyses. The isotopic ratio analyses displayed lower RSDs; only δ^{13}C-DIC analysis U was slightly above 20%, reaching 23.54%. The expanded uncertainty of δ^{13}C and δ^{15}N analyses were both below 10% (2.88 and 9.2%) meaning that both sampling and analysis components showed low levels of uncertainty.

The parameters measured in leachate for this study are not regulated for environmental quality compliance, but are useful to a more thorough understanding of the environmental conditions and for the proper interpretation of the targeted data with legislation thresholds. However, even for these data it is relevant to be aware of the measurement uncertainty i.e. the range of values within which the true value of the measurand might lie (Figure 5), to ponder the reliability of the information they provide. When this range is too wide, additional information may be required. Also, the quality of the values is determined by the size of the intrinsic uncertainty of the measurement. The "Appropriate Sampling for Optimized Measurement" (ASOM) approach (Ramsey, 2016), considers the whole measurement process, including the primary sampling, but is not limited to it. The author argues that the advantages of this approach is particularly evident when it is applied to in situ measurement techniques, as was the case of the physicochemical ancillary parameters, since this type of measurement integrates the steps of both sampling and analysis into one measurement process. He continues stating that only the top-down empirical approach, as the duplicate method used here, can estimate the effects of all types of sampling "errors" on measurement uncertainty, including the effects of those errors that cannot be quantified separately. The leachate is a highly heterogeneous media and this, consequently, introduces an important random error in the primary sampling. In Figure 5 it can be seen in ORP measurements, for example, that apart from SP3, the sampling points range of certainty of the measurements practically overlap, therefore their distinction based solely on this parameter is not sound. On the other hand, it is possible to verify that SP3 is consistently distinct from the other ponds based on ORP measurements as well as that SP1 and SP3 are reliably different from the other ponds based on Electrical Conductivity and Turbidity. δ^{13}C and δ^{15}N analyses, which showed narrower ranges of uncertainty, allowed the differentiation of most ponds from each other. This is an important finding from the forensic point of view, because demonstrates a possibility of tracking a suspect spill to a specific pond in this case.

The leachate of this landfill was the pollution source in an important environmental crime that took place in Rio Grande do Sul State, Brazil that led to a mass fish death in Sinos River. Augustin and Viero (2012) reported that chemical analytical data show chloride, ammonia nitrogen and chromium as the main pollutants from the landfill. The importance of Cr as the main metal pollutant related to the landfill leachate can be observed in Figure 6. Although the high pollution potential of the leachate regarding Cr is clear, there were other tanneries that discharged its Cr enriched effluents in the same stream and, therefore, the attribution of the responsibility for the pollution to the HWL could not rely only in metal analyses.

The landfill's infrastructure does not comply to regulated technical standards; it does not present correct sealing of the bottom and the cells are uncapped (Augustin and Viero, 2012). As a result of these malpractices, it is expected that the underlying leachate collection systems mix the drainages from different cells with different types of residues and ages explaining the differences in the different ponds concerning physicochemical parameters, carbon and nitrogen elemental and isotopic measurements and metals as demonstrated in Figure 5.

The heterogeneity of the leachate composition makes it difficult to assign a signature for this potential pollutant based on C/N ratios and/or metal content ratios, because they can vary a great deal with the type and age of the refuse. Nevertheless, δ^{13}C and δ^{15}N analyses have been increasingly demonstrating to be useful in identifying leachate discharges because of their distinctive highly enriched in the heavier C and N isotopes characteristic (North et al., 2006; Engelmann et al., 2018; Del Rey et al., 2020) and, additionally, due to the relatively narrow uncertainty range of these measurements. This assures the importance of these analyses as tools for landfill leachate pollution source identification in highly impacted environments where other types of discharges occur.

Conclusions

In the environmental crime case that involved the HWL, isotopic analyses were not performed, which jeopardized the proper assessment, investigation and judgement since there were similar sources of metal contamination in the surroundings.

The hazardous waste landfill leachate, a potential pollutant and the pollution source in the mentioned case, was characterized by its isotopic signature (δ^{13}C, δ^{15}N, and δ^{13}C-DIC) and physicochemical parameters in different leachate ponds of the HWL and a distinctive signature, highly enriched in the heavier isotopes, was found. δ^{13}C and δ^{15}N analyses in the impacted area could have enhanced the robustness of the forensic evidence and even allowed source apportionment of the major contaminants responsible for the massive fish death that would have supported determinations of responsibilities and application of penalties for the perpetrators.

The uncertainty of the measurements, including the sampling component disclosed the “range of certainty” where the results of the analysis and measurements of this study lie and was reported with the measurements’ results as expanded uncertainty, with 95% confidence. This is relevant information for environmental investigations, especially forensic ones, and key to the correct interpretation of the results from environmental samples where there is often substantial heterogeneity, especially at polluted sites. Most expanded uncertainties were below or slightly above 20% which can be considered fit-for-purpose for a great deal of environmental surveys related to environmental forensics.

The implementation of the Forensic Metrology approach illustrates that often different measurement data can be, in fact, undistinguishable when considered the media heterogeneity posing a problem to their proper interpretation. The top-down method using range statistics employed is a practical strategy that aggregates quality and reliability to environmental assessments.

Availability of data and material

All data used and analyzed in this study are available from the corresponding author on reasonable request.

Authors’ contributions

Cristina Barazetti Barbieri: conceptualization, investigation, experiments, formal analysis, methodology, writing-original draft. Jorge Eduardo de Souza Sarkis: supervision, writing-review. Luis Frederico Rodrigues: methodology, analysis, writing-review.

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ORCID

Cristina Barazetti Barbieri http://orcid.org/0000-0003-3747-9740
J. E. S. Sarkis http://orcid.org/0000-0002-6745-8185
L. F. Rodrigues http://orcid.org/0000-0001-5960-4843

References

Abu-Elala, N. M., Abd-Elsalam, R. M., Marouf, S., Abdelaziz, M., and Moustafa, M. 2016. Eutrophication, ammonia intoxication, and infectious diseases: Interdisciplinary factors of mass mortalities in cultured Nile tilapia. *Journal of Aquatic Animal Health* 28(3): 187–198.

Andrews, J. E., Greenaway, A. M., and Dennis, P. F. 1998. Combined carbon isotope and C/N ratios as indicators of source and fate of organic matter in a poorly flushed, tropical estuary: Hunts Bay, Kingston Harbour, Jamaica. *Estuarine, Coastal and Shelf Science* 46(5): 743–756.

Atekwana, E. A., and Krishnamurthy, R. V. 1998. Seasonal variations of dissolved inorganic carbon and δ13C of surface waters: Application of a modified gas evolution technique. *Journal of Hydrology* 205:265–278.

Augustin, P. V., and Viero, A. P. 2012. Environmental impact and geochemical behavior of soil contaminants from an industrial waste landfill in Southern Brazil. *Environmental Earth Sciences* 67(5):1521–1530.

Baettker, E. C., Kozak, C., Knapik, H. C., and Aisse, M. M. 2020. Applicability of conventional and non-conventional parameters for municipal landfill leachate characterization. *Chemosphere* 251:126414.

Barbieri, C. B., and Sarkis, J. E. S. 2018. Estimating the uncertainty from sampling in pollution crime investigation: The importance of metrology in the forensic interpretation of environmental data. Forensic Science International *288*:14–22.

Chen, R., Teng, Y., Chen, H., Hu, B., and Yue, W. 2019. Groundwater pollution and risk assessment based on source apportionment in a typical cold agricultural region in Northeastern China. *The Science of the Total Environment* 696(19):1–12.

Coplen, T. B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Communications in Mass Spectrometry: RCM* 25(17):2538–2560.

Del Rey, G. O., Santos, R. V., Soares da Cunha, L., and Ferreira, G. S. 2020. Seasonal variations of geochemical parameters for a tropical landfill: Implications for landfill stabilization. *Applied Geochemistry* 121:104686.

Eddy, F. B. 2005. Ammonia in estuaries and effects on fish. *Journal of Fish Biology* 67(6):1495–1513.

Engelmann, P. M., dos Santos, V. H. J. M., Barbieri, C. B., Augustin, A. H., Ketzer, J. M. M., and Rodrigues, L. F. 2018. Environmental monitoring of a landfill area through the application of carbon stable isotopes, chemical parameters and multivariate analysis. Waste Management *76*:591–605.

Fleming, I. R., Rowe, R. K., and Cullimore, D. R. 1999. Field observations of clogging in a landfill leachate collection system. *Canadian Geotechnical Journal* 36(4): 685–707.

Gautam, P., Kumar, S., and Lokhandwala, S. 2019. Advanced oxidation processes for treatment of leachate from hazardous waste landfill: A critical review. *Journal of Cleaner Production* 237():1–14. https://doi.org/10.1016/j.jclepro.2019.117639.

Haarstad, K., and Maehlum, T. 2013. Tracing solid waste leachate in groundwater using δ13 C from dissolved inorganic carbon. *Isotopes in Environmental and Health Studies* 49(1):48–61.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9.

Han, Z., Ma, H., Shi, G., He, L., Wei, L., and Shi, Q. 2016. A review of groundwater contamination near municipal solid waste landfill sites in China. *Science of the Total Environment* 570(1):1255–1264.

Imwinkelried, E. J. 2012. Forensic metrology : The new honesty about the uncertainty of measurements in scientific analysis. Research Paper No. 317. Davis, CA. Available at: https://ssrn.com/abstract=2186247.

Kangur, K., Kangur, A., Kangur, P., and Laugaste, R. 2005. Fish kill in Lake Peipsi in summer 2002 as a synergistic effect of cyanobacterial bloom, high temperature and low water level. *Proceedings of the Estonian Academy of Sciences. Biology Ecology* 54 (1):67–80.

Leite, V. J., de Oliveira, E. C., and Aucélio, R. Q. 2021. Impact of the sampling process on the measurement uncertainty, a case study: Physicochemical parameters in diesel. *Accreditation and Quality Assurance* 26(1):1–9.

Lisk, D. J. 1991. Environmental effects of landfills. *Science of the Total Environment* 100:415–468. https://doi.org/10.1016/0048-9697(91)90387-T.

Magnusson, B., Krysell, M., Sahlin, E., and Näykki, T. 2020. Uncertainty from sampling. Nordtest Report TR 604 (2nd) 2020, ISBN 978-91-89167-31-5. Available from www.nordtest.info.

Mohammadzadeh, H., Clark, I., Aravena, R., Bourbonnais, A., and Middlestead, P. 2006. Isotopic analysis of ammonium (δ15N), nitrate (δ18O & δ15N) and dissolved carbon (δ13C) in landfill leachate plume. *Environmental Science & Technology Proceedings II* 2(3): 145–150.

North, J., Frew, R., and Hale, R. 2006. Can stable isotopes be used to monitor landfill leachate impact on surface waters? *Journal of Geochemical Exploration* 88(1–3): 49–53.

North, J. C., Frew, R. D., and Peake, B. M. 2004. The use of carbon and nitrogen isotope ratios to identify landfill leachate contamination: Green Island Landfill, Dunedin, New Zealand. *Environment International* 30: 631–637.

Owens, N. J. P. 1987. Natural variations in 15N in the marine environment. *Advances in Marine Biology* 24(C):389–451. https://doi.org/10.1016/S0065-2881(08)60077-2.

Raja, S., Khalifa, U., Ebenezer, V., Kumar, A., Sanjeevi, P., and Murugesan, M. 2019. Mass mortality of fish and water quality assessment in the Tropical Adyar Estuary, South India. *Environmental Monitoring and Assessment* 191(8):512–524.

Ramsey, M. H. 1998. Sampling as a source of measurement uncertainty: Techniques for quantification and comparison with analytical sources. *Journal of Analytical Atomic Spectrometry* 13: 97–104.

Ramsey, M. H. 2002. Appropriate rather than representative sampling, based on acceptable levels of uncertainty. *Accreditation and Quality Assurance* 7:274–280. https://doi.org/10.1007/978-3-662-05173-3_30

Ramsey, M. H. 2016. Appropriate sampling for optimised measurement (ASOM), rather than the theory of sampling (TOS) approach, to ensure suitable measurement quality: A refutation of Esbensen and Wagner (2014). *Geostandards and Geoanalytical Research* 40(4): 571–581.

Ramsey, M. H., Rostron, P., and Ellison, S. L. R. 2019. *Eurachem/EUROLAB/CITAC/Nordtest/AMC guide: Measurement uncertainty arising from sampling: A guide to methods and approach*, 2nd Ed. Available at: https://www.eurachem.org/images/stories/Guides/pdf/UfS_2019_EN_P2.pdf

Thornton, S. F., and McManus, J. 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science* 38(3): 219–233.

Trabelsi, I., Horibe, H., Tanaka, N., and Matsuto, T. 2000. Origin of low carbon/nitrogen ratios in leachate from old municipal solid waste landfills. *Waste Management and Research* 18(3):224–234.

Vosk, T. 2016. Forensic metrology: Its importance and evolution in the United States. *Journal of Physics: Conference Series* 772:012018. https://doi.org/10.1088/1742-6596/772/1/012018.

Wimmer, B., Hrad, M., Huber-Humer, M., Watzinger, A., Wyhlidal, S., and Reichenauer, T. G. 2013. Stable isotope signatures for characterising the biological stability of landfilled municipal solid waste. *Waste Management (New York, N.Y.)* 33(10):2083–2090.

Xu, Y., Xue, X., Dong, L., Nai, C., Liu, Y., and Huang, Q. 2018. Long-term dynamics of leachate production, leakage from hazardous waste landfill sites and the impact on groundwater quality and human health. *Waste Management (New York, N.Y.)* 82:156–166.

Ya, X., Xiangshan, X., Lu, D., Changxin, N., Yuqiang, L., and Qifei, H. 2018. Long-term dynamics of leachate production, leakage from hazardous waste landfill sites and the impact on groundwater quality and human health. Waste Manag*ement* 82:156–166. https://doi.org/10.1016/j.wasman.2018.10.009

Ziyang, L., Youcai, Z., Tao, Y., Yu, S., Huili, C., Nanwen, Z., et al. 2009. Natural attenuation and characterization of contaminants composition in landfill leachate under different disposing ages. *Science of the Total Environment* 407(10):3385–3391.