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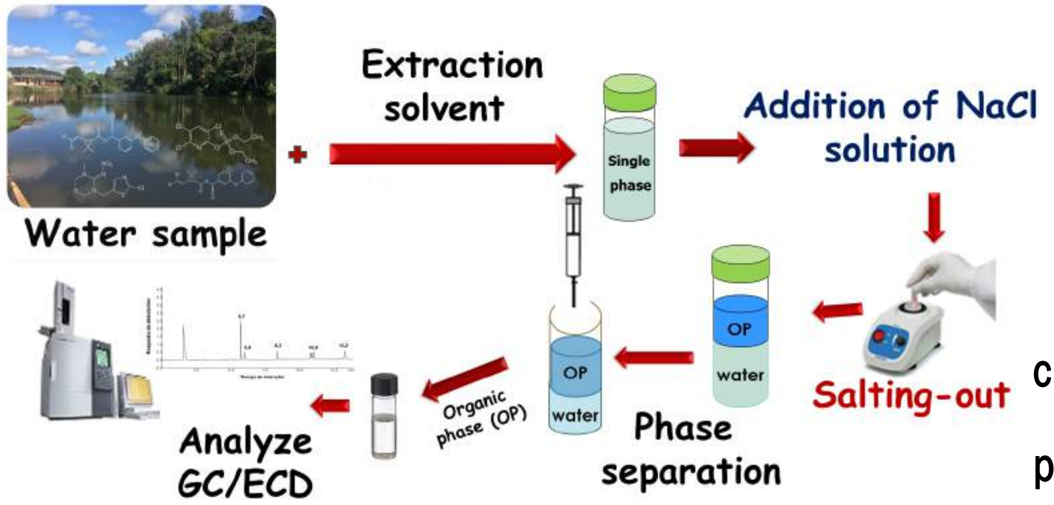

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Editorial

VIII Brazilian Meeting of Environmental Chemistry (ENQAmb)

The Brazilian Meeting of Environmental Chemistry (ENQAmb) has been consolidated as a biannual event and it is the main national forum for scientific and academic discussions focused on the relationship between chemistry and environment.

The first edition of the ENQAmb was organized by researchers from the Institute of Chemistry of the State University of Campinas (IQ-Unicamp) and was performed in 1990 in Campinas (SP). After the first meeting, the Division of Environmental Chemistry was consolidated in the Brazilian Society of Chemistry (SBQ) including a significant number of researchers involved in environmental issues. In 2003 the second edition of ENQAmb was performed in Brasília (DF) with support of the Division of Environmental Chemistry. There was a significant number of abstracts submitted suggesting the need of periodic continuity of the meeting. Since 2006 the ENQAmb has been performed biannually.

The eighth edition of ENQAmb was realized from 3 to 6 October of 2017 in Federal Technological University of Parana, Curitiba Campus. The thematic chosen was: "The chemical and environmental challenges in today's society" and the main goal of the event was provided a forum of interactions between researchers from academia and industry presenting their research results and activities. This meeting was organized by Federal Technological University of Parana (UTFPR), Federal University of Paraná (UFPR); State University of Ponta Grossa (UEPG) and the Swedish Meteorological and Hydrological Institute (SMHI).

The meeting was the most important edition of this event considering the significant number of inscriptions and abstract submitted. The scientific program of the 8th edition of ENQAmb was composed by national and international speakers. There were 245 inscriptions, 221 papers presented, being 146 abstracts and 75 complete abstracts. Additionally, for the first time the meeting offered the opportunity for researchers publishing their results in Eclética Química Journal.

Profa. Dra. Erika Pereira Felix (Guest Editor)
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Editor's note

The articles published in this special issue went through all the standard procedures used by the Eclética Química Journal and fulfilled all the qualitative requirements of selection, peer review and editing.

The readers can see the papers cover the Environmental Chemistry area, in line with the editorial profile of EQJ, with the following subjects: salting-out assisted liquid-liquid extraction method; micropollutants in surface waters; removal of toxic metal ions from water; interaction of nanoparticles with metals in the presence of humic substances; total and labile metals in surface sediments; degradation of dye effluent by photo-Fenton process and determination of lead in stools of animals.

Prof. Dr. Assis Vicente Benedetti
Editor-in-Chief

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[2] Wendlant, W. W., Thermal Analysis, Wiley-Interscience, New York, 3rd ed., 1986, ch1.

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[3] Ferreira, A. A. P., Uliana, C. V., Souza Castilho, M. de, Canaverolo Pesquero, N., Foguel, N. V., Pilon dos Santos, G., Fugivara, C. S., Benedetti, A. V., Yamanaka, H., Amperometric Biosensor for Diagnosis of Disease, In: State of the Art in Biosensors - Environmental and Medical Applications, Rinken, T., ed., InTech: Rijeka, Croatia, 2013, Ch. 12.

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SUMMARY

ORIGINAL ARTICLES

Eclética Química Journal

Original article
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Optimization and validation of the salting-out assisted liquid-liquid extraction method and analysis by gas chromatography to determine pesticides in water

Alessandra Aparecida Zinato Rodrigues[1], Antônio Augusto Neves[1], Maria Eliana Lopes Ribeiro de Queiroz[1], André Fernando de Oliveira[1], Lucas Henrique Figueiredo Prates[2], Elisa Helena da Costa Morais[1]

[1] Federal University of Viçosa (UFV), Center of Exact and Technological Sciences, Peter Henry Rolfs Av, Viçosa, Minas Gerais, Brazil
[2] Federal University of Viçosa (UFV), Center of Agricultural Sciences, Peter Henry Rolfs Av, Viçosa, Minas Gerais, Brazil

+Corresponding author: Maria Eliana Lopes Ribeiro de Queiroz, e-mail address: meliana@ufv.br

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ABSTRACT: The salting-out assisted liquid-liquid extraction (SALLE) was developed for the analysis of four pesticides (cypermethrin, chlorpyrifos, deltamethrin, and thiamethoxam) in water samples. For its optimization, a 2^3 factorial design was used to evaluate the simultaneous behavior of three factors: sample and extraction solvent ratio, saline concentration, and stirring mode. 1.0 mL saline solution (NaCl) was added to the single-phase mixture of water and extraction solvent to separate the phases. The mixture was stirred, allowed to stand and the top organic phase containing the pesticides of interest was analyzed by gas chromatography coupled with electron capture detector (GC/ECD). The optimized method was validated for some merit figures. The limit of detection of the method ranged from 0.15 to 5.5 μg L^{-1} and the limit of quantification from 5.5 to 18.2 μg L^{-1}. The method showed satisfactory results for the linearity ($R^2 \geq 0.990$), precision (CV < 9 %) and accuracy (72 % ≤ recovery ≤ 90 %) for all the assessed analytes. The method showed to be simple, efficient and inexpensive for the extraction of pesticide residues. The validated method was applied to ten samples of water collected in the Zona da Mata region of the state of Minas Gerais, Brazil.

1. Introduction

Indiscriminate use and improper application of pesticides can generate residues of potentially toxic substances in the environmental compartments: soil, water, and air, as well as food, thus causing serious problems to the environment and to human health[1, 2, 3].

The use of pesticides is the second largest cause of contamination of rivers in Brazil, second only to domestic sewage[4]. Contamination of water by pesticides can occur through the transmission of aerial sprays, through the erosion of contaminated soils, surface runoff, leaching, and the disposal and washing of tanks and product packaging[5, 6].

Therefore, special attention should be given to drinking water, as daily consumption of water contaminated with pesticide residues can cause toxic effects such as cancer and damage to the central nervous system, depending on the type of compound and the amount ingested. In Brazil, the norms on the drinking water potability and quality for human consumption are established by the Ministry of Health in which allowed values are presented for microbiological, organoleptic, physical and chemical characteristics[7].

Pesticide residues analyses in water samples are difficult to be executed since these compounds have very different physicochemical properties and occur at extremely low concentrations in the presence of high concentrations of interfering compounds[8].

The first step for analysis and monitoring of pesticide residues in water involves the development of an extraction technique. The goal of this step is to remove selectively the pesticide residues from the samples and promote their pre-concentration[9]. Thus, techniques for the determination of pesticide residues in different matrices have significantly evolved in simplification, improvement of extraction and purification of samples, with the main objectives of minimizing sample and solvent consumption[10].

Several studies in the literature reported the determination of pesticide residues in water using the techniques of sample preparation solid-phase extraction – SPE[11]; solid-phase microextraction – SPME[1, 12]; liquid–liquid extraction with low temperature partitioning - LLE-LTP[13, 14]; liquid phase microextraction – LPME[15, 16]; dispersive liquid–liquid microextraction – DLLME[17, 18]; single drop microextraction – SDME[19]. Despite the high number of existing techniques, the salting-out assisted liquid-liquid extraction (SALLE) technique emerged as a new alternative for the extraction of pesticide residues in water based on its advantages of simplicity of operation, rapidity (brief time for phase separation), low volume of both sample and solvent, low cost, safety and good sensitivity. In addition, sample preparation is the largest source of errors in trace analysis, thus a rapid and simple extraction step improves the method as a whole.

The extraction technique is based on the partitioning of the compounds between the initially miscible aqueous and organic phases, which are separated by the addition of salts. This behavior can be explained in terms of solvation of the ions by the water molecules, facilitating the migration of pesticides to the organic phase. This salt addition effect is known as salting-out and may cause a decrease in the solubility of certain analytes in the aqueous phase, in favor of the extraction[20, 21].

After the extraction step, the determination of the pesticides residues on several matrices is traditionally carried out by chromatographic techniques due to the ease of separation, identification, and quantification of the substances present in the sample. Furthermore, gas chromatography is specially used for pesticide residues determination due to the low limits of detection that can be achieved[22].

In Brazil some pesticides widely used for pest control belong to chemical groups of organophosphates, pyrethroids and neonicotinoids. In this context, the aim of this study was to optimize and validate the salting-out assisted liquid-liquid extraction (SALLE) technique followed by gas chromatography analysis (SALLE – GC/ECD) for the determination of four pesticides belonging to these chemical groups (Figure 1) in water. The proposed method was applied to 10 samples of water collected in the Zona da Mata of Minas Gerais, Brazil.

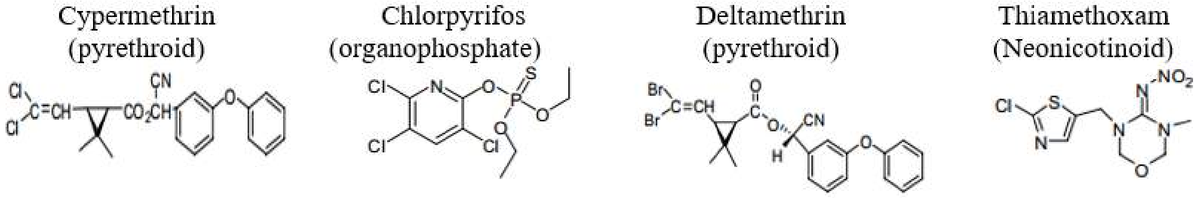

Figure 1. Chemical groups and structures of the pesticides cypermethrin, chlorpyrifos, deltamethrin, and thiamethoxam.

2. Experimental

2.1 Chemicals and reagents

Analytical standards of chlorpyrifos (99.0 % w/w), cypermethrin (92.4 % m/m), and deltamethrin (99.0 % w/w) were purchased from Chem Service (West Chester, PA, USA), thiamethoxam (99.7 % w/w) was purchased from Sigma Aldrich (Germany) and the bifenthrin (92.2 % w/w), used as internal standard, was purchased from FMC do Brasil. Stock solutions of 500.0 mg L^{-1} of all analytical standards in acetonitrile (Mallinckrodt/ HPLC) were prepared and stored in freezer. Diluted solutions were prepared from the stock solutions to obtain the working solutions at different concentrations with the four pesticides. Sodium chloride (Reagen – P.A. 99.5 % w/w) solutions were used to break the equilibrium (salting-out effect).

2.2 Chromatographic conditions

Identification and quantification of the pesticides extracted from the samples were done with a gas chromatograph equipped with an electron capture detector system (GC/ECD) (Shimadzu GC-2014, Kyoto, Japan), and autoinjector AOC – 20i. The separations were performed on an HP-5 capillary column, 30 m long, 0.25 mm inner diameter, and 0.10 µm film thickness (Agilent Technologies, Palo Alto, CA, USA), with the stationary phase consisting of phenyl 5 % and dimethylsiloxane 95 %. Nitrogen (99.999 %, Air Products, Brazil) was used as carrier gas at 1.2 mL min^{-1}. Injector and detector temperatures were set at 280 and 300 °C, respectively. The initial column oven temperature was 150 °C, with a heating rate of 20 °C min^{-1} up to 250 °C, and 10 °C min^{-1} up to 290 °C maintained for 5 min. The injected volume was 1 µL at a split ratio of 1:5. The total running time was 14 min.

2.3 Fortified samples

Samples of distilled water (pesticides free) fortified with the working solutions containing the four pesticides were used in different concentrations for the optimization and validation of the method. Fortified water samples were allowed to stand for 3 h, at room temperature for solvent evaporation, prior to use. The technique was submitted to a multivariate optimization with the evaluation of three parameters (i) volume ratio of sample: extraction solution, (ii) saline solution concentration, and (iii) stirring mode.

2.4 Experimental design

The salting-out assisted liquid-liquid extraction (SALLE) method was optimized using a factorial design 2^3 to evaluate the influence of the variables on the extraction yield of the four pesticides. The following factors were evaluated: ratio between the water volume and the volume of the solvent extractor, acetonitrile and ethyl acetate, (4.5/6.5 or 3.0/6.0 mL); sodium chloride solution concentration (1.0 or 2.0 mol L^{-1} of NaCl); stirring mode (40 s at the vortex or 10 min at the orbital shaker with 175 rpm and 25 °C). The data corresponding to the (−) and (+) levels of factorial design are shown in Table 1. The tests in duplicate allowed to calculate the average recovery percentages of pesticides (Table 1). The levels of the three factors used in factorial planning were established in previous experiments.

After stirring, the solutions were allowed to stand and the upper organic phase containing the analytes of interest was withdrawn with a volumetric pipet. The volumes of the extracts were adjusted with acetonitrile in a 5.0 mL volumetric flask containing 100 µL of bifenthrin solution at 5.0 mg L^{-1} (internal standard). The extracts were analyzed by gas chromatography coupled to an electron capture detector (CG). The best conditions of the factorial design were evaluated according to the chromatographic responses (areas) obtained in each experiment. The obtained data were analyzed using the Excel® and OriginPro®.

Table 1. Factorial design 2³ and evaluated factors: (F1) ratio between the water and extraction solvent volume, (F2) sodium chloride solution concentration, (F3) stirring mode.

	Coded factors			Real factors		
Experiment	F(1)	F(2)	F(3)	(1) H_2O:solvent ratio (v/v)	(2) Saline solution conc. (mol L^{-1})	(3) Stirring mode
1 and 2	-	-	-	4.5/5.0/1.5 (A)	1.00	Vortex (40 s)
3 and 4	+	-	-	3.0/5.0/1.0 (B)	1.00	Vortex (40 s)
5 and 6	-	+	-	4.5/5.0/1.5 (A)	2.00	Vortex (40 s)
7 and 8	+	+	-	3.0/5.0/1.0 (B)	2.00	Vortex (40 s)
9 and 10	-	-	+	4.5/5.0/1.5 (A)	1.00	Orbital shaker (10 min)
11 and 12	+	-	+	3.0/5.0/1.0 (B)	1.00	Orbital shaker (10 min)
13 and 14	-	+	+	4.5/5.0/1.5 (A)	2.00	Orbital shaker (10 min)
15 and 16	+	+	+	3.0/5.0/1.0 (B)	2.00	Orbital shaker (10 min)

F1 = Ratio v/v (water:acetonitrile:ethyl acetate).

2.5 Method validation

The validation of the optimized SALLE method for pesticide residue determination in water was assessed with the following merit parameters: selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision), and accuracy (recovery and methods comparison). The validation procedures followed the recommendations of the International Conference on Harmonization (ICH)[23], the Brazilian Health Regulatory Agency (ANVISA)[24], and the National Institute of Metrology, Quality and Technology (INMETRO)[25].

2.6 SALLE application in water samples

The optimized and validated SALLE method for determination of pesticides in water was applied in eight samples of water collected in rivers at the Zona da Mata of Minas Gerais State. The sampling was done at the municipalities of Manhuaçu, Matipó, Raul Soares and Rio Casca, where there is a major planting of coffee and citrus fruits. Also, two samples of tap water were taken from the water distribution network of the water treatment station of the Universidade Federal de Viçosa (municipality of Viçosa, Minas Gerais, Brazil).

3. Results and discussion

3.1 Chromatographic analysis

The chromatogram of the standard solution with the four pesticides at 100 µgL^{-1} in acetonitrile is presented in Figure 2. The third peak, with retention time (R_t) of 8.3 min, corresponds to the internal standard, bifenthrin. The multiple peaks observed at the chromatogram are related to the conversion to isomers of the pyrethroids (cypermethrin and deltamethrin) in the chromatograph injector. The quantification of these two pyrethroids was done considering the total area of both peaks of the isomers, both at the standard analysis and at samples analysis.

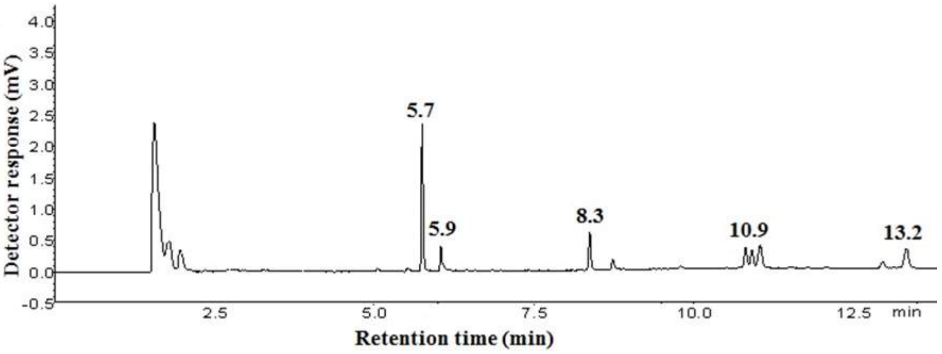

Figure 2. Chromatogram of the standard solution with the four pesticides and internal standard at 100.0 µg L^{-1} in acetonitrile, where: R_t = 5.7 min: chlorpyrifos, R_t = 5.9 min: thiamethoxam, R_t = 8.3 min: bifenthrin (IS) R_t = 10.9 min: cypermethrin, and R_t = 13.2 min: deltamethrin.

3.2 Optimization of the salting-out assisted liquid-liquid extraction method

The optimization of the extraction technique evaluated three factors simultaneously: the ratio between sample and extraction solvent volumes, the NaCl concentration, and the stirring mode of the mixture. In this evaluation, a factorial design 2^3 was conducted and the effects evaluated by recovery percentages. The tests, performed in duplicate, generated 16 responses that allowed to estimate the experimental errors associated to the determination of each mean response. Table 2 highlights the trials that obtained the best recovery percentages for the four pesticides analyzed in water samples.

Table 2. Percentages of mean recovery and estimation of the experimental error obtained in the experiments of the factorial design for water samples containing the pesticides chlorpyrifos, thiamethoxam, cypermethrin, and deltamethrin extracted by salting-out assisted liquid-liquid extraction method.

	Coded factors			Recovery (%) ± estimation of the experimental error			
Experiment	F(1)	F(2)	F(3)	Chlorpyrifos	Thiamethoxam	Cypermethrin	Deltamethrin
1 and 2	-	-	-	89 ± 8	50 ± 12	69 ± 10	72 ± 11
3 and 4	+	-	-	67 ± 6	49 ± 11	70± 8	72.1 ± 0.6
5 and 6	-	+	-	86 ± 4	67 ± 16	76 ± 9	85 ± 19
7 and 8	**+**	**+**	**-**	**88.9 ± 0.2**	**66 ± 3**	**93 ± 9**	**90 ± 5**
9 and 10	-	-	+	84 ± 7	56 ± 23	73 ± 2	63 ± 22
11 and 12	+	-	+	79 ± 1	66 ± 17	42 ± 18	57 ± 5
13 and 14	-	+	+	52 ± 7	49 ± 14	56.3 ± 0.8	57 ± 8
15 and 16	+	+	+	70 ± 3	53 ± 6	72 ± 7	81 ± 1

F1 = Ratio v/v (water:acetonitrile:ethyl acetate), F2 = NaCl concentration (mol L^{-1}), and F3 = Stirring mode.

The replicates were used to calculate the average recovery percentages, the effects of each factor and the interactions between the factors in the extraction of each of the pesticides. The calculations were done with the Excel® and OriginPro® programs. The errors associated with each effect and their interactions were evaluated by t-test for 95 % probability (α = 0.05) and 8 replicates (n = 8). Some results of the statistical analysis are presented in Table 3.

Table 3. Average recovery percentages, effects of each factor and interactions between the factors (± estimate of the experimental error) in the extraction of each of the pesticide, obtained in the factorial design experiments for water samples, by the SALLE method.

	Chlorpyrifos	Thiamethoxam	Cypermethrin	Deltamethrin
Mean recovery	76 ± 1	57 ± 3	69 ± 2	72 ± 3
(1) Ratio (H_2O:solvent)	-2 ± 3	2 ± 6	1 ± 5	6 ± 5
(2) NaCl conc.	**- 7 ± 3***	3 ± 6	11 ± 5	**12 ± 5***
(3) Stirring mode	**-12 ± 3***	-2 ± 6	**-16 ± 5***	**-15 ± 5***
(1) and (2)	**11 ± 3***	-2 ± 6	**15 ± 5***	**9 ± 5***
(1) and (3)	**7 ± 3***	4 ± 6	-8 ± 5	3 ± 5
(2) and (3)	**-16 ± 3***	-14 ± 6	-4 ± 5	-3 ± 5
(1), (2), and (3)	-1 ± 3	-1 ± 6	8 ± 5	6 ± 5

***(in bold)** Statistically significant effect at the 95 % probability level by t-test ($t_{tab} > t_{0.05} = 2.306$).

The results obtained from the factorial design showed that the main effect of the ratio between the sample and the extractive mixture (F1) did not have a significant effect on the recovery percentage. The variation of the NaCl concentration presented a significant positive effect for the extraction of deltamethrin and negative for the chlorpyrifos. The stirring mode used to homogenize the mixture had a significant negative effect on the extraction of all pesticides, except for thiamethoxam. Thus, stirring mode by vortex (F3, level -) was the most efficient for extraction of the pesticides.

According to the results obtained from the statistical analysis, tests 7 and 8 (Table 2) showed higher recovery percentages and lower mean dispersion for the analyzed pesticides. Therefore, the sample proportion ratios: extraction solvent (3.0 mL of water, 5.0 mL of acetonitrile and 1.0 mL of ethyl acetate), NaCl at 2.0 mol L^{-1} and stirring with the vortex mixer for 40 s were chosen as the best conditions.

3.3 Optimized SALLE method

The optimized method for the extraction of pesticides consists of placing 3.00 mL of the water sample, 6.0 mL of the extraction mixture (5.0 mL of acetonitrile and 1.0 mL of ethyl acetate) in transparent glass bottles (22 mL) to form a single phase. To break this equilibrium (salting-out effect) should be added 1.0 mL of NaCl solution at 2.0 mol L^{-1}. The mixture is homogenized in the vortex mixer for 40 s and kept at rest (about 1 min), forming a two-phase system. The upper organic phase is withdrawn with an automatic pipette, and its volume adjusted in volumetric flask to 5.0 mL. The extract was stored in the freezer for further analysis by gas chromatography.

3.4 Method validation

The SALLE method for determination of pesticides in water was validated and the results are adequate, according to the validation standards of several regulatory agencies. The values of the retention time of the compounds, calibration curve equation, and coefficient of determination, linear range, limit of detection, and limit of quantification for the developed method to analyze pesticides residues in water are represented in Table 4.

Table 4. Retention time (R_t), calibration curve equation, the coefficient of determination (R^2), linear range, limit of detection (LOD), and limit of quantification (LOQ) of SALLE method.

Pesticide	R_t (min)	Calibration curve equation (y = ax + b)	R^2	Linear range (µg L^{-1})	LOD (µg L^{-1})	LOQ (µg L^{-1})
Chlorpyrifos	5.7	y = 0.087x - 0.033	0.994	0.5 – 6.0	0.15	0.5
Thiamethoxam	5.9	y = 0.005x + 0.360	0.990	18 - 450	5.50	18.2
Cypermethrin	10.9	y = 0.024x + 0.813	0.992	5.0 - 60	1.50	5.0
Deltamethrin	13.2	y = 0.005x + 0.155	0.993	12 - 150	3.70	12.0

The selectivity of the method was evaluated by applying the optimized conditions in pesticide-free water samples (white). Subsequently, these samples were fortified with the pesticides under study and again submitted to the extraction and analysis method. No interferences were observed in the retention time of the studied pesticides, proving the selectivity of the method.

The limits of detection (LOD) and quantification (LOQ) of the SALLE method for the water samples were determined considering the value of three and ten times the area of the baseline signal (noise), respectively. The LOD values for the four pesticides ranged from 0.15 to 5.5 µg L^{-1}, whereas LOQ ranged from 0.5 to 18.2 µg L^{-1} (Table 4).

The LOQs found for the pyrethroids (cypermethrin and deltamethrin) are below the maximum residue limit (MRL) established by the Brazilian Ministry of Health (Portaria N° 2914/2011)[7], for permethrin, which is 20 µg L^{-1}, a pesticide of the same class. According to the Ministry of Health, the MRL allowed for chlorpyrifos in water is 30 µg L^{-1}, being much higher than the LOQ (0.5 µg L^{-1}) found for this pesticide in this study. Although the MRL for thiamethoxam is not established in Ministry of Health, the drinking standard was established for other health-risk pesticides such as mancozeb, carbendazim, diuron, alachlor, endosulfan and trifluralin, profenofos and glyphosate, which is a value $\geq$ 20 µg L^{-1}, higher than the LOQ found for this pesticide in this study.

Similar results were found by Vieira *et al.* (2007) when analyzing pyrethroids in water samples using liquid-liquid extraction with low temperature partitioning (LLE/LTP) and GC-ECD analysis. In that study, the limit of detection and quantification obtained for cypermethrin was 1.9 and 2.7 µg L^{-1} and for deltamethrin it was 2.9

and 5.5 µg L^{-1}, respectively[26]. In addition, the use of this same technique to extract pesticides in water has found LOD equal to 3.4 µg L^{-1} and LOQ equal to 10 µg L^{-1} for chlorpyrifos[14]. When analyzing the pesticides residues in pineapple samples using the SLE-LTP method, Costa Moraes *et al.* (2014) obtained the LOD equal to 15 µg kg^{-1} and LOQ equal to 50 µg L^{-1} for thiamethoxam, and LOD equal to 12 µg kg^{-1} and LOQ equal to 40 µg L^{-1} for deltamethrin[10]**.** However, for all the studied analytes the LOQs found for the proposed method are lower than others in the literature.

The linearity of the method was evaluated by an analytical curve equation obtained with eight points, by means of regression, including the first point of the analytical curve equals to the LOQ of the method for each tested pesticide. The extracts from samples that were fortified with the pesticides obtained using the SALLE method was injected and analyzed by GC-ECD. The analytical curves were made using the ratios between the areas of the analytes and the internal standard, thus obtaining regression equations and coefficients of determination. The coefficients of determination (R^2) for the analytical curves of the four pesticides were higher than 0.99. These results are shown in Table 4.

The precision was evaluated in terms of repeatability and intermediate precision and expressed by the coefficient of variation (CV) associated with the relative responses of the pesticides in each test. (Table 5). The repeatability of the method was determined in samples of water fortified with the standards of cypermethrin (30.0 µg L^{-1}), chlorpyrifos (3.0 µg L^{-1}), deltamethrin (72.0 µg L^{-1}) and thiamethoxam (110 µg L^{-1}) at the 6 x LOQ concentration of the method, with seven replicates and submitted to the SALLE method. Intermediate precision was evaluated by the same analyst, using the same instrument and on three distinct days (1st, 7th and 30th), with seven repetitions in the concentration referring to 6 x LOQ of the method (Table 5). The accuracy of the method was evaluated in triplicate by recovery assays at four concentration levels (1, 3, 6, and 12 x LOQ). Significance tests using the Student's t-test were also performed to evaluate the veracity of the method.

Table 5. Recovery and coefficient of variation of the SALLE extracted and analyzed fortified water samples.

Pesticide	Accuracy				Repeatability (Intra-day)	Intermediate precision (Inter-day)
	Recovery (%)				Coefficient of variation (%)	
	LOQ (µg L^{-1})	3 x LOQ (µg L^{-1})	6 x LOQ (µg L^{-1})	12 x LOQ (µg L^{-1})	6 x LOQ (µg L^{-1})	6 x LOQ (µg L^{-1})
Chlorpyrifos	81.3	79.7	83.1	81.4	6.8	6.4
Thiamethoxam	72.2	74.8	77.9	74.1	8.1	8.3
Cypermethrin	87.8	89.6	87.4	88.8	5.7	6.7
Deltamethrin	87.8	88.1	86.7	90.1	5.4	5.5

*LOD: limit of detection; LOQ: limit of quantification

Considering the obtained results, the proposed SALLE method for the four pesticides is simple, with good extraction efficiency (72-90 %) and coefficients of variation for intermediate accuracy and repeatability less than 9 %.

The results obtained using the SALLE method for determination of chlorpyrifos, thiamethoxam, cypermethrin and deltamethrin pesticides in water were compared with those obtained by two other methods: method 1 – liquid–liquid extraction with low temperature partitioning – LLE-LTP[26] and method 2 – liquid–liquid extraction – LLE[27]. All water samples were fortified to obtain an extract with a final concentration equal to 6 x LOQ of the equivalent method to each target analyte (Table 6).

Table 6. Recovery (%) and coefficients of variation (%) obtained from the extraction of water samples with the pesticides chlorpyrifos (3.0 µg L^{-1}), thiamethoxam (110.0 µg L^{-1}), cypermethrin (30.0 µg L^{-1}) and deltamethrin (72.0 µg L^{-1}) in triplicates using the SALLE method and two methods used as reference.

	Recovery (%) and CV (%)			
	Chlorpyrifos	Thiamethoxam	Cypermethrin	Deltamethrin
Method 1	80; 5	76; 9	87; 6	83; 5
Method 2	83; 9	79; 6	75; 5	83; 6
SALLE	86; 6	77; 8	91; 6	89; 6

Method 1 - liquid–liquid extraction with low temperature partitioning - LLE-LTP[26] and method 2- liquid–liquid extraction – LLE[27].

Considering the proximity between the results obtained by the SALLE method and methods 1 and 2, it can be concluded that both methodologies did not show large dispersion of the coefficients of variation. The recovery percentages obtained for chlorpyriphos, thiamethoxam and deltamethrin did not differ statically (95 % probability) in the three methods studied, with the exception of cypermethrin, which presented higher extraction efficiency in the SALLE method (91 %) than method 2 (75 %). When comparing method 1 with SALLE, both are efficient, using small volumes of sample and solvents. The SALLE method requires less time for phase separation (one minute) while method 2 requires 12 h for forming a two-phase system. The method 2 has the advantage of preconcentrating the analyte from water samples, approximately equal to 3 times. However, it is important to note that during this process, matrix impurities are concentrated together with the analyte. In addition, a relatively large volume of samples (25.0 mL) and solvents (135.0 mL) are required in method 2, generating greater problems of environmental contamination and risks to the health of the analyst. On the other hand, the SALLE method requires a small amount of sample (3.0 mL) and solvent (6.0 mL) for the determinations (Table 6).

The optimized and validated SALLE method was applied to samples of water collected in rivers of the Zona da Mata of the State of Minas Gerais (Brazil) and in points of the water treatment station of the Universidade Federal de Viçosa (Viçosa, Minas Gerais, Brazil). However, none of the four pesticides investigated were detected in any of the analyzed samples or were in concentrations below the limit of detection of the method. Considering that the half-life, mainly of the pyrethroids, is relatively short[26], a way to increase the probability of detecting contamination in the water resources would be collecting the samples in the period in which the application of the pesticides coincided with the rainy season, when it is expected an increase in the pesticide application.

After obtaining the results of the analysis of the water collected in the rivers of the Zona da Mata and verified the exemption of the pesticides under study, three samples of water from rivers that presented a more turbid aspect due to the particulate material were contaminated with known amounts of the four pesticides. Subsequently, without any previous treatment, these fortified natural samples were submitted to the optimized and validated extraction method in order to evaluate the matrix effect and its selectivity. Comparatively, the recovery rates and the coefficients of variation of the analyzes are statistically similar, with no matrix effect in the results (Table 7).

Table 7. Recovery (%) and coefficients of variation (%) obtained from the extraction of distilled and fortified natural water samples with the pesticides chlorpyrifos (3.0 µg L^{-1}), thiamethoxam (110.0 µg L^{-1}), cypermethrin (30.0 µg L^{-1}) and deltamethrin (72.0 µg L^{-1}) using the SALLE method.

	Recovery (%) and CV (%)			
	Chlorpyrifos	Thiamethoxam	Cypermethrin	Deltamethrin
Distilled water	83; 7	76; 7	90; 7	85; 6
Sample 1	81; 6	77; 9	91; 8	90; 6
Sample 2	82; 6	80; 9	90; 7	88; 5
Sample 3	85; 6	76; 8	88; 5	87; 6

4. Conclusions

The optimized and validated salting-out assisted liquid-liquid extraction (SALLE) method for analyzing by gas chromatography (GC/ECD) is simple, inexpensive, fast, efficient, with high recovery percentages and good repeatability, allowing its use to detect pesticides in environmental samples.

The recovery percentages were higher than 72 % and the coefficient of variation was lower than 9 %. The main advantage of the SALLE method followed by GC/ECD analysis was the low consumption of sample (3.0 mL) and solvent (6 mL), and shorter time for phase separation (1 min), allowing its use in routine analysis of the pesticides in water samples. Hence, it seems possible to extend this method to extract the compounds of interest in other similar samples by varying the extraction conditions.

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Pharmaceuticals and personal care products as emerging micropollutants in Brazilian surface waters: a preliminary snapshot on environmental contamination and risks

Fernando Fabriz Sodré[1+], Priscila Mendonça Dutra[1], Viviane Portela dos Santos[1]

[1] University of Brasília (UnB), Institute of Chemistry, Darcy Ribeiro Campus, Brasília, Distrito Federal, Brazil

[+]Corresponding author: Fernando Fabriz Sodré, e-mail address: ffsodre@unb.br

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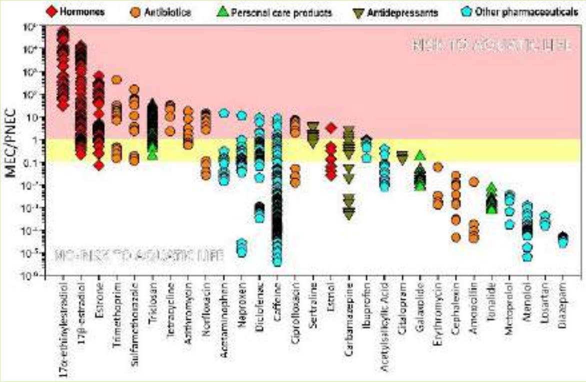
ABSTRACT: This work aimed to present a preliminary snapshot on the contamination and risks of Brazilian surface waters concerning the presence of hormones, pharmaceuticals and personal care products. Data gathered in the literature for Measured Environmental Concentrations (MEC) in Brazilian surface waters were compared with the most restrictive Predicted No-Effect Concentration (PNEC) available in the literature. A risk assessment was performed revealing that hormones, antibiotics drugs and triclosan should be prioritized to subsidize the generation of water quality standards to protect aquatic life.



1. Introduction

Emerging environmental micropollutants, such as pharmaceuticals and personal care products, are potentially toxic substances of recent interest to academics, regulatory agencies, and sectors of society[1, 2]. Information on the occurrence and effects of such micropollutants in environmental compartments is critical to assess their risks under natural conditions. Although there are some isolated requirements for surface water monitoring in Brazil, as a result of the first studies published in the country during the last decade, there are still no quality standards that guarantee the safe use of water for different purposes[3]. Among the many uses of water is the protection of aquatic life, probably one of the most noble since it aims to guarantee the integrity and survival of organisms from different trophic levels. For most micropollutants, legislated or not, restrictive criteria have been reported to protect aquatic life, since the protection targets are organisms extremely sensitives to changes in water quality caused by the input of exogenous substances, even at trace concentrations[4, 5].

There are many strategies described in the literature to assess environmental risks of chemicals[6-8]. One of them, as well as the simplest, is based on the comparison of predicted or measured environmental concentrations with predicted no-effect concentration (PNEC) for a given substance[9, 10]. The PNEC can be described as the concentration limit which harmful effects on organisms will most likely not occur. For aquatic systems, a PNEC should be derived that, if not exceeded, ensures an overall protection of the

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environment[11]. In the literature, as well as in many reports produced by environmental organizations, several approaches to derive PNEC are described. Most of them are based on a primordial data, mainly obtained from acute or chronic toxicity tests. Standard acute toxicity tests, based on short-term exposure, are commonly expressed as EC_{50} or LC_{50}, i.e., the concentration required to cause short-term effects or to kill, respectively, half the organisms of a tested population after a specified exposure time. On the other hand, long-term exposure tests provide toxicity data related to the lowest observable effect concentrations (LOEC) or to no-observable effect concentrations (NOEC).

For some emerging micropollutants, data from both short and long-term toxicity tests can be assessed. However, for other substances, only acute toxicity data may be available. Considering the availability of toxicological data, some extrapolation concerning the aquatic environment has to be done to represent the whole ecosystem sensitivity. Under these circumstances, assessment factors (AF) are used to derive PNEC as follows:

$$PNEC = \frac{Toxicological\ data}{AF} \qquad (1)$$

Both acute (EC_{50} or LC_{50}) and chronic (LOEC or NOEC) toxicological data, derived from single-species toxicity tests, are used in the literature to calculate PNEC for aquatic systems. In this case, the lowest figures for these tests, representing the worst-case scenario, are commonly be used. The use of AF was advocate to establish a concentration below which adverse effects will most likely not occur. The magnitude of AF is inversely proportional to the amount and quality of toxicological data available. For example, according to the European Commission technical guidelines[11, 12] an AF of 1000 is often used when at least one short-term data is available for one of the three organisms representing different aquatic trophic levels, i.e., a fish, a microcrustacean (generally *Daphnia*), and an alga. When one long-term NOEC data for either fish or *Daphnia* is available, an AF of 100 or 50 is used. Assessment factors varying from 1 to 10 are used as more long-term NOECs from two to three trophic levels are available. When species sensitivity distribution (SSD) for multi-species data is available the AF must be reviewed on a case by case basis. The same approach is used when field or model ecosystems data are assessed. For the latter approach, modeling software such as QSAR or ECOSAR, based on structure activity relationships (SAR), generate PNECs assuming a common mode of action (narcosis) and may be useful to identify links between micropollutant groups and environmental toxicity[6, 13].

In the literature, risk assessment using PNECs are commonly investigated by the risk quotient (RQ) calculated between the predicted environmental concentration (PEC) and PNEC[6,9,14]. When available, measured environmental concentrations (MEC) reported in the literature may also be used (Equation 2), but in most cases MEC data are scarce for a meaningful analysis[9].

$$RQ = \frac{PEC\ or\ MEC}{PNEC} \qquad (2)$$

The predicted concentration of a micropollutant in surface waters can be calculated using information such as the amount of the target substance used per year, the removal efficiency in wastewater treatment plants (WWTP), the volume of wastewater entering a WWTP for a given period, a dilution factor in the environment, amid others[15, 16]. Simple PEC calculation may assume that the amount used/consumed is equally distributed over the year, the target substance is used/consumed throughout the region investigated (city, hydrographic basin, country, etc.), it is constantly diluted in the environment without metabolization, transformation, sorption, etc. On the other hand, as pointed out by de Bruijn *et al.*[11], data on measured levels provides useful information regarding specific steps of the exposure assessment, such as background levels, concentration ranges and distribution. Also, although measured data present uncertainties associated with temporal and spatial variations, the concentration range may reflect different patterns observed during manufacturing or use of the target substances that are not considered in the modelling procedures. In summary, both PEC and MEC data are important for risk assessment complementing each other during the analysis.

In 2009, Souza *et al.*[17] carried out an environmental risk assessment of antibiotics using PEC estimates for 21 substances in Brazil. Consumption data was assessed from reports of an intensive care unit and from hospital pharmacies of the city of Curitiba. One can consider that the most challenging aspect in order to calculate PEC values for pharmaceuticals and personal care products in Brazil is the assessment of consumption data. Baldoni *et al.*[18] reviewed 620 pharmacoepidemiologic studies produced in Brazil and observed that forms and interviews were frequently used as the main source instead of secondary sources such as prescription data and electronic databases. Locatelli *et al.*[19] investigated environmental concentrations for the eight most consumed antibiotics in Brazil according to data obtained from the National Health Surveillance Agency (ANVISA). However, as pointed out by the authors, there is a large number of pharmacies and drugstores selling controlled drugs without prescription throughout the country making the available data uncertain.

In the face of the problems surrounding data gathering for PEC calculations, this work aimed to provide a snapshot on the environmental contamination and risks of pharmaceuticals and personal care products in Brazil, based on the few data available for MEC in surface waters throughout the country. Therefore, our intention is not to perform a complete risk assessment of emerging micropollutants, but rather to provide a scenario consistent with the contamination of Brazilian waters considering the concentration ranges of the most investigated substances as well as their possible risks to the aquatic life.

2. Experimental

The most restrictive PNEC values reported in the literature, at the best of our knowledge were selected. In some cases, PNECs were estimated in this work using assessment factors suggested by the European Commission[11] as well as toxicological data available elsewhere[20-24]. Table 1 shows a list of the most commonly investigated emergent micropollutants in Brazil and PNEC values obtained in the literature or estimated in the present work.

Table 1. Substances selected in this work and their respective PNEC values.

Main group	Substance	PNEC (ng/L)	PNEC Calculation	Reference
Hormone	Estrone	3	NOEC and LOEC; AF=5	[25]
	17β-estradiol	1	LOEC; AF=10	[25]
	Estriol	60	Vitellogenin assay; AF=10	[26]
	17α-ethinylestradiol	0.1	NOEC e LOEC; AF=5	[25]
Antibiotic	Amoxicillin	100000	NOEC; AF=10	[14]
	Azithromycin	19	Derived from EC$_{50}$[20]; AF=1000	This work
	Cephalexin	100000	NOEC; AF=10	[14]
	Ciprofloxacin	50	Derived from NOEC[21]; AF=100	This work
	Erythromycin	27530	EC$_{50}$; AF=1000	[27]
	Norfloxacin	20	Derived from NOEC[21]; AF=100	This work
	Sulfamethoxazole	10	Derived from NOEC[21]; AF=100	This work
	Oxytetracycline	230	ECOSAR Database	[15]
	Tetracycline	5	Derived from NOEC[21]; AF=100	This work
	Trimethoprim	16	Derived from NOEC[21]; AF=100	This work
Anti-inflammatory	Acetylsalicylic acid	61000	Derived from EC$_{50}$; AF=100.	[16]
	Diclofenac	1000	Derived from NOEC[22]; AF=100	This work
	Ibuprofen	2300	Derived from EC$_{50}$[20]; AF=1000	This work
	Naproxen	2000	Derived from NOEC[23]; AF=10	This work
	Acetaminophen	1000	Derived from NOEC[23]; AF=10	This work
Antidepressant	Carbamazepine	250	NOEC; AF=10	[28]
	Citalopram	360	ECOSAR Database	[10, 29]
	Sertraline	43	Derived from EC$_{50}$; AF=1000	[30]
Anthelmintic	Mebendazole	98000	Derived from LC$_{50}$[24]; AF=1000.	This work
Anxiolytic	Diazepam	14100	EC$_{50}$; AF=1000.	[16]
Beta blocker	Atenolol	77700	ECOSAR Database	[15]
	Metoprolol	7900	EC$_{50}$; AF=1000.	[31]
	Propranolol	10	NOEC; AF=50	[22]
	Losartan	78000	NOEC; AF=10	[32]
Stimulant	Caffeine	5200	NOEC; AF=100	[28]
Personal care product	Triclosan	12	Derived from EC$_{50}$[20]; AF=1000	This work
	Galaxolide	310	ECOSAR Database	[33]
	Tonalide	4000	NOEC; AF=100	[34]

Details on the toxicological assays used to estimate the PNEC values portrayed in Table 1 can be obtained from the respective references. Table 2 shows a compendium of results generated in Brazil from 1999 to 2018 on the presence of emerging micropollutants in surface waters, notably hormones, pharmaceuticals and personal care products[19, 35-59]. The total number of samples and the concentrations reported above limits of quantification (LOQ) is shown. Table 2 also shows the concentration range and the classification of the samples in the three different ranges referring to the risk quotients calculated according Equation 2.

Table 2. Concentration and risks of emerging micropollutants frequently investigated in Brazilian Surface waters.

Main group	Substance	Total Samples[a]	Positivee results[b]	Min[c]	Max[c]	Median[c]	RQ[d]≥1	1>RQ[d]≥0,1	RQ[d]<1
Hormone	Estrone	278	57	0.21	1800	7.7	49	7	1
	17β-estradiol	324	81	0.21	13450	51	68	13	0
	Estriol	30	9	1.5	182	7.7	1	4	4
	17α-ethinylestradiol	190	44	3.0	5900	305	44	0	0
Antibiotic	Amoxicillin	10	5	1.3	1284	8.9	0	0	5
	Azithromycin	8	8	10	313	26.2	5	3	0
	Cephalexin	19	11	2.4	2422	150	0	0	11
	Ciprofloxacin	18	12	0.6	350	208	9	0	3
	Erythromycin	8	4	35.3	1586	66	0	0	4
	Norfloxacin	18	13	0.5	276	114	9	1	3
	Sulfamethoxazole	42	13	1.1	1529	106	10	3	0
	Oxytetracycline	24	1	44	44	44	0	1	0
	Tetracycline	18	7	11	153	104	7	0	0
	Trimethoprim	18	14	2.3	6376	150	9	5	0
Anti-inflammatory	Acetylsalicylic acid	89	15	12.2	20960	2096	0	3	12
	Diclofenac	196	54	0.02	8250	118	5	24	25
	Ibuprofen	28	10	326	2094	1659	0	10	0
	Naproxen	80	28	0.02	21285	174	1	12	15
	Acetaminophen	99	17	13.4	13440	35	1	5	11
Antidepressant	Carbamazepine	33	17	0.12	652	58	3	6	8
	Citalopram	16	5	48	79	55	0	5	0
	Sertraline	16	8	30	164	62	6	2	0
Anthelmintic	Mebendazole	10	1	14	14	14	0	0	1
Anxiolytic	Diazepam	17	12	0.38	0.71	0.52	0	0	12
Beta blocker	Atenolol	28	25	0.48	90	7.1	0	0	25
	Metoprolol	19	7	1.3	28	14	0	0	7
	Propranolol	3	1	0.56	0.56	0.56	0	0	1
	Losartan	10	3	12	32	16	0	0	3
Stimulant	Caffeine	175	162	0.02	41590	3.1	6	13	143
Personal care product	Triclosan	151	85	2.2	415	77	72	13	0
	Galaxolide	17	17	2.4	53	5.6	0	1	16
	Tonalide	17	17	2.8	28	6.3	0	0	17

[a]Total of samples investigated. [b]Number of samples with concentrations reported above method LOQ. [c]Reported data considering only positive results. [d]Risk quocient = MEC/PNEC.

3. Results and discussion

Figure 1 portrays the most frequently quantified emerging micropollutants reported in studies carried out in Brazilian surface waters between 1999 and 2018. The personal care products galaxolide and tonalide, together with the antimicrobial azithromycin, were in the top of the list. However, is important to point out that the number of samples investigated for the abovementioned substances is relatively low in comparison with data available for substances such caffeine and triclosan. Also, in the present work, the characteristics of the different sampling points, such as land use and potential pollution sources were not explored. Thus, aquatic systems with different degrees of anthropic impact were selected without distinction. Consequently, it is possible that the most frequently quantified substances were investigated only in a few samples as well as in more polluted aquatic systems.

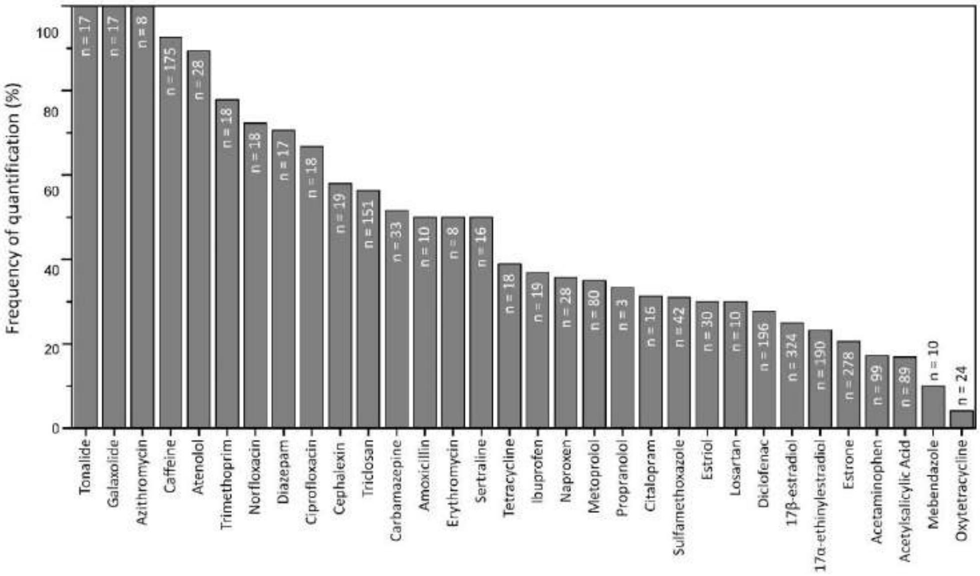

Figure 1. Percentage of samples presenting results reported above LOQ. The total of samples investigated (n) for each substance is also shown.

In Figure 1, it is also noticed that caffeine was the most frequently quantified substance (92.6 %) among the most investigated ones, followed by triclosan (56.3 %) and naproxen (35 %). The higher frequency of quantification for caffeine is in accordance with the important role of this stimulant drug as an indicator of anthropic activities, notably in Brazilian urban regions[60-62].

Another important aspect is the diversity of analytical methods and techniques employed in the studies carried out in Brazil. As a result, different method LOQs are reported, which may imply in a possible underestimation of the contamination degree. For example, Figure 2 shows the evolution of method LOQs reported for the determination of 17α-ethinylestradiol and 17β-estradiol in surface waters from the region of Campinas (São Paulo) using different analytical techniques.

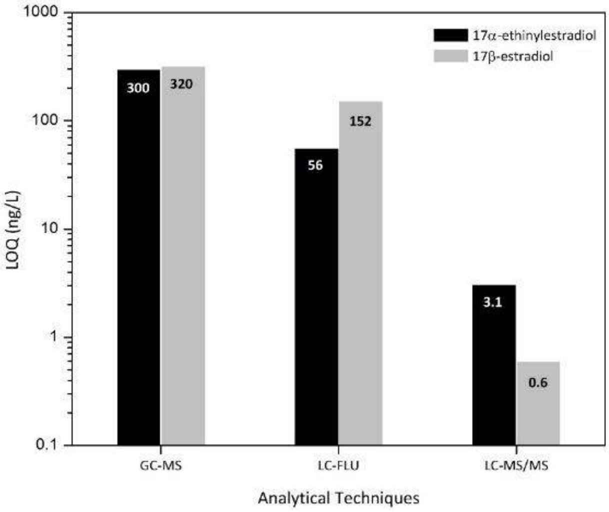

Figure 2. Method limits of quantification (LOQ) for 17β-estradiol and 17α-ethinylestradiol obtained in different analytical systems. GC-MS: gas chromatography coupled to mass spectrometry, LC-FLU: liquid chromatography with fluorescence detector, LC-MS/MS: liquid chromatography coupled to tandem mass spectrometry[36, 45, 60].

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) provides higher quantification capacity, i.e., lower LOQ values, when compared to other analytical techniques. Among the 25 studies selected in the present work, 60 % used LC-MS/MS systems to assess the concentrations of the target substances. Others employ liquid chromatography with fluorescence (LC-FLU) or diode array detectors (LC-DAD) as well as gas chromatography coupled to mass spectrometry (GC-MS) with or without derivatization steps. Considering the information portrayed in Figure 2, it is expected higher frequencies of quantification when data were produced using only LC-MS/MS systems.

On the other hand, one can consider that the frequency of quantification may also vary if more studies were selected covering different Brazilian regions and aquatic systems with different degrees of pollution.

Finally, it is important to mention that the studies developed in Brazil are not yet equally distributed throughout the country territory. Most of the data generated, about 44 %, was obtained in the state of São Paulo, followed by the states of Rio Grande do Sul (15 %), Minas Gerais (11 %) and Rio de Janeiro (11 %). These four states are among the most populous in Brazil, accounting for 45 % of the country's population. The rest of the work was developed in Paraná, Mato Grosso do Sul, Federal District and Amazonas. It is interesting to note that, to the best of our knowledge, no scientific paper has yet been published on the presence of pharmaceuticals and personal care products in surface waters from Northeast Region of Brazil. This region has nine states and accounts for 28 % of the Brazilian population, being the second most populous. The Southeast Region, where the states of São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo are located, is the most densely populated one.

In a recent work, Machado *et al.*[42] aimed to perform a nationwide survey on the presence of emerging micropollutants in drinking and source waters. Although data on the presence of caffeine and atrazine were produced for drinking water samples from almost all Brazilian capitals, only surface waters from São Paulo, Minas Gerais and Rio Grande do Sul were investigated.

Figure 3 portrays the concentrations of pharmaceuticals and personal care products in Brazilian surface waters.

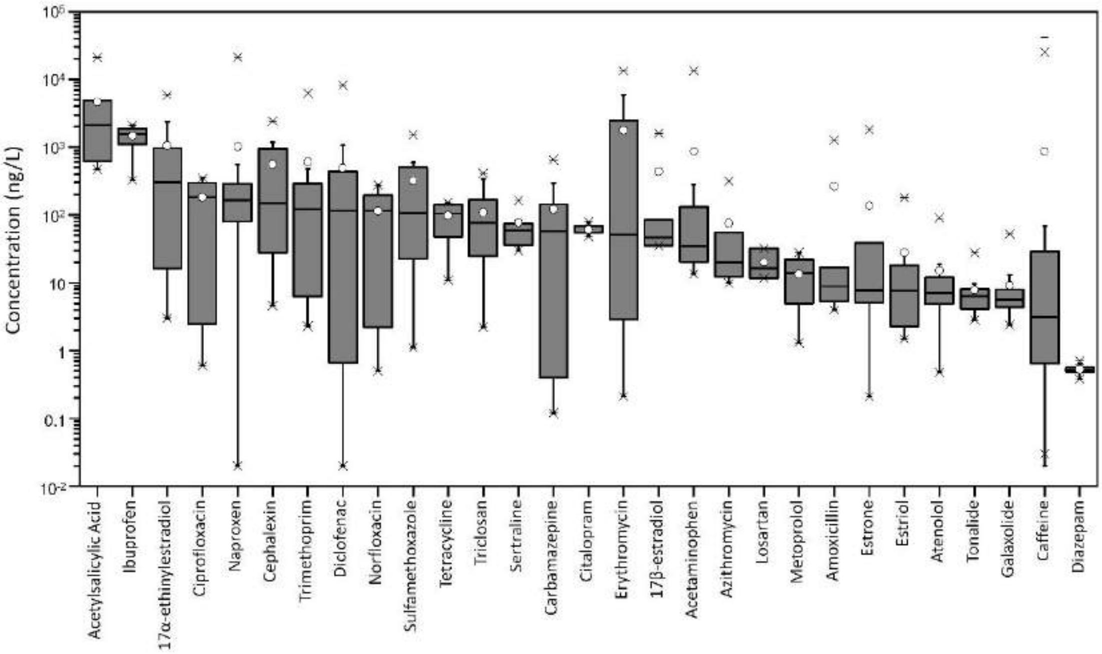

Figure 3. Box-plots of pharmaceuticals and personal care products concentrations in Brazilian surface waters. Open circles are the arithmetic means. The large box represents the 25th percentile, median, and 75th percentile; error bars indicate the 5th and 95th percentiles; ×-symbols represent the 1st and 99th percentiles; whiskers are the minimum and maximum values.

Concentration ranges for the majority of the micropollutants present in Figure 3 had high amplitude, evidencing the diversity of polluted aquatic environments investigated in Brazil so far. For many substances, mean and median values are coincident or close together, whereas for the drugs amoxicillin and caffeine, for example, mean values are up to two orders of magnitude higher than medians. This behavior shows that some of the concentrations reported are exceptionally high, reflecting the influence of more polluted aquatic environments. It is interesting to note that caffeine, investigated in 175 samples and quantified in most of them, presents the highest variability of concentrations, corroborating with its role as an indicator of anthropic activities, since it differentiates aquatic environments with different degrees of pollution.

In general, the levels of pharmaceuticals and personal hygiene products in Brazilian surface waters are higher than those found in other countries, as already reported in recent literature reviews[1, 63]. Higher median concentrations are noticed for acetylsalicylic acid, followed by ibuprofen and 17α-ethinylestradiol. The first two substances have been found at high concentrations not only in Brazilian waters, but also in other countries[64-66]. The same observation, however, cannot be made for 17α-ethinylestradiol, as well as for other substances investigated in Brazil.

Quadra *et al.*[63] evidenced that Brazilian surface waters present higher concentrations of 17α-ethinylestradiol, 17β-estradiol, and caffeine in comparison with other locations. In the present study, it should be noted that most of the results involving the occurrence of hormones were obtained by Machado *et al.*[53] and Montagner *et al.*[36] in the Upper Iguaçu River Basin (Paraná) and in the region of Campinas (São Paulo), respectively. In both studies, mean concentrations of 17α-ethinylestradiol and 17β-estradiol were 1916 and 3689 ng/L, respectively. These values are also higher than those reported in other Brazilian regions. For example, in Minas Gerais, the average concentration of 17α-ethinylestradiol in waters nearby the capital, Belo Horizonte, is 206 ng/L, considering 15 positive data (34 % of the total)[46, 47]. In the region of São Carlos (São Paulo), Campanha *et al.*[39] reported an average value of 2 ng/L for 17β-estradiol in 24 positive data out of a total of 81 (30 %). Again, these results demonstrate the great variability of Brazilian surface waters concerning their degree of pollution, certainly influenced by sewage discharges, as pointed out earlier[1, 42, 60].

Data for MEC portrayed in Figure 3 can be used to perform a preliminary risk assessment on the presence of pharmaceuticals and personal care products in Brazilian surface waters using Equation 2, as well as PNEC data presented in Table 1. As suggested by Komori *et al.*[28] risk quotients (MEC/PNEC) greater than 1 imply risk while values lower than 0.1 indicate no-risk. Intermediate values indicate potential risk and, consequently, the need for further studies. Figure 4 shows individual MEC/PNEC ratios for the substances selected in the present work.

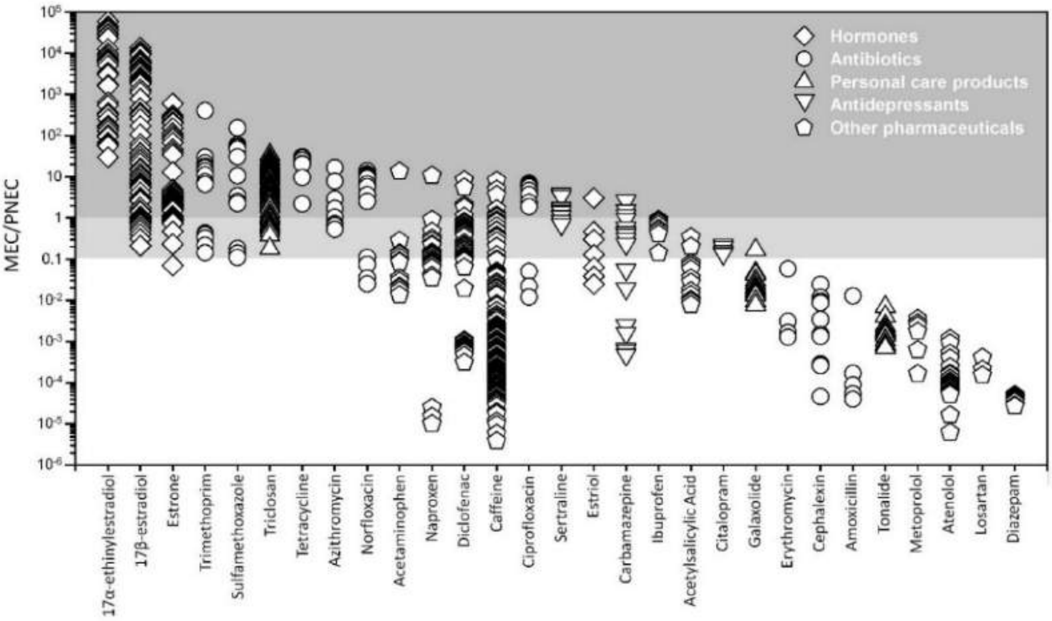

Figure 4. Environmental risk quotients of pharmaceuticals and personal care products in Brazilian waters.

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Higher risk quotients are noticed in Figure 4 for hormones and antibiotic drugs, evidencing that micropollutants of both classes should be prioritized to ensure the protection of aquatic life. Most of the risk quotients for the hormones 17α-ethinylestradiol, 17β-estradiol and estriol are 1000 to 10000 times greater than the first threshold (MEC/PNEC > 1), due to consequence of their high concentrations as well as to the risk that these substances may pose to aquatic biota. Triclosan should also be prioritized, since all results generated in the country so far evidence risk or possible risk. An extensive investigation on triclosan in Brazilian waters has already put this substance as a strong candidate in a priority list that must be regulated in Brazil to preserve aquatic environments[57].

Non-prescribed drugs such as acetaminophen, diclofenac and naproxen, as well as the antidepressants carbamazepine and sertraline are not in the top of the rank but may also be candidates to further investigations.

Galaxolide and tonalide present the highest frequencies of quantification but are not in the top of rank due to their high PNECs which leads, therefore, to low risk quotients. These substances, however can be used as indicators of anthropic pollution, as pointed out by Glassmeyer *et al.*[67].

Caffeine is also frequently quantified in Brazilian waters but presents low risk to aquatic biota. It is true that some risk quotients for caffeine were above the first threshold, certainly due to extremely high values reported in waters impacted by direct inputs of domestic sewage. Although most MEC/PNEC values indicate low risk for caffeine, it is important to reinforce the role of this substance as an indicator of anthropic pollution. In addition to this use approach, caffeine has recently been used to rank Brazilian surface waters presenting different levels of estrogenic activity[61].

Finally, Figure 4 shows positive risk in all samples investigated for tetracycline and 17α-ethinylestradiol. In this case, it is important to mention that the former presented a frequency of quantification of 39 % while the hormone 17α-ethinylestradiol could be quantified in 23 % of the samples. Even so, for both substances, all samples reported with positive values (>LOQ) present risk. Thus, although such substances are obviously of concern in terms of aquatic life protection, representative results may be obtained by improving method LOQs.

4. Conclusions

Data on the occurrence of pharmaceuticals and personal care products in Brazilian surface waters were gathered in order to provide a preliminary snapshot on environmental contamination and risks. Although substances such as tonalide, galaxolide, atenolol and caffeine were frequently quantified, higher risks were accounted for hormones and antibiotics, as well as for triclosan, a biocide present in personal hygiene products. Key aspects including the behavior of micropollutants, as well as the influence of land use and occupation, degree of anthropic pollution, diffuse and point sources, among others, may be considered in future investigations. However, such discussions may be improved as a more uniform and broad database is available. Thus, it is evidenced the need for more studies on the diagnosis of Brazilian surface waters for the presence of several micropollutants of recent interest, legislated or not. It should be mentioned that other emerging micropollutants, such as pesticides, illicit drugs, and industrial-derived substances, were not included in this study, but they can be evaluated in future investigations using the same strategy presented here.

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Use of vermicompost for the removal of toxic metal ions of synthetic aqueous solutions and real wastewater

Luane Patricia Guedes Barbosa[1], Tácila Oliveira Pinto de Freitas[1], Madson de Godoi Pereira[1+]

[1] Bahia State University, Department of Exact and Earth Sciences, 2555 Silveira Martins St, Salvador, Bahia, Brazil

[+]Corresponding author: Madson de Godoi Pereira, e-mail address: madson.pereira444@gmail.com

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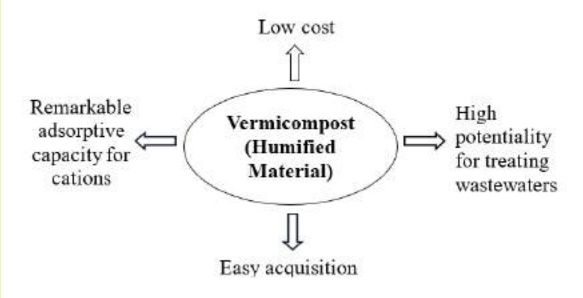
ABSTRACT: Samples of vermicompost were used to decontaminate aqueous media containing Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and/or Zn^{2+} ions at concentration up to 10X higher than the limits of National Council of the Environment (Resolution 430/2011). For this purpose, 50.00 mL of synthetic solutions (pH 0.9 just after preparation) and wastewater from a chemical laboratory were stirred with dried vermicompost (~10.0000 g), without sieving or with diameter ≤ 0.053 mm. Aqueous media were treated with vermicompost, either at rest (24 to 96 h) or under mechanical agitation (2 to 10 h). All elements were spectrometrically determined in the different supernatants. In the synthetic solutions, except for Mn^{2+} and Zn^{2+}, concentrations were lower than the legal limits or than the limits of detection, when vermicompost without sieving was used. After treating synthetic solutions with sieved vermicompost, Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} e Zn^{2+} ions concentrations were undetectable, while Mn^{2+} concentrations were too close to the legal limits. In the wastewater, $Cd^{2+}$and Pb^{2+} concentrations, which were 1.5 and 3.8X higher than the legal limits, respectively, were undetectable after treatment with vermicompost without sieving. Besides its efficiency of retaining the evaluated cations, vermicompost was able to increase the aqueous media pH from 0.9 to 6.0 ± 0.5.



1. Introduction

Human activities have caused harmful impacts on the environment, with emphasis on aquatic ecosystems, which are frequently recipients of several types of liquid disposal. Of the various aquatic pollutants in this disposal, metallic elements deserve special attention due to their high potential for bioaccumulation and biomagnification, as well as the fact that these elements are responsible for the appearance of various diseases in humans[1]. Therefore, aqueous disposal contaminated with metals must be efficiently treated and, among the available treatment modalities, those that use adsorption stand out for their operational simplicity and high efficiency. When adsorbents of natural origin are used, adsorption processes also have a very low cost[2].

Many naturally occurring adsorbents have been successfully used for the removal of metal cations from aqueous media, including clays[3], plant biomass[4] and material enriched with humified organic matter[5, 6]. Humified organic matter is formed by the chemical and microbiological decomposition of different organic precursors, such as proteins, carbohydrates and lipids. These precursors are fragmented, and the fragments are randomly recombined to form highly stable and complex molecular structures[2].

Humified organic matter is divided into three classes, according to solubility criteria against the pH of the medium. Thus, humics are insoluble irrespective of pH, whereas fulvic acids have a

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high solubility over a wide pH range. In turn, humic acids are soluble in alkaline or slightly acidic media. The structures of humics, in relation to the structures of fulvic acids, have higher carbon content, while the number of hydrophilic groups is considerably lower. The structure of humic acids has an intermediate percentage of both carbon and hydrophilic groups[7].

Among the different ways of obtaining humified materials is vermicomposting, in which certain earthworm species ingest a mixture of soils and organic residues (e.g., cattle manure). In the digestive tract of these animals, a broad and diverse microbial population is responsible for accelerating the fragmentation of organic molecules and the formation of humified compounds. After excretion of the ingested and metabolized material by the worms, the vermicompost is obtained, with high levels of humified organic matter, in addition to high concentrations of nutrients such as calcium, magnesium, potassium and sodium. Additionally, the vermicompost has a high surface area and this property favors adsorption processes[8].

In view of the above, this study evaluated the efficiency of vermicompost in adsorbing Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions from aqueous media. Therefore, mixed synthetic solutions were used, as well as real wastewater (potentially contaminated with these metal cations) originated from an atomic absorption spectrometry laboratory. This study was conducted under high acidity conditions, which is characterized as a limiting factor for adsorption efficiency. Thus, this study differs from others for offering an interpretation of the adsorption potential of vermicompost under conditions commonly found in complex matrices of many wastewaters generated by chemical laboratories.

The six elements chosen for this study are of environmental relevance, as they can all cause serious damage to human health, if they are discarded at high concentrations in water sources, with their consequent pollution. In this context, cadmium and lead are very toxic, and can lead to infertility, leukemia, bone weakness and/or psychiatric disorders. Although copper, manganese, nickel and zinc are essential within certain limits, the excessive ingestion of these elements can lead to blindness (in the case of copper), while high doses of manganese in the human body lead to neurological diseases. In turn, nickel and zinc contaminations cause humans to develop different types of tumors[1].

2. Experimental

2.1 Instruments and reagents

The following instruments were used: water purification unit (Gehaka Master P & D, Brazil), horizontal shaker table (Quimis Q225M, Brazil), potentiometer (Tecnal Tec-3MP, Brazil) and flame atomic absorption spectrometer (GBC SensAA, Malaysia).

Individual stock solutions of cadmium, lead, copper, manganese, nickel and zinc ions at 1000 mg L^{-1} (SPC, USA) were used daily to calibrate the flame atomic absorption spectrometer, as well as to prepare mixed synthetic solutions of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions. Adjustments of pH were made with HNO_3 solutions, which were prepared from dilution of 14 mol L^{-1} HNO_3 (Merck, Germany).

2.2 Procedures

Vermicompost sample was characterized[9] for pH, humidity, total organic matter, and ashes. All analyses were performed in triplicate.

All adsorption tests described in this section were performed with vermicompost previously oven-dried (70 °C) to constant mass. The vermicompost was produced in the city of Lauro de Freitas-BA and purchased in the local commerce of Salvador-BA. Adsorption tests were performed with analytical blanks, using four replicates, and the concentrations of cadmium, lead, copper, manganese, nickel and zinc ions were quantified by flame atomic absorption spectrometry (F AAS). Thus, the spectrometer was operated with an air-acetylene flame at the respective flow rates of 8.0 and 2.0 L min^{-1}. All analyses were performed with background radiation correction, using a deuterium lamp, and the hollow cathode lamps were operated at the following wavelengths (nm): 228.8 (Cd), 324.8 (Cu), 279.5 (Mn), 232.0 (Ni), 283.3 (Pb) and 213.9 (Zn). Calibration curves were built at the following concentration ranges (mg L^{-1}): 0.1 to 1.5 (Cd), 0.5 to 5.0 (Cu, Mn and Ni), and 1 to 6 (Pb and Zn).

In the first phase of the experiments, approximately 10.0000 g of vermicompost (without going through any sieving step) were placed in contact with 50.00 mL of mixed solution of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (pH 0.9 just after preparation) at the respective

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concentrations of 2.0; 10.0; 10.0; 20.0; 5.0 and 50.0 mg L^{-1}. The contacts of the vermicompost with mixed solution aliquots were performed under mechanical stirring (2, 4, 6, 8 and 10 h) at 200 rpm, as well as at rest (24, 48, 72 and 96 h).

In a second phase of the experiments, 50.00 mL aliquots of mixed solution of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions (pH 0.9 just after preparation), at the respective concentrations of 2.0; 10.0; 2.0; 20.0; 5.0 and 10.0 mg L^{-1}, were stirred (200 rpm) with approximately 10.0000 g of vermicompost (without going through any sieving step) for 10 h. Volumes of 50.00 mL of the aforesaid mixed solution were also placed in contact with about 10.0000 g of vermicompost for 24, 48, 72 or 96 h, at rest.

In the third stage of the experiments, approximately 10.0000 g of vermicompost sieved in a 0.053-mm mesh were placed in contact with 50.00 mL of mixed solution of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions (pH 0.9 just after preparation), at the respective concentrations of 2.0; 10.0; 10.0; 20.0; 5.0 and 50.0 mg L^{-1}. The vermicompost masses and portions of the mixed solution were contacted via mechanical stirring (10 h at 200 rpm) or at rest (24, 48, 72 and 96 h).

In the fourth and last experimental stage, about 10.0000 g of vermicompost, without sieving, were mechanically stirred for 10 h at 200 rpm, with 50.00 mL of real wastewaters generated by the atomic absorption spectrometry laboratory of the Department of Exact and Earth Sciences at the State University of Bahia, Salvador Campus. This wastewater was treated at its natural pH (pH = 5.0), and after its acidification with drops of HNO_3 solution at 1.0 mol L^{-1} until pH 1.0.

After the different procedures described above, the supernatants were filtered on qualitative filter paper, stored in polyethylene bottles (previously decontaminated in a 10% v/v HNO_3 bath for 24 h) and refrigerated at 4 °C, until the determinations of cadmium, lead, copper, manganese, nickel and zinc by F AAS, under the previously described experimental conditions. In the case of tests with the wastewater from the atomic spectrometry laboratory, they were analyzed by F AAS, before and after the treatment with the vermicompost. At the end of the first experimental phase, supernatants were also analyzed for pH by immersing a combined glass electrode.

3. Results and discussion

The vermicompost, which was used in this study, had the following results (Mean ± standard deviation, N = 3) concerning the characterization analyses: pH (6.4 ± 0.2), humidity (52.9 ± 2.2 %, wt./wt.), total organic matter (55.6 ± 0.7 %, wt./wt.), and ashes (44.4 ± 0.7 %, wt./wt.). The average pH indicates deprotonated acidic organic chemical groups (carboxylates, for example), and the high humidity levels corroborate with the presence of these groups, which can establish hydrogen bonds with water molecules. Moreover, the high amounts of hydrophilic organic chemical groups in the considered vermicompost sample can be related to its expressive contents of organic matter. In turn, 44.4 ± 0.7 % (wt./wt.) of ashes highlight the use of soils in the vermicompost production[9]. Thus, the results of vermicompost characterization support its promising results for treating aqueous media (synthetic solutions and real wastewater) containing Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions as discussed below.

The concentrations of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions in the mixed solutions, which were used in the first and third experimental phases, corresponded to concentrations 10 times higher than the maximum limits established by Resolution 430/2011 of the National Environmental Council (*Conselho Nacional do Meio Ambiente*, CONAMA)[10], whose values are (mg L^{-1}): 0.2 (Cd), 1.0 (Cu), 1.0 (Mn), 2.0 (Ni), 0.5 (Pb) and 5.0 (Zn). On the other hand, in the second experimental phase, the concentrations of Mn^{2+} and Zn^{2+} were modified for the reasons discussed throughout this section.

All adsorption tests, whose results are discussed below, were performed with 50.00 mL mixed solutions, since it was found that this volume was sufficient to completely cover vermicompost particles.

The detection limits for cadmium, lead, copper, manganese, nickel and zinc were calculated by analyzing 15 independent blanks. The standard deviation of the analytical signals generated by the blanks was multiplied by three and divided by the slope of the calibration curve for each element[11]. The following detection limits (mg L^{-1}) were obtained: 0.05 (Cd), 0.08 (Cu), 0.1 (Mn), 0.06 (Ni), 0.2 (Pb) and 0.05 (Zn).

Table 1 lists the concentrations of cadmium, lead, copper, manganese, nickel and zinc in the supernatants obtained after the first phase of the experiments. Considering the limits of

CONAMA[10], it was concluded that all conditions adopted (under mechanical stirring or at rest) were efficient for the removal of cadmium, copper and nickel. For lead, mechanical stirring tests were successful, while tests at rest were less efficient. This fact indicates that the diffusion of lead, from the mixed solution to the vermicompost particles, is slow, thus requiring constant movement of the liquid mass. In turn, all the conditions tested in the first experimental phase were not successful in reducing the concentrations of manganese in the mixed solution. For zinc, except for the experiments under mechanical stirring for 2 h, all the other experiments yielded unsatisfactory results in relation to the legal limit[10].

Table 1. Concentrations (mg L^{-1}) of cadmium, copper, manganese, nickel, lead, and zinc after the first experimental phase*, N = 4. Values expressed as Mean ± standard deviation.

Experimental condition	Cation					
	Cd^{2+}	Cu^{2+}	Mn^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
VA** 2h	0.12±0.01	0.10±0.03	>5.0*****	1.4±0.1	<0.2	1.3±0.2
VA 4h	0.11±0.01	0.08±0.01	>5.0	1.1±0.2	<0.2	7.0±0.9
VA 6h	0.10±0.01	0.09±0.01	>5.0	1.1±0.1	<0.2	7.1±0.6
VS 8h	< 0.05****	0.03±0.01	>5.0	0.50±0.03	<0.2	4.5±0.2
VA 10h	< 0.05	0.05±0.01	>5.0	1.1±0.2	<0.2	6.9±0.9
VR*** 24 h	0.14±0.06	0.10±0.03	>5.0	1.3±0.4	1.7±0.2	8±2
VR 48 h	0.06±0.06	0.10±0.03	3.5±0.3	0.6±0.5	0.6±0.2	5±3
VR 72 h	0.05±0.01	0.04±0.02	3.5±0.1	0.4±0.1	0.6±0.1	4.9±0.5
VR 96 h	0.08±0.01	0.06±0.01	>5.0	0.7±0.2	0.7±0.2	6.0±0.7

*Experimental conditions: mechanical agitation of vermicompost without sieving (10.0000 g) at 200 rpm with 50.00 mL aliquots of mixed solution of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (pH 0.9), at the respective concentrations of 2.0; 10.0; 10.0; 20.0; 5.0 and 50.0 mg L^{-1} **Vermicompost under mechanical agitation. ***Vermicompost at rest. ****Lower than the detection limit of the analytical method. *****Higher than the highest concentration of the calibration curve.

The concentrations in Table 1, as well as in Tables 2 and 3, are associated with Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions, since the composition of the synthetic solutions was exclusively aqueous and did not contain binding molecules and/or complex-forming binding ions. This statement can be extended to the wastewater generated by the atomic absorption spectrometry laboratory, whose composition was also exclusively aqueous, derived from the use of standard metal solutions for the calibration of flame atomic absorption spectrometers, in addition to the analysis of samples previously decomposed in strongly oxidizing media.

Some means listed in Table 1 are associated with high standard deviation values, which can be explained by the heterogeneity of the vermicompost matrix. Although the vermicompost was previously homogenized, it was not possible to avoid such deviations considering the vermicompost without going through any sieving step. However, due to the large distancing of the concentration means in the supernatants in relation to the limits established by CONAMA for Cd^{2+}, Cu^{2+} and Ni^{2+} [10], standard deviation values associated with the concentration means of these cations in the supernatants do not interfere in the interpretation of the adsorbent efficiency of the vermicompost. This statement is also valid for most Pb^{2+} ions concentrations after the tests under resting conditions. Specifically, for Zn^{2+} ions, the standard deviation values listed in Table 1 do not render interpretation unfeasible, since the remaining concentrations in the supernatants are higher than or very close to the limit established by CONAMA, which is 5.0 mg L^{-1} [10].

The initial pH of the mixed solution used in the first experimental phase was 0.9. However, after the different adsorption procedures, the pH values of the supernatants ranged from 4.7 ± 0.3 (resting tests for 24 h) to 6.0 ± 0.5 (resting tests for 72 h), considering the analysis of four replicates. Thus, in addition to the cations evaluated, the vermicompost also adsorbed H_3O^+ ions from the mixed solution, with the consequent increase in pH. In turn, this increase in pH favors the appearance of negative charges on the surface of vermicompost particles, with the consequent potentiation of physical (electrostatic) adsorption among negatively charged adsorption sites and the cations present in the mixed solution. These results demonstrate the efficiency of the vermicompost in simultaneously adjusting the pH of aqueous media to the recommended range (5.0 to 9.0)[10] and leading to significant reductions in the concentrations of different cations.

Hydrated ionic radius (nm) of cadmium, lead, copper, manganese, nickel and zinc are, respectively, 0.426, 0.401, 0.419, 0.438, 0.404 and 0.430[8, 12]. The lower the hydrated ionic radius, the higher the ion charge density, considering the same charge as in the case of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions. Thus, for adsorption processes in which electrostatic interactions contribute significantly, as in adsorption processes in the vermicompost matrix[2], cations with the highest charge densities will be preferentially adsorbed. Therefore, considering the values of hydrated ionic radius, it is possible to observe why the vermicompost presented lower adsorption efficiencies for Mn^{2+} and Zn^{2+}, when compared to Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}. It should be noted that, although electrostatic interactions play a significant role in adsorbents such as the vermicompost, the formation of covalent bonds with humified organic matter cannot be neglected[7]. In addition to the humified organic matter, the vermicompost matrix has a high aluminosilicate content, with marked cation exchange capacity[3].

Although the tests of the first experimental phase were not successful for the cations Mn^{2+} and Zn^{2+}, the retentions of other cations occurred more efficiently when vermicompost particles were under mechanical stirring for a period of 10 h. Thus, mechanical stirring for 10 h was used in the adsorption tests of the second experimental phase, in which the initial concentrations of Mn^{2+} and Zn^{2+} decreased in the mixed solutions, respectively, to 2.0 and 10.0 mg L^{-1}. The decrease in these initial concentrations was adopted to compensate for the lower affinities of the adsorption sites of the vermicompost in relation to both cations. To investigate if smaller initial amounts of Mn^{2+} and Zn^{2+} would improve the adsorption efficiency of the vermicompost without mechanical stirring, the second experimental phase also adopted the resting times of 24, 48, 72 and 96 h.

The results obtained after the second experimental phase are in Table 2, emphasizing that the analyses were performed to quantify only manganese and zinc. This strategy was adopted since, during the tests conducted in the first experimental phase, the concentrations of Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} ions had already been much lower than the respective legal limits[10]. Therefore, the decrease in Mn^{2+} and Zn^{2+} concentrations in the second experimental phase could not decrease the satisfactory adsorption of Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} ions, which had been previously observed.

Data in Table 2 demonstrated that the decreases in Mn^{2+} and Zn^{2+} ions concentrations to 2.0 and 10.0 mg L^{-1}, respectively, were sufficient to guarantee adsorptions capable of framing treated aqueous media within the requirements of quality standards for effluents[10]. It is important to observe that, although the initial concentrations of Mn^{2+} and Zn^{2+} ions decreased in the mixed solution, they remained 2 times higher than their respective legal limits[10].

Table 2. Concentrations (mg L^{-1}) of manganese and zinc after the second experimental phase*, N = 4. Values expressed as Mean ± standard deviation.

Experimental condition	Cation	
	Mn^{2+}	Zn^{2+}
VA** 10 h	0.80 ± 0.04	< 0.05****
VR*** 24 h	0.90 ± 0.07	< 0.05
VR 48 h	0.50 ± 0.06	< 0.05
VR 72 h	0.40 ± 0.01	< 0.05
VR 96 h	0.6 ± 0.1	< 0.05

*Experimental conditions: mechanical agitation of vermicompost without sieving (10.0000 g) at 200 rpm with 50.00 mL aliquots of mixed solution of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (pH 0.9), at the respective concentrations of 2.0; 10.0; 2.0; 20.0; 5.0 and 10.0 mg L^{-1}. **Vermicompost under mechanical agitation. ***Vermicompost at rest. ****Lower than the detection limit of the analytical method.

The results from the third experimental phase (use of 0.053 mm mesh, sieved vermicompost) are listed in Table 3. Here, the experiments were carried out with a mechanical stirring time of 10 h, for reasons previously explained for the procedures of the second experimental phase.

The results in Table 3 showed that the decrease in the diameter of vermicompost particles (granulometry ≤ 0.053 mm) provided a very effective adsorption for Zn^{2+} ions, since its initial concentration in the mixed solutions was 50 mg L^{-1}, or 10 times higher than the legal limit[10]. This observation is also valid for the other cations. Therefore, it is concluded that the increase in the surface area of vermicompost particles considerably minimized the competition effects observed in the first experimental phase for Zn^{2+}.

For Mn^{2+} ions, the concentrations in the supernatants after the treatment with 0.053 mm sieved vermicompost were also effective, considering that the initial concentration of Mn^{2+} in the mixed solutions was 20 mg L^{-1}. However, for most experimental conditions tested, the concentrations of manganese ions were higher than or very close to the maximum limit of this cation (1.0 mg L^{-1})[10]. Despite these results, the adsorption of Mn^{2+} ion was much more effective in the third experimental phase than in the first phase, and small increases in the mass of the sieved vermicompost using a 0.053 mm mesh would be sufficient to adjust Mn^{2+} concentrations to the limit of CONAMA[10]. The presence of high standard deviation values for some of the means listed in Table 3 can also be interpreted as previously discussed for Table 1.

The results of the first, second and third experimental phases indicated that relatively reduced vermicompost masses can remove significant amounts of the six elements investigated. Considering that the ton of vermicompost can be purchased for about US$ 100, it becomes feasible to use larger amounts of that adsorbent, if it is necessary to treat higher volumes of aqueous media. It should be noted, however, that the tests performed used aqueous media at very high concentrations (up to 10 times higher than the legal limits) of the six elements evaluated, so that, in general, real wastewaters have considerably lower concentrations. As discussed below, this was the case of the wastewater of an atomic spectrometry laboratory.

Table 3. Concentrations (mg L^{-1}) of cadmium, copper, manganese, nickel, lead, and zinc after the third experimental phase*, N = 4. Values expressed as Mean ± standard deviation.

Experimental condition	Cation					
	Cd^{2+}	Cu^{2+}	Mn^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
VA** 2h	< 0.05****	< 0.08	1.4 ± 0.2	< 0.06	< 0.2	0.4 ± 0.03
VA 10h	< 0.05	< 0.08	1.6 ± 0.8	< 0.06	< 0.2	0.2 ± 0.04
VR*** 24 h	< 0.05	< 0.08	1.4 ± 0.2	< 0.08	< 0.2	< 0.05
VR 48 h	< 0.05	< 0.08	1.0 ± 0.2	< 0.06	< 0.2	< 0.05
VR 72 h	< 0.05	< 0.08	< 0.1	< 0.06	< 0.2	< 0.05
VR 96 h	< 0.05	< 0.08	0.9 ± 0.1	< 0.06	< 0.2	< 0.05

*Experimental conditions: mechanical agitation of vermicompost (10.0000 g) sieved in a 0.053 mm mesh at 200 rpm with 50.00 mL aliquots of mixed solution of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} (pH 0.9), at the respective concentrations of 2.0; 10.0; 10.0; 20.0; 5.0 and 50.0 mg L^{-1}. **Vermicompost under mechanical agitation. ***Vermicompost at rest. ****Lower than the detection limit of the analytical method.

Table 4 lists the concentrations of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions in the wastewater, whose natural pH was 5.0. Considering the results listed in Table 4 and those of the first three experimental phases, vermicompost with its natural granulometry and mechanical stirring for 10 h were used.

Table 4. Concentrations of cadmium, copper, manganese, nickel, lead, and zinc (mg L^{-1}) in a real wastewater (pH 5.0) derived from an atomic spectrometry laboratory, N = 4. Values expressed as Mean ± standard deviation.

Cation					
Cd^{2+}	Cu^{2+}	Mn^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
0.3 ± 0.02	< 0.08*	0.15 ± 0.09	< 0.06	1.9 ± 0.1	0.15 ± 0.04

*Detection limit of the analytical method.

Before the treatment with the vermicompost, the wastewater already had concentrations of Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} ions below the respective legal limits[10]. However, the concentrations of Cd^{2+} and Pb^{2+} were, respectively, 1.5 and 3.8 times higher than these limits[10]. After the treatment, the concentrations of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} ions were below the respective detection limits of the analytical methods. The same results were observed when wastewater was acidified with HNO_3, thus highlighting the viability of the vermicompost for the treatment of wastewater under different conditions of pH, i.e., at pH 5 (natural pH) and 1 (after acidification with HNO_3).

In a review[13] concerning the use of vermicompost to treat aqueous media, it is possible to observe a scarcity of studies related to the use of vermicompost to adsorb metallic cations under conditions of high acidity and strong competition among the adsorbates (mixed solutions). However, studies using vermicompost and similar materials (composted solid residues) to retain metallic cations from single aqueous solutions can be used to compare adsorption affinities. In this context, Zhu *et al.*[14] evaluated the adsorptive efficiency of vermicompost to retain Cd^{2+} and Pb^{2+} ions, and a preferential adsorption of Pb^{2+} was observed, thus corroborating this study. In turn, two composted municipal solid residues were used to retain Cu^{2+}, Pb^{2+} and Zn^{2+} [15], and the following adsorption order was observed: $Pb^{2+} > Cu^{2+} > Zn^{2+}$. These results are also in agreement with this study, thus indicating that composted materials (including vermicompost) have similar adsorption profiles, independently of their sources.

Recently, vermicompost derived from cow dung was used to retain Cu^{2+}, Fe^{2+}, Mn^{2+}, and Zn^{2+} ions from effluents of different types of industries (beverage, distillery, and paper mill)[16], and the following adsorption order was observed: $Cu^{2+} > Mn^{2+} > Fe^{2+} > Zn^{2+}$. Similarly, to this study, the cited research[16] indicated a preferential affinity of Cu^{2+} ions in relation to the chemical groups of the vermicompost, whose masses were of up to 100 g. In general, 100 g of vermicompost resulted in the highest adsorption percentages, thus underlining that large masses of vermicompost are able to minimize strong chemical competitions in relation to adsorptive chemical groups. The need for higher vermicompost masses was also observed in our investigation, but in a minor scale, since 10 g of vermicompost were sufficient to assure satisfactory adsorptions for the most investigated metallic cations.

When vermicomposts are submitted to partial extractions of humified substances, humified insoluble solids are produced. The production of this type of solid is important to compare the adsorptive efficiency among vermicomposts with and without chemical modifications. In this context, a humified insoluble solid was used to adsorb Ni^{2+} and Pb^{2+} ions from effluents of a white clay factory[17], and the authors verified adsorptions of 98 % and 96 % for Ni^{2+} and Pb^{2+}, respectively, considering adsorbent masses of up to 20 g and pH 7. This outcome revealed a great difference between the cited research[17] and our study, in which 10 g of vermicompost (not submitted to chemical modifications) were sufficient to assure simultaneous and satisfactory adsorptions of the most of six metallic cations (Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) even considering high acidity conditions.

Treatment procedures for aqueous media based on adsorption processes, such as the one presented in this study, generate an environmental liability, which is characterized by the adsorbent mass enriched with various metals. Therefore, safe alternatives for the allocation of such environmental liabilities should be carefully evaluated. Thus, the destination of the vermicompost enriched with the execution of this study will be object of future investigations, which will be based on procedures already tested. In this context, previous studies[9] pointed out that metallic cations adsorbed in the vermicompost can be completely desorbed by percolation of acidic eluents, as 1 mol L^{-1} HNO_3 solution. As for the recovery of desorbed metal ions, the literature[18] points to successful reuse alternatives in the scope of practical classes.

4. Conclusions

The results obtained in this study extended the understanding of the interaction forms between metallic cations and the vermicompost, one of the most complex commercially available humified materials. In this context, the use of the vermicompost was very suitable for the removal of Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} cations from aqueous media, even considering the high acidity of the mixed solutions tested. This study also showed the suitability of the vermicompost for the treatment of a real wastewater from an atomic spectrometry laboratory.

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Influence of the diameter of nanoparticles in complexes metal-aquatic humic substances

Daniele Frasson Vieira[1+], Nicolas Henrique Furquim[1], Wander Gustavo Botero[2], Luciana Camargo de Oliveira[3], Danielle Goveia[1]

[1] São Paulo State University "Júlio de Mesquita Filho" (UNESP), Experimental Campus of Itapeva, 519 Geraldo Alckmin St, Itapeva, São Paulo, Brazil
[2] Federal University of Alagoas (UFAL), Campus of Arapiraca, Manoel Severino Barbosa Av, Arapiraca, Alagoas, Brazil
[3] Federal University of São Carlos (UFSCAR), Sorocaba Campus, João Leme dos Santos Hwy, Km 110 - SP-264, Sorocaba, São Paulo, Brazil

[+]Corresponding author: Daniele Frasson Vieira, e-mail address: dani.frasson@hotmail.com

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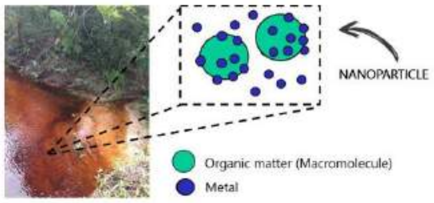
ABSTRACT: Nanoparticles are emerging as the object of research in all fields of chemistry, their special properties are matter for concern, because a considerable portion of these materials are eliminated in the environment. A key point of the discussion is how nanoparticles will interact with other components in natural waters. In this project, the main objective will be to study the interactions of nanoparticles with metallic ions in the presence of humic substances in environmental systems. It is intended to differentiate free and labile metal ions using nanoparticles and organic matter in the form of aquatic substances (extracted from samples collected on the coast of São Paulo). It is intended to simulate the environmental systems and to verify the competition between the complexants. The differentiation of the free and complexed ions will be done using an ultrafiltration system equipped with polyethersulfone membrane (1KDa) and determination of the metals by atomic absorption spectrometry with flame atomization.



Metals can be found free (more toxic) or complexed with organic matter. Nanoparticles when discarded will interact with this system.

1. Introduction

By providing more efficient, lightweight, suitable and mainly low cost final products, many nanomaterials are in the commercialization stage. The increasing production and application of nanomaterials has provoked a wide discussion about the potential risks of these materials to the environment and to human health. Some studies suggest that, due to their small size, nanomaterials may have a greater permeability through the skin, mucous membranes and cell membranes, and may have their toxic effect exalted because they have a higher reactivity caused mainly by the increase of surface area[1, 2]. As an example, gold, which is a practically inert metal but which in the form of nanoparticles becomes highly reactive[3].

The properties of nanoparticles, which make them so valuable, are of concern in the analysis of the ecosystem since a significant portion will be eliminated in the environment. In this way, its chemical interactions, its cycle and destiny, are evolving into an important topic of discussion in all fields of chemistry. A key point of the discussion is how nanoparticles will interact with the other components in natural waters, such as metal ions, dissolved organic matter and microorganisms. In aquatic environments, organic matter exerts a great influence on the behavior of metals due to their physicochemical polyelectrolyte properties[4].

Approximately 40 % to 60 % of the organic matter is in the form of Aquatic Humic Substances (SHA) that represent the main class of natural complexants present in natural waters. One study demonstrated that humic and fulvic acids can significantly alter the capacities and the mechanisms of adsorption of metals in silica nanoparticles. In this study, it was clear that humic material could increase the amount of metal bound to the nanoparticle, influencing the behavior of metals and nanoparticles in the environment[5, 6].

Only a small fraction of the total dissolved metal is present as free ions, most of which are in the form of stable complexes with dissolved inorganic or organic binders and organic particles[7]. Free metal ions are more toxic than complexed or adsorbed ions to some substance or surface of particles. So, the more strongly attached the less bioavailable and the lower the toxicity of the metal[8].

In this system composed of metals, SHA and nanoparticles, there are two possibilities of action of the humic material: it can complex the metals and decrease the bioavailability and reactivity in a process of competition with the complexing sites of the nanoparticles, as well as interacting with the surface of the nanoparticle, increasing the absorption capacity of the metal and the ability to cross the cell wall carrying the metal into the cell, which translates into a greater toxicological risk to the environment. Thus, it becomes even more important to know the dynamic nature of the complexes[9, 10]. Our focus will be to understand the impact of silica nanoparticles on the matrix, that is, how they interfere in the speciation of iron and manganese metal ions in environmental systems, originally bound in organic matter.

2. Experimental

2.1 *Obtaining Aquatic Humic Substances*

In February 2017, approximately 100 liters of water samples were collected from the Juréia River, located in the city of São Sebastião-SP, due to the high content of organic matter present. After the collection, the extraction of the Aquatic Humic Substances was carried out. An adsorptive column chromatography system was mounted using the DAX-8 superlite microporous resin which was suspended in deionized water and stored in a glass column, forming an exchanger bed. Then, by gravity, the samples were percolated through the glass column.

After saturation with SHA, as verified by the darkened color of the resin, a 0.1 mol L^{-1} NaOH solution was used for the elution of SHA. The humic extract was collected at the base and taken to the oven and dried at a temperature of approximately 55 °C.

The *in situ* characterization of the source was carried out by means of determinations of pH, temperature, dissolved oxygen and conductivity, from the calibration of the equipment, using reference standard solutions. The SHA were characterized by ultraviolet-visible spectrophotometry, where 2.0 mg of dry SHA sample was dissolved in 10 mL of 0.05 mol L^{-1} $NaHCO_3$ and the reading the absorbance of the sample on the Spectaphotrometer Specta 50 at wavelengths 465 and 665 nm to determine the E4 / E6 ratio based on the optical densities obtained at the respective wavelengths.

2.2 *Evaluation of metal-binder interactions*

The sample was digested to determine the concentration of the total metal and the free metal present in the water *in natura*. 300 mL of plate water was digested at approximately 120 °C using 10 ml of nitric acid. The digest was quantitatively transferred to volumetric flask and the volume adjusted with deionized water to 50 mL.

Subsequently, 250 mL solutions containing Fe and Mn ions were prepared in a concentration of approximately 2.0 mg L^{-1}. The extracted SHA was added at a concentration of approximately 100 mg L^{-1}. These solutions were for the ultrafiltration system (Figure 1), using peristaltic pump, tygon tubes, 1 kDa and 47 mm diameter porosity membrane (Ultracel Ultrafiltration Discs) and pressure controllers.

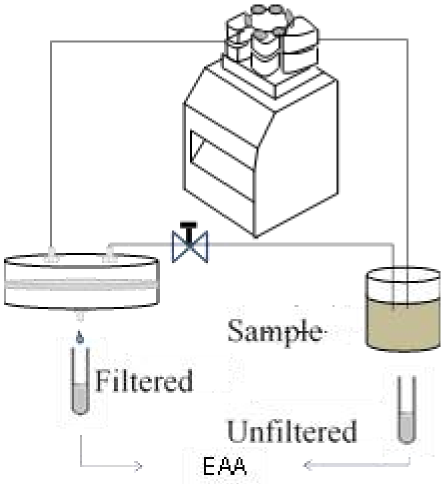


Figure 1. Ultrafiltration system used to evaluate complexes formed between metal ions and binders (chemicals and nanoparticles).

After passing through the 1 kDa membrane, an aliquot (time zero) was withdrawn. The metal determined in this aliquot is the free metal and the metal complexed to the fraction of organic matter <1kDa. Considering that this fraction of organic matter is very small in relation to the fraction> 1kDa and to facilitate the understanding, we will consider this aliquot as free metal. LUDOX silica nanoparticles were then added. LUDOX silica nanoparticles are sold commercially for equipment calibration, so they have specific and regular sizes and are in suspension form in water. Three suspensions were chosen for analysis: LUDOX LS30, LUDOX TM40 and LUDOX TM50. These nanoparticles used in these experiments were characterized in earlier studies (Table 1)[9]. Were added to the solutions 800 μL of LS30, 1070 μL of TM40 and 1330 μL of TM50 all in the proportion in moles of 1: 1.

Table 1. Characterization of silica nanoparticles in different sizes.

Type of silica	Diameter (nm)	Surface area (m^2/g_{silica})	Number of particles/g_{silica}
LS30	8	215	3.86E+17
TM40	17	140	3.74E+16
TM50	31	140	5.73E+15

The filtrate aliquots were withdrawn from time to time for 24 h and the metal determinations were made by flame atomization atomic absorption spectrometer (novAA 400 Analytik Jena), following the manufacturer's recommendations.

3. Results and Discussion

Considering that the SHA structure is directly related to its origin, it is necessary to carry out the preliminary characterization of the environment where the samples were collected. The results of the *in situ* characterization are shown in Table 2.

Table 2. Preliminary characterizations of water samples collected in the tributary of Juréia River in February/2017.

Parameters analyzed	Sample of water from Juréia River	Sample of water from the Itapanhaú River [11]
pH	5.38	5.20
Temperature (ºC)	27.00	22.20
Conductivity (μS cm^{-1})	60.30	73.20
Dissolved oxygen (mg L^{-1})	5.20	3.70

The National Environmental Council (CONAMA) in Resolution No. 357, dated March 17, 2005[12] classifies the water bodies and environmental guidelines for its setting and establishes the conditions and standards for effluent releases, and makes other provisions from of parameters. Analyzing the dissolved oxygen parameter, the water sample can be classified as class 2, since the concentration is greater than 5 mg L^{-1} and less than 6 mg L^{-1}. However, the pH presented a value below the ideal considered according to CONAMA (pH of 6.0 to 9.0), but the value obtained is characteristic of water bodies rich in organic matter. The results presented similarities with rich source of organic matter evaluated in previous studies, as can be observed in Table 2.

The absorbance values found in UV-VIS spectrophotometry at wavelengths 465 and 665 nm, respectively, were 0.02753 and 0.0268. Therefore, the ratio of E4/E6 is equal to 1.027. This ratio indicates how high is the degree of condensation of the sample, that is, if the obtained value of the ratio decreases, the sample shows a high degree of structural condensation, being associated directly to the degree of humification. If the values obtained are high, it indicates a less condensed structure and, consequently, a lower degree of humification[13]. In this case we can infer that SHA does not present such a high degree of structural condensation, considering high values less than 1.0.

Table 3 presents the result of the determination of the total metal present in the water *in natura*, considering all the metal detected in the sample after the acid digestion. The free metal is that obtained in the membrane filtered solution of 1kDa.

Table 3. Total metal concentration in the Juréia River *in natura* and concentration of free metal.

Concentration of metals		
	Fe (mg L^{-1})	**Mn (mg L^{-1})**
Total	37.20	2.70
Free	0.20	0.20

Comparing the concentrations of free iron with the *in natura* concentration, it is verified that the iron present in the organic matter is almost totally forming Fe-SHA complexes. With the manganese the same behavior is observed, but to a lesser extent, considering the amount of this metal in the well.

After the differentiation of the free and complexed metals to SHA and addition of the silica nanoparticles, the concentration of the free metals was evaluated from time to time until 24 h. The impact that the nanoparticles could cause on the complexes formed was evaluated by varying the size of the nanoparticle. Tables 4, 5 and 6 show the values obtained for the metal concentrations complexed with the SHA> 1kDa, and the free metal quantified in the filtrate (SHA fraction <1kDa).

Table 4. Fractionation of the complexed metal ions to the Aquatic Humic Substances. Complexation time: 24 h after addition of LUDOX LS30% nanoparticle (8 nm in diameter).

Organic Matter		Fe (mg L^{-1})	Mn (mg L^{-1})
SHA-Juréia	Total metal	1.7	2.2
	Free metal	0.8	1.0
	LUDOX LS30%	1.3	0.5

Table 5. Fractionation of the complexed metal ions to the Aquatic Humic Substances. Complexation time: 24 h after addition of the LUDOX TM40% nanoparticle (17 nm diameter).

Organic Matter		Fe (mg L^{-1})	Mn (mg L^{-1})
SHA-Juréia	Total metal	1.8	1.9
	Free metal	0.3	0.4
	LUDOX TM40%	0.2	0.3

Table 6. Fractionation of the complexed metal ions to the Aquatic Humic Substances. Complexation time: 24 h after addition of LUDOX TM50% nanoparticle (31 nm diameter).

Organic Matter		Fe (mg L^{-1})
SHA-Juréia	Total metal	1.8
	Free metal	0.2
	LUDOX TM50%	0.2

It was possible to observe that the size of the nanoparticle and the surface area alter the stability of the SHA-Metal complexes. The LUDOX LS30 nanoparticle has a smaller diameter and, consequently, a larger surface area. When added to the solution increases the proportion of free metal, providing the labile metal, which is interacting weakly with the organic matter. By evaluating the data presented by the addition of the larger nanoparticles (TM40 and TM50) it was verified that the amount of free metal does not change. There are two strands here, one of which indicates that the larger diameter nanoparticle does not interfere with the formation of the SHA-Metal complexes, that is, it does not increase the bioavailability of the labile metal. Another possibility, which cannot be evaluated by studying only the free metal, is the stability of the nanoparticle-Metal complexes.

The kinetics of the reaction were evaluated by time and the results quantified in the aliquots were plotted in the subsequent figures. Figure 2 shows the behavior of the free iron concentration over a period of 24 h of complexation in the presence of nanoparticles of different diameters, showing its influence on the Fe-SHA complexes for SHA extracted from the Juréia River.

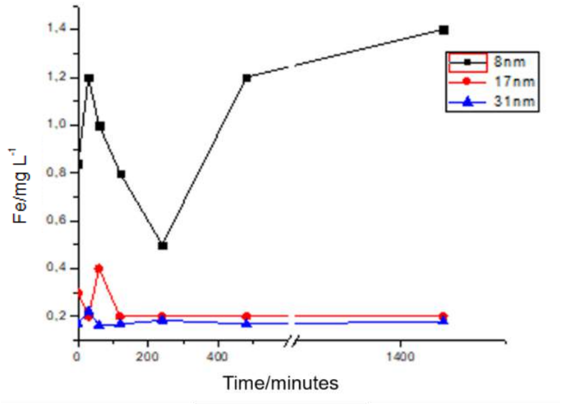


Figure 2. Free iron determined in the filtrate after addition of LUDOX LS30 (8 nm), TM40 (17 nm) and TM50 (31 nm) nanoparticles to the Metal-SHA complex, SHA extracted from the Juréia River.

It is worth mentioning that time zero corresponds to the moment before the addition of the silica nanoparticles. It is noted that in the first minutes after the addition of LUDOX LS30% silica nanoparticle, an increase in free iron concentration occurs. Probably the ions originally complexed to SHA may be available in solution, breaking the interaction between the formed complex (SHA metal) by influence of the nanoparticle. SHA may also be interacting with the nanoparticle, masking the complexing sites and thus leaving the metals available. In the sequence a considerable drop occurs in this concentration, showing that the free iron ions are complexing with the nanoparticles and / or SHA. As after a longer time, the concentration of free ions returns to increase, we can infer that the metal-SHA interaction is stronger than the metal-nanoparticle interaction.

Observing the behavior of the concentration of free iron ions after the addition of the nanoparticles of silica TM40% and TM50%, it is observed that it remained practically constant throughout the time. This indicates that the metal-SHA complex is not influenced by these nanoparticles. Figure 3 shows the results of the concentration of free manganese in 24 h of complexation, showing the influence of the nanoparticles in the Mn-SHA complexes for SHA extracted from the Juréia River.

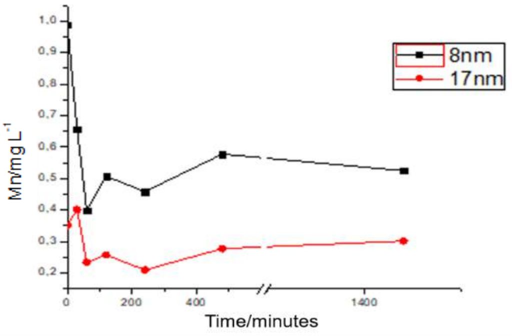


Figure 3. Free Mn determined in the filtrate after addition of the LUDOX LS30 (8 nm) and TM40 (17 nm) nanoparticles to the Metal-SHA complex, SHA extracted from the Juréia River.

In the presence of LUDOX LS30%, in the first minutes the concentration of Mn falls, possibly the free ions formed complexes with the nanoparticle. However, after 120 min, there is a small increase and subsequent stabilization of the concentration of free ions. In the presence of LUDOX TM40%, it is verified that in the first minutes after the addition of the nanoparticle nothing happens, the value of the concentration is the same as in time zero. In the sequence, it presents the same behavior observed with the LS30, that is, a drop of free manganese ions and then small variations.

More information is needed to make sure the ion is complexing with the nanoparticle or SHA. This doubt can be evidenced when we change the binder addition sequence, that is, when we add SHA to the stable complexes M-nanoparticles.

4. Conclusion

The results obtained so far have shown to be promising in relation to the impact of the nanoparticles on the environment, indicating that the size and surface area interfere with the behavior and availability of the metals in the environment, and the lower the nano-silica, the higher the metal complex, complexed prior to SHA.

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Evaluation of pseudo-total and labile metals (Cu^{2+}, Cr^{3+}, Ni^{2+}, Mn^{2+}, Pb^{2+} and Zn^{2+}) in surface sediments at the mouth of tributaries of the Itaipu Reservoir, Brazil

Ismael Laurindo Costa Junior[1+], Thiara Reis Lopes[1], Bruna Ataide Barros Fonseca[1], Mariane Butik[2], Juliana Bortoli Rodrigues Mees[1], Adelmo Lowe Pletsch[1], Yohandra Reyes Torres[2]

[1] State University of Midwest (Unicentro), Department of Chemistry, 875 Salvatore Renna St, Guarapuava, Paraná, Brazil
[2] Federal Technological University of Paraná (UTFPR), Department of Chemistry, 4232 Brasil Av, Medianeira, Paraná, Brazil

[+]Corresponding author: Ismael Laurindo Costa Junior, e-mail address: ismael@utfpr.edu.br

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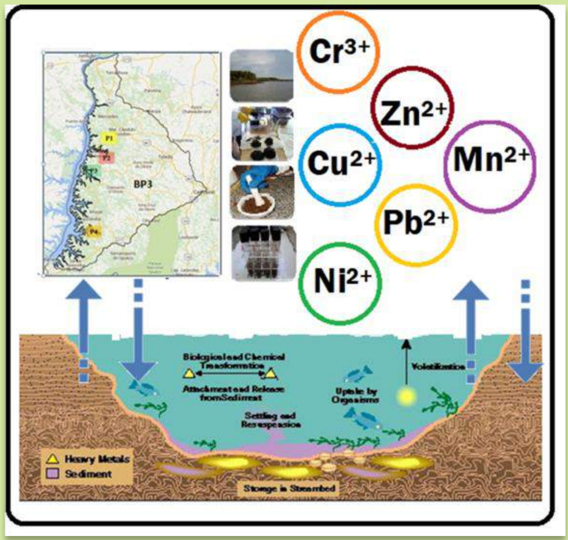
ABSTRACT: Sediments are reservoirs for a variety of substances widely used as indicators of pollution in aquatic environments. In this research it was carried out the evaluation of the concentration of metals in the pseudo-total and labile fractions in surface sediments at the mouth of four main tributaries of the Itaipu Reservoir. Three samplings were performed between February 2014 and March 2015 using Petersen type dredger. The determination of metals occurred by atomic absorption spectrometry and physicochemical parameters by gravimetric and potentiometric techniques. The samples presented neutral to slightly alkaline character and homogeneous pH values. It was observed the predominance of the clay fraction and the occurrence of variable organic matter between the points. In the labile fraction, only the copper content reached concentrations higher than the thresholds effects levels (TEL) at all sampling points. In the pseudo-total fraction, some sites presented for Cr^{3+}, Pb^{2+} and Zn^{2+} TEL level. For Ni^{2+}, values of probable effects level (PEL) were verified and for Cu^{2+}, concentrations in severe effect level (SEL) were reported according to the international sediment classification criteria adopted by the National Oceanic and Atmospheric Administration (NOAA).



1. Introduction

The technological advancement resulting from population and industrial growth contributes to increasing the use and deterioration of ecosystems. Water is one of the main natural resources and after being used in various purposes, it returns to the environment with different quality from the original. Several contaminants, such as metals, are incorporated, thus favoring the pollution of water bodies and consequent accumulation of these substances in sediments and biota.

Artificial reservoirs and natural lakes are important surface water resources with great relevance for water supply, flood control, irrigation, aquaculture, climate regulation and maintenance of the ecological balance[1]. In this context, the Itaipu Reservoir is one of the largest artificial lakes in the world, whose main purpose is the generation of electricity, in addition to aquaculture and navigation. By its location and

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extension, it exerts a direct influence on the local ecosystem[2].

The sediments comprise the unbound material, formed by particles of different sizes, shapes and chemical composition, carried by water, air or ice, distributed along the valleys of the drainage system and oriented from the processes of weathering and erosion[3].

Increased concentrations of metals in sediments are related to anthropogenic activities, such as industrial effluent discharges, agriculture, land transport, lack of adequate sewage treatment and solid waste, which can result in contamination of the food chain and biological diversity[4, 5].

The high capacity in the retention of sediment contaminants is a factor that highlights the importance of this matrix for the evaluation of pollution levels. Its composition reflects the environmental characteristics of both the sampling moment and the remote memory of the aquatic environment[6]. With this, it can be considered a key compartment that reports the environmental history, through the permanent record of the effects of contamination[7].

The objective of this study was to evaluate the pseudo-total and labile metal fractions extracted from sediments sampled at the mouth of tributaries of the Itaipu Reservoir and to compare the results with the National Oceanic and Atmospheric Administration (NOAA)[8].

2. Experimental

2.1 Location of Collection Points

The study was carried out in the Paraná Basin 3 (BP3). BP3 has an area of approximately 8,000 km², involving 28 municipalities in the State of Paraná and one in Mato Grosso do Sul[9] (Figure 1).

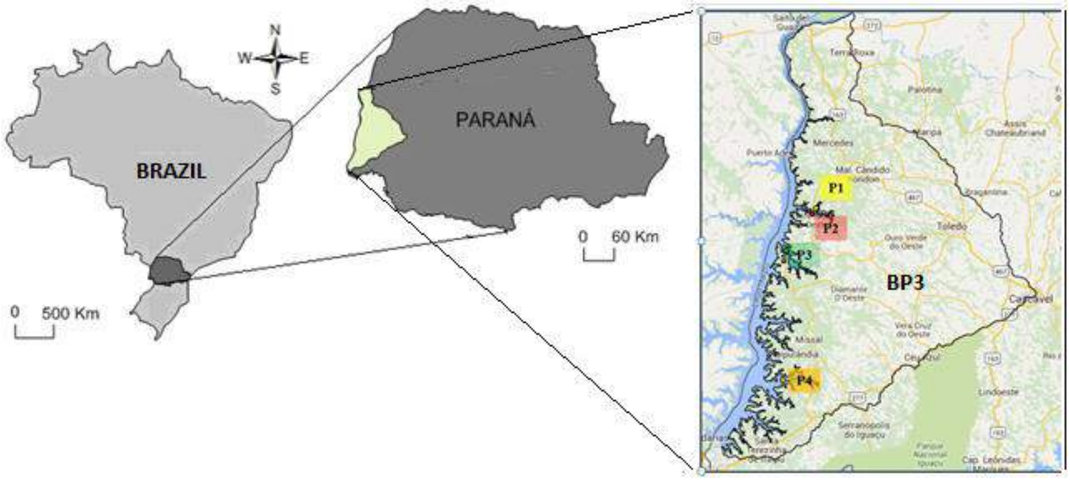

Figure 1. Delimitation of the Paraná River Basin 3 (BP3) and Sampling Points.

Sampling points were chosen at the mouth of the Arroio Fundo River, São Francisco Verdadeiro River, São Francisco Falso River and Ocoí River to characterize the tendency of contamination through the concentration levels of metals in the recently deposited sediments (Table 1).

Table 1. Location of sediment sampling points.

Point	Location	River	Figure
P1	24° 39´55”S 54°13´01”W	Arroio Fundo	
P2	24°41’00”S 54°14’10”W	São Francisco Verdadeiro	

P3	24°50’52”S 54°19’25”W	São Francisco Falso	
P4	25°13’56”S 54°13’24.4” W	Ocoí	

2.2 Sample Collection, Preparation and Physicochemical Characterization

Three samples were taken between February 2014 and March 2015. The sediments were collected between 0-10 cm depth with the use of a Pettersen type dredger. All glassware and polypropylene material were soaked in 10 % (v/v) HNO_3 for 24 h and rinsed thoroughly with ultrapure water before use. Ultrapure water was obtained from a Human UP 900 purification system, which was also used to prepare all of the solutions. After collection, samples were refrigerated for transport. The physical and chemical determinations were performed with the moist sediments. For the determination of metals, some of the material was being air-dried at room temperature (25 ± 1 °C) for 2 weeks and then disaggregated with the use of mortar and pistil, sieved in a mesh of 500 μm, stored in glass flask.

Determination of the texture of sediment samples (e.g., silt, sand, clay) was performed using densitometry[2]. The pH was determined in potentiometer with suspension of 1.0 g of sediment in KCl 1 mol L^{-1} [10]. Total solids (TS), Total Fixed Solids (FTS) and total volatile solids (VTS) were determined by gravimetry, by drying 5.0 g of sample in an oven at 105 °C until constant weight and then ignition in furnace at 500 °C for 4 h[10]. Total organic carbon (TOC) content was calculated by the empirical formula Van Bemmelen factor: TOC = VTS/1.724[2, 11].

2.3 Metals Determination

The analysis of labile metals in sediments is defined as the fraction extracted by diluted acid attack. Approximately 1.0 g dry sediment was added in Erlenmeyer flask, together with 25.0 mL of HCl (Biotec Ltda., São Paulo, SP, Brazil) 0.10 mol L^{-1} and kept under vigorous agitation using a rotary table (Orbital®) at 200 rpm for 2 h at room temperature (25 ± 1 °C)[12].

The pseudo-total metals were extracted by acid digestion according to Environmental Protection Agency (EPA) method 3050B: 0.50 g dry sediment were added in Erlenmeyer flask containing 15.0 mL HNO_3 (Neon, Suzano, SP, Brazil) and heated for 4 h at 60 °C. Subsequently, 8.0 mL of H_2O_2 (Neon, Suzano, SP, Brazil) were added and heated for 30 min. After extraction, samples for pseudo-total and available metals were gravity filtered by washing the filter paper with ultrapure water and completed to 50 mL and stored at 4 °C[2, 13].

Metallic-ion quantification was performed using flame atomic absorption spectrometry (FAAS; model SpectraAA-220, equipped with hollow cathode lamps and deuterium lamp for background correction (Varian, Palo Alto, CA). All absorbance measurements were determined by integration area; the samples were prepared in triplicate; and the signal of the blank experiment was discounted. Table 2 lists the FAAS operating conditions for the determination of metallic ion.

Table 2. FAAS operating conditions for determination of metallic ions.

Metals	λ (nm)	i (mA)	Slit (nm)	Flame	Range (mg L^{-1})
Cu^{2+}	324.7	4	0.5	Ar/C_2H_2	0.03–8.0
Cr^{3+}	357.9	7	0.2	Ar/C_2H_2	0.06–20.0
Mn^{2+}	279.5	5	0.2	Ar/C_2H_2	0.02–6.0
Pb^{2+}	217.0	5	1.0	Ar/C_2H_2	0.1–20.0
Zn^{2+}	213.9	5	1.0	Ar/C_2H_2	0.01–2.0
Ni^{2+}	232.0	4	0.2	Ar/C_2H_2	0.1–8.0

The FAAS was calibrated with analytical curves prepared with aliquots of standard solutions of 1000 mg L^{-1} of the Cu^{2+}, Cr^{3+}, Mn^{2+}, Pb^{2+}, Zn^{2+} and Ni^{2+} metal ions. The method was validated by means of linearity, with regression analysis of the calibration curves for each metal, by determination of the limits of quantification (LOQ) and detection (LOD) and by a comparative recovery test to the certified reference material (MRC-PACs-2, National Research Council of Canada). For the linearity, the coefficient of determination (R^2) was considered being adequate when close to 100 %. Limits of detection (LODs) and quantification (LOQs) were calculated using signal-to-noise ratios of 3 and 10, respectively, as well as parameters from the linear equation[14]. The accuracy of the method was verified considering the recovery in relation to the values measured for the MRC: Accuracy (%) = $100 \times C_{med}/C_{MRC}$, where C_{med} is the concentration obtained experimentally and C_{MRC} is the concentration declared in the certificate.

2.4 Analyses of Correlations Among the Parameters Evaluated

The statistical tool used was principal component analysis (PCA). PCA permits evaluation of the data set by decreasing the dimension of the data and conserving the most useful statistical information present in the original data. PCA is performed through the determination of distances among the sample data, which can be calculated considering the closest K-neighbor or mean of the samples. The data can be either the original (scaled or centered on the mean) data or the scores generated by the PCA[15, 16]. The statistical operations were performed using the Statistica 10.0 software[17]. Statistical analysis was performed using analysis of variance (ANOVA).

3. Results and Discussion

3.1 Physical-Chemical Characteristics of the Sediment

The pH values of the sediments indicated a neutral to slightly acid character. The greatest difference between the values occurred between points 2 and 3 (0.53 pH units). This indicates that the studied sediments have relatively homogeneous pH. This characteristic can favor the accumulation of metals in sediments because, the increase of the pH favors the precipitation of the dissolved metals[6].

The largest fraction of FTS occurred in P3 and the lowest was observed in P4. The concentrations of VTS and total carbon were higher in P4, followed by P1 and P2. This higher VTS concentration indicates a higher occurrence of organic matter at these sampling points, which may favor the retention of metals in this matrix (Table 3). Sediments of fine granulometry, clayey, rich in nutrients and organic matter constitute one of the main adsorbents of several metals that may be present in the aquatic environment.

Table 3. Results of the analysis of physicochemical parameters of sediment samples.

Sample	pH	TS (%)	FTS (%)	VTS (%)	TOC (%)	Clay (%)	Silt (%)	Sand (%)
P1	6.83±0.04	52±2	88±1	12±2	6±1	44	14	42
P2	7.19± .02	59±3	89.8±0.3	10.2±0.3	5.7±0.2	44	14	42
P3	6.66±0.04	68±1	91.0±0.2	9,0± 0.2	5.0±0.1	22	8	70
P4	7.10±0.05	28±1	3.8± 0.6	16.2±0.6	9.0±0.3	42	8	50

P1 - Arroio Fundo River; P2 – São Francisco Verdadeiro River; P3 – São Francisco Falso River; P4 – Ocoí River.

The samples of P1, P2 and P4 presented equivalent results, with the predominance of the clay fraction. Sediments with predominance of this fraction provide a higher concentration of metals due to the high tendency of adsorption in groups of fine granulometry. Sediments with smaller particles have higher adsorption capacity. Consequently, they have a greater potential of environmental contamination, because the metals retained in these sites are more labile[18, 19].

The lowest fraction of clay occurred in P3, with only 22 %, at this point the predominance of the sand fraction can be justified by the proximity of the artificial beach of Santa Helena, which is periodically coated with this material and which is transported to the bed of the reservoir. When the sediments present values of organic matter above 10 % are considered organic, and below 10 % are considered inorganic or mineral sediments. In this study all sediments were classified as primarily mineral[20, 21].

3.2 Metals determination validation parameters

The validation parameters for the metals determinations regarding linearity, limit of detection, limit of quantification and accuracy were considered satisfactory (Table 4). All linear regression equations, obtained by means of solutions of known concentration of the studied metals and their respective absorbances, presented determination coefficients in the range of 99.1 % to 100 %, indicating a high relation between the concentrations and respective absorbances. The LOD and LOQ values ranged from 0.009 to 0.072 mg L^{-1} and 0.029 to 0.23 mg L^{-1} respectively. The lowest values are for Cu^{2+} and the highest values for Mn.

Table 4. Determination of metal ions in CRM by the method of pseudo-total digestion and validation parameters.

Metal	Equation	R² (%)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Certified (mg kg^{-1})	Measured (mg kg^{-1})	Extracted (%)
Cu^{2+}	ABS = 0.15×[Cu] + 0.03	100	0.009	0.029	310± 12	291 ± 5	93.9
Cr^{3+}	ABS = 0.02×[Cr] + 0.0005	99.6	0.022	0.074	91± 5	86 ± 6	94.4
Mn^{2+}	ABS = 0.17×[Mn] – 0.03	100	0.072	0.239	440 ± 19	381 ± 25	86.6
Pb^{2+}	ABS = 0.04×[Pb] + 0.003	99.9	0.025	0.082	183± 12	147 ± 15	80.5
Zn^{2+}	ABS = 0.08×[Zn] + 0.19	100	0.039	0.061	364 ± 23	336 ± 13	92.2
Ni^{2+}	ABS = 0.13×[Ni] – 0.0003	99.9	0.005	0.016	40 ± 2	36 ± 2	91.1

For the recovery study, the methodology used in the pseudo-total extraction of the metals ions in sediment samples was compared with the MRC (PACs 2). Certified values and percent recovery after application of the metal extraction procedure in the pseudo-total sediment fraction were satisfactory (Table 4), indicating which method used provided the analytes to be quantified by FAAS and that the determined concentrations are consistent with that certified in the reference material.

3.3 Monitoring Study

Higher concentrations were found in the three samples at Cu^{2+}, Mn^{2+}, Pb^{2+} and Zn^{2+} at points 2 and 4 (Table 5).

Table 5. Metal concentrations metals in sediment samples.

Labile Fraction (mg kg^{-1})							
Sample		**Cu^{2+}**	**Cr^{3+}**	**Mn^{2+}**	**Pb^{2+}**	**Zn^{2+}**	**Ni^{2+}**
P1	**Feb. 2014**	31 ± 4	12.3 ± 0.2	400 ± 1	10.3 ± 0.7	25 ± 2	4.1 ± 0.2
	Aug. 2014	**57 ± 2**	14.9 ± 0.6	407 ± 3	13.8 ± 0.7	31 ± 2	5.3± 0.3
	Mar. 2015	**71.9 ± 0.3**	17 ± 1	391 ± 2	13.5 ± 0.4	24.5 ± 0.7	4.6 ± 1.2
P2	**Feb. 2014**	**79.9 ± 9.3**	12.8 ± 0.2	406.8 ± 0.7	14 ± 2	32 ± 3	3.2 ± 0.3
	Aug. 2014	**73 ± 3**	15.0 ± 0.4	407.7 ± 0.2	14.9 ± 0.5	42 ± 2	5.5 ± 0.5
	Mar. 2015	**66 ± 3**	17.1 ± 0.7	401.2 ± 0.9	14 ± 2	25 ± 2	3.6 ± 0.2
P3	**Feb. 2014**	18 ± 1	12.2 ± 0.4	403 ± 3	9.4 ± 0.7	4.0 ± 0.6	0.4 ± 0.2
	Aug. 2014	17.4 ± 0.9	15.3 ± 0.4	385 ± 6	18 ± 1	4 ± 2	0.4± 0.2
	Mar. 2015	**67 ± 3**	19.2 ± 0.7	330 ± 2	17.4 ± 0.7	39 ± 2	3.0 ± 0.6
P4	**Feb. 2014**	**74 ± 6**	16.2 ± 0.9	410.4 ± 0.4	13.0 ± 0.6	43 ±4	3.3 ± 0.3
	Aug. 2014	**55 ± 3**	16.7 ± 0.2	405 ± 2	12 ± 1	35.2 ± 0.9	5.3 ± 0.6
	Mar. 2015	**65 ± 3**	3.0± 0.4	406 ± 3	15 ± 2	36 ± 2	3.2 ± 0.2
Pseudo-total Fraction (mg kg^{-1})							
Sample		**Cu^{2+}**	**Cr^{3+}**	**Mn^{2+}**	**Pb^{2+}**	**Zn^{2+}**	**Ni^{2+}**
P1	**Feb. 2014**	**188 ± 2**	**77 ± 1**	929 ± 6	**45.4 ± 0.8**	**212 ± 3**	**57 ± 2**
	Aug. 2014	**159 ± 25**	**68 ± 2**	945.1 ± 0.9	30 ± 3	**167± 14**	**41 ± 3**
	Mar. 2015	**179 ± 3**	**81 ± 3**	939 ± 9	**41 ± 9**	**181 ± 4**	**43.1 ± 0.7**
P2	**Feb. 2014**	**163 ± 2**	**104 ± 2**	947.5 ± 0.4	**64 ± 2**	**178 ±13**	**72 ± 8**
	Aug. 2014	**238±14**	**88±2**	944 ± 4	**62 ± 2**	**249 ± 9**	**58 ± 5**

	Mar. 2015	**144 ± 5**	**57 ± 5**	904 ±12	**40 ± 7**	**152 ± 8**	**36.3 ± 0.7**
P3	**Feb. 2014**	**70 ± 2**	**85 ± 4**	959 ± 3	**76 ± 8**	90 ± 20	**31.4 ± 0.8**
	Aug. 2014	**126 ± 14**	**124 ± 7**	933 ± 8	**79 ± 8**	103 ± 4	**46 ± 4**
	Mar. 2015	**130 ± 2**	**46 ± 4**	612 ±16	**51.7 ± 0.2**	94 ±10	**29 ± 2**
P4	**Feb. 2014**	**208.4 ± 0.5**	**71 ± 4**	957 ± 8	**57 ± 5**	**192 ± 2**	**43 ± 3**
	Aug. 2014	**264 ± 5**	**97.6 ± 0.8**	955.4 ± 0.7	**57 ± 2**	**240 ± 4**	**58 ± 2**
	Mar. 2015	**228 ± 6**	**78 ± 3**	962 ± 3	**66 ± 4**	**247 ±23**	**50 ± 7**
TEL[a]		35.7	37.3	-	35.0	123.0	18.0
PEL[a]		97.0	90.0	-	91.3	315.0	36.0
SEL[a]		110.0	110.0	1100.0	250.0	820.0	75.0

P1 - Arroio Fundo River; P2 –São Francisco Verdadeiro River; P3 – São Francisco Falso River; P4 –*Ocoí River*.

[a] Quality guidelines of segment trace metals in sediment adopted by NOAA (mg kg-1 dry basis)

TEL - *Thresholds Effects Level*; PEL - *Probable Effect Levels*; SEL - *Severe Effect Level*

In the pseudo-total fraction, in February 2014, in the samples P1 and P2, the concentrations of Pb^{2+}, Zn^{2+} and Ni^{2+} were higher when compared to the other samples. At the collected station there was a lower volume of rainfall. This may have contributed to the preconcentration of some metal ions at these two points. The analyte concentrations in the pseudo-total fraction were always higher than in the environmentally available fraction. The toxic potential of metals is related to their bioavailability. This shows the importance of determining the concentration of metals in the total and labile fractions in the sediment. However, these lower levels of labile fraction, compared to pseudo-total, do not exempt sediments from negative environmental impacts[22].

The lowest copper content in the available fraction was observed in the sediments sampled in the year of 2014 in P3 and P1, only these samples had concentrations below the TEL level (values below which adverse effects are rarely expected) 35.7 mg kg^{-1}. In 2015, the concentrations of this element in the same fraction were higher than the TEL level at all sampling points. This indicates the increase of this analyte in the sediments during the period studied. The labile fraction represents the dynamics of exchange between the compartments and the organisms. This indicates potential risk due to higher TEL values. Although it is an essential element in lesser amounts, in larger doses it is toxic. Similar results were obtained in sediment samples from the Itaipu Reservoir in similar studies, where Cu^{2+} concentrations higher than the TEL were reported at some points[7].

In the pseudo-total fraction, all sediment samples had concentrations higher than the TEL level. The sediment samples collected in August 2014 at point 2 and all samples at point 4 showed concentrations of Cu^{2+} that exceeded the PEL limit (probable effects levels), 197.0 mg kg^{-1}.

The highest concentrations of Cu in P1, P2 and especially in the sediments of the mouth of the Ocoí River (P4) may be related to the intensive agriculture that are predominant in this region of study. In these activities are applied various compounds containing copper, as fungicides. In addition, the low solubility of the oxides of this metal increases the possibility of increment of this element in the sediments of the studied sites[23]. Pork farming is another activity intensely performed in the region of study that may contribute to the increase of Cu^{2+} and Zn^{2+} in the environment. This is related to the supply of highly concentrated rations, which means that the manure from this activity presents elevated levels of these elements. The disposal of these residues as fertilizer can provide the contamination of the water bodies, through the lixiviation surface runoff[24].

Concentrations of Cu^{2+}, both labile fraction and pseudo-total fraction, were higher at points 1, 2 and 4, as well as concentrations of VTS and TOC (Table 3). This indicates a higher occurrence of organic matter at these points, which may have provided greater retention of metals. Sediments of lower granulometry, rich in clay, nutrients and organic matter are one of the main adsorbents of several metals that may be present in the aquatic environment[6]. The concentrations of Cu^{2+} obtained were close to the values found in the surface sediment of the Cachoeira River in Bahia, where there was variation between 4.9 and 206.3 mg kg^{-1} [22].

The chromium concentration for the labile fraction presented a uniform distribution at all the points sampled. Concentrations below the threshold where adverse effects are rarely expected (TEL), 37.3 mg kg^{-1} have been found. In the pseudo-total fraction, at all points Cr^{3+} concentrations were higher than the TEL level, 37.3 mg kg^{-1}. The sediment samples collected in 2014, in February in point 2 and August in points 3 and 4, exceeded the limit of PEL, 90.0 mg kg^{-1}. In August 2014, concentrations in the pseudo-total fraction at point 3 were above the severe effects (SEL) levels, 110.0 mg kg^{-1}. These results are similar to concentrations already reported in sediments of the Itaipu Reservoir, in the Cr^{3+} concentrations of 6.4 to 71.9 mg kg^{-1} [7].

The concentrations of manganese presented similar concentrations between points and collections, both in the fraction labile and in the pseudo-total fraction, except for point 3, where a reduction in the concentration of Mn^{2+} occurred in the sample collected in March 2015. In all fractions Mn concentrations were lower than the SEL level of 1100.0 mg kg^{-1}. Mn concentrations in the pseudo-total fraction were close to and higher than those determined in sediment samples from the Itaipu Reservoir, Paraná, where concentrations of Mn^{2+} were reported between 182.0 and 543.0 mg kg^{-1} [7].

In general, the Pb^{2+} concentration was low for the fraction labile. The highest levels were verified in August 2014 and March 2015. In the pseudo-total fraction, all the collection points had Pb^{2+} concentrations higher than the TEL level, 35.0 mg kg^{-1}, except for point 1 in August 2014. Pb^{2+} is poorly soluble in water. This can certainly contribute to its increase in sediment. Lead can have several origins, such as old paint chips, ceramics, plastics, gasoline, lead salts used in hair dye preparations[25]. The Pb^{2+} concentration in the pseudo-total fraction, when compared to another study in the same region (12.0 to 38.1 mg kg^{-1}), was higher than that reported[7].

For the Zn^{2+}, in the labile fraction all collection points had concentrations lower than the TEL level, 123.0 mg kg^{-1}. In the pseudo-total fraction, they exceeded the limits of TEL, except in point 3. The high concentrations of Zn^{2+}, with emphasis on the pseudo-total fraction at points 1, 2 and 4, may be related to pork farming in region of study and that can certainly contribute with the increase of Zn^{2+}, similar to that observed for Cu^{2+} [24].

All nickel concentrations of the labile fraction were lower than the TEL (18.0 mg kg^{-1}) level, the lowest concentrations occurred at point 3. In the pseudo-total fraction, Ni^{2+} levels exceeded the TEL level. At points 1, 2 and 4 the concentrations were higher than the PEL level, 36.0 mg kg^{-1}. The occurrence of Ni^{2+} in the study sites may contribute to agricultural activities, since fertilizers and correctives applied in soils usually have residual impurities of their production and represent an important source of micronutrients, such as Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+} and Ni^{2+} [26].

3.4 Multivariate Analysis and Correlation Study

Associations that reveal information about processes that influence the distribution of elements in the sediments were evaluated through a multivariate principal component analysis (PCA). It was verified that direct and positive correlations were higher than 0.6 for pseudo-total metals and physicochemical parameters Cu^{2+}-pH, Cu-VTS, Cu^{2+}-C total, Cu^{2+}-clay, Cr^{3+}-sand, Mn^{2+}-pH, Mn^{2+}-VTS, Mn^{2+}-TOC, Mn^{2+}-clay, Pb^{2+}-sand, Zn^{2+}-pH, Zn^{2+}-VTS, Zn^{2+}-TOC, Zn^{2+}-clay, Ni^{2+}-pH and Ni^{2+}-clay. Cu^{2+}-FTS, Cu^{2+}-sand, Cr^{3+}-clay, Cr^{3+}-silt, Mn^{2+}-ST, Mn^{2+}-FTS, Mn^{2+}-sand, Pb^{2+}-clay, Pb^{2+}-silt, Zn^{2+}-TS, Zn^{2+}-FST, Zn^{2+}-sand and Ni^{2+}-sand.

For the labile metals and physicochemical parameters, direct correlations were observed between Cu^{2+}-pH, Cu^{2+}-clay, Cr^{3+}-TS Cr^{3+}-FTS, Mn^{2+}-pH, Mn^{2+}-VTS, Mn^{2+}-TOC, Mn^{2+}-clay, Pb^{2+}-TS, Pb^{2+}-FTS, Pb^{2+}-sand, Zn^{2+}-pH, Zn^{2+}-VTS, Zn^{2+}-TOC, Zn^{2+}-clay, Ni^{2+}-pH, Ni^{2+}-clay and Ni^{2+}-Silt. Mn^{2+}-TS, Mn^{2+}-FST, Mn^{2+}-sand, Pb^{2+}-VTS, Pb^{2+}-TOC, Pb^{2+}-clay, Zn^{2+}-TS, Zn^{2+}-FTS, Cr^{3+}-TOC, Cr^{3+}-TOC, Cr^{2+}-TOC, Zn^{2+}-sand, Ni-sand.

These relationships indicated that pH and TOC are related to the occurrence of some elements in the sediments. The relationship between pH increase and the concentration of some metal ions in sediments may be related to the fact that more alkaline pH favors the precipitation of the dissolved metals in the form of insoluble oxides and hydroxides, especially with Fe and Mn oxides[6].

The low affinity of the sand in the retention of metallic species is one of the factors that explain its inverse effect for the majority of the metals studied. It was possible to observe among the metals of the pseudo-total fraction that Mn^{2+}-Cu^{2+}, Pb^{2+}-Cr^{3+}, Zn^{2+}-Cu^{2+}, Zn^{2+}-Mn^{2+}, Ni^{2+}-Cu^{2+}, Ni^{2+}-Mn^{2+} and Ni^{2+}-Zn^{2+} obtained direct correlations and higher than 0.6. The occurrence of inverse correlation was observed between Pb^{2+}-Mn^{2+}. In the available fraction Mn^{2+}-Cu^{2+}, Zn^{2+}-Cu^{2+}, Zn^{2+}-Mn^{2+}, Ni^{2+}-Cu^{2+}, Ni^{2+}-Zn^{2+} and Ni^{2+}-Mn^{2+} presented direct correlation.

The inverse correlation was observed between Mn^{2+}-Cr^{3+}, Pb^{2+}-Mn^{2+}, Zn^{2+}-Cr^{3+} and Ni^{2+}-Pb^{2+}. The good correlations of Mn^{2+} with trace metals indicate the presence of iron and manganese oxides associated with these metals. The precipitation of hydrated oxides of Fe and Mn is associated with the transport and availability of trace metals, which influence the changes in the water-sediment interface[27]. A similar study with samples collected in the same region also reported the occurrence of positive correlations between Cu^{2+}-Mn^{2+}, Fe^{2+}-Mn^{2+} and Cr^{3+}-Pb^{2+} in surface sediments[7].

The concentration of Zn^{2+} in sediments of the Terengganu River basin in Malaysia also showed a relation with the Fe concentration, which can be explained by the adsorption of Zn^{2+} by secondary Fe oxides and hydroxides[28]. The relationships between the collection points with the physical-chemical variables and metals studied can be better visualized in Figure 3, where the PCAs are plotted considering the pseudo-total and labile fraction.

For de labile fraction, in the PCA (Figure 3a and 3b) the first and second principal components captured 41.04 and 26.13 % of the variance of the experimental data, respectively, together totaling 67.17 % of variance captured. The first and second principal components (Figure 3a) did not separate the samples by collections (years), but by points sampled. All samples of P3 and P4 were placed in the positive quadrant of the y-axis (PCA factor 2), indicating similarity of sediments sampled at these sites. The samples of P1 and P2 appeared in the negative quadrant of the y axis (PCA factor 2) also showing similarity between these points and indicating differences in relation to P3 and P4.

In Figure 3b (PCA factor 1), it can be seen that metallic ions show a strong correlation with pH, clay and silt, indicating a preference of adsorption by sediment of smaller particle sizes, which shows that the distribution of metals is controlled by the transport and sedimentation of fine particles[2, 28, 29].

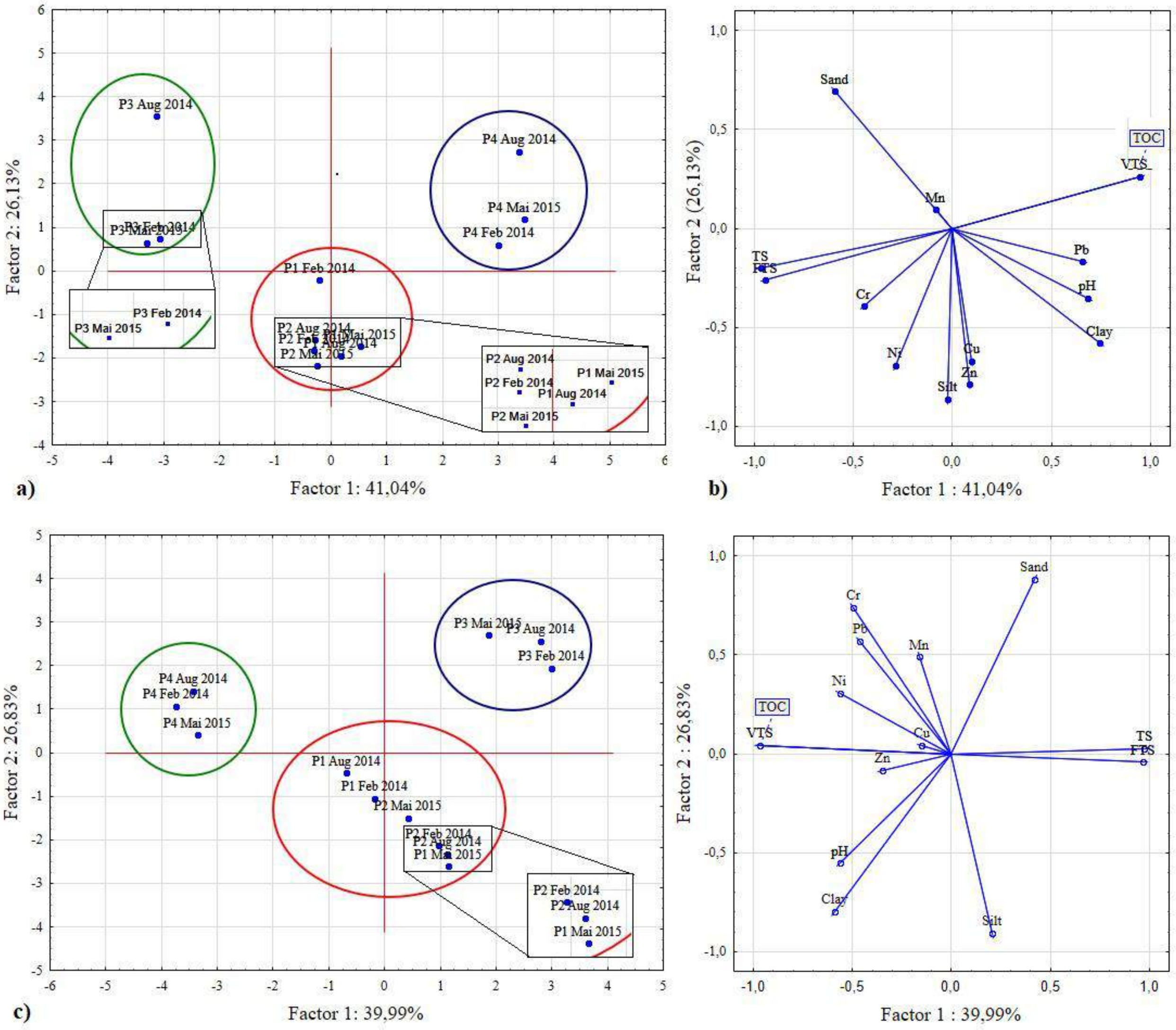

Figure 3. Scores of sampling sites (a) and (c) and loadings of variables (b) and (d) on the plane defined by the principal factor of PCA analysis for physical-chemical variables and metals.

For the pseudo-total fraction, in the PCA (Figure 3c and 3d) the first and second principal components captured 39.99 and 26.83 % of the variance of the experimental data, respectively, together totaling 66.83 % of variance captured. In this fraction, it was also observed behavior similar to labile metals, where the separation occurred because of the points sampled and not of the annual collections.

The grouped samples in the positive y-axis presented a high correlation with the variables Pb^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{3+}, and TOC, whereas the sample located in the negative y-axis correlated with Zn^{2+}, pH and clay. In the positive quadrant of the x-axis, a group was formed by sampling of P3, these collection points showed lower concentrations of metal ions evaluated and a strong correlation with sand, FTS and TS.

Samples from the same point are more clustered in PCA for pseudo-total metals (Figure 3c), indicating variation between collection campaigns. For the bioavailable fraction (label) it was observed a greater dispersion of the data at each point (Figure 3a). This was confirmed at points P3 (Aug 2014), P4 (Aug 2015) and P1 (Feb 2014) which are further away from the others.

4. Conclusions

The sediments of the mouth of some of the main tributaries of the Itaipu Reservoir, in relation to the physicochemical parameters evaluated, presented neutral to slightly alkaline character. The concentrations of VTS and TOC were higher in P4, followed by P1 and P2. This higher concentration of VTS indicated the occurrence of organic matter, which may favor the retention of metals in the compartment. Regarding the granulometry, the predominance of the clay fraction was observed. Point 3 presented the smallest fraction of this mineral, only 22 %.

In the fraction labile, only Cu content reached concentrations higher than the TEL level at all sampling points. Regarding the pseudo-total fraction, some study sites reached concentrations for Cr^{3+}, Pb^{2+} and Zn^{2+} metals at the TEL level, below which adverse effects are rarely expected. For Ni^{2+}, PEL values were verified, being this the level of effects probable and for Cu^{2+} concentrations in SEL were reported where there is possibility of severe effects with rich in potentials on the quality of the environment, according to the international criteria of sediment classification adopted NOAA.

The PCA was useful to evaluate the correlation between the sampling points, between collections and the interaction, with the physical-chemical variables and the fractions of metals studied, indicating that the anthropogenic activity exerts influence on the contents of metals in the sediment at the mouth of tributaries of the Itaipu Reservoir.

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Discoloration of red 5B and reactive blue 5G dyes in synthetic textile dye effluent by photo-Fenton process

Leandro Finger[1+]; Ismael Laurindo Costa Junior[1]; Poliana Paula Quitaiski[1]; Juliana Bortoli Rodrigues Mees[1]

[1] Universidade Tecnológica Federal do Paraná (UTFPR) Campus Medianeira, Paraná, Brazil

[+]Corresponding author: Leandro Finger, e-mail address: fingerleandro@hotmail.com

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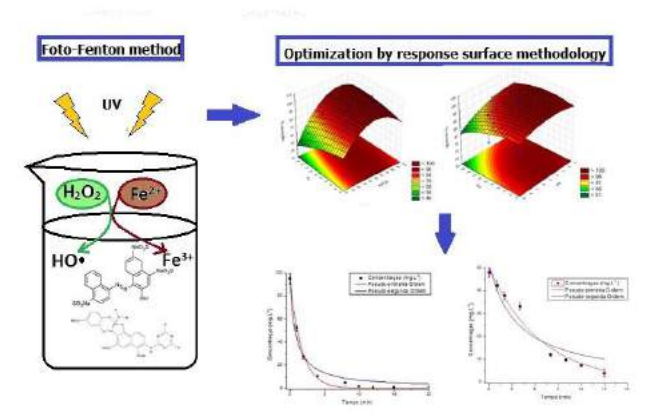
ABSTRACT: Effluents from textile industries are difficult to treat because they are loaded with non-biodegradable dyes. In this context, the Advanced Oxidation Processes (AOPs) are presented as alternatives to be studied for the treatment of these effluents. The objective of this work was to evaluate the efficiency of the discoloration of red 4B and 5G blue dyes in synthetic solution by means of the advanced oxidation Photo-Fenton. The best pH for discoloration of Red 4B was pH 3 and for Blue 5G dye was pH 1.6. The obtained results report a good discoloration when using H_2O_2 and Fe^{2+} ions. With a H_2O_2 concentration of 66.80 mg L^{-1}, Fe^{2+} of 9.66 mg L^{-1} and pH 5.81 with a predicted percentage discoloration of 100.01 % for Red 4B dye. And for Blue Reactive 5G dye a concentration of H_2O_2 of 55.04 mg L^{-1}, Fe^{2+} ions of 10.34 mg L^{-1} and pH 2.59 with an expected percentage discoloration of 100.56 %. The kinetics was that pseudo-first order with k_1 of 0.597 min^{-1} and $t_{1/2}$ of 1.16 min for red 4B and k_1 of 0.150 min^{-1} and $t_{1/2}$ of 4.60 for blue 5G. The results indicate the application of photo-Fenton as promising for the dyes discoloration in aqueous solutions.



1. Introduction

The environmental contamination has global proportions due, especially the continuous emission of large volume of domestic and industrial wastewater, with high polluting potential when not treated in a proper way. Regarding industrial activities, textile fiber dyeing operations deserve attention due to the high volumes of liquid waste, with high organic load and intense coloration[1].

The interest of environmental research related to the treatment of effluents from the textile industry is increasing, since these are highly complex, because they have different physical and chemical compositions, great diversity of synthetic dyes and different toxicity potentialities, thus making them difficult treatment and high pollution capacity[2].

In this sense, the advanced oxidation processes (AOPs) have been gaining visibility as a consequence of the great contribution potential with the conventional processes of effluent treatment, since the hydroxyl radicals generated have high reactivity and low selectivity, being able to act in the chemical oxidation of a large number of substances[3]. The photo-Fenton process ($UV/H_2O_2/Fe^{+2}$) has been prominent among AOPs, since it provides oxidations in shorter times when compared to others, besides being simple,

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since it is based on the reaction between ferrous salts (Fe^{2+}) and hydrogen peroxide (H_2O_2), associated with UV irradiation[4].

In this sense, the objective of this work was to evaluate the efficiency of the discoloration of red 4B and blue 5G in synthetic solution by means of the photo-Fenton reaction ($UV/H_2O_2/Fe^{+2}$), by optimizing a discoloration condition for the variables pH, Fe^{2+} ions concentration and H_2O_2 concentration and kinetic study of the process.

2. Experimental

2.1 Solutions and Analytical Determinations

Stock standard solutions of red 4B and blue 5G dyes were prepared using ultrapure water at 1000 mg L^{-1} concentration. The pH adjustments by 0.1 mol L^{-1} HCl (Biotec) and 0.01 mol L^{-1} NaOH solutions (Vtec) were also prepared. For the photo-Fenton process, H_2O_2 1000 mg L^{-1} (Alphatec) and $FeSO_4$ $7H_2O$ 100 mg L^{-1} (Merck) were used.

For the quantification, initially scans of the solution of each dye were performed to determine the absorption maximum. The calibration curve was constructed by absorbance readings in the maximum length of solutions with known dye concentrations (0.5 to 50 mg L^{-1}) using the dual beam scanning UV/VIS molecular absorption spectrophotometer, Perkin Elmer brand, model Lambda 45, in the range of 400 to 800 nm with resolution of 2 nm and quartz cuvettes with optical path of 1 cm.

2.2 Optimization of Discoloration Conditions

The experiments with the synthetic effluent were carried out in a laboratory-scale reactor, operating in a batch system, consisting of a beaker with 250 mL of capacity and a magnetic stirrer to homogenize the solution, positioned inside a wooden box (80 cm x 40 cm x 60 cm) coated with aluminum foil to increase the radiation incidence in the solution and equipped with a UV source (high pressure Mercury vapor lamp without the bulb – 250W) fixed at the top about 20 cm from the solution. The inner temperature, after 20 min, was maintained at about 45 °C (Figure 1).

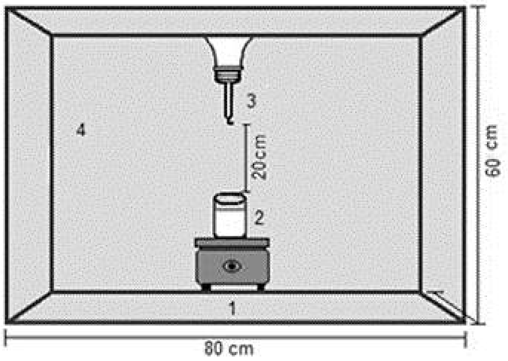


Figure 1. Photo-Fenton process reactor.
Caption:
1 – Magnetic stirrer.
2 – Reactor.
3 – UV source.
4 – Wooden box coated with aluminum foil.

Samples were withdrawn at pre-established intervals, 0-20 min for red 4B dye, and 0-15 min for blue 5G dye, and immediately the remaining concentration in the solution was determined using the dual beam scanning UV/VIS molecular absorption spectrophotometer by the calibration curve.

In order to verify the influence of the H_2O_2 concentration, Fe^{2+} ions concentration and pH and to obtain an optimum discoloration condition, a central composite rotational design (CCRD) 2^3 was elaborated with 2 replicates at the central point, using Statistica 8.0 software and the desirability function as tool. As the response variable in the statistical analysis the percentage of discoloration of the analytes (Red 4B and Blue 5G dyes) was used. The experimental data were adjusted to the linear and quadratic models it was evaluated using the Analysis of Variance (ANOVA) at the 95 % confidence level.

2.3 Discoloration Kinetic Study of Dyes in Water

In the study of kinetics of dye discoloration, solutions were used with concentration of 100 mg L^{-1} for red 4B and 50 mg L^{-1} for blue 5G in 250 mL beakers. Aliquots were taken in the time intervals of 0; 1; 2; 4; 8; 10; 12; 15 and 20 min to the red 4B dye, and 0; 1; 2; 4; 8; 10; 12 and 15 min to the blue 5G dye, with determination based on the previously determined maximum wavelength.

The discoloration was evaluated using the photo-Fenton process ($UV/H_2O_2/Fe^{+2}$) by kinetic models. The order of the reaction is the dependence of the speed of the reaction with the concentration. With C_0 being the initial concentration of the reagent, and C the concentration of the reagent elapsed at time t of reaction and n the order of the reaction. When n = 1 the reaction is of pseudo-first order (Equation 1) and n = 2 of pseudo-second order (Equation 2).

$$\ln C = \ln C_0 - k_1.t \;\therefore\; C = C_0.e^{-k_1 t} \qquad (1)$$

$$\frac{1}{C} = \frac{1}{C_o} + k.t \;\therefore\; C = \frac{C_o}{1+C_{o.}.k.t} \qquad (2)$$

The experimental data obtained in the discoloration assays were fitted to these models in order to evaluate their decay during the experiment time, as well as to determine the half-life of each analyte according to the equation of pseudo-first order (Equation 3) and of pseudo-second order (Equation 4).

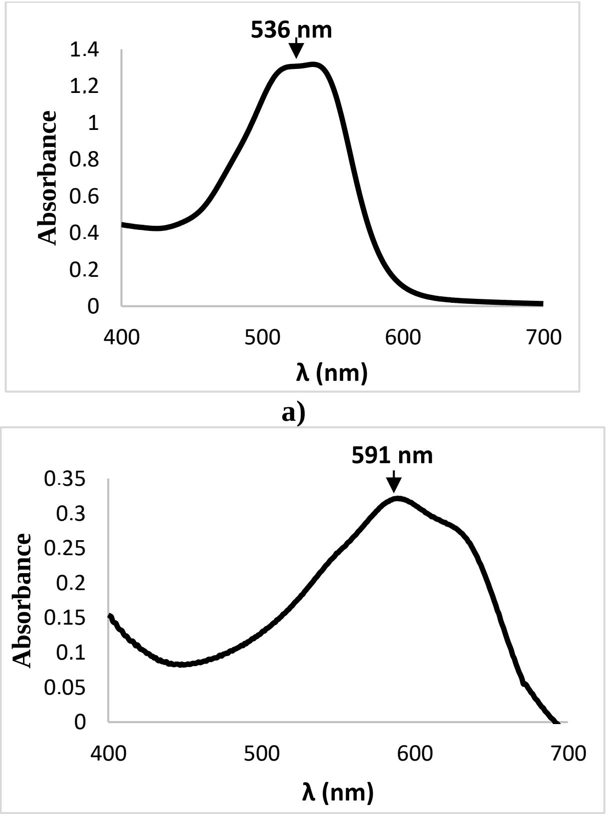
$$t_{\frac{1}{2}} = \frac{ln2}{K_1} \qquad (3)$$

$$t_{\frac{1}{2}} = \frac{1}{K.C_o} \qquad (4)$$

3. Results and Discussions

3.1 Spectrophotometric Evaluation

In order to determine the amount of dye removed, the optimum wavelength definition for the red 4B and blue 5G dyes reagent was first determined. This definition was obtained by double beam spectrophotometer scanning, where the molecular absorption spectrum was obtained, indicating the maximum absorbance band at 536 nm for red 4B dye and at 591 nm for blue 5G dye (Figure 2).



a)

b)

Figura 2. a) UV absorption spectrum in aqueous solution at 50 mg L^{-1} concentration for red 4B dye and b) blue 5G dye.

3.2 Optimization of Discoloration Conditions Employing Photo-Fenton Process

The experimental matrix for the 2³ central composite rotational design (CCRD) delineation is presented in Table 1, with the levels of each factor, the actual values and the response variable obtained for the discoloration of red 4B and blue 5G dye in the photo-Fenton process (UV/H_2O_2/Fe^{+2}) in the course of 16 runs performed randomly.

Table 1. CCDR 2^3 planning matrix with the factors coded (and real) and responses regarding the discoloration efficiency of 4B red and 5G blue dyes.

Assays	**Factors**			**Variable Response**	
	[H_2O_2]* (mg L^{-1})	**[Fe]* (mg L^{-1})**	**pH***	**Red 4B discoloration (%)**	**Blue 5G discoloration (%)**
1	-1 (25.0)	-1 (5.0)	-1 (3.0)	95.31	86.35
2	-1 (25.0)	-1 (5.0)	+1 (7.0)	87.41	18.34
3	-1 (25.0)	+1 (15.0)	-1 (3.0)	85.76	81.16
4	-1 (25.0)	+1 (15.0)	+1 (7.0)	74.18	55.67
5	+1 (75.0)	-1 (5.0)	-1 (3.0)	100.00	100.00
6	+1 (75.0)	-1 (5.0)	+1 (7.0)	95.61	39.84
7	+1 (75.0)	+1 (15.0)	-1 (3.0)	98.75	73.75
8	+1 (75.0)	+1 (15.0)	+1 (7.0)	92.61	78.82
9	-1.68 (8.0)	0 (10.0)	0 (5.0)	65.04	72.64
10	+1.68 (92.0)	0 (10.0)	0 (5.0)	98.20	86.79
11	0 (50.0)	-1.68 (1.6)	0 (5.0)	95.06	16.24
12	0 (50.0)	+1.68 (18.4)	0 (5.0)	96.22	77.60
13	0 (50.0)	0 (10.0)	-1.68 (1.6)	95.12	100.56
14	0 (50.0)	0 (10.0)	+1.68 (8.4)	95.68	80.62
15	0 (50.0)	0 (10.0)	0 (5.0)	98.60	80.57
16	0 (50.0)	0 (10.0)	0 (5.0)	98.31	82.67

More effective discolorations were observed in assays 5, 7, 10, 14, 15 and 16 for both dyes, where H_2O_2 concentrations were applied at higher levels and pH was more acidic.

With the experimental results obtained in the discoloration of the dyes by photo-Fenton from CCDR 2^3, the values of the estimated effects of each parameter (concentration of H_2O_2 and Fe^{2+} ions, and pH) on the response variables presented in Table 1 were obtained, values that presented p-value less than 0.05 were considered significant for the 95 % confidence interval. From the significant values, the mathematical equation of the quadratic regression model and its respective determination coefficients (R^2) were obtained (Table 2).

Table 2. Mathematical models and determination coefficients (R^2) of the models adjusted for discoloration of the red 4B and blue 5G dyes.

Response	Model	R^2 (%)
Red 4B discoloration (%)	$98.37 + 7.33H_2O_2 - 5.78(H_2O_2)^2 - 1.84Fe^{2+} - 2.13pH - 0.89(pH)^2 + 2.31H_2O_2.Fe^{2+} + 1.12\ H_2O_2.pH$	88.56
Blue 5G discoloration (%)	$82.13 + 5.47H_2O_2 + 10.84Fe^{2+} - 13.49(Fe)^2 - 13.34pH + 13.47Fe.pH$	87.42

For the discoloration of the red 4B and blue 5G dyes respectively the R^2 values show that 88.56 % and 87.42 % of the responses were explained by the models, and the linear effects of pH and concentrations of [H_2O_2] (mg L^{-1}) and [Fe^{2+}] (mg L^{-1}) were the most important to describe the behavior of discoloration of the analytes by the photo-Fenton process.

To verify the fit quality of the model, the analysis of variance (ANOVA) was used, evaluating the determination coefficients (R^2) and the F test for both discolorations (Table 3).

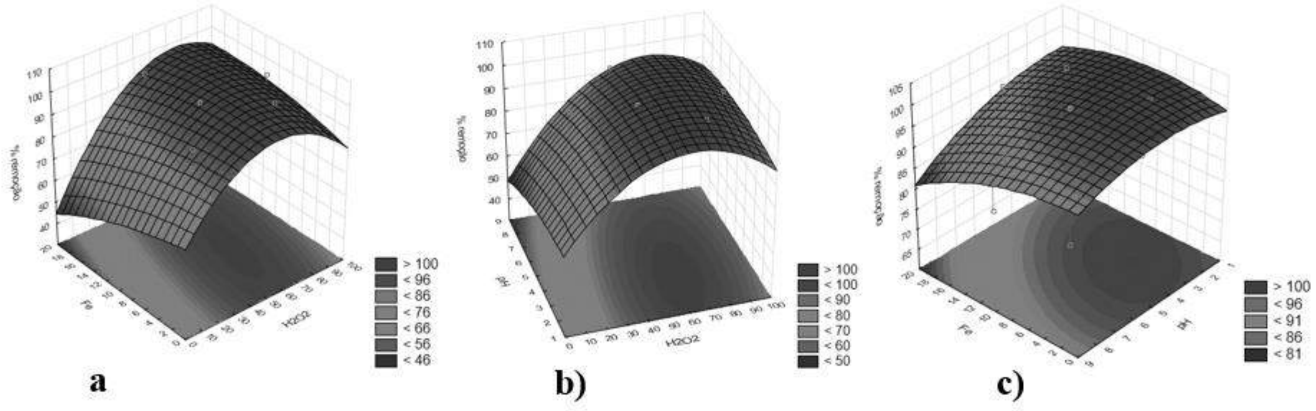
Table 3. ANOVA of the quadratic model to remove red 4B and blue 5G dyes.

	Sources of Variation	SQ	GL	MQ	F_{cal} (95%)	F_{tab} (95%)	F_{cal}/F_{tab}
Red 4B	Regression (model)	1249.64	9	138.85	5.16	4.10	1.26
	Residuals	161.34	6	26.89	-	-	-
	Lack of Adjusted	161.30	5	32.26	786.83	230.00	3.42
	Error	0.041	1	0.041	-	-	-
	Total	1410.98	15				
Blue 5G	Regression (model)	8591.58	9	954.62	5.10	4.10	1.24
	Residuals	1122.36	6	187.06	-	-	-
	Lack of Adjusted	1120.14	5	224.03	101.23	230.00	0.44
	Error	2.21	1	2.21	-	-	-
	Total	9713.93	15				

It is observed in Table 3 that the ratio of F_{cal} to F_{tab} for the regression presented a statistically significant value for the equation of discoloration of both dyes, a fact evidenced by a value higher than 1. Moreover, for the blue 5G dye, it was not observed (Figure 2). In this study, the F_{cal}/F_{tab} ratio for the lack of fit was lower than 1[5].

On the other hand, for the discoloration of red 4B dye the regression model generated was significant ($p \leq 0.05$) because $F_{cal} = 5.10$ was higher than the $F_{tab} = 4.10$. However, the lack of fit was also significant ($F_{cal} = 786.83 > F_{tab} = 230$), although the ideal was a value of $F_{cal} < F_{tab}$, and therefore not significant. However, since the means at the central points were very close and the pure error very low (reasons for the F of the lack of high fit), the model was considered valid for predictive purposes[5].

The Figure 3 shows the behavior of the process regarding the discoloration efficiency of red 4B according to the relation of the dependent variables by the surface response graph.



a

b)

c)

Figure 3. Response surface for discoloration efficiency red 4B dye by a) Fe^{2+} (mg L^{-1}) and H_2O_2 (mg L^{-1}); b) pH and H_2O_2 (mg L^{-1}); c) Fe^{2+} (mg L^{-1}) and pH.

In Figure 3, it can be observed that the discoloration efficiency of red 4B dye presented mean values between 65.04 % and 100.00 %. The best discoloration efficiency values, 98.75 % and 100.00 %, were obtained with pH 3, H_2O_2 concentration of 75 mg L^{-1}, Fe^{+2} concentration of 5 and 15 mg L^{-1}, respectively.

The Figure 4 shows the behavior of the process regarding the discoloration efficiency of the blue 5G dye according to the relation of the dependent variables by the surface response graph.

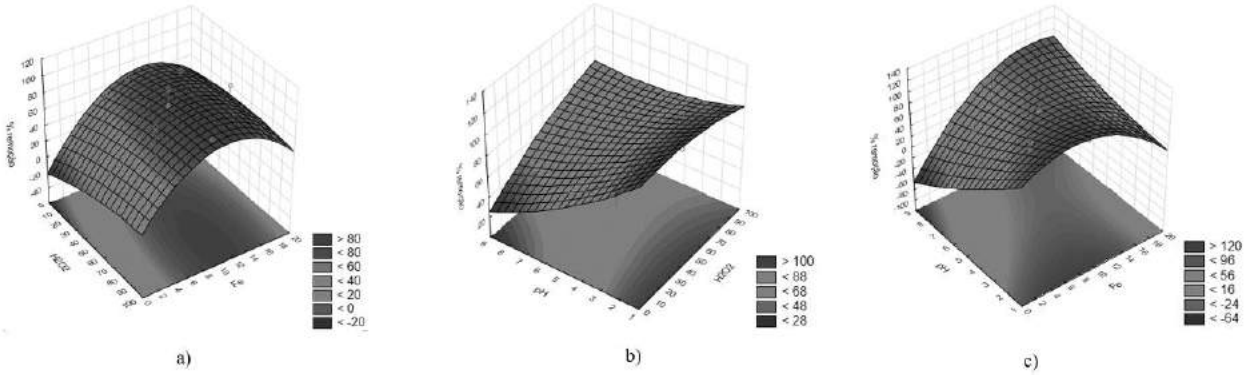


Figure 4. Response surface for blue 5G dye discoloration efficiency by a) Fe^{2+} ions (mg L^{-1}) and H_2O_2 (mg L^{-1}); b) pH and H_2O_2 (mg L^{-1}); c) Fe^{2+} ions (mg L^{-1}) and pH.

In Figure 4 it is observed that the discoloration efficiency of the blue 5G dye presented average values between 16.24 % and 100.00 %. The best discoloration efficiency values, both at 100.00 %, were obtained with pH 3 and 1.6, H_2O_2 concentration of 75 mg L^{-1} and 50 mg L^{-1}, and Fe^{+2} concentration of 5 and 10 mg L^{-1}.

Analyzing Figures 3 and 4 it is possible to verify that the concentration of H_2O_2 and Fe^{2+} ions showed a positive effect on the discoloration efficiency of red 4B and blue 5G dyes and that the pH variable had a negative effect on the discoloration efficiency, indicating that the highest discolorations were achieved with the decrease of this variable.

Škodič *et al.* demonstrated that the additions of moderate concentrations of H_2O_2 and Fe^{+2} ions catalyst during the AOPs obviously increased the decolorization efficiencies within the first few minutes of the processing time (5–10 min) for Reactive Blue 4 and Reactive Blue 268 dyes[4].

As a more favorable response to the discoloration of dyes in aqueous solution, the optimization tool employing the desirability function indicated a H_2O_2 concentration of 66.80 mg L^{-1}, Fe^{2+} ions of 9.66 mg L^{-1} and pH 5.81 with a percentage of 100.01 % discoloration to red 4B dye. For the blue 5G dye, a H_2O_2 concentration of 55.04 mg L^{-1}, Fe^{2+} ions of 10.34 mg L^{-1} and pH 2.59 with a predicted percent discoloration of 100.56 %.

3.3 Discoloration Kinetics Employing the Photo-Fenton Process (Uv / H_2O_2 / Fe^{+2} ions)

The optimum conditions were used in the kinetic study for both dyes. The pseudo-first order and pseudo-second order models fitted to the experimental data are shown in Figure 5.

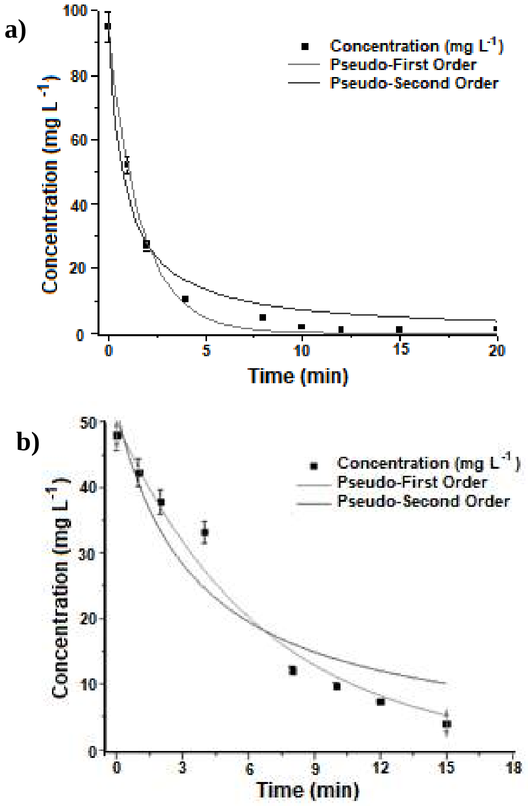


Figure 5. Adjustment of the experimental data of dye discoloration to the kinetic models of Pseudo-first order and Pseudo-second order. a) Red 4B; b) Blue 5G.

By means of this adjustment, it was possible to obtain the kinetic constant of pseudo-first order (k_1) and pseudo-second order (k_2), correlation coefficient (R^2) and half-life time ($t_{1/2}$), indicated in Table 4, for the discoloration of the red 4B and blue 5G dyes.

Santana *et al.* also obtained a good kinetic adjustment for the pseudo-first order for the removal of reactive blue dyes 5G and remazol red RB 133 % by photo-Fenton process[6].

Table 4. Kinetic data for the photo-Fenton process ($UV/H_2O_2/Fe^{+2}$) of the red 4B and blue 5G dyes.

Dye	**pH**	**Model**		
		Pseudo-First Order		
		K_1 (min^{-1})	**$t_{1/2}$ (min)**	**R^2**
Red 4B	5.8	0.597	1.16	0.99
Blue 5G	2.6	0.150	4.60	0.97

Half-life times of 1.16 min for red 4B and 4.60 min for blue 5G dyes were observed. It was also verified that there was a greater speed of dye discoloration in the pseudo-first order model, besides a better adjustment in both treatments.

According to Núñes *et al.*, taking into account that the concentration of the reactive species needs to reach a steady state during the process, and once the oxidant concentration can be considered constant, the kinetics of the discoloration process can be treated as pseudo-first order, in terms of the consumption of the organic compound, in this case, the dye[7]. Generally, advanced discoloration processes obey the pseudo-first order kinetics[8].

4. Conclusions

The advanced photo-Fenton oxidative process ($UV/H_2O_2/Fe^{+2}$) proved to be very efficient in the discoloration of the synthetic solutions of red 4B and blue 5G dyes, allowing to eliminate the color of the aqueous media in times less than 20 min.

By means of the kinetic models evaluated, it was verified that the mathematical model of pseudo-first order was the one that best represented the experimental data of discoloration. However, it was observed that for red dye 4B the discoloration was faster, as indicated by the kinetic constant, that is, the half-life time was lower for the red4B compared to the blue 5G dye. It was also found that the concentrations of H_2O_2 and Fe^{2+} ions exhibited a positive effect on the discoloration for the studied dyes, increasing efficiency.

Further studies on the application of this process in real textile effluent are required, as well as in larger effluent volumes to compare the results with those obtained in this study. Studies using advanced chromatography techniques or total organic carbon are also necessary to verify possible degradation of the dyes in these effluents.

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Determination of lead (Pb) in feces of *Lontra longicaudis* (Olfers, 1818) by flame atomic absorption spectrometry (F AAS)

Nubya Gonçalves Cavallini[1+], Rodrigo Arantes Reis[1], Juliana Quadros[1], Andrea Oliveira[1], Mariana Gallucci Nazário[1], Liliani Marilia Tiepolo[1]

[1] Federal University of Paraná (UFPR), Litoral Campus, 512 Jaguariaíva St, Matinhos, Paraná, Brazil

[+]Corresponding author: Nubya Gonçalves Cavallini, e-mail address: nubyacavallini@gmail.com

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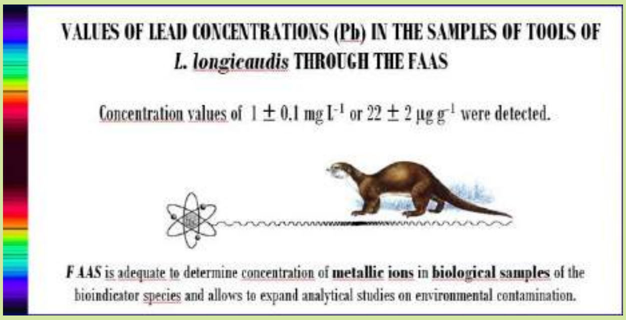
ABSTRACT: The determination of metallic ions by flame atomic absorption spectrometry reaffirms the success of the technique for ecological and toxicological studies of environmental contamination. In order to contribute to the scientific research on anthropogenic contamination, the study of lead in feces of *Lontra longicaudis* (Olfers, 1818) in the Guaraguaçu River Basin aims to understand the anthropogenic environmental contamination of the local ecosystem, located in one of the largest port areas in Brazil. Six samples were collected along the Guaraguaçu River. To determine the lead concentration by flame atomic absorption spectrometry method, samples were extracted with HNO_3 1 % v/v. Lead concentration values about 1 mg L^{-1} were detected in feces samples, indicating abnormalities. Thus, the analytical method allows the interpolation of fecal analysis of *L. longicaudis* in order to quantify the inorganic contaminants in water, secondary food web items and complementary data of elements that do not yet exist in normative tables. Thus, it converges to deepen the development of possible actions to control, monitor and inspect this element in the environment and in its main anthropogenic sources of contamination and for the presentation of possible conservation strategies of the ecosystemic biodiversity.



VALUES OF LEAD CONCENTRATIONS (Pb) IN THE SAMPLES OF TOOLS OF *L. longicaudis* THROUGH THE FAAS

Concentration values of 1 ± 0.1 mg L^{-1} or 22 ± 2 μg g^{-1} were detected.

F AAS is adequate to determine concentration of **metallic ions** in **biological samples** of the bioindicator species and allows to expand analytical studies on environmental contamination.

1. Introduction

The Guaraguaçu River Basin is an area characterized by divergent configurations of space where there are discordant relationships between social and natural determinants in relation to land occupation and the economic aspects of its use[1, 2]. The set of actions developed in its area of insertion destined to the production, distribution, consumption and financial market, mainly related to the agribusiness sector associated with the largest port complex in the region, represent a serious indication of environmental crisis[1-4]. Thus, the problem that drives the development of this work is based on the contamination of the physical and biological compartments of the Guaraguaçu River by metallic ions from the productive, logistic and operational processes of the Port of Paranaguá, located in the homonymous bay, on the coast of Parana. Also, it is considered that the coast of Parana does not currently present detailed knowledge about sanitary problems and ecological imbalance arising from metallic ions resulting from this type of contamination[5].

The analytical knowledge about the dynamism between the socioeconomic and socioenvironmental establishment for the occupation and use of the coastal space contributes to identify and to characterize territorial tendencies of risks to the ecosystemic health by productive processes potentially polluting. As a

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primary condition for the search for territorial development and quality of the natural space, such knowledge is strategic so that preventive, control, monitoring, inspection and remediation procedures are desired at the local level.

Thus, the use of biological material from the bioindicator species allowed not only to carry out the quantitative determinations of contamination by Pb from the productive activities of the local agricultural and port sector, but also to expose the understanding of the complexity and inseparability of the environmental impacts over the use and the disorderly and indiscriminate exploitation of common property.

With all the progress recently achieved, atomic absorption spectrometry is reaffirmed as an important and successful technique for the determination of metals and semi-metals in biological and environmental samples[6, 7]. As an available tool for assessing the environmental and toxicological effects of individual metallic ions available in water, sedimentological and/or biological compartments, its use has been widely inserted in environmental monitoring and control contexts.

In this scenario, through the use of the flame atomic absorption spectrometry technique in the determination and analysis of lead in the stools samples of *Lontra longicaudis*, the objective of this study was to quantify the impacts of the environmental contamination originated from the Port of Paranaguá in the study area, as well as their direct and indirect interference relations on the organisms of the local ecosystem. It is possible to provide such a diagnosis considering that the bioindicator species, as a carnivorous predator of the chain, has its biological and ethological data coinciding space-temporally with other groups of species in the regional scope. Its traces reveal the integrality of the flows and dynamics of the metals in the environmental compartments in which it inhabits and shares with other groups of organisms.

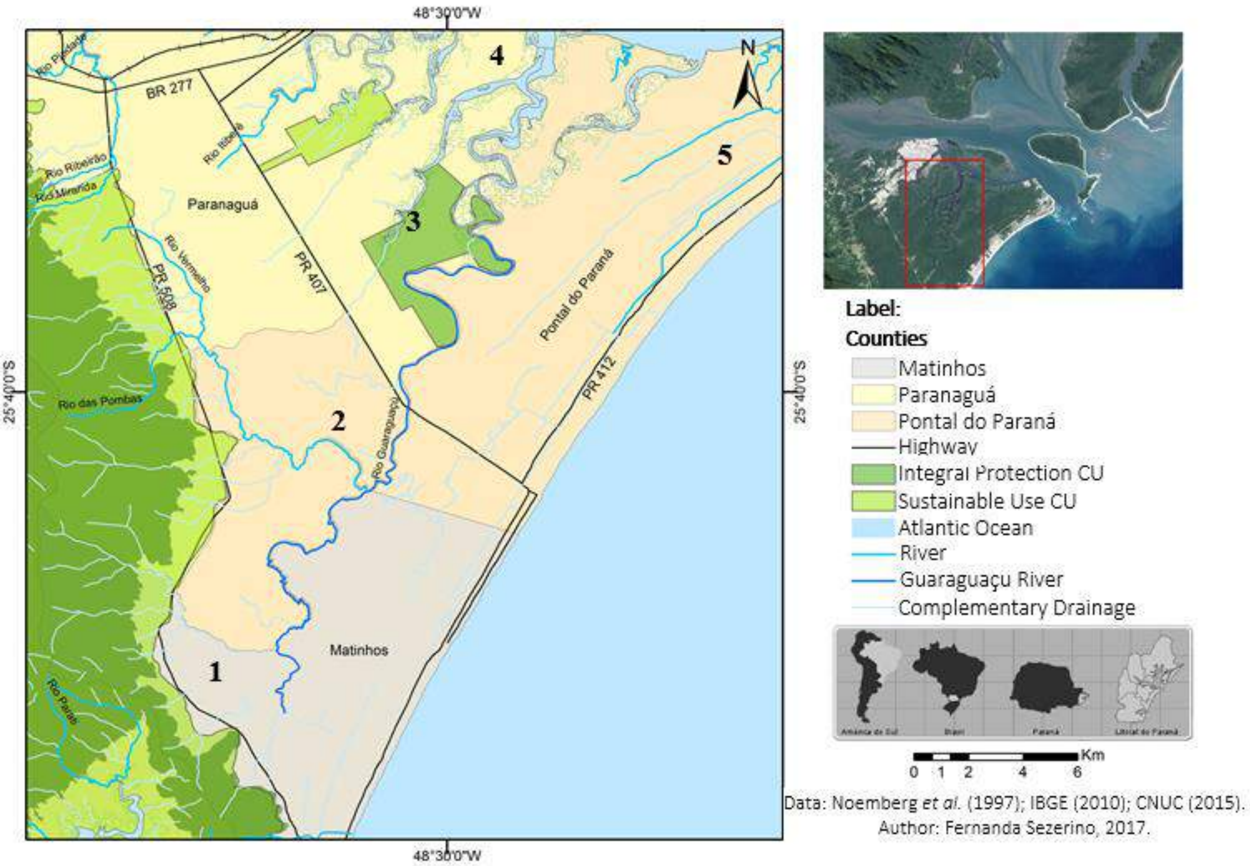
Therefore, the research had a quantitative support and a qualitative understanding regarding the contamination of the Guaraguaçu River by inorganic contaminants from the use of an animal bioindicator and, according to the trophic web, in a regional way, of the animal species. That is, the results included the provision of data applicable to environmental surveillance and ecosystem health in the areas of direct and indirect influence of the Guaraguaçu River Basin, and the identification of irregular aspects for the future development of conservation strategies of specific and ecosystem diversity.

Therefore, it subsidizes the continuity of studies that aim at monitoring ecosystem and species conservation actions at a regional level, taking into account conservation plans for the species at the state and national levels.

2. Experimental

2.1 Study area and sampling points

Fieldwork and sampling campaigns consisted of the active fecal search along the 60 km of Guaraguaçu River, which were classified according to the influence of the tides. In order to understand quantitatively the impact of the environmental contamination originating from the Port of Paranaguá and, consequently, the possible scenarios under the different physicochemical conditions related to the local dynamics of tidal intake and tide, the points of collection were segmented according to a division in five areas: Area 1, control, mountain slope: without tidal influence; Area 2: low tidal influence; Area 3: intermediate tidal influence; Area 4, mouth in estuarine environment of the Bay of Paranaguá: high tidal influence; and, Area 5, Ditch or Watercourse DNOS: high tidal influence (Figure 1).



Data: Noemberg *et al.* (1997); IBGE (2010); CNUC (2015).
Author: Fernanda Sezerino, 2017.

Figure 1. Map of location of the Guaraguaçu River in the coastal basin and sampling areas numbered from 1 to 5.

Each fecal sample found was referenced in a field book, identified and stored in plastic bags. All handling procedures ensured that samples were not contaminated by the use of utensils, packaging and tools. After the collection, identification and storage, the samples were sent to the Laboratories of Chemical and Biological Pre-Analysis and Physical-Chemical Analysis of the Federal University of Paraná - Littoral Sector for the appropriate specific laboratory procedures.

2.2 Sample preparation

Samples of feces of *Lontra longicaudis* contain variable water contents in their composition, as well as a great heterogeneity of components. As a result, the samples were dried in a conventional oven at 80 ºC until constant weight, fragmented and homogenized. The treatment procedures of the samples were established from the development complete factorial design 2^3 [8]. The multivariate statistical technique is based on the application of a fixed effects model for the optimization of factors and levels from a small number of experimental trials, as well as in the evaluation of interdependence effects and interrelations that they can cause in response of the final combinations[8, 9].

In the present work, the procedure of the purpose method was based on an experiment employing three factors (milling, HNO_3 volume and digestion time) and with each one of the variables tested in two levels. The response variable was evaluated from duplicate experiments leading to the 16 experiments. Replicas are of significant importance since they serve to determine experimental errors that can be evaluating the reproducibility of the method, as well as of the instrumental analysis[8].

The Table 1 indicates the factors and levels chosen to define the factorial design, and Table 2 shows the experimental conditions performed randomly as indicated by software Statistica® 7.0.

Table 1. Factorial design 2³ for optimization of the conditions of sample treatment.

	MILLING	HNO_3 mL^{-1}	DIGESTION TIME/h
LEVELS	Hammer + Strainer	4	3
	Willey type knife mill	2	1.5

Table 2. Full factor design (2³) for reactional conditions optimization in the sample treatment.

EXPERIMENTS	MILLING	HNO_3 mL^{-1}	TIME DIGESTION/h
1	Hammer + Strainer	2	1.5
9	Hammer + Strainer	2	1.5
5	Willey type knife mill	2	1.5
13	Willey type knife mill	2	1.5
7	Hammer + Strainer	2	3
15	Hammer + Strainer	2	3
3	Willey type knife mill	2	3
11	Willey type knife mill	2	3
2	Willey type knife mill	4	1.5
10	Willey type knife mill	4	1.5
6	Hammer + Strainer	4	1.5
14	Hammer + Strainer	4	1.5
4	Hammer + Strainer	4	3
12	Hammer + Strainer	4	3
8	Willey type knife mill	4	3
16	Willey type knife mill	4	3

The results of the lead concentrations obtained from random variables showed no significant difference between the levels tested. The feces samples were fragmented and homogenized by automated method of the Willey-type knife mill, due to its greater agility, economy and safety.

Sample treatment step was also optimized employing full factorial design 23 (Table 1). Again, due to significant non-differentiation between the volumes tested, the sample treatment with 2.00 mL of HNO_3 65 % (v/v) during 1.5 h in a digestion block was chosen due to the characteristics of economy and agility. The previously prepared samples were directed to the wet decomposition step with oxidant acid - HNO_3 65 % (v/v) - and hydrogen peroxide - H_2O_2 30 % (m/m). After optimized conditions the samples, about 450 mg, were treated with 2.00 ml of HNO_3 65 % (v/v) and allowed to stand for 30 minutes at room temperature. Then, 1.00 mL of 30 % H_2O_2 (w/w) and 2.00 mL H_2O were added on. The flasks were semi-sealed with parafilm protector and placed in a digester block at 80 °C for 1.5 h. After complete mineralization and cooling, the extracts were diluted to 10.00 mL with ultrapure water, filtered with 0.45 μm Teflon membrane filter and kept under refrigeration until the analysis of the samples by F AAS.

Automated Instrumental Analysis - F AAS after the acid digestion process, the extracts were sent to the Automated Instrumental Analysis Laboratory of the Federal University of Parana - Litoral Sector for analysis on AA240 Varian atomic absorption spectrometer and determination of the flame Pb contents in the following operating conditions: gas compressed air and acetylene in flow of 5 L min-1; lamp current equal to 12 mA; wavelength equal to 217 nm; slit equal to 1 nm and background correction with Deuterium lamp. The atomic absorption spectrophotometer analyzes followed the basic protocols of analytical chemistry and previous validation of the method, considering that the whole experimental process was reproducible, sensitive, precise, accurate and linear in the range of the analytical calibration curve. The range of the analytical calibration curve constructed using lead standard solutions in HNO_3 1 % v/v at eight calibration levels, were: 0.25; 0.50; 0.75; 1.5; 2.0; 2.5; 3.0; 4.0 mg L^{-1}. The linearity was evaluated by linear regression equation obtaining the determination coefficient ≥ 0.995 (Figure 2).

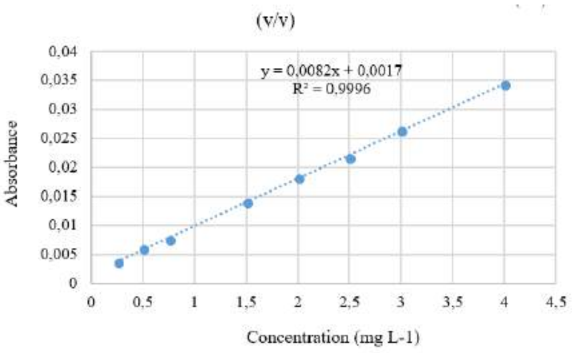


Figure 2. Analytical calibration curve for the lead (Pb) in HNO_3 1 % (v/v).

2.3 Sample treatment

From the data shown by the graph, the functional relationship between the variables was characterized and expressed by the present calibration analytical curve which, as a straight equation, is calculated from the measured signals at concentrations previously established through the linear regression model[7, 10, 11, 12]. Thus, it was possible to determine the concentrations of Pb in the sample, since its results were totally dependent and directly proportional to the concentration of the analyte in the sample[7, 10, 11, 12]. In all cases, the correlation coefficients showed a satisfactory linearity. That is, it showed direct proportionality to the concentration of the analyte in the sample, within the specific concentration range, and it can be proved from the values presented by the determination coefficients ($\mathbf{R}^2$ = 0.9996).

3. Results and Discussion

The results of the concentrations obtained through the F AAS technique are presented in Table 3.

Table 3. Values of lead concentrations (Pb) obtained in the feces samples of *Lontra longicaudis* by F AAS

Samples	Area	Pb mg L^{-1}	Mean ± Std Dev	Pb mg kg^{-1}	Mean ± Std Dev
1	Intermediate tide influence	0.89	0.98 ± 0.09	19.78	22 ± 2
2	Intermediate tide influence	1.06		23.58	
3	Without tide influence	1		22.22	
4	Without tide influence	0.90		20.05	
5	Without tide influence	0.94		20.87	
6	Without tide influence	1.11		24.66	

The analysis of the results of the concentration values was based on comparative checks and knowledge of acknowledgments with the legislation of reference organisms, such as ANVISA[10], MAPA[11], CONAMA[12] and MERCOSUL[13] (Table 4 and 5). These legislations with their respective standards and values regarding inorganic contaminants in water and food samples are used as guiding parameters since specimens of this nature make reference to the biology, habitat and way of life of the species. In addition, there is still no data to ensure the maximum limits allowed in *Lontra longicaudis* feces. That is, the comparative proposal is based on confronting the concentration values found in feces with the maximum values allowed by current legislation in samples that incorporate part of the ecosystem, biology and ethology of the bioindicator species.

Therefore, the determination and analysis of the Pb ions metal in the feces samples of *Lontra longicaudis* involved a certain complexity about the concentrations as a function of the different spatialities between the areas determined as points of sampling and collection, as well as in relation to the nature of the samples and the comparative values of the reference legislations.

Despite the gap in the results, significant non-differentiation points to the fact that environmental contamination in the study area may not be only timely for the activities of the Port of Paranaguá. There are also some other activities such as agriculture, livestock, fish farming, tourism, leisure and navigation, which are already described in the literature as potentially contaminating activities due to their uncontrolled and irrational use[1-4]. This context of insertion of the collection and sampling points will be investigated through the study between detailed examinations of the region of the study area and their respective surroundings, concomitantly with the data obtained by the analytical technique of flame atomic absorption spectrometry.

Table 4. Guidelines and guidelines on maximum lead limits (Pb) in foods.

Part	Regulatory Agency	Normative	Samples	Maximum
MERCOSUL[13]	Ministério da Saúde (MS) Agência Nacional de Vigilância Sanitária (ANVISA)[10]	Resolution Nº 12/11	Fishes	0.3 mg kg^{-1}
			Cephalopod mollusks	1 mg kg^{-1}
			Bivalve mollusks	1.5 mg kg^{-1}
			Crustaceans	0.5 mg kg^{-1}
SDA/MAPA[11]	Defesa Agropecuária (SDA) Ministério da Agricultura, Pecuária e Abastecimento (MAPA)[11]	Normative Instruction Nº 09/17	Catch fish	0.3 mg kg^{-1}
			Growing fish	0.3 mg kg^{-1}
			Shrimp	0.5 mg kg^{-1}
Present study	-	-	Feces extract	21.9 mg kg^{-1}

Table 5. Guidance standards and values on lead limits (Pb) in fresh, salt and brackish water.

Part	Regulatory Agency	Normative	Samples	Maximum limit
CONAMA[12]	Conselho Nacional do Meio Ambiente (CONAMA)	Resolution Nº 357/05	Class Sweet Waters 1 and 2	0.01 mg L^{-1}
			Class Sweet Waters 3	0.033 mg L^{-1}
			Salt water class 1	0.01 mg L^{-1}
			Salt water class 2	0.21 mg L^{-1}
			Brackish water class 1	0.01 mg L^{-1}
			Brackish water class 2	0.21 mg L^{-1}
Present study	-	-	Feces extract	21.86 mg kg^{-1}

It is also known that in matrices of different natures there are variations to the maximum limits allowed by virtue of the unique biogeochemical and physicochemical characteristics. In sediment samples and organic materials, the tendency is to have higher concentrations due mainly to the adsorption process, because the adsorption variability influences the mobility, availability, retention and, therefore, the concentration of metallic ions and their toxicity. In the case of lead, toxicology consists of the interaction of its ionic form with organic groups present in the soil, sediments and biological tissues, and the introduction into the organism through atmospheric air, contact with soil, water and through skin[14]. The toxicity of lead results mainly from its interference in the functioning of cell membranes and enzymes, since it is capable of forming stable complexes with binders containing sulfur, phosphorus, nitrogen or oxygen, for example with the groups $-SH_2$, $-H_2PO_3$, $-NH_2$ and -OH, which function as electron donors[14, 15]. In this way, most of the lead, initially present in the blood, goes up to reach a plateau. The excess penetrates the soft tissues and organs, among which the brain stands out, and finally it deposits in the bones, teeth and hair[14, 15], where it replaces the calcium due to the similarity of size between the Pb^{2+} and Ca^{2+} [15]. From this perspective, therefore, the high concentrations of lead in the samples of this work are consistent when considering that the constitution of the feces samples is mostly composed of bones and scales originating from the food items of a diet such as otter, predominantly piscivorous. In this way, it is possible to provide a diagnosis considering the absorption by the alternative routes to the digestive route and, finally, the incorporation of metallic ions in the food web since the bioindicator species is a chain-top carnivore. When comparing the results and comparing the Pb analyzed in related studies, a significant equivalence of the data can be observed when analyzed on the normalized form of minimum and maximum values of the determined concentrations: the routing to a worsening of the ecosystem health due to natural processes and/or potentially polluting. As with the guideline values of the maximum limits allowed and established by SDA/MAPA[11], CONAMA[12] and MERCOSUL[13], the presence of irregularities is mainly correlated with the determinations obtained in all mentioned studies (Table 6)[14].

Table 6. Pb Concentrations in *Lontra longicaudis* in related studies

Species	Matrix	El	Conc. (min. - max.)	References
Lutra lutra	Feces	Pb	12.4 - 20.9 mg kg^{-1}	Mason and MacDonald (1986)[16]
Crysocyon brachyurus	Pelage		0 - 4.3 mg kg^{-1}	Brait, Antoniosi Filho and Furtado (2009)[17]
Cerdocyon thous	Pelage		0.9 - 3.6 mg kg^{-1}	Brait, Antoniosi Filho and Furtado (2009)[17]
Leopardus pardalis	Pelage		0.3 - 2.5 mg kg^{-1}	Brait, Antoniosi Filho and Furtado (2009)[17]
Cathorops spixii	Dorsal muscle		0.004 - 0.48 mg kg^{-1}	Trevizani (2011)[18]
Lontra longicaudis	Feces		1.35 - 27.327 mg kg^{-1}	Present study (2018)

In order to emphasize the study developed by Trevizani (2011)[18] in comparison with the present study (2018), both carried out in the Bay of Paranaguá, it is possible to investigate and recognize the minimization of the threats and risks involved, as well as the concealment for future trends and projections regarding territorial development and the quality of natural territorial spaces. In addition, the analytical knowledge about the dynamism between the socioeconomic and socioenvironmental establishment for the occupation and use of the coastal space contributed to identify and characterize the externalities to the ecosystemic health by potentially polluting productive processes, as is the case of the Port Terminal of Paranaguá. Thus, the use of biological samples from the bioindicator species allowed not only to carry out the quantitative determinations of Pb contamination from the productive activities of the local port sector, but also to explain the understanding of the complexity and inseparability of environmental impacts for long periods in the disorganized and indiscriminate use and exploitation of natural property in common use. Therefore, the importance of detailed knowledge about sanitary problems and ecological imbalance from metallic ions that rely on the direct and indirect relations of interference on all the environmental segments of the territory, among them the hydric, sedimentological and/or biological compartments.

4. Conclusions

Quantitatively, there are no significant differences in the Pb concentrations between collection points. This requires more in-depth studies based on a larger sample size as a function of the main anthropogenic sources of contamination for each of the sampling and collection areas. It also needs to investigate potential contaminants in water, sediment, food items such as fish and crustaceans, and biological tissues such as liver, brain, kidneys and lungs of dead animals. As well, to identify individuals or populations through DNA analysis aiming the clarification of the mobility by a certain area and, consequently, of the provenance of the feces.
However, the determination of lead in feces of *Lontra longicaudis* by flame atomic absorption spectrometry (F AAS) was adequate to determine concentrations of metal ions in biological samples of the bioindicator species. This fact makes it possible to use these procedures to extend the analytical studies on contamination in animal samples present in the territory.

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