

Chemical Analysis of Interstitial Water in Rivers of Centro Experimental Aramar Area

Erika Matoso^{1,2}, Julia Rosa Gonçalves¹, Solange Cadore²

¹Centro Tecnológico da Marinha em São Paulo
Centro Experimental Aramar (CTMSP-CEA)
Rodovia Sorocaba - Iperó, km 12.5 s/n
18560-000 Iperó, SP
ematoso@hotmail.com

²Universidade Estadual de Campinas,
Instituto de Química, Departamento de Química Analítica,
Caixa Postal 6154,
13084-971 Campinas, SP

ABSTRACT

This work presents the results from analysis of samples of interstitial waters for the following chemical parameters: F⁻, Cl⁻, NO₂⁻, Br⁻, NO₃⁻, PO₄³⁻, SO₄²⁻ by Ionic Chromatography, Na, K by Flame Photometry, Al, Cd, Pb, Cu, Cr, Fe, Mn, Ni, Zn by ICP OES, pH and the biological parameter: toxicity by natural bioluminescent bacterium (*Vibrio fischeri*) bioassay. The samples were obtained from sediments collected in 6 different sampling locations, in a ratio of 10-km-long from Centro Experimental Aramar (CEA). The rivers where the samples came from were: Ipanema River, Sorocaba River and Ribeirão do Ferro River. The interstitial water was extracted by centrifugation (3000 rpm, 20 min, 4°C). Analysis for metal concentrations were carried out after acid digestion and other tests proceeded in the sample after filtration without further treatment. These data will contribute to evaluate the distribution of contaminants and nutrients in these collecting points and this toxicity status. The release of soluble substances from sediments to interstitial water provides one way for bioaccumulation of these compounds and may affect the survival or development of aquatic organisms. The analysis in interstitial water has never been evaluated at this sampling points and the importance of this study is collecting data providing a better knowledge of the hydrological conditions in which Centro Experimental Aramar is located.

1. INTRODUCTION

The increasing of industrial and agricultural activities, sometimes in a unsustainable way, can lead to a contamination of hydrological resources, such as overlaying and underground water, mostly by discharges of wastes, resulting a decrease of water quality, besides an accumulation of contaminants in sediments [1]. The metal contaminants remain mainly insoluble in the sediments, but some conditions may lead to the dissolution of them to water phase, such as: pH, salinity, redox potential and organic ligands[2,3,4,5].

Interstitial waters are located between the sediments pores, carrying out chemical exchanges with them [6]. Water represents the dynamic equilibrium between the release of elements from sediments to water phases (sediment-water interface or overlying water) [7]. The exchanges occurs by diffusion and their intensity depends in particular on the gradient

concentration in the interstitial water [8,9]. This contaminants diffusion provides one pathway for bioaccumulation in the food chain [7] and represents the best expression of toxicity to benthic organisms [10]. Analysis of interstitial water contribute to a better understanding of the hydrochemical variations in the subsurface water. In fact, there is an increasing interest of this studies of groundwater pollution [11] to evaluate the partition water-sediment in order to determinate the bioavailability of contaminants [12].The interstitial water can be extracted from sediments by centrifugation in low temperatures (usually 4°C).[11,13,14,15]

The Centro Tecnológico da Marinha (CTMSP) is a research center which goals are developing the nuclear fuel cycle for energetic purposes and navy submarine propulsion. Its shead-office is located at São Paulo University and this Experimental branch, called Centro Experimental Aramar (CEA) is located at Iperó at São Paulo State[16,17]. Since the rivers Ipanema and Ribeirão do Ferro cross through CEA and drain into Sorocaba River, sample points of these three rivers were chosen for this work. The environmental sampling locations are showed in Figure 1.

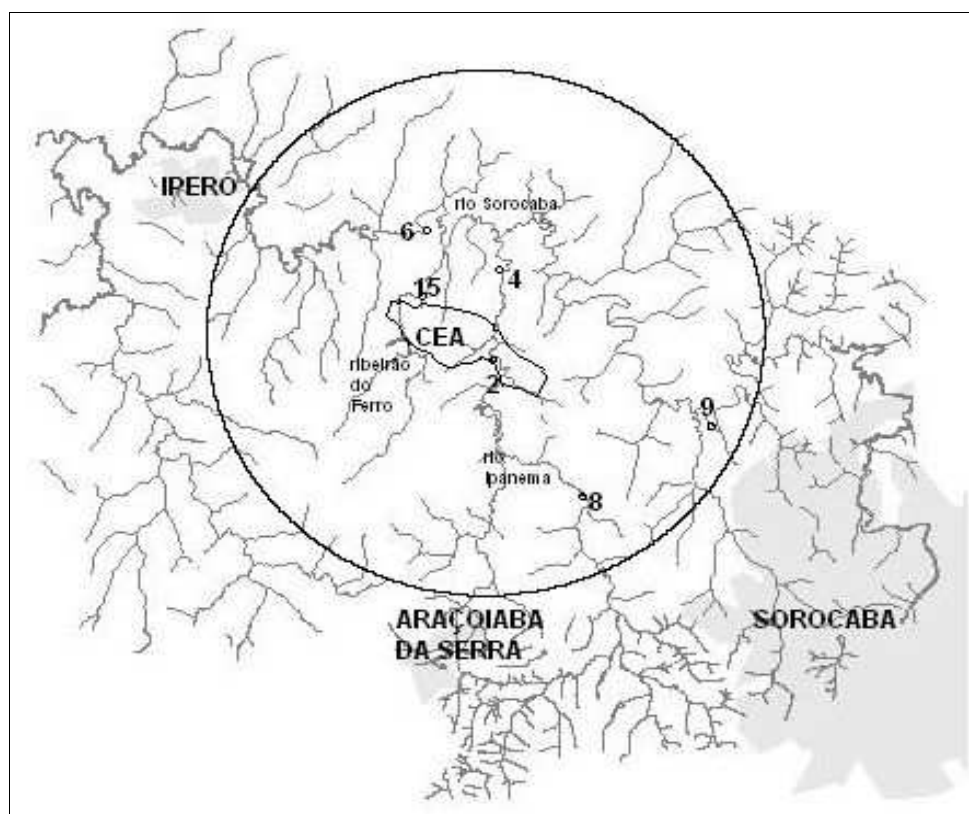


Figure 1: Interstitial water sampling locations

The importance of this study is to collect new data, since interstitial water has never been evaluated before in these sampling locations. Regarding metal concentration in this samples, even when the individual concentration of each contaminant is low, it must be considered the addition effect of different metals, which may cause toxicity to benthic organisms [15].

2. MATERIALS AND METHODS

2.1. Sampling locations

The samples were collected at the sampling locations showed in Table 1.

Table 1: Sampling locations Identification

Sample Identification	River	Coordinates
AI02	Ipanema	235051E, 7409710N
AI04	Ipanema	235290E, 7412106N
AI06	Sorocaba	232708E, 7413615N
AI08	Ipanema	238303E, 7403542N
AI09	Sorocaba	242876E, 7406164N
AI15	Ferro	2311720E, 7409390N

2.2. Sampling

The sediments samples were collected every two months and the supernatant water were discharged. The humid samples were placed in centrifuge tubes and spun in a refrigerated ultracentrifuge (4°C, 3000rpm, 20 minutes) to separate interstitial water from sediment. The interstitial water was filtered (0,45µm filter paper) in an ice bath and aliquots were separated for analysis.

2.3. Sample preparation

The analysis by ionic chromatography, pH and toxicity were carried out in the filtered aliquots without any other pretreatment. The metals analysis by ICP OES and flame photometry were carried out at digested samples, following the procedure: 0.5 mL nitric acid p.a. were added to 25 mL of sample which was heated (100°C) in a hot plate, until volume reduction to 5mL. The digested aliquot was transferred to a volumetric flask and the volume was completed to 25mL with deionized water.

2.4. Analytical techniques

The anions determinations (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , PO_4^{3-} , SO_4^{2-}) were carried out on a Dionex ICS-900 ionic chromatograph. The pH determinations were carried out on a Hanna HI2221 pHmeter, While for Na and K determination a Micronal B262 flame photometer was used. The metallic ions determination (Al, Cd, Pb, Cu, Cr, Fe, Mn, Ni, Zn) was carried out on

a Perkin Elmer Optima 3000DV (axial view) inductively coupled plasma optical emission spectrometer (ICP OES). The toxicity determinations by natural bioluminescent bacterium (*Vibrio fischeri*) bioassay were carried out on a SDI Microtox 500 Analyser.

3.RESULTS

3.1.Anions determination

Tables 2 to 5 show the results of anions determinations in samples collected every two months from August/2012 to February/2013. The chloride and sulfate presented higher concentration values. For chloride, the range was from 2 to 37 mg/L and for sulfate from 0.2 to 163 mg/L, depending on the sampling point and/or the collecting time.

Figures 2 and 3 particularly show the results of chloride and sulfate determinations. Figure 2 shows that, except for AIO4 sample, collected at February/2013, values of chloride concentrations were below 25 mg/L for all samples. Figure 3 shows that only four samples, collected in different times, presented values of sulfate concentration above 20 mg/L.

The Brazilian National Environmental Council (CONAMA) do not define regulatory values for interstitial water anion content in rivers. However, values for quality control of water surface are stated by this agency and were used in this work as control references. For both chloride and sulfate this value is set up to 250 mg/L for Class II water, (classification of the three rivers from where samples were collected). The higher sulfate concentrations were obtained in samples AI15 and AI06 (150 and 160 mg/L, respectively) and both were below the CONAMA reference. For nitrate, the higher values were obtained for sample AI09 (October and December sampling) and both are below the reference value allowed for nitrogen (calculated as nitrate) from CONAMA. For all other samples, nitrate concentrations were below to 3 mg NO₃⁻/L.

Table 2: August/2012 sampling Anions Determination

Anions	AI02	AI04	AI06	AI08	AI09	AI15	LOQ
F ⁻ (mg/L)	0.073	0.020	0.234	0.097	0.510	0.205	0.002
Cl ⁻ (mg/L)	3.23	1.64	10.85	21.48	11.38	3.98	0.01
NO ₂ ⁻ (mg/L)	< 0.01	<0.01	<0.01	<0.01	0.04	<0.01	0.01
Br ⁻ (mg/L)	0.03	0.01	0.06	0.06	0.01	0.07	0.01
NO ₃ ⁻ (mg/L)	0.09	< 0.01	0.06	0.23	2.24	0.05	0.01
PO ₄ ³⁻ (mg/L)	< 0.02	< 0.02	<0.02	<0.02	<0.02	<0.02	0.02
SO ₄ ²⁻ (mg/L)	0.49	1.42	0.21	0.50	8.06	0.23	0.01

Note: LOQ = Limit of quantification

Table 3: October/2012 sampling Anions Determination

Anions	AI02	AI04	AI06	AI08	AI09	AI15	LOQ
F ⁻ (mg/L)	0.180	0.040	0.310	(*)	0.570	0.150	0.002
Cl ⁻ (mg/L)	8.20	8.55	18.41	(*)	17.21	1.79	0.01
NO ₂ ⁻ (mg/L)	0.01	0.01	< 0.01	(*)	0.01	< 0.01	0.01
Br ⁻ (mg/L)	0.02	0.03	0.04	(*)	< 0.01	0.04	0.01
NO ₃ ⁻ (mg/L)	0.16	0.28	0.13	(*)	32.95	0.12	0.01
PO ₄ ³⁻ (mg/L)	< 0.02	< 0.02	< 0.02	(*)	0.04	< 0.02	0.02
SO ₄ ²⁻ (mg/L)	3.79	0.60	12.35	(*)	62.91	0.31	0.01

Note: (*) It was impossible to extract interstitial water from sample. There were just rocks.

Table 4: December/2012 sampling Anions Determination

Anions	AI02	AI04	AI06	AI08	AI09	AI15	LOQ
F ⁻ (mg/L)	0.076	0.020	0.245	(*)	0.105	0.038	0.002
Cl ⁻ (mg/L)	3.73	2.00	8.43	(*)	14.01	2.34	0.01
NO ₂ ⁻ (mg/L)	< 0.01	< 0.01	< 0.01	(*)	0.11	< 0.01	0.01
Br ⁻ (mg/L)	0.07	< 0.01	0.21	(*)	< 0.01	0.03	0.01
NO ₃ ⁻ (mg/L)	2.57	0.11	0.18	(*)	42.89	0.87	0.01
PO ₄ ³⁻ (mg/L)	< 0.02	< 0.02	< 0.02	(*)	< 0.02	< 0.02	0.02
SO ₄ ²⁻ (mg/L)	1.30	1.37	0.18	(*)	90.88	147.39	0.01

Note: (*) It was impossible to extract interstitial water from sample. There were just rocks.

Table 5: February/2013 sampling Anions Determination

Anions	AI02	AI04	AI06	AI08	AI09	AI15	LOQ
F ⁻ (mg/L)	0.275	0.177	0.142	0.139	0.454	0.175	0.002
Cl ⁻ (mg/L)	22.32	36.92	13.50	12.80	16.25	3.24	0.01
NO ₂ ⁻ (mg/L)	0.12	0.05	0.01	< 0.01	< 0.01	< 0.01	0.01
Br ⁻ (mg/L)	0.09	0.01	< 0.01	0.08	0.05	0.08	0.01
NO ₃ ⁻ (mg/L)	0.89	0.57	2.62	0.34	0.20	0.24	0.01
PO ₄ ³⁻ (mg/L)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	0.02
SO ₄ ²⁻ (mg/L)	5.96	4.97	162.97	1.03	0.40	4.71	0.01

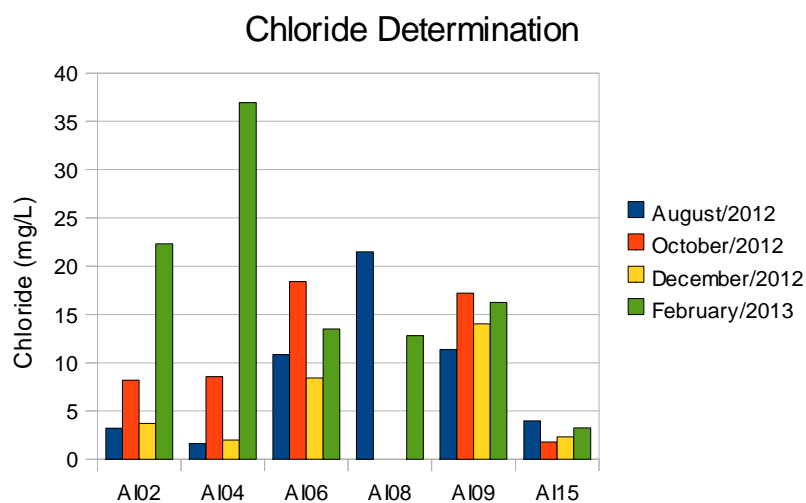


Figure 2: Results for Chloride determination for all sampling

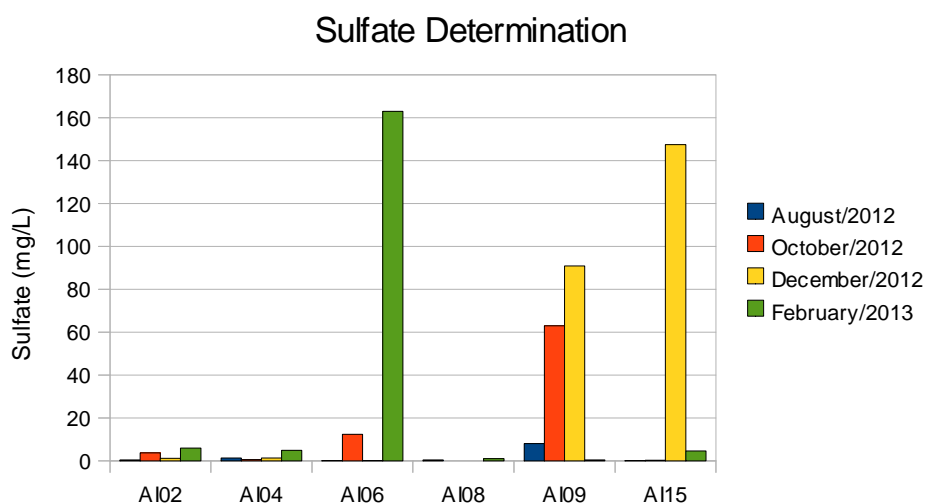


Figure 3: Results for Sulfate determination for all sampling

3.2.pH determination

Table 6 show the results for pH determinations of all samples. Figure 4 shows that the pH varying approximately from 6 to 8, but most of the results are near to pH 7. The sample with the largest range was AI04 (2.02 pH units). All pH values obtained are in accordance with CONAMA quality reference, considering the reference values defined for surface water by this agency.

Table 6: pH Determination

Sampling date	AI02	AI04	AI06	AI08	AI09	AI15
August/2012	6.83	7.90	6.94	7.56	7.60	7.21
October/2012	6.71	6.40	6.86	(*)	6.82	7.18
December/2012	7.82	7.63	6.98	(*)	7.15	7.10
February/2013	6.81	5.88	6.44	6.76	7.23	7.07
Range	1.11	2.02	0.54	0.80	0.78	0.14

Note: (*) It was impossible to extract interstitial water from sample. There were just rocks.

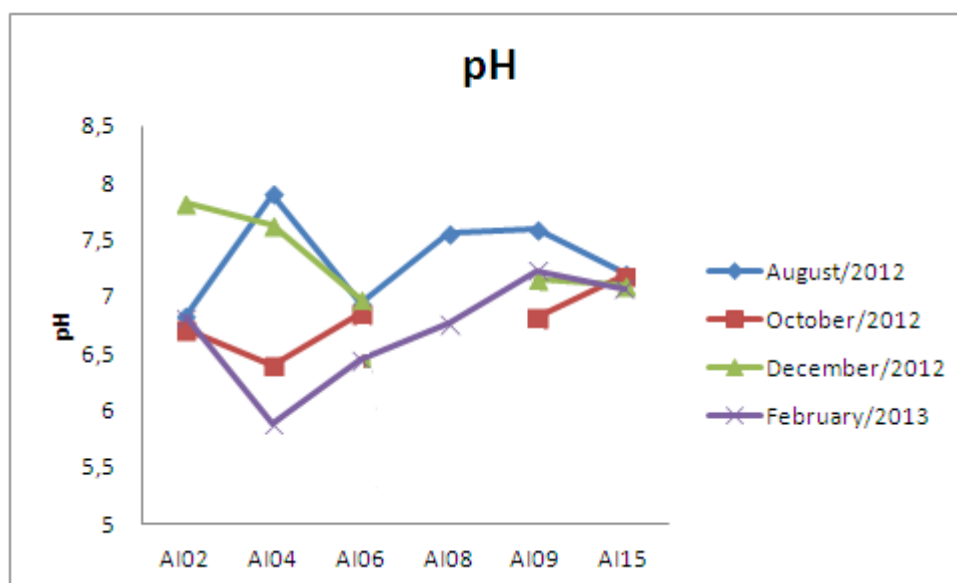


Figure 4: Results for pH determination

3.3.Flame photometry determination (Na and K)

Table 7 shows that the range for sodium concentration goes approximately from 3 to 28 mg/L and for potassium from 1 to 16 mg/L. The sample with the highest variation for potassium was AI06 and for sodium was AI08. Sample AI08 needs additional investigation, since two sampling dates were missed, due to the impossibility to extract interstitial water. Figures 5 and 6 show that samples from AI06 to AI09 have higher concentration of sodium and sample AI06 has also higher concentration of potassium.

Table 7: Na and K Determination

Sampling date	AI02 Na / K (mg/L)		AI04 Na / K (mg/L)		AI06 Na / K (mg/L)		AI08 Na / K (mg/L)		AI09 Na / K (mg/L)		AI15 Na / K (mg/L)	
	Na	K	Na	K	Na	K	Na	K	Na	K	Na	K
August/2012	8.0	2.8	3.2	1.1	19.5	14.4	27.5	2.2	11.4	2.4	11.1	6.9
October/2012	9.2	3.8	6.7	2.6	16.5	7.0	(*)	(*)	13.4	2.5	8.2	6.1
December/2012	8.3	3.4	3.0	2.7	15.2	16.3	(*)	(*)	24.1	7.8	9.5	5.0
February/2013	7.2	10.8	5.7	5.3	18.1	10.8	10.3	8.9	15.2	8.8	9.7	5.0

Note: (*) It was impossible to extract interstitial water from sample. There were just rocks.

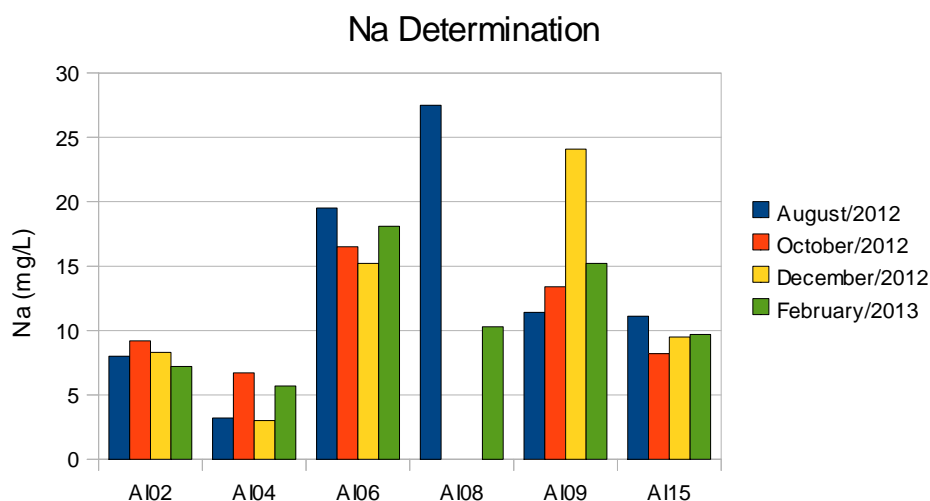


Figure 5: Results for Na determination for all sampling

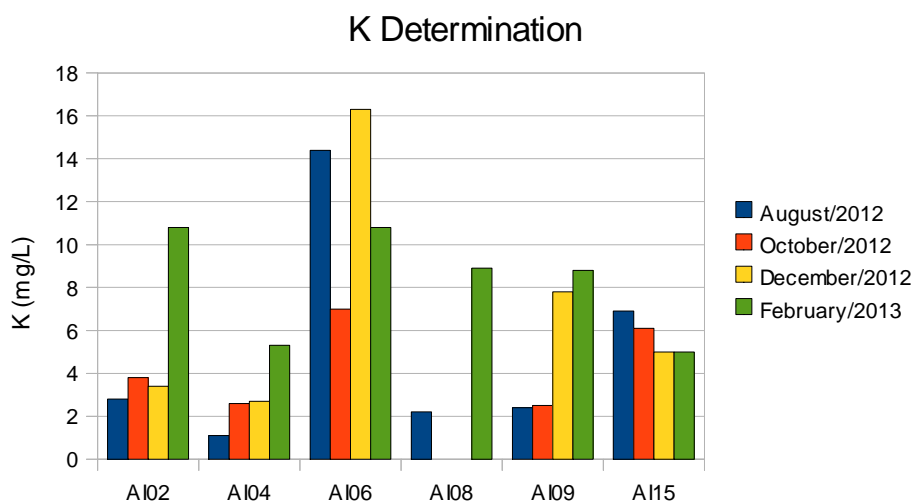


Figure 6: Results for K determination for all sampling

3.4.ICP OES Determination

The analyzed elements detected in higher concentrations were iron and manganese, which ranging from 0.03 mg/L to 67 mg/L for iron and 0.03mg/L to 16mg/L for manganese. The concentrations for Cd, Pb, Cu, Cr and Ni were below the limit of quantification. Sample AI06 presented the highest results for iron and sample AI15 for manganese, as showed in Figures 7 and 8. The higher results for iron are coherent with a previous work[19] where total iron content was analyzed in sediments at 02, 04 and 06 sampling points. In that work, iron concentrations were determined by neutron activation analysis and the results were for 1 % w/w to 4% w/w.

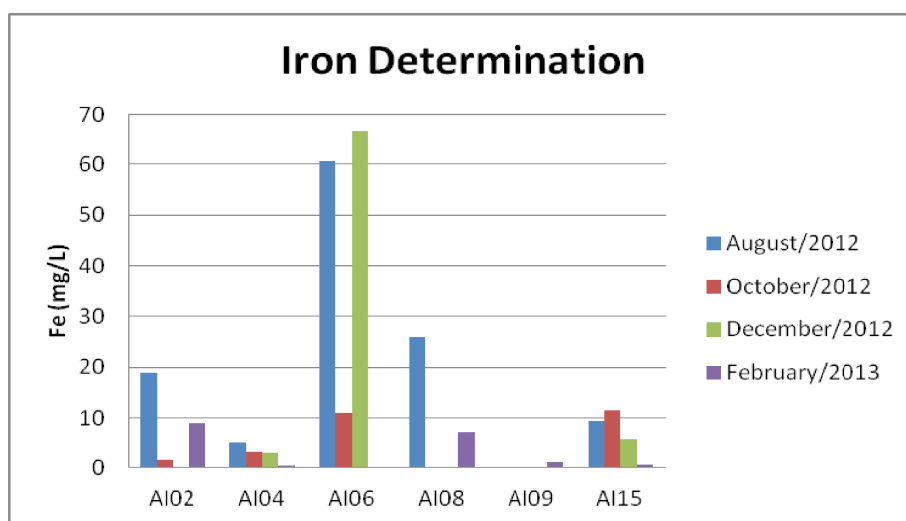


Figure 7: Results for Fe determination for all sampling

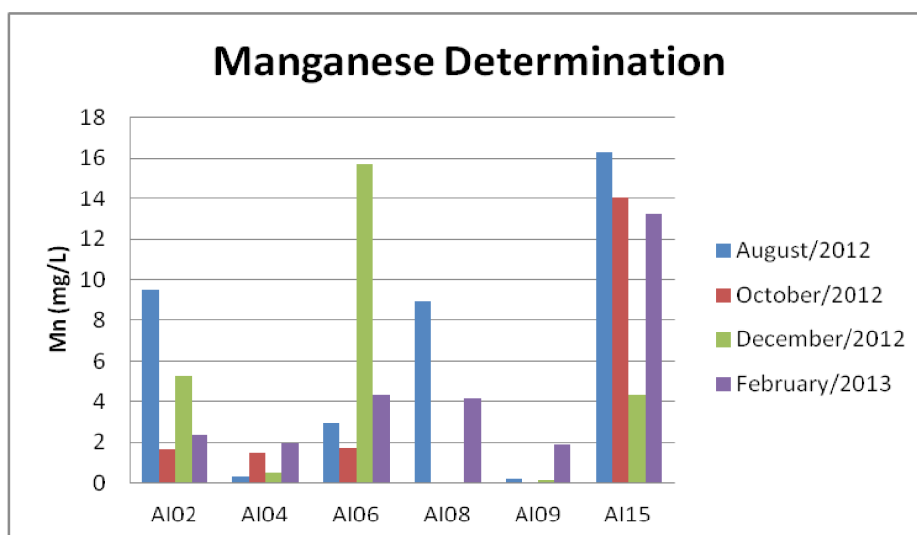


Figure 8: Results for Mn determination for all sampling

Table 8: August/2012 sampling ICP OES Determination

Elements	AI02	AI04	AI06	AI08	AI09	AI15
Al (mg/L)	0.36	0.739	< 0.05	< 0.05	< 0.05	< 0.05
Cd (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb (mg/L)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Cu (mg/L)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Cr (mg/L)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Fe (mg/L)	18.82	5.14	60.58	25.96	0.03	9.47
Mn (mg/L)	9.51	0.31	2.94	8.92	0.25	16.32
Ni (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Zn (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Table 9: October/2012 sampling ICP OES Determination

Elements	AI02	AI04	AI06	AI08	AI09	AI15
Al (mg/L)	0.25	0.05	3.24	(*)	0.09	< 0.05
Cd (mg/L)	< 0.01	< 0.01	< 0.01	(*)	< 0.01	< 0.01
Pb (mg/L)	< 0.05	< 0.05	< 0.05	(*)	< 0.05	< 0.05
Cu (mg/L)	< 0.02	< 0.02	< 0.02	(*)	< 0.02	< 0.02
Cr (mg/L)	< 0.02	< 0.02	< 0.02	(*)	< 0.02	< 0.02
Fe (mg/L)	1.48	3.28	11.1	(*)	0.09	11.53
Mn (mg/L)	1.65	1.49	1.71	(*)	0.03	14.04
Ni (mg/L)	< 0.01	< 0.01	< 0.01	(*)	< 0.01	< 0.01
Zn (mg/L)	< 0.01	< 0.01	< 0.01	(*)	< 0.01	< 0.01

Note: (*) It was impossible to extract interstitial water from sample. There were just rocks.

Table 10: December/2012 sampling ICP OES Determination

Elements	AI02	AI04	AI06	AI08	AI09	AI15
Al (mg/L)	0.09	0.12	< 0.05	(*)	< 0.05	< 0.05
Cd (mg/L)	< 0.01	< 0.01	< 0.01	(*)	< 0.01	< 0.01
Pb (mg/L)	< 0.05	< 0.05	< 0.05	(*)	< 0.05	< 0.05
Cu (mg/L)	< 0.02	< 0.02	< 0.02	(*)	< 0.02	< 0.02
Cr (mg/L)	< 0.02	< 0.02	< 0.02	(*)	< 0.02	< 0.02
Fe (mg/L)	0.29	3.02	66.57	(*)	0.11	5.81
Mn (mg/L)	5.25	0.50	15.71	(*)	0.15	4.33
Ni (mg/L)	< 0.01	< 0.01	< 0.01	(*)	< 0.01	< 0.01
Zn (mg/L)	0.02	< 0.01	0.05	(*)	0.09	0.03

Note: (*) It was impossible to extract interstitial water from sample. There were just rocks.

Table 11: February/2013 sampling ICP OES Determination

Elements	AI02	AI04	AI06	AI08	AI09	AI15
Al (mg/L)	4.08	< 0.05	< 0.05	0.29	0.14	< 0.05
Cd (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb (mg/L)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Cu (mg/L)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Cr (mg/L)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Fe (mg/L)	8.93	0.50	0.29	7.12	1.01	0.59
Mn (mg/L)	2.39	1.97	4.33	4.15	1.93	13.25
Ni (mg/L)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Zn (mg/L)	0.12	0.06	0.28	0.06	0.06	0.03

3.5. Bioluminescent bacteria (*Vibrio fischeri*) Bioassay [20,21,22]

The bioluminescent bacterium (*Vibrio fischeri*) bioassay is usually used for the determination of the acute toxicity. The bacterium naturally emits light in suitable aquatic environments. Although the bacterium is from marine origin, it is also possible to use it to assess the toxicity of samples of fresh water and sediment, after osmotic adjustment. In the presence of toxic compounds, the bacterium metabolism is affected and the light emission decreases. The results are expressed as effective concentration 20 (EC₂₀), which is the sample concentration (%w/w or mg / L) that causes a 20% reduction in the light emission from *Vibrio fischeri*, after 15 minutes of exposure time of. Thus, the smaller the EC₂₀, the more toxic is the sample. The sample is classified as showed in Table 12.

Table 12: Classification of acute toxicity test with *Vibrio fischeri* (System Microtox ®)

Sample evaluation	EC ₂₀ result
Non-Toxic	EC ₂₀ > 81.9%
Moderately Toxic	50% < EC ₂₀ = 81.9%
Toxic	25 < EC ₂₀ = 50%
Very Toxic	EC ₂₀ = 25%

All samples were analyzed and the results obtained were not toxic, even for those with higher contaminants concentration.

4. CONCLUSIONS

The importance of this work was to collect chemical information from interface sediment-water from rivers of CEA surroundings to start a database, comparing with toxicological results. So far, all toxicological analysis showed results *Not Toxic* with the level of chemical compounds quantified.

For future work, the chemical analysis will be continued in order to find a range of normal conditions in which no toxicological effects are observed.

5. REFERENCES

1. V.K.Saraiva, “*Critérios para avaliação da Qualidade de Sedimentos – estudo de caso: Sub-Bacia do Ribeirão Espírito Santo, Afluente do Rio São Francisco*” - Dissertação de Mestrado, CNEN, Belo Horizonte (2007).
2. J.S.Santos, M.L.P Santos, E.Oliveira, “Estudo da mobilização de metais e elementos traços em ambientes aquáticos de semiárido brasileiro aplicando análise de componentes principais”, *Quim. Nova*, **31**, pp.1107-1111 (2008).
3. M.A Hortellani, J.E.S.Sarkis, D.M.S.Abessa, E.C.P.M.Sousa, “Avaliação da contaminação por elementos metálicos dos sedimentos do estuário Santos-São Vicente”, *Quim. Nova*, **31**, pp.10-19 (2008).
4. V.K. Saraiva, M.R.L Nascimento, H.E.L. Palmieri, V.M.F. Jacomino, “Avaliação da Qualidade de Sedimentos – Estudo de Caso: Sub-Bacia do Ribeirão Espírito Santo, Afluente do Rio São Francisco”, *Quim. Nova*, **32**, pp.1995-2002 (2009).
5. M.Antunes, D.B Dillon, J.SCrespo, M.Giovanela,. “Avaliação dos parâmetros físico-químicos e do teor de metais em amostras de sedimentos de uma microbacia gaúcha”, *Geochimica Brasiliensis*, **22**, pp.178-188 (2008).
6. W.F.L.Gonçalves, “*Sazonalidade Geoquímica em águas intersticiais, superficiais e sedimentos estuarinos de Cubatão (SP), impactados por atividades industriais*”, Dissertação de Mestrado, Instituto de Geociências – Universidade Estadual de Campinas – São Paulo (2009).
7. A.J.Paulson, S.E.Cox, “Release of elements to natural water from sediments of Lake Roosevelt, Washington, USA”, *Environmental Toxicology and Chemistry*, **26**, 2550-2559 (2007).
8. B.Garban, D.Ollivon, M.Poulin, V.Gaultier,, A.Chesterikoff, “Exchanges at the sediment-water interface in the river Seine, downstream from Paris”, *Wat. Res*, **29**,pp. 473-481 (1995).
9. P.Bonanni, R.Caprioli, E.Ghiara, C.Mignuzzi, C.Orlandi, G.Paganin, A.Monti, “Sediment and interstitial water chemistry of the Orbetello lagoon (Grosseto, Italy); nutrient diffusion across the water-sediment interface”, *Hydrobiologia*, **235/236**, pp.553-568 (1992).
10. A.Davis, D.Atkins, “Metal distribution in Clark Fork river sediments”, *Environ. Sci. Technol*, **35**, pp.3501-3506 (2001).

11. M.J.Spence, S.F.Thornton, S.H.Bottrell, K.H.Spence, “Determination of interstitial water chemistry and porosity in consolidated aquifer materials by diffusion equilibrium-exchange”, *Environ. Sci. Technol.*, **39**, pp.1158-1166 (2005).
12. E.C.Nizoli, W.L.Silva, “O papel dos sulfetos volatilizados por acidificação no controle potencial de biodisponibilidade de metais em sedimentos contaminados de um estuário tropical, no sudeste do Brasil”, *Quim. Nova*, **32**, pp.365-372 (2009).
13. J.Liu, X.Feng, G.Qiu, H.Yao, L.Shang, H.Yant, “Intercomparison and applicability of some dynamic and equilibrium approaches to determine methylated mercury species in pore water”, *Environ. Toxicol. Chem.*, **30**, pp.1739-1744 (2011).
14. A.T.Fisher, E.Giambalvo, J.Sclater, M.Kastner, B.Ransom, Y.Weinstein, P.Lonsdale, “Heat flow, sediment and pore fluid chemistry, and hydrothermal circulation on the east flank of Alarcon Ridge, Gulf California”, *Earth and Planetary Science Letters*, **188**, pp.521-534 (2001).
15. A.A.Mozeto, G.A.Umbuzeiro, W.F.Jardim, “*Métodos de Coleta, Análises Físico-Químicas e Ensaio Biológicos e Ecotoxicológicos de Sedimentos de Água Doce*”, Editora Cubo, São Paulo (2006).
16. “CTMSP - Centro Tecnológico da Marinha em São Paulo” http://www.mar.mil.br/ctmsp/conheca_centro.html (2013).
17. “Programa Nuclear da Marinha”, <http://www.mar.mil.br/pnm/pnm.htm> (2013).
18. Resolução CONAMA N°357, de 17 de Março de 2005 (Padrões de Qualidade Água Doce – Classe 2)
19. D.R.Arine, “Análise de águas de superfície e sedimentos de rios da região de Iperó, SP, por espectrometria de absorção atômica e por ativação neutrônica”, IPEN, São Paulo (2000).
- 20 - Norma Técnica – L5.227 – Teste de toxicidade com bactéria luminescente *Vibrio fischeri*: método de ensaio.
21. S.Girotti, E.N.Ferri, M.G.Fumo, E.Maiolini, “Monitoring of environmental pollutants by bioluminescent bacteria”, *Analytica Chimica Acta*, 608, 2-29, 2008.
22. “Classificação do teste de toxicidade aguda com *Vibrio fischeri* (Sistema Microtox®)”, <http://www.cetesb.sp.gov.br/userfiles/file/agua/aguas-superficiais/aguas-interiores/documentos/indices/09.pdf> (2013).