

EFFECT EVALUATION OF URANIUM MINING EFFLUENTS ON THE DENSITY AND COMPOSITION OF THE PHYTOPLANKTON COMMUNITY

Cláudio V. Roque¹, Maria José Dellamano-Oliveira², Heliana de Azevedo¹, Armando L. Bruschi¹, Carla R. Ferrari¹, Leilane B. Ronqui¹, Michelle B. Campos¹, Marcos Roberto L. Nascimento¹ and Suzelei Rodgher¹

¹Poços de Caldas Laboratory, Brazilian Nuclear Energy Commission
Rodovia Poços de Caldas - Andradas, Km 13
37701-970 Poços de Caldas, MG- Brazil
cvroque@cnen.gov.br
hazevedo@cnen.gov.br
abruschi@cnen.gov.br
cferrari@yahoo.com.br
leilanebio@yahoo.com.br
michele_borato@hotmail.com
marcos@cnen.gov.br
surodgher@uol.com.br

²Department of Ecology and Evolutionary Biology –
Federal University of São Carlos
Rodovia Washington Luiz-SP 310, Km 235
13565-905 São Carlos, SP - Brazil
pdeol@ig.com.br

ABSTRACT

Located in the region of the Poços de Caldas Plateau, the Osamu Utsumi mine is the first uranium extraction and production mine to have its deposits explored in Brazil and it is situated on the premises of the Brazilian Nuclear Industries Ore Treatment Unit (UTM/INB). Within the UTM/INB installations, water samplings were carried out every three months (from October 2008 to July 2009) in three points (P1, P2 and P3): P1 (pit mine), P2 (Tailings Management Facility/TMF) and P3 (environment). The objective of the current study was to evaluate density and composition of the phytoplankton community, as well as chemical characteristics of water samples from UTM/INB effluents, which present different pH levels (ranging from acidic to alkaline). In the current study, values of pH, total nitrogen (TN), total phosphorus (TP), silicate (Si), sulfate (SO_4^{-2}), fluoride (F), uranium (U), thorium (Th) and chlorophyll *a* (Chl *a*) were determined, as well as composition and density of the phytoplankton community. After comparing the three sampling points, it was verified that Cyanophyceae presented greater tolerance to chemical conditions of the water such as elevated concentrations of sulfate, fluoride, uranium and thorium, as well as pH variations, since this class was detected in all studied environments.

1. INTRODUCTION

One of the environmental problems resulting from mining activities is the occurrence of Acid Mine Drainage (AMD), which can compromise the quality of hydric resources on the region of its occurrence [1].

The AMD is the result of the natural oxidation of metal sulfides when exposed to hydrological (oxygen) or biological weathering (chemoautotrophic bacteria), which leads to the formation of sulfuric acid, promoting conditions such as: low pH values, presence of dissolved ion sulfate, elevated concentrations of some metals (As, Cu, Fe, Mn, Pb, Zn among others), low alkalinity, as well as elevated values of electrical conductivity [2]. Thus, the AMD is related to the formation of acid effluents, being characterized by a variety of contaminants, which represent risk of contamination once they are related to pollution in aquatic ecosystems [3]. In addition, these effluents can reach aquatic bodies and freatic ground water, compromising the quality of the latter, thus preventing their use and altering the aquatic flora and fauna. This way, analysis of dispersion and distribution of toxic elements become essential, especially when their concentrations are elevated, when compared to the natural background of the environment [4].

It is worth emphasizing that there is a limited number of records about the ecology of mining ponds (pit mines), especially those related to phytoplankton communities [5]. The phytoplanktonic organisms are considered important in the aquatic ecosystems, since they play an important role on circulation of materials and energy flow in the environments.

In this context, the Osamu Utsumi Mine, located in the Plateau region of Poços de Caldas, is the first uranium ore extraction and production mine in Brazil, situated on the premises of the Brazilian Nuclear Industries Ore Treatment Unit (UTM-INB). In this mining area, the uranium deposits occur associated to iron sulfide (FeS_2), which in adequate conditions lead to the occurrence of AMD [6].

In the current study the phytoplankton composition and density, as well as the physical and chemical variables were evaluated in two points located inside the UTM-INB installations (P1 and P2), in addition to a point interfaced with the environment (P3). The results generated in the current study will contribute to the knowledge of the behavior of this community in these ecosystems, once there is a lack of information about environments impacted by uranium mines. Furthermore, such information can be useful in the sense of contributing with actions relating to the decommissioning of this mining area.

2. MATERIALS AND METHODS

In the current study, quarterly collections of water samples were carried out from October 2008 to July 2009, in three points located in the UTM-INB: point P1 ($21^\circ 56' 46''\text{S}$ and $46^\circ 30' 06, 7''\text{WO}$), which receives treated effluents generated by the AMD process; point P2 ($21^\circ 58' 16,60''\text{S}$ and $46^\circ 29' 43,4''\text{WO}$), which corresponds to the Tailings Management Facility and point P3 ($21^\circ 58' 20,6''\text{S}$ and $46^\circ 29' 31,3''\text{WO}$), corresponding to the interface with the environment. Water samples were collected with the aid of a 5 liter-capacity Van Dorn bottle and sent to the Radioecology Laboratory of the Brazilian Nuclear Energy Commission

(LAPOC/CNEN), in order to perform the physical, chemical and biological analyses (chlorophyll *a* and qualitative and quantitative analyses of phytoplankton).

2.1. Physical and Chemical Analyses

In the laboratory, the determination of pH values in the water samples was performed using a selective electrode (Analion). The determinations of total nitrogen and total phosphorus were performed according to the methodology described by [7] and [8], respectively. Silicate was determined according to [9]. The sulfate analyses were conducted by UV-Visible spectrometry according to the method described by ASTM [10]. Fluoride was determined by the potentiometer method with a selective ion electrode [11]. Analyses of uranium and thorium (spectrophotometry with arzenazo II) were performed according to [12].

2.2. Biological Analyses

Chlorophyll *a* concentrations were determined in water samples in accordance with the methodology described by [13]. In order to identify the phytoplankton community, samples were collected using a plankton net of 10 μm pore opening and fixed with 1% acetic lugol. For the identification of phytoplanktonic organisms, a specialized literature [14], [15], [16] and [17] was employed. For a quantitative analysis of the phytoplanktonic organisms, samples were counted according to the methodology described to [18], while the densities were determined according to APHA [19] with an inverted microscope (Zeiss, model Axiovert).

3. RESULTS AND DISCUSSION

The results referring to the physical and chemical variables determined in water samples of the evaluated points are presented on Table 1. The recorded pH values indicate distinct environments which varied from acid - due to AMD at point P1 (pH value around 4) - to basic at point P2 (pH=9.5) where the tailings are deposited after being treated with calcium hydroxide and hydrated lime. The point P3 (pH=7.5) corresponds to the point of release in the environment. While analyzing effluent samples from UTM-INB, [20] also verified similar pH values to those recorded in the current study, that is: P1 and P2 with average values equal to 4.0 and 8.62, respectively and at point P3 with average value of 7.3.

The highest value of total nitrogen was recorded in Jan/09 at point P2 ($2.170 \mu\text{gL}^{-1}$), while the lowest value was detected in samples from point P3 in July/09 ($560 \mu\text{gL}^{-1}$). The lowest value of total phosphorus ($1.35 \mu\text{gL}^{-1}$) was recorded at points P1 and P3 in Jan/09, while the highest value was detected in samples from point P1 in April/09 ($17.95 \mu\text{gL}^{-1}$). The lowest silicate value, that is, 2.30 mg L^{-1} was obtained in samples from point P2 in July/09, while the lowest value was recorded at samples from point P1 and P3 in Oct/08 (0.20 mgL^{-1}). Regarding the sulfate, there were records of the highest value at point P1 (1.832 mgL^{-1}) in Oct/08, while the lowest value was detected in samples from point P3 in July/09 (69.0 mgL^{-1}). Regarding the fluoride, the lowest concentration was recorded at point P3 (1.72 mgL^{-1}), while the most elevated value was found in samples from point P1 (75.1 mgL^{-1}). In a study conducted in acid mining lakes in the region of Lusatia, Germany, [21] recorded close sulfate ($1100\text{-}1800 \text{ mg L}^{-1}$) and total phosphorus values ($<5\text{-}7 \mu\text{gL}^{-1}$) to those detected at point P1, corresponding to the pit mine.

Concerning uranium, the samples from P1 presented the highest concentrations of this element in Oct/08 (4.25 mgL⁻¹). On the other hand, points P2 and P3 presented the lowest concentrations of this element (<0,004 mgL⁻¹). Records of the highest thorium concentration (0,3 mg L⁻¹) were observed at point P1 in April/09, while the lowest concentrations were detected in samples from points P2 and P3 in April/09 as well (<0,002 mgL⁻¹).

Table 1. Values of physical, chemical and biological variables analyzed in water samples from the UTM/INB, Caldas, MG.

Variables	P1				P2				P3			
	Oct/08	Jan/09	Apr/09	Jul/09	Oct/08	Jan/09	Apr/09	Jul/09	Oct/08	Jan/09	Apr/09	Jul/09
pH	4.1	3.7	3.6	3.9	9.50	9.50	9.60	9.83	7.30	7.80	7.90	6.04
TN (µg L ⁻¹)	700	770	840	700	700	2170	910	980	700	700	980	560
TP (µg L ⁻¹)	5.6	1.35	17.95	13.1	3.65	4.05	6.35	9.15	6.15	1.35	3.10	4.65
Si (µg L ⁻¹)	0.2	0.5	1.3	1.2	0.20	1.10	1.05	2.30	0.20	0.50	1.25	0.30
SO ₄ ⁻² (mg L ⁻¹)	1832	1653	1800	366.6	754	391.5	346.1	83.2	508	357.9	378.7	69.1
F ⁻ (mg L ⁻¹)	75.1	33.4	*	*	4.08	*	*	2.04	2.9	*	*	1.72
U (mg L ⁻¹)	4.25	3.17	4.23	0.12	<0.05	*	<0.004	<0.01	<0.05	*	<0.004	<0.01
Th (mg L ⁻¹)	0.1	0.11	0.3	0.1	<0.05	<0.05	<0.002	<0.01	<0.05	<0.05	<0.002	<0.01
Chl a (µg L ⁻¹)	*	9.94	10.75	9.37	1.97	1.23	1.12	3.72	0.0	0.70	1.11	4.19

*Samples not analyzed.

Concerning the phytoplankton community, the number of taxa recorded in samples from P1, P2 and P3 were respectively, 25, 15 and 20. Upon comparing the three points analyzed, the greatest number of taxa (20) was verified in a close to neutrality pH value, that is, in samples from point P3, with pH values ranging from 6.04 to 7.90. In a study conducted by [21] in the coal mining lake ML Felix in the region of Lusatia, Germany, in which pH values ranged from 3.4 to 3.8, these authors recorded the presence of 25 taxa – a much greater number when compared to point P1, where we can observe a similar pH value range (3.6 to 4.1) but only identify 14 taxa.

At points P1, P2 and P3, seven Classes were recorded (Cyanophyceae, Chlorophyceae, Euglenophyceae, Bacillariophyceae, Zygnematophyceae, Dinophyceae Cryptophyceae), with exception to Chlorophyceae, which was not recorded at point P2 (that presented basic pH values). Within the seven Classes registered, the most qualitatively representative ones were: Bacillariophyceae, Cyanophyceae and Chlorophyceae. It was observed that at point P1 the classes Bacillariophyceae (8), Chlorophyceae (7) and Cyanophyceae (4) were the ones who

contributed the most in the species composition of the phytoplankton community. In samples from points P2 and P3, Cyanophyceae stood out with 7 and 6 taxa, respectively (Table 2).

The highest density values were recorded in samples from P1 in Jan/09 (373 ind mL^{-1}), followed by points P3 (377 ind mL^{-1}) in Oct/08 and P2 (221 ind mL^{-1}) in April/09. It was also verified that at point P1 the species density was more heterogeneous, since the Classes Bacillariophyceae (31%), Cyanophyceae (23%), Chlorophyceae (22%) and Cryptophyceae (12%) were predominant in relative density. In samples from P2 the Class Cyanophyceae (73%) was predominant in density while at point P3 the Dinophyceae (64%) was quantitatively predominant (Figure 1).

The results obtained in the current study have shown that Cyanophyceae was the most representative Class in density values in samples from points P1 (236 ind mL^{-1}), P2 (198 ind mL^{-1}) and P3 (250 ind mL^{-1}), independently from the pH values obtained in this study. Organisms belonging to this Class are known for their metabolic plasticity and their capacity to develop in ecosystems of different chemical characteristics, which could justify their occurrence at point P1 (acid medium, with high concentration of stable and radioactive metals) and at points P2 (basic pH) and P3 (neutral pH associated to low metal concentration) [22]. In addition, filamentous cyanobacteria of the genus *Oscillatoria*, *Limnothrix* and *Spurulina* were found present on mining lakes of the region of Lusatia, Germany, with pH value equal to 2.7 [23,24]. On the other hand, [25] concluded that such organisms do not develop in a lower than 4 pH value, thus not corroborating with the results reported by [24]. In the present study, the presence of *Limnothrix sp* was recorded in samples from point P1 in Jan/09 with a pH value around 4, in accordance to the results by [24].

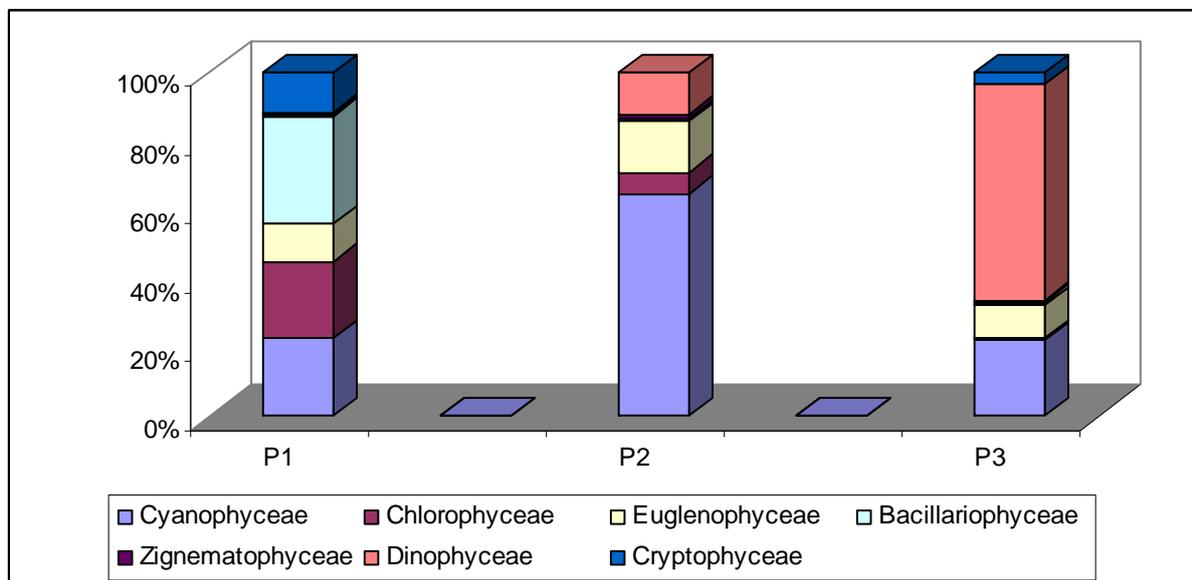


Figure 1. Relative density (indmL^{-1}) of the Phytoplankton Community recorded at points P1, P2 and P3 during the evaluated period.

Table 2. Species Composition of the Phytoplankton Community at points P1, P2 and P3.

Taxons	P1	P2	P3
Cyanophyceae			
<i>Aphanocapsa sp.</i>		X	
<i>Anabaenopsis sp.</i>			X
<i>Células livres de Microcystis sp.</i>	X	X	
<i>Chroococcus minimus</i>	X		
<i>Geitlerinema unigranulatum</i>		X	X
<i>Limnothrix sp</i>	X	X	X
<i>Microcystis sp.</i>	X		X
<i>Oscillatoria cf. splendida</i>		X	
<i>Phormidium spp.</i>		X	
<i>Pseudanabena mucicola</i>			X
<i>Pseudanabaena spp.</i>		X	X
Chlorophyceae			
<i>Botriococcus sp.</i>	X		
<i>Chlamydomonas sp.</i>	X		
<i>Cylindrocystis sp.</i>	X		
<i>Coelastrum microporum</i>			
<i>Coelastrum reticulatum</i>	X		
<i>Desmodesmus sp.</i>	X		
<i>Dictyosphaerium pulchellum</i>	X		
<i>Eudorina sp.</i>			X
<i>Monoraphidium sp.</i>			X
<i>Scenedesmus opoliensis</i>	X		
Euglenophyceae			
<i>Euglena sp.</i>	X		X
<i>Phacus curvicauda</i>			X
<i>Trachelomonas volvocina</i>	X	X	X
<i>Trachelomonas sp.</i>	X	X	X
Bacillariophyceae			
<i>Aulacoseira granulata</i>	X		X
<i>Aulacoseira sp.</i>	X	X	X
<i>Cyclotella sp.</i>	X		
<i>Eunotia sp</i>	X	X	X
<i>Fragilaria spp.</i>	X		
<i>Navicula spp.</i>	X	X	X
<i>Pinnularia sp</i>	X		
<i>Rizosolenia sp.</i>	X		
Zygnematophyceae			
<i>Closterium sp.</i>			X
<i>Cosmarium sp.</i>			
<i>Mougeotia sp.</i>	X	X	X
Dinophyceae			
<i>Peridinium sp.</i>	X	X	X
Cryptophyceae			
<i>Cryptomonas sp.</i>	X	X	X
Total taxa number	25	15	20

4.CONCLUSIONS

After comparing the three sampling points, it was verified that Class Cyanophyceae presented greater tolerance to the chemical conditions of the water such as elevated concentrations of sulfate, fluoride, uranium and thorium, as well as pH variations, since this Class was detected in all sampling points.

ACKNOWLEDGMENTS

The authors would like to thank FAPEMIG - Fundação de Amparo à Pesquisa do Estado de Minas Gerais

REFERENCES

1. A.C. Rebouças, B. Braga, J.G. Tundisi, “*Águas Doces do Brasil: Capital Ecológico. Uso e Conservação*”, 3ed. São Paulo: Escrituras, pp. 748 (2006).
2. J. Harding, I. Boothroyd, Impacts of mining, In: J.S. Harding, P. Mosely, C. Pearson, B. Sorrell (eds.) “*Freshwaters of New Zeland*”, Christchurch: New Zeland Limnological and Hydrological Societies, (2004).
3. G.G. Pyle, S.M. Swanson, D.M. Lehmkuhl, “Toxicity of uranium mine receiving waters to early life stage fathead minnows (*Pimephales promelas*) in laboratory”, *Environ. Pollut*, **Vol. 116** pp. 243-255 (2002). doi 10.1016./S(01)00130-0. PMID:11806452
4. J.M.S. Oliveira, J. Farinha, J.X. Matos, P. Ávila, C. Rosa, M.J.C Machado, “Diagnóstico Ambiental das principais áreas minerais degradadas do país”, *Boletim de Minas*, **Vol. 2** pp.30 (2002).
5. B. Nixdorf, K. Wollmann, R. Deneke, “Ecological Potentials for Planktonic development and food web interactions in extremely acidic mining lakes in Lusatia”. In W. GELLER, H. KLAPPER, W. SALOMONS, (eds) *Acidic mining lakes*, pp.147-167 (1998).
6. D.K. Nordstrom, J.A.T. Smelle, M. Wolf, “*Chemical and isotopic composition of groundwater and their seasonal variability at the Osamu Utsumi mine and Morro do Ferro analogue study sites Poços de Caldas Brazil*” Sweden: Swedish Nuclear Fuel and Waste Management CO, pp.111 (1990).
7. J.C. Valderrama, “The simultaneous analysis of total nitrogen and total phosphorous in natural waters”, *Marine chemistry*, **Vol. 10** pp.109-222 (1981).
8. F.J.H. Mackereth, J. Heron, J.F. Talling, “*Water analysis: some revised methods for limnologists*”, Freshwater Biological Association. Scientific Publication. Kendall: Titus Wilson & Son Ltd, 117p. (1978).
9. H.L. Goltermann, R.S. Clymo, M.A.M. Ohnstad, “*Methods for chemical analysis of freshwater*” Oxford: Blackwell Scientific Publications, pp.213 (1978).
10. AMERICAN PUBLIC HEALTHY ASSOCIATION (APHA). *Standart methods for the examination of water and wastewater*, 18 ed. Washington: American Water Works association and Water Association Federation, (1992).
11. M.R.L. Nascimento, H.T. Fukuma, M.A. Hortellani. Projeto Itataia- Controle de processo produção de ácidos fosfáticos e urânio Poços de Caldas INB, “*Manual de Métodos e Análises Químicas*”, pp.143 (1998).

12. H.T. Fukuma, E.A.N. Fernandes, M.R.L. Nascimento, A.L. Quinelato, "Separation and spectrophotometric determination of thorium contained in uranium concentrates", *Journal of Radioanalytical and Nuclear Chemistry*, **Vol. 249** pp.549-553 (2001).
13. C.J. Lorenzen. "Determination of chlorophyll and phaeopigments: spectrophotometric equations". *Limnol Oceanogr.*, **Vol. 12** pp.343-346 (1967).
14. K. Anagnostidis, J. Komárek, "Modern approach to the classification system of Cyanophytes: Oscillatoriales", *Archiv für Hydrobiologie Supplement*," **Vol. 80**, n.1-4 pp. 327-472 (1988).
15. K. Anagnostidis, J. Komárek, "Modern approach to the classification system of Cyanophytes. 4: Nostocales", *Algological Studies*, **Vol. 80** pp.327-472 (1989).
16. J. Komárek, K. Anagnostidis, *Süßwasserflora Von mitteleuropa band 19/1: Cyanoprocarota 1. Teil: Chroococcales*. Gustav Fisher. Stuttgart (1999).
17. C. V. Hoek, D. G. Mann, H. M. Jahns, *Algae an introduction to Phycology* Cambridge University Press, Cambridge & United Kingdon (1995).
18. H. Utermöhl, "Zur Vervollkommnung der quantitativen Phytoplankton Methodik". *Mitteilungen*", *Internationale Vereinigung fuer Theoretische und Angewandte Limnologie*, **Vol. 9** pp.1-38 (1958).
19. American Public Health Association; American Water Work Association; Water Control Federation APHA, *Standard methods for the examination of water and wastewater*, 19.ed, New York (1995).
20. H.A.Gomes, Jr.O. Garcia, Ocorrência de *Thiobacillus ferroxidans* e *Thiobacillus thiooxidans* em efluentes e botas foras de minas de extração de Urânio. In: V ENAN 2000. Rio de Janeiro: Proceedings of V ENAN (2000).
21. K. Wollmann, R. Deneke, B. Nixdorf, G. Packroff, "Dynamic of planktonic food webs in thre mining lakes across a pH gradient (pH 2-4)", *Hydrobiologia*, **Vol. 433** pp. 3-14 (2000).
22. E. Shubert. "*Algae as Ecological Indicators*", London: Academic Press, London (1984).
23. C.E.W. Steinberg, H.Schaefer, W.Beisker, "Do acidtolerant cyanobacterial exist?", *Acta Hydrochim. Hidrobiol.*, **Vol. 26** pp. 13-19 (1998a).
24. C.E.W. Steinberg, H. Schaefer, J. Tittel, W. Beisker, Phytoplankton composition and biomass spectra created by flow cytometry and zooplankton composition in mining lakes of different states of acidification. In: W. Geler, H. Klapper, W. Salomons (eds). "*Acidic Mining Lakes*", Berlin: Springer, pp. 127-145 (1998b).
25. B.A. Whitton, Soils and rice-fields. In: B.A. Whitton, M. Potts (eds). "*The Ecology of Cyanobacteria*", Dordrecht: Kluwer Academic Publishers, pp.233-255 (2000).