# Microbial communities in an oligotrophic reservoir influenced by high levels of ionizing radiation

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**Abstract.** In this study, we investigated the microbial community (bacteria and protozooplankton) of a Brazilian reservoir that receives neutralized acid mine drainage flowing from piles of earth produced during uranium extraction. Thus, this research represents an attempt to fill a gap in the information on the ecology of freshwater microorganisms in tropical oligotrophic habitats, in particular in systems affected by high levels of the uranium. During the study, we observed very low chlorophyll *a* contents and protozooplankton cell densities and biomass. These cell densities were between 0 and 89 cells $L^{-1}$ and were lower than those frequently reported for oligotrophic freshwater lakes. In contrast, bacterial densities were normal or even slightly high, between 1.85 to $6.0 \times 10^9$ cells $L^{-1}$. The present study has demonstrated very low protozooplankton cell densities and biomass in oligotrophic reservoir under the influence of acidic mining effluents and of high levels of ionizing radiation. It is likely that the ciliate cell densities and biomasses recorded in this study, which fall significantly below the previously published values, can be explained by the chronic fractionated exposure of these protozoa to the high levels of uranium to this system.

## 1. INTRODUCTION

Bacteria and protozoa are components of the microbial food web of the plankton and benthos in freshwater habitats. The bacteria are recognized as important decomposers and remineralizers of inorganic nutrients in aquatic ecosystems, as well as channeling carbon and energy, acquired from the dissolved organic matter excreted by algae, to organisms at higher trophic levels in the food chain [16, 17]. Few experimental or descriptive studies have been published on microbial food webs in oligotrophic and ultraoligotrophic temperate lakes, compared to their eutrophic and mesotrophic counterparts [18, 19]. According to Laybourn-Parry [19], the oligotrophic systems are harder to study, since they contain highly-stressed organisms at low densities. The Antas Reservoir (Minas Gerais state, Brazil), oligotrophic system, is located in a tropical region of raised background radioactivity (due to local uranium and thorium anomalies) and receives the neutralized acid leach solution draining from waste heaps generated by uranium mining. This study was a season-by-season analysis of the abundance and biomass of ciliated protozoa among the plankton of Antas Reservoir and their interactions with other components of the microbial food web (density and biomass of the dinoflagellate *Peridinium* sp., density of bacterial plankton and biomass of phytoplankton) and with the physical and chemical characteristics of the water. Thus, this research represents an attempt to fill a gap in the information on the ecology of freshwater

microorganisms in tropical oligotrophic habitats, in particular in systems affected by high levels of the uranium.

## 2. MATERIALS AND METHODS

Antas Reservoir, is in the Brazilian state of Minas Gerais, at 1291 m above sea level, in a region that has dry, cool winters (May to August) and hot, wet summers (October to March). Samples were taken from the surface at three sites on the reservoir (A1, A2 and A3-S) and from the bottom at one of these sites (A3-B; depth 6.5 m), at intervals of about 3 months: January, April, July and October 2006 and February 2007. Water was collected for all tests physical, chemical and biological (density and biomass of Protozoa ciliates and the dinoflagellate *Peridinium* sp. and bacteria density). Water transparency was measured with a Secchi disk. The pH values were measured using pH meter (model DM-21, Digimed) and electrical conductivity values were measured using electrical conductivity meter (model DM-31, Digimed) with selective electrode. Total suspended solids were analyzed by a method described by [14]. Water hardness ($Ca^{2+}$ and $Mg^{2+}$), manganese and zinc were with a Liberty RL sequential ICP-OES (Liberty RL Varian, USA). Fluoride was estimated potentiometrically with an ion-selective electrode [12]. Sulfate was analyzed by UV-Vis spectrophotometry, as described in ASTM [2]. To analyze uranium, was measured by absorbance at 650 nm in a UV-Vis spectrophotometer [7]. Thorium was determined by a method similar to that used for uranium, according Fukuma et al. [7]. The chemical data collected on the water sampled from the reservoir were compared with the limits adopted in the Brazilian guidelines (CONAMA Resolution 357) [4].

Ciliated protozoa and dinoflagellate *Peridinium sp.* were fixed water samples in the laboratory and specimens counted in Sedgwick-Rafter chambers under an optical microscope (Orthoplan, Ernest Leitz Wetzlar, Germany). The ciliated protozoa and the dinoflagellates were identified, counted and their densities recorded. In addition, linear measurements were made on about 20 specimens of each morphological type, in order to calculate the total biovolume of each type at each site and collection time, using the mathematical formulae most suited to the geometrical shape of the organism [14]. To estimate the biomass of the ciliates and dinoflagellate, the biovolumes were multiplied by the carbon conversion factors estimated by [13] and [3], respectively. The ciliated protozoa and dinoflagellates observed in the samples were grouped into nutritional types with a view to classifying them into trophic levels, as defined by [6] and [10], respectively. Aliquots of 1 mL of each fixed sample were stained with an aqueous solution of acridine orange [5]. After 5 minutes of staining, the sample was filtered on to a black polycarbonate membrane of pore size 0.22 $\mu$m (Millipore) [8] and examined with a Zeiss-Axioplan 2 epifluorescence microscope (Zeiss, Germany). To calculate bacterial density, 300 microscope fields were counted on each membrane, at a magnification of 1000×. Chlorophyll *a* content in the 5 L water samples was determined as recommended by Marker et al. Its concentration in the extract was calculated from spectrophotometer readings by the equations provided by [11]. To estimate the biomass of the phytoplankton, the chlorophyll *a* content (w/v) was multiplied by a carbon conversion factor of 67 [1]. An analysis of variance (ANOVA) was used to detect significant differences between water samples from different sites and collection times, in the results of the physical, chemical and biological analyses. The Principal Component Analysis (PCA) was applied in order to compare the distribution of environmental parameters, that is, physical and chemical variables as well as the biological variable chlorophyll *a* on sites A1, A2, A3-S and A3-B, located in the Antas Reservoir. The Canonical Correlation Analysis (CCA) was applied in order verify the existence of a correlation between the environmental parameters and density and biomass of the microbial communities. In order to verify the significance level of environmental parameters as well as density and biomass, the Monte Carlo test was applied with 999 random permutations, with p value lower or equal to 0,5 ($p \leq 0,5$). The Principal Component Analysis (PCA) as well as the Canonical Correlation Analysis (CCA) were performed with the aid of the software CANOCO 3.12 [9].
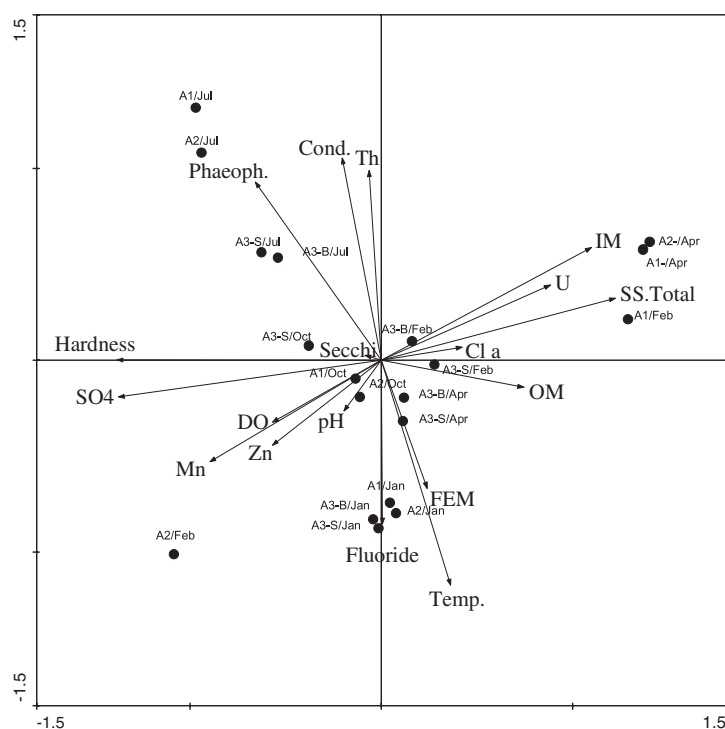
**Figure 1.** PCA ordination diagram of the A1, A2, A3-S and A3-B sites (Antas reservoir) in January, April, July and October 2006 and February 2007. SO4 = sulfate, Fluoride, Cond. = electrical conductivity, hardness = water hardness, U = uranium, Th = thorium, DO = dissolved oxygen, Temp = water temperature, pH = hydrogenionic potential, Cla = Chlorophyll a, Phaeophytin = Phaeoph.., IM = inorganic matter, OM = organicmatter, SS. Total = total suspended solids, Secchi = water transparency, FEM = oxidation reduction potential, Zn = Zinc and Mn = manganese.

## 3. RESULTS

Firstly, regarding the seasonal variations, it was noted that during the rainy season (February samples), the lowest mean values of pH and highest suspended solid contents were recorded. In fact, the February pH at all the sites, except A2, fell below the lower limit permitted by the regulatory body (CONAMA, 2005) in its Resolution 357. On the other hand, in the cool, dry season (July), the lowest mean suspended solid content and highest mean electrical conductivity were observed. The latter ($411\,\mu S.cm^{-1}$) was significantly higher ($P < 0.05$) than the conductivities measured in October ($192\,\mu S.cm^{-1}$) and January ($144\,\mu S\,cm^{-1}$). The water collected from site A2 was significantly harder ($P < 0.05$) than that from the site A1.

When the chemical variables under study (concentrations of $F^-$, $SO_4^{-2}$, Mn, Zn and U) were compared among collection sites, A2 stood out in terms of the number of maximum values observed there. Fluoride levels exceeded the limits set by Conama in Resolution 357 (CONAMA, 2005) in all samples except those from A1 and A2 in April and from A1 in February, and sulfate concentrations were also found to be above the Conama limit ($250\,mg\,L^{-1}$) at site A2, in July ($278.7\,mg\,L^{-1}$) and February ($412.3\,mg\,L^{-1}$). All the manganese concentrations sufficiently high to be detected by the method used in this study were, in fact, above the Conama limit ($0.1\,mg\,L^{-1}$) for this metal (CONAMA, 2005). Among these measurable values, the mean concentration at A2 ($5.13\,mg\,L^{-1}$) was significantly
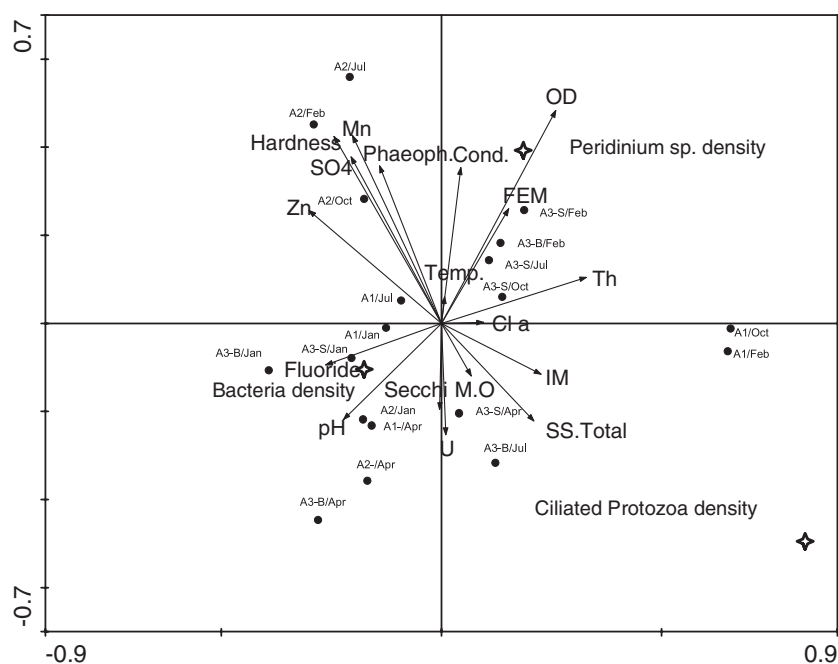
**Figure 2.** CCA ordination diagram of the Ciliated Protozoa density, Bacteria density and the dinoflagellate *Peridinium* sp. density at the A1, A2, A3-S and A3-B sites (Antas reservoir) in January, April, July and October 2006 and February 2007. SO4 = sulfate, Fluoride, Cond. = electrical conductivity, hardness = water hardness, U = uranium, Th = thorium, DO = dissolved oxygen, Temp. = water temperature, pH = hydrogenionic potential, Cla = Chlorophyll a, Phaeoph = Phaeophytin, IM = inorganic matter, OM = organic matter, SS.Total = total suspended solids, Secchi = water transparency, FEM = oxidation reduction potential, Zn = Zinc and Mn = manganese.

higher ($P < 0.05$) than those at A3-S ($1.70\,\mathrm{mg\,L^{-1}}$) and A1 ($0.76\,\mathrm{mg\,L^{-1}}$). Zinc levels in the water taken from site A2, in February ($2.03\,\mathrm{mg\,L^{-1}}$) and July ($0.21\,\mathrm{mg\,L^{-1}}$), were also above the limit ($0.18\,\mathrm{mg\,L^{-1}}$) stipulated in Resolution 357 (CONAMA, 2005). Finally, the uranium concentrations recorded in April, at A1 and A2 ($0.11\,\mathrm{mg\,L^{-1}}$), and in October, at A1 ($0.08\,\mathrm{mg\,L^{-1}}$), A2 and A3-S ($0.06\,\mathrm{mg\,L^{-1}}$), were above the reference limit published in the Brazilian law based on Resolution 357 ($0.02\,\mathrm{mg\,L^{-1}}$) (figure 1). No limits are as yet defined in Brazilian Law for thorium concentration in freshwater, but the highest levels were detected in January at A1 ($0.03\,\mathrm{mg\,L^{-1}}$) and in July at A3-F ($0.06\,\mathrm{mg\,L^{-1}}$).

The plankton bacteria counted in the water samples from Antas Reservoir exhibited the following mean densities (in units of $10^9\,\mathrm{ind\,L^{-1}}$): 2.90 in January, 6.00 in April, 6.00 in July, 5.74 in October and 1.85 in February. Regarding the densities of ciliates and dinoflagellates, the mean value for ciliated protozoa varied from $7.5\,\mathrm{ind\,L^{-1}}$ in January to $37.3\,\mathrm{ind\,L^{-1}}$ in October, while the number of *Peridinium* dinoflagellates ranged from $2{,}437\,\mathrm{ind\,L^{-1}}$ in April to $44{,}197\,\mathrm{ind\,L^{-1}}$ in October. The average biomasses observed ranged from $0.01\,\mu\mathrm{g\,C\,L^{-1}}$ (April) to $0.05\,\mu\mathrm{g\,C\,L^{-1}}$ (October) for the ciliates and from $0.18\,\mu\mathrm{g\,C\,L^{-1}}$ (April) to $3.26\,\mu\mathrm{g\,C\,L^{-1}}$ (February) for the dinoflagellates.

Turning now to the distribution of these microorganisms along the reservoir, the highest ciliate densities were found in two of the five field collections, in water collected from the head of the reservoir (A1) in October ($87.0\,\mathrm{ind\,L^{-1}}$) and February ($89\,\mathrm{ind\,L^{-1}}$), while the lowest were recorded in three collections, in water from A2 (near the mine affluent) in July ($3.0\,\mathrm{ind\,L^{-1}}$), October ($6.0\,\mathrm{ind\,L^{-1}}$) and February ($3.0\,\mathrm{ind\,L^{-1}}$). On the other hand, the highest densities of plankton bacteria were seen in water
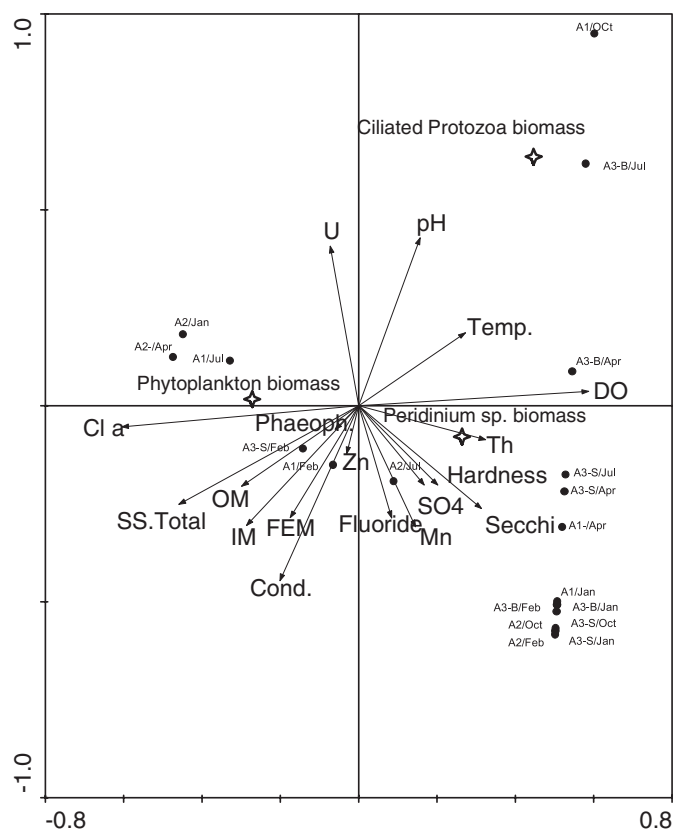
**Figure 3.** CCA ordination diagram of the ciliated protozoa biomass, phytoplankton biomass and the dinoflagellate *Peridinium* sp. biomass at the A1, A2, A3-S and A3-B sites (Antas reservoir) in January, April, July and October 2006 and February 2007. SO4 = sulfate, Fluoride, Cond. = electrical conductivity, hardness = water hardness, U = uranium, Th = thorium, DO = dissolved oxygen, Temp = water temperature, pH = hydrogenionic potential, Cla = Chlorophyll a, Phaeophytin = Phaeoph., IM = inorganic matter, OM = organic matter, SS.Total = total suspended solids, Secchi = water transparency, FEM = oxidation reduction potential, Zn = Zinc and Mn = manganese.

taken from A2 in April ($8 \times 10^9$ ind L$^{-1}$) and October ($9 \times 10^9$ ind L$^{-1}$), while the lowest were recorded at A3-S and A3-F ($1.1 \times 10^9$ ind L$^{-1}$), in February. Finally, the highest densities of *Peridinium* sp. were found in water from A1 in October (79,380 ind L$^{-1}$) and February (58,510 ind L$^{-1}$), while the lowest was observed in the sample taken from site A3-F in April (690 ind L$^{-1}$) (figure 2).

The two highest biomasses of ciliated protozoa were recorded in water samples taken from the head of the reservoir (A1) in October (1.21 $\mu$g C L$^{-1}$) and from site A3-S in July (0.075 $\mu$g C L$^{-1}$), while the lowest ciliate biomasses were found in water collected in January, from the sites A3-F (0.001 $\mu$g C L$^{-1}$) and A3-S (0.003 $\mu$g C L$^{-1}$). The two largest *Peridinium* sp. biomasses were observed in water from A3-S in February (6.53 $\mu$g C L$^{-1}$) and from A1 in October (6.08 $\mu$g C L$^{-1}$) and two smallest masses for this dinoflagellate occurred in the water collected in April, at A3-F (0.04 $\mu$g C L$^{-1}$) and A2 (0.10 $\mu$g C L$^{-1}$). The mean phytoplankton biomass (over time) at the site A2 was significantly greater ($P < 0.05$) than in samples collected at the other sites. The two largest phytoplankton biomass values were recorded in water from site A3-S in February (33.66 $\mu$g C L$^{-1}$) and from A2 in January (20.03$\mu$g C L$^{-1}$) (figure 3).

## 4. CONCLUSIONS

The present study has demonstrated very low protozooplankton cell densities and biomass in oligotrophic reservoir under the influence of acidic mining effluents and of high levels of ionizing radiation. It is likely that the ciliate cell densities and biomasses recorded in this study, which fall significantly below the previously published values, can be explained by the chronic fractionated exposure of these protozoa to the high levels of uranium to this system.

   In addition, the microbial communities in the Antas Reservoir were influenced by another chemical stress effect related to the release of treated radioactive effluents from the uranium mine. Among the studied groups (ciliated protozoa biomass and density, bacterioplankton density, *Peridinium* sp biomass and density), the protozooplankton was the most sensitive to the synergistic effect due to fractionated chronic exposition to uranium and the release of radioactive effluents treated by uranium milling.

### Acknowledgments

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