

TESTATE AMOEBAE AS BIO-INDICATORS OF CONTAMINATION BY TRACE ELEMENTS IN THE RESERVOIR OF SALTO GRANDE AMERICANA-SP, BRAZIL

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Abstract

This work aims to evaluate the degree of sediment contamination and the response of Testate amoebae populations through a sediment core taken from the Salto Grande Reservoir (RSG), situated in Americana, in São Paulo State (SP), Brazil. It is based on the analysis of the trace elements geochemistry (by ICP-OES) and Testate amoebae data (by stereomicroscopic morphometric analysis). The indices of homogeneity and richness (Simpson 1-D and Margalef) were calculated. Sediment contamination was evaluated according to VGQS (TEL and PEL). The results evidenced that the sediments of Facies 3 are highly contaminated since the beginning of RSG filling. Contamination caused significant decline of Testate

amoebae populations dimension and diversity. In sedimentary facies with lower concentrations of trace elements diversity and size of Testate amoebae populations are higher and are composed of different species. The results of this work indicate that Testate amoebae populations showed differentiated responses to different environmental conditions. So, these amoeboid protists can be considered good indicators of pollution caused by trace elements in fresh water aquatic environments.

Keywords: Trace Elements. Testate amoebae. Bio-Indicators. Pollutants. Reservoir.

1. Introduction

The Reservoir of Salto Grande (RSG) is located in the municipality of Americana, São Paulo State, Southeastern of Brazil (Fig. 1). It was built to provide electric energy supply to industries of the region and to develop local economy (Espíndola et al., 2004). It began to operate in 1950 and today its electricity production has ceased due to the use of part of the water of the Atibaia watershed, where it is located, to supply the city of São Paulo. The Atibaia River is the main contributor to the reservoir. The RSG receives domestic and industrial sewage of 20 cities, in the highly industrialized Campinas Metropolitan Region, including for instance petrochemical, textile, fertilizer, pharmaceutical and food industries. Also, agriculture (sugar cane and fruits plantation, livestock) is very expressive on land close to the dam and along the watershed of the Atibaia River (Espíndola et al., 2004; Fonseca and Matias, 2014). So, since the 50's the RSG experienced an input of heavy polluted water that accompanied the growth of the Campinas Metropolitan Region.

1.1 Testate amoebae

The thecamoebians can be defined as a paraphyletic group of amoeboid protozoa, endowed with shells, placed in the Super Group Amoebozoa, Group Tubilinea, Sub-Group Testate, Family Arcellenidea (Adl et al., 2005).

Thecamoebians are known in the fossil record since 800 million years, over a long period of Earth's History (Porter et al., 2003). Its successful evolutionary history should be due to the alternation of generations, sexual reproduction alternated with asexual one. This type of life cycle is well known in some species such as *Arcella vulgaris*, *Trinemalineare*, *Corythiondubium*, *Centropyxis orbicularis*, *Corythion delamarei* (Raikov et al., 1989; Mignot and Raikov, 1992; Yudina and Sukhanova, 2000).

The Testate amoebae are protected by unilocular test (Medioli and Scott, 1983; Pchelin, 2010). Tests have the function of providing mechanical protection and resistance against predation and adverse environmental conditions (Booth, 2001).

Thecamoebians are found in different latitudes and environments and therefore they are considered cosmopolitan (Pénard, 1902; Medioli and Scott, 1983; Finlay et al., 1996; Cruz, 2004). Pénard (1902) and Decoître (1953) proposed several dispersal mechanisms, such as birds, aerosol events and river currents. Their tests can be classified as autogeneic or xenogeneic. The former tests are secreted by the organism itself and are smooth and proteinic, and may be endowed with siliceous or, rarely, calcareous plaques. These thecamoebians are hardly preserved in the fossiliferous record.

Xenogeneic tests are formed by agglutination of particles (e.g., diatom frustules, quartz grains and other silicate mineral particles) on autogeneic cement. Unlike the autogeneic, they are resistant to low pH conditions, being often fossilized (Medioli and Scott 1988; Török, 2001; Gomes e Souza, 2008).

Thecamoebians have different sizes (10 μm to 900 μm) and shapes. They are mostly classified by their test morphology. According to Gomes and Souza (2008), Testate amoebae can present variations in their shape and size, depending on the environment and/or biotic and/or abiotic stress where they live. Many works on the ecology of the Thecamoebians mention several correlations of these organisms with their habitat. And they point to different interactions relating morphology, size, abundance and richness of the species with the environment (Lansac-Tôha et al., 1999; Duleba et al., 2004; Ferreira et al., 2006; Alves et al., 2007).

Testate amoebae have been used as bio-indicators of polluted sediments in rivers and lakes worldwide (Dias-Britto et al., 1988; Eichler and Bonetti, 1995; Patterson et al., 1996; Duleba, 1999; Oliveira 1999; Patterson and Kumar, 2000 a, b; Booth 2001; Onianwa, 2001; Duleba et al., 2005; Xu et al. 2005; Nguyen-Vieta et al., 2008; Meyer et al., 2012).

Currently due to concerns about the impacts of climate change, especially about water conditions, the use of ecological indicators as an instrument to be applied in water quality monitoring programs becomes urgent.

1.2 Trace elements

Trace elements are serious contaminants of sediments and, in some cases, can be mobilized to the water column becoming a threat to the biota (Esteves, 2011). Once they have accumulative effect in the food chain their effects will eventually reach humans, through consumption of fish and other animals (Guilherme et al., 2005; Esteves, 2011).

Several features may influence trace elements mobilization, which vary from biotic, such as natural chelator substances, to abiotic factors such as synthetic chelators substances, pH variations and degree of eutrophication (Jenne, 1968; Zitko and Carson, 1972; Chau and Shiomi, 1972; Lockwood and Chen, 1973).

1.1 The main goals

In this study, the variations of Thecamoebians populations were related to the variations of trace elements concentrations (As, Cr, Cu, Fe, Mn, Ni, Pb, Sc and Zn; Sc was used to normalize the elements concentrations) and sediments grain size, aiming to test the hypothesis of these organism's populations and communities are bioindicators of pollution.



Fig. 1. Sampling point (Red Star) in the Reservoir of Salto.

2. Geological setting of the study area

The Reservoir of Salto Grande (RSG) is located in the municipality of Americana, São Paulo State, Southeastern of Brazil (22°44' S and 47°19' W; Fig. 1). The RSG is located in the São Paulo peripheral depression in the middle Tietê area and is part of the Piracicaba, Capivari and Jundiaí river basin, formed by the damming of the Atibaia River. The Atibaia River, the main tributary of the reservoir, is born from the confluence of the Atibainha and Cachoeira rivers, both sources of the Greater São Paulo Metropolitan Region and are the main constituents of the Cantareira System.

The Atibaia River runs a length of 154 km until its impoundment, attending 20 municipalities (Fig. 1). Along its course, the river is influenced by twelve tributaries. At the head of the Atibaia River, starting from the municipality of Nazaré Paulista, the Atibaia river intersects metamorphic geological units associated to deformed sequences of the upper Neoproterozoic. The lithology comprises ortho and

para-derived gneisses and migmatites, belonging to the Vargínia-Guaxupé complex, in addition to intrusive granites of several ages.

The Atibaia River runs a stretch of approximately 133 km (77% of its extension to the dam) in crystalline units of the Precambrian basement. At the municipality of Campinas (near the dam), this river reaches the Paleozoic sedimentary units of the Paraná Basin, represented by sandstones and shales of the Itararé Group and basalts of the Serra Geral Formation. From this point, the river runs approximately 40 km until reaching the dam, at the municipality of Americana (Fonseca and Matias, 2014).

3. Material and methods

A sediment core of 235 cm was taken from the RSG (22°43'29.4"S and 47°13'51.3"W) at a point shown in Figure 1, approximately 1300 m from the west bank and 900 m from

the east bank and 14 m deep. The core was collected during the dry season on May 27, 2015, by means of witness of gravity, following the sampling technique of SubGeo - Commerce of Nautical Equipment and Submarine Technical Services. In this technique, the core collection is performed from the percussion of a weight which is thrown against a transverse rod attached to the sampler tube. The progressive elevation of this weight as well as the removal of the toss was controlled by divers. The transport of the core was carried out with extreme care to avoid contamination of the lower layers by elements of the upper layers that could percolate between the sediments. The core was taken to the laboratory of the Instituto Oceanográfico of Universidade of São Paulo (IO-USP) where it was open and sliced; the first slice, beginning at the depth of 8 cm until 16 cm, the first centimeters were sliced every 8 cm due to fluidity presented by sediments, after 16 cm it was sliced every 4 cm until 24 cm and in sequence, to the base of the core, it was sliced every 2 cm.

The slices were put in plastic vials and taken to the refrigerator (4°C) until lyophilizing. The vials were previously washed with "extran" (MERK), rinsed in osmolized water and then submerged into HNO₃ 10% solution for 24 hours and dried.

Chemical analysis to detect trace elements were carried out at the Laboratório de Química Inorgânica Marinha (LaQI Mar), from IO-USP. A partial digestion of the sediments was carried out following the protocol 3050B (USEPA, 1996). To quantify trace elements a ICP-OES model MPX 710-ES from VARIAN was used according to the method SW-846 USEPA 6010c (USEPA, 2000). Concentrations levels of As, Cr, Cu, Fe, Mn, Ni, Pb and Zn were analyzed. Concentrations were calculated according to the formula (Skoog et al., 1998):

$$[E] = \frac{Vp \times Fd \times Vf}{m}$$

Where:

[E]: Trace element concentration mg/kg

Vp: Value obtained by the plasma, mg/l

Fd: Dilution factor

Vf: Final volume, ml

m: mass, g

Each sample was measured 3 times and the values correspond to the mean of the measures.

Granulometric analyses were carried out at the Laboratório de Sedimentologia, of Instituto de Geociências, of Universidade de São Paulo (IGC-USP). The equipment used was a Mastersizer 2000 from Malvern. The degree of sediments selection was classified according to Folk and Ward (1957). The methodology initially consists of preparing the sample: macerate and homogenize the sediment and then

remove organic material and carbonates by attack with hydrogen peroxide. The sample is then oven dried and homogenized. An aliquot of 2 grams of the dry sediment in 1 liter of deionized water under constant stirring and 5 drops of dispersant (sodium pyrophosphate) are added, keeping the system under ultrasound to disaggregate the particles which are directed to a reader of laser diffraction. The data obtained by Malvern are sent to a program that expresses the measured values graphically, using the percentages of different granulometric fractions.

In order to evaluate the anthropic intervention in the RSG system, demographic growth data were compared with the concentrations of trace elements. Population growth data were obtained through published IBGE-2014 reports

Testate amoebae species were identified using a Zeiss stereomicroscope with a 3.5 Mb digital camera. Photos were taken in order to enable measurements, when necessary. As the communities of Testate amoebae represent thanatocoenoses, the only way to taxonomically identify a species is through its morphology, once no DNA or RNA molecular information is available. The organism's identification was based on the morphology of Testate amoebae and using as reference the work of several authors (Asioli et al., 1996; Lahr, 2006; Gomes e Souza, 2008). Although this is not the most ideal scenario to detect the different species, information obtained through thanatocoenoses, in turn, allows the detection of biotic and abiotic variations on a broader temporal scale (Duleba et al., 2004).

Density was calculated using the number of tests per 10cc. Simpson 1-D and Margalef D_{Mg} were also determined in order to infer the homogeneity of Testate amoebae communities and to access the species richness, respectively, and were used as diversity indices.

D_{Mg} was calculated according to the following formula:

$$D_{Mg} = \frac{(S-1)}{\ln N}$$

Where,

D_{Mg}=Species richness

S= Number of sampled species

N= Total number of individuals in a population.

D was calculated according to the formula:

$$D = \sum \left(\frac{n_i [n_i - 1]}{N [N - 1]} \right)$$

Where,

n_i= number of specimens in the i_{th} species.

N= Total number of specimens.

Simpson index is thus 1-D in order to facilitate the interpretation of data. The highest values of this index are indicative of more homogeneous communities (Magurran, 2004).

In order to evaluate the degree of sediments contamination the sediment quality guide values adopted by the Brazilian legislation, CONAMA n° 454 of November 1st 2012 to dredge sediments (CONAMA, 2012), which is based on the Canadian classification of pollutants (TEL and PEL) was used (Canadian Environmental Quality Guidelines-CCME, Summary Table; <http://st-ts.ccme.ca/en/index.html>), so TEL values (Threshold Effect Level) contaminant levels under which no harm is expected to the biota and PEL (Probable Effect Level) contaminant levels in which it is expected to occur adverse biological effects.

3.1 Statistical analyses

Principal Correspondence Analysis (PCoA) and Pearson correlation (r) from the main component analysis (PCoA) were used to identify patterns of association between the analyzed variables (granulometry, trace elements and Testate amoebae species). Also, Non - Metric Multidimensional Scaling (NMDS) was performed based on diversity, richness and density indices, allowing the use of any type of scale between the analyzed variables and the species of Testate amoebae. When the obtained stress values are <0.05 , they indicate very low incompatibility between the classification order of data distance. But values >0.3 indicate an arbitrary ordering which makes very difficult the results interpretation. Both PCoA and NMDS are ordination analyzes that allow the exploration of environmental variables in a gradient searching that can explain the distribution of the species along the gradient.

The PAST 3.12 program (Hammer et al., 2001) was used to perform the Grouping, PCoA and NMDS analysis. Regarding NMDS, we chose to use the Gower method, as the variables are on different scales.

4. Results

Grain size curves of sand, silt and clay fractions are shown in Figure 2. Sand, silt and clay fraction represent, in average, 3.5%, 71.2% and 25.3% respectively, in the upper 160 cm of the sediment core as shown in Figure 2.

The results of trace elements concentrations measured in the sediments along the studied core are presented in Table 1 and plotted in Figure 3. Concentrations varied for: As between 2.97 - 12.16 mg/kg; Cr between 16.5 - 90.8 mg/kg; Cu between 38 - 522 mg/kg; Fe between 49 - 71910 mg/kg; Mn between 52.6 - 1200 mg/kg; Ni between 6.50-35.72 mg/kg; Pb between 11.78-38.51 mg/kg and; Zn between 35 - 164 mg/kg.

Facies 3 (154 - 94 cm depth) was the most representative in terms of contamination since it keeps the highest concentration values of all analyzed elements: As- 12 mg/kg; Cr - 90 mg/kg; Cu - 522 mg/kg; Fe - 64 g/kg; Mn - 1200 mg/kg; Ni - 36mg/kg; Pb - 38mg/kg and; Zn - 164 mg/kg.

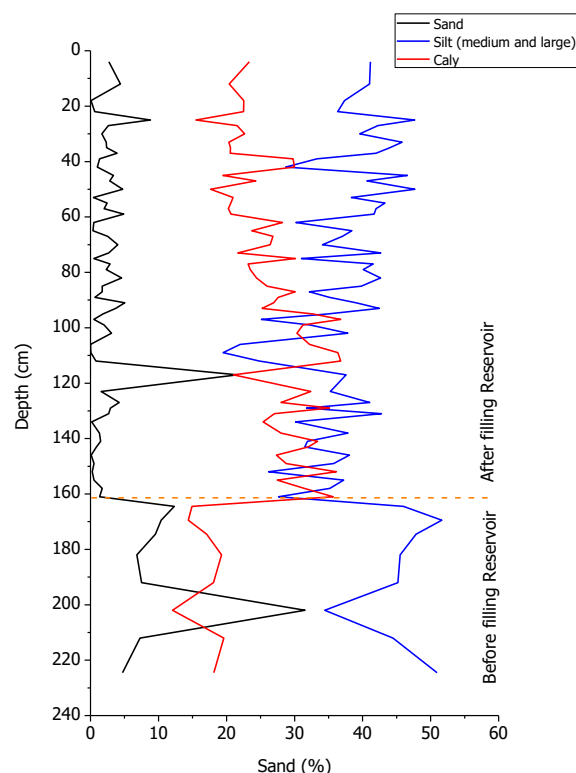


Fig. 2. Percentage of silt, clay and sand in contrast to depth of sediments in the core. Dotted traced line represent the moment at which the RSG was filled.

The relations of trace elements and grain size are shown in PCoA analysis (Appendix 1), where the first axis explains 95.86% of the variance of the majority of trace elements and clay and sand fractions is mainly related to coordinate 2.

Pearson correlations (r) between grain size and trace elements are shown in Table 2. The majority of trace elements have high correlation with clay fraction. Arsenic, Cr, Cu and Pb presents the highest correlation with clay fraction and; Zn has the highest correlation with sand fraction; only Mn shows high correlation with silt.

In Table 4, colored Facies indicate changes in Testate amoebae community composition. Figure 6 correlates the density, richness and abundance of Testate amoebae populations with textural characteristics of different sedimentary facies of the core.

Facies 1 (230-177 cm deep): gradual transition to clay levels well consolidated with light grey color with varied texture formed by spots of ocher color followed by a mottled aspect. Facies 1 is characterized by the total absence of thecamoebians.

Tab. 1. Trace elements concentrations (mg/kg) and TOC percentage (%) along the studied core. Values in red indicate concentrations above PEL and TEL.

Depth (cm)	As mg/Kg	Cd mg/Kg	Cr mg/Kg	Cu mg/Kg	Ni mg/Kg	Pb mg/Kg	Zn mg/Kg	Fe mg/Kg	Mn mg/Kg	TOC %
8	4.3	0.4	16.6	38.7	6.6	11.8	57.1	55299.0	690.3	3.4
16	3.9	0.4	21.9	53.6	12.6	14.4	82.6	63590.0	825.1	2.7
20	4.0	0.5	25.7	58.2	13.9	16.1	112.5	60001.0	920.6	2.0
24	3.3	0.5	25.5	54.1	13.2	16.6	107.9	58861.0	760.7	2.2
26	3.3	0.4	16.9	48.5	8.0	12.6	81.9	59096.0	867.0	3.2
28	4.2	0.4	20.4	44.2	11.4	14.0	89.5	57426.0	768.8	2.6
32	4.3	0.5	25.2	53.2	14.9	16.3	92.1	54782.0	968.0	2.6
34	3.4	0.3	18.3	38.1	8.5	13.0	64.3	49259.0	691.7	2.0
36	4.3	0.4	23.4	47.7	12.0	14.1	79.7	57808.0	771.0	2.5
38	4.7	0.6	28.5	56.6	16.3	15.6	87.9	56630.0	939.5	3.3
40	4.2	0.5	29.0	51.7	14.2	16.0	85.5	63499.0	799.7	1.5
44	3.9	0.5	26.9	48.4	12.5	15.5	95.9	58040.0	867.5	2.3
46	3.8	0.4	22.8	42.9	9.4	13.4	90.1	57677.0	851.6	2.6
48	4.9	0.6	30.6	60.2	15.9	17.3	111.4	56495.0	891.2	1.7
52	5.1	0.6	25.7	59.0	14.8	16.3	108.5	55506.0	882.1	2.5
54	3.0	0.5	20.0	39.7	8.3	14.0	72.2	62516.0	1002.7	2.1
56	3.7	3.2	21.0	47.2	11.6	13.8	82.7	57974.0	869.2	1.8
58	5.6	0.6	28.7	59.0	17.6	16.7	104.3	57038.0	888.7	2.6
60	3.8	0.6	22.3	46.0	13.4	15.1	85.6	56699.0	887.3	2.3
64	4.9	0.5	26.5	48.8	13.3	16.3	90.9	58963.0	1176.9	2.3
66	3.3	0.4	18.5	39.1	7.6	13.3	66.1	57380.0	928.1	2.1
68	6.5	0.7	27.4	62.5	15.5	17.5	105.4	62707.0	972.8	2.5
72	5.7	2.3	24.8	51.7	11.6	16.3	77.7	59857.0	1127.0	3.6
74	4.7	0.4	19.4	39.8	8.7	13.6	60.9	54578.0	809.5	2.4
76	4.1	2.7	21.7	43.1	9.6	15.8	69.4	55151.0	1135.7	1.7
78	4.9	2.3	20.3	47.3	11.0	15.5	67.5	55433.0	1103.4	2.6
80	5.5	0.5	27.3	49.5	16.3	18.5	80.6	58978.0	851.8	2.9
84	5.5	0.7	29.2	59.2	19.1	20.4	94.7	71910.0	1110.7	1.9
86	4.7	2.1	22.0	50.9	12.2	18.4	70.4	58035.0	843.5	2.8
88	5.5	0.6	25.7	65.9	16.2	19.7	82.0	56011.0	878.9	1.5
90	3.8	3.2	16.5	49.8	7.7	14.7	47.1	55423.0	729.3	1.4
92	5.2	2.3	19.9	61.6	10.8	17.4	60.5	59049.0	869.0	2.3
94	4.1	3.6	22.2	71.5	11.2	16.9	60.6	57576.0	960.9	4.5
96	6.0	0.7	42.4	154.9	20.8	21.7	87.6	63710.0	912.5	2.1
100	4.1	3.8	21.8	209.2	12.8	15.6	57.9	54508.0	527.6	3.1
104	4.9	5.0	27.1	331.6	8.8	19.0	68.6	59607.0	633.8	2.9
108	5.8	22.0	45.6	212.4	15.9	36.1	96.8	55840.0	1199.9	3.2
114	5.3	7.1	47.0	220.4	24.7	29.2	89.1	60295.0	752.0	2.0
120	12.2	6.8	72.1	522.2	35.7	38.5	163.6	59019.0	324.9	2.3
126	7.9	9.4	60.9	301.2	20.1	29.3	80.6	56213.0	589.8	3.2
128	8.3	6.6	90.9	414.0	21.6	32.0	90.3	60241.0	441.1	2.3

Tab. 1. (cont.) Trace elements concentrations (mg/kg) and TOC percentage (%) along the studied core. Values in red indicate concentrations above PEL and TEL.

Depth (cm)	As mg/Kg	Cd mg/Kg	Cr mg/Kg	Cu mg/Kg	Ni mg/Kg	Pb mg/Kg	Zn mg/Kg	Fe mg/Kg	Mn mg/Kg	TOC %
132	10.7	16.8	70.0	409.3	20.6	37.2	96.2	59162.0	360.7	2.1
136	4.4	10.7	34.5	164.4	15.9	22.3	80.8	60433.0	781.6	3.1
140	7.8	6.7	46.1	226.3	21.6	29.8	100.7	64329.0	604.7	4.8
148	5.9	6.2	42.2	67.2	16.0	24.5	128.4	61964.0	608.3	5.3
154	5.6	4.5	40.6	46.6	11.8	23.7	120.9	65459.0	1095.2	3.1
162	6.7	4.9	42.9	59.7	13.9	24.1	79.7	58027.0	365.8	3.1
167	4.6	4.8	32.6	44.3	10.4	20.9	51.6	25775.0	164.3	1.1
177	3.3	4.3	30.0	38.4	9.7	20.6	35.7	16268.0	101.0	0.8
233	6.3	9.6	33.2	79.7	10.4	22.1	49.3	26179.0	52.6	0.9

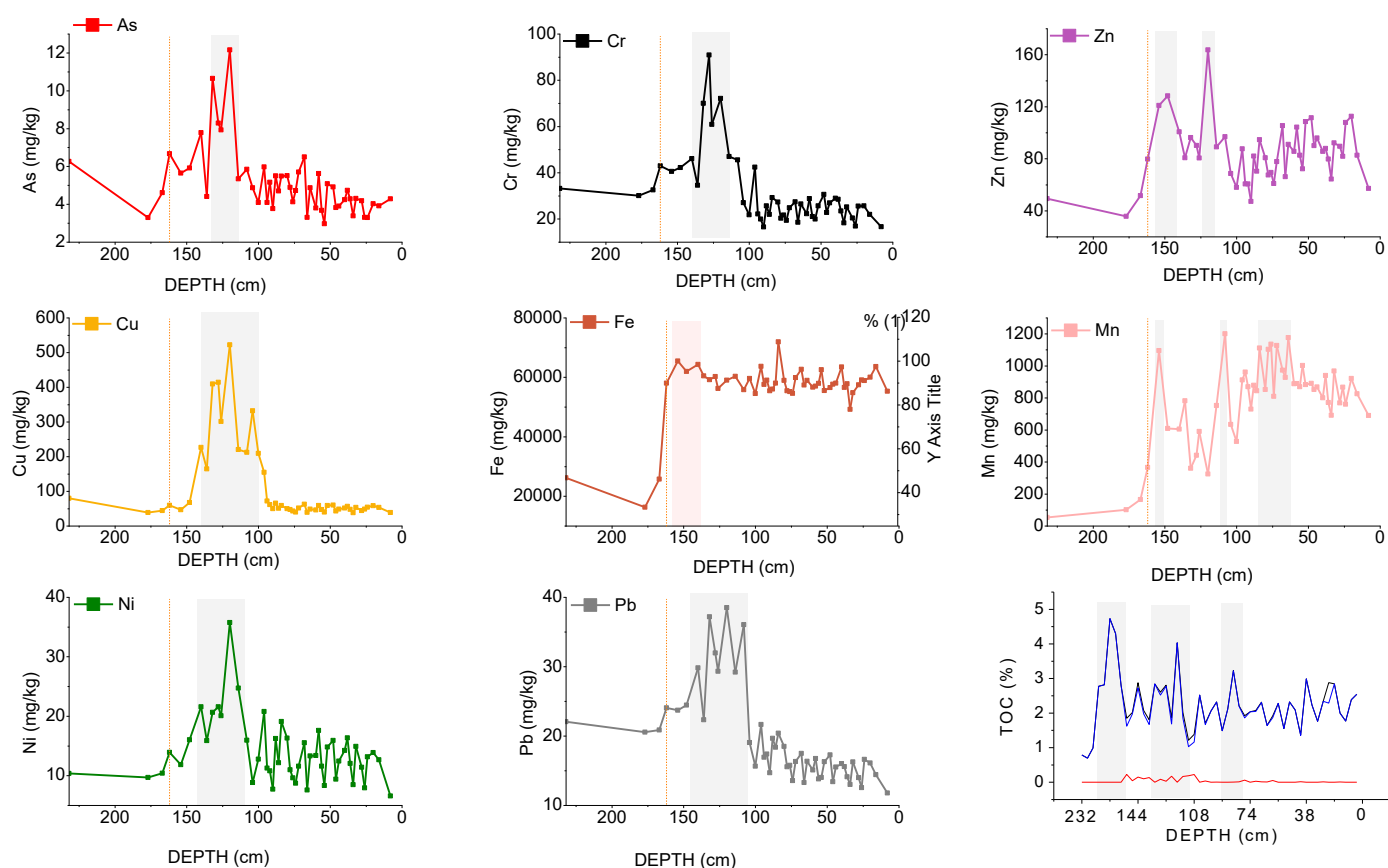


Fig. 3. Trace elements against depth. TOC (black line) organic carbon (blue line) and inorganic carbon (red line) concentrations against depth. Dotted vertical lines represent the moment of RSG filling.

Facies 2 (177-154 cm deep): erosion unconformity marking the contact between the facies of mud-clay grey color to the facies with a clearer color gradation in direction to the base of the core, with high concentration of roots that decrease towards the base, suggestive of soil; this facies characterizes the beginning of the presence of

thecamoebians, the most prevalent species are *Diffugia corona*, *Diffugia oblonga*, *Diffugia gramen* and *Arcella gibbosa*.

Facies 3 (154-94 cm deep): gradual transition to a more consolidated and more homogeneous sediment with a clearer brown color; it is possible to see a subtle plan-parallel stratification; it presents higher index of contamination by

metals; the dominant species are *Arcella megastoma*, *A. gibbosa* and *D. corona*.

The Facies 4 (94-42 cm deep): is the most representative facies of the core. It is composed by more consolidated sediments; it has plan-parallel stratification at centimetric level, with intercalations of black and brown mud; it has greater abundance, richness and diversity of the of Testate amoebae populations, which are mainly composed by

Centropyxis aculeata discoid and *D. corona* followed by *A. megastoma*, *D. gramen* and *Netzelia oviformis*.

Facies 5 (34-8 cm deep): is composed by very fluid unconsolidated sediments, with dark brown color; near the top is characterized by a new decline in the abundance, diversity and richness of the Testate amoebae populations; the predominant species is *C. aculeata discoides*. The species found in the present work are shown in Figures 7 and 8.

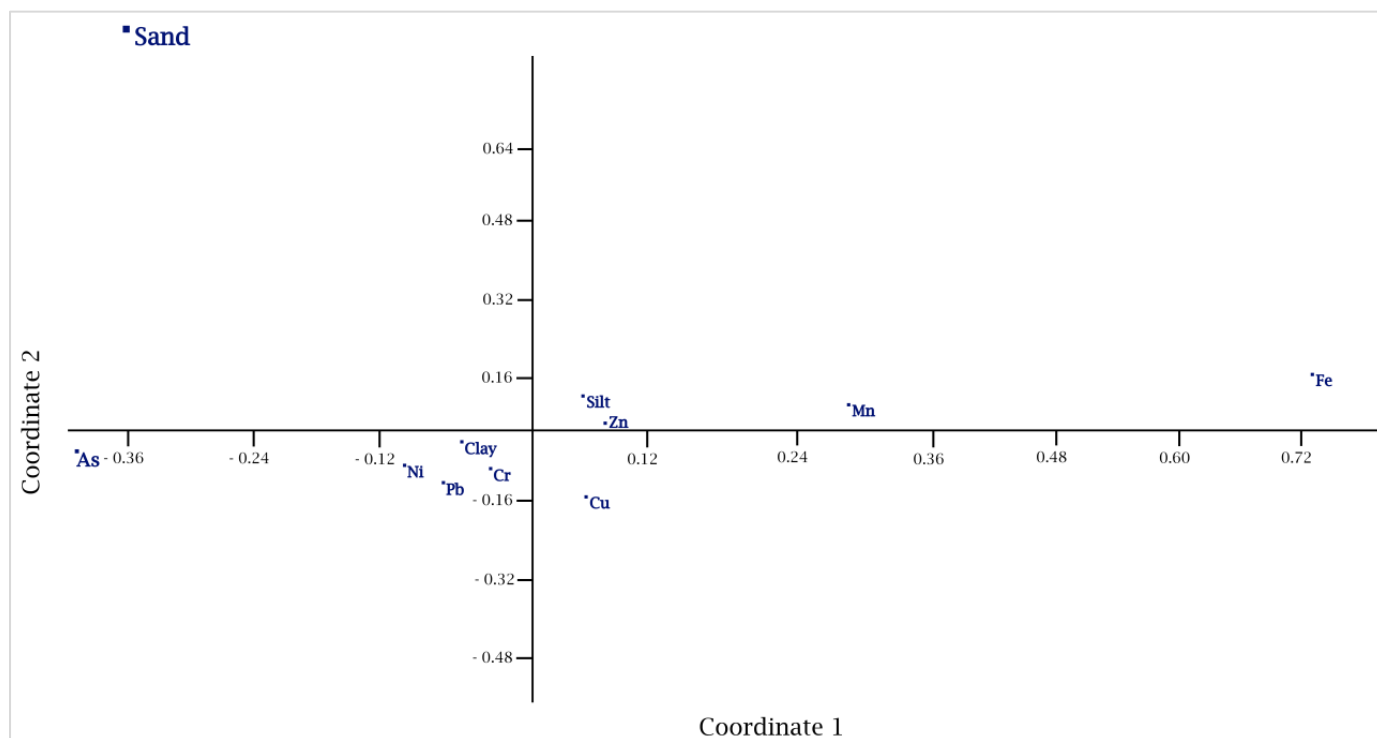


Fig. 4. Graphic representation of environmental variables (trace elements and grain size) by PCoA. Values is in Log₂.

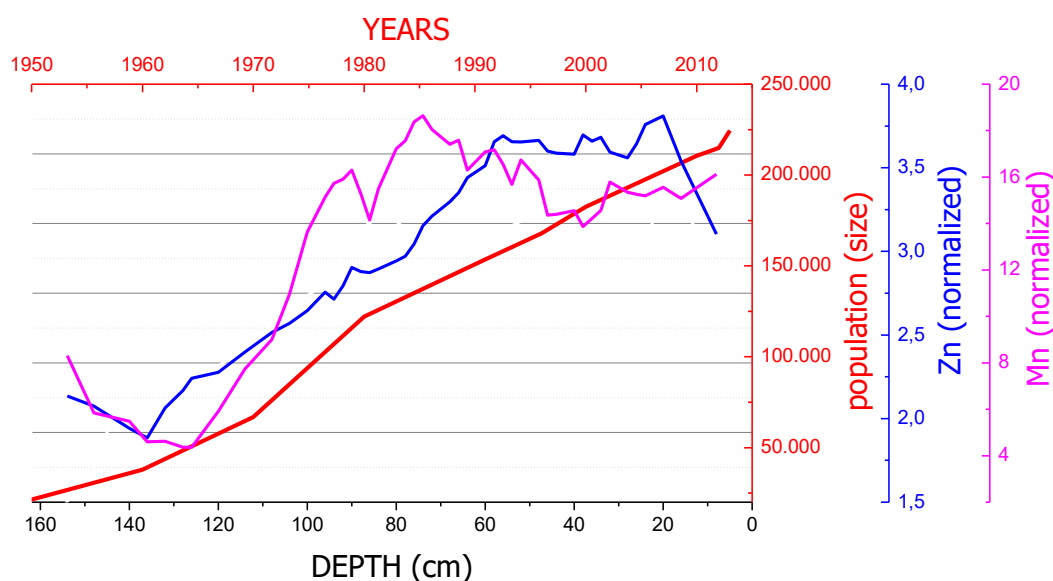


Fig. 5. Demographic growth curve followed by the concentration curves of Mn and Zn mg/kg.

The depth plot of As, Cr, Cu, Ni, Pb and Zn and the number of Testate amoebae specimens are shown in Figure 9. The Pearson correlations between Testate amoebae abundance of specimens and potentially toxic trace elements, such as As, Cr, Cu, Ni, Pb and Zn showed that the most negative correlations occurred with Cr, Cu and Pb (Table 5).

The NMDS plot (Fig. 10) correlates the density, richness and abundance of the populations of Testate amoebae with trace elements concentrations and core depth. The variables of Table 4 were used to generate the NMDS plot; the obtained stress value was 0.06204 (Fig. 11). It shows that Facies 5 (red) and Facies 3 (purple) are overlapped as well as Facies 4 (blue) and 1 (green). This overlapped pattern of Facies is related to the correlation between Testate amoebae populations distribution and trace elements concentrations distribution as suggested by the data in Table 5. This table shows negative Pearson correlation between number of specimens of Testate amoebae and trace elements concentration.

Tab. 2. Pearson r between sand, silt and clay fractions and trace elements.

Variables	Sand	Silt	Clay
Sand	-	0.02	-0.03
Silt	0.02	-	-0.94
Clay	-0.03	-0.94	-
As	0.39	-0.61	0.67
Cr	0.40	-0.55	0.59
Cu	0.59	-0.5	0.55
Ni	0.53	-0.49	0.52
Pb	0.38	-0.67	0.71
Zn	0.20	-0.16	0.03
Fe	-0.28	-0.40	0.41
Mn	-0.27	0.44	-0.52

5. Discussion

In the present work is analyzed the composition of Testate amoebae, granulometry and geochemical data, obtained along the sediment core that covers a period of the flooding of the in the reservoir of Salto Grande (RSG; Fig. 1) in the municipality of Americana-SP, Brazil, until the present, a time spanning ≈65 years. The splicing of the core followed a 2-cm interval, practically an annual resolution, considering a sedimentation rate of 2 cm/year estimated for this area by Leite (2002).

Granulometric parameters presented small variation throughout the core. In the core section 230-170 cm, the

granulometric curves presented no oscillation, indicating a stable hydrodynamic environment. From 170 cm to the core top, the granulometric curves present variations of low amplitude, related probably to frequent meteorological and other disturbing events. The granulometric curves suggest the occurrence of four main disturbing events. The most representative event is recorded in the core section 170-160 cm, where a strong inflection in the granulometric curves is observed, related to be the moment of the reservoir filling. All three events indicate an increase in fine sand and diminution of silt and shale. The granulometric analyzes of the RGS core showed predominance of silt-shale sediments (Fig. 2).

Sedimentary TOC content, depends on the amount of organic matter supplied to the bottom, deposited and preserved in the sediments. So, and although it is not correct to infer about the trophic state of a lacustrine system only based on the evaluation of the sedimentary carbon content, the obtained results in this work allow to do some considerations on the contribution of the influx of nutrients to the RSG. In general, TOC concentrations are relatively lower (Table 1), than those obtained from other Dams of São Paulo state, such as Billings, Rasgão, Bariri, Barra Bonita, Promissão (Silveiro, 2003). Although the RGS should not be considered a highly eutrophized environment, it is noteworthy that in the last 65 years of existence, the carbon values oscillated a lot probable due to the influx of terrigenous contributions, associated with river floods and the supply of macrophyte remains. According to Meyer et al. (2012), macrophytes are among the highest contributors of organic carbon in lake systems. Nevertheless, at specific intervals, it was detected a relation between the nutrients and the Testate amoebae population. For example, at Facies 3 (core section 154-94 cm), where conspicuous increase in the values of TOC and trace elements occurs, a deep decline in the abundance and richness of the Testate amoebae populations is observed, suggesting increased stress by contamination and excess of organic matter deposition in the sediments.

To evaluate the influence of anthropic activities on the RSG, a series of trace elements normalized by Sc were used (Table 1 and Fig. 3), the curves obtained show a marked event between 140-90 cm depth (Facies 3). In this core section, all the trace elements increase their concentration, except Mn, that presents lower concentration. From this level to the core top (Facies 4 and 5), the trace elements concentrations are moderate, suggesting periods of major intervention of public policies and social actions through ecological groups that took place in the 1980s and 1990s. Data of IBGE (2010) also corroborate this interpretation. They point to major investments in the treatment of domestic and industrial effluents by the municipalities of Atibaia river (main tributary of RSG). Concentrations of Zn and Mn has a gradual tendency to increase towards the core

top. Zn is a chemical tracer indicative of contributions from soil leaching both in the surrounding area and in the drainage basin and industrial and domestic effluents contribution.

Trace elements such as As, Cr, Cu, Ni and Pb present the highest concentrations in Facies 3. Toward the top of the sediment core: Facies 3 have moderate contamination by As, Cr, Ni and Pb; high contamination by Cu which reaches 500 mg/kg; Facies 4 and 5 have more moderate contamination by trace elements; low contamination was found in the soil portion (Facies 1).

The high concentrations of Cu present in the sediments should be related to the intensive use of algicide to control cyanobacteria and macrophytes flowering and in agricultural activity as a pesticide (Bordeaux), in both cases in the form of CuSO_4 .

Thus, the use of the surrounding soil, population growth and variation of the rain rate exert a strong control of the contribution of Cu, Zn and Mn in the RSG and reflect the contribution of the anthropic activities (Fig. 5).

Tab. 3a. Application of sediment quality guideline values according to CONAMA 454/2012 resolution for the section between 8-100 cm. Values in mg/kg.

Elements	RSG (0-100 cm)		TEL	PEL	World Shales
	Min	Max			
As	3.0	6.5	<5.9	37	13
Cr	16.5	42.4	<37.3	90	90
Cu	38	209	<35.7	197	45
Fe	49259	71910	SVR	SVR	46.7
Mn	528	1177	SVR	SVR	850
Ni	6.6	20.8	<18.0	35.9	68
Pb	11.8	21.7	<35.0	91.3	20
Zn	47.1	112.5	<123	315	95

Tab. 3b. Application of sediment quality guideline values according to CONAMA Resolution 454/2012 for the section between 100 - 148 cm. Values in mg/kg.

Elements	RSG (100-148 cm)		TEL	PEL	World Shales
	Min.	Max			
As	4.4	12.2	<5.9	37	13
Cr	27.1	90.9	<37.3	90	90
Cu	164	522	<35.7	197	45
Fe	55840	64329	SVR	SVR	46.7
Mn	325	1200	SVR	SVR	850
Ni	8.8	35.7	<18.0	35.9	68
Pb	19.0	38.5	<35.0	91.3	20
Zn	68.6	163.6	<123	315	95

The elements Fe and Mn are kept in high concentrations throughout the core, but these elements are found naturally in high concentrations. The statistical analysis of PCoA and Pearson correlations (Fig. 4 and Table 2), indicate positive relationship between the analyzed elements and fine-grained sediments, namely clay fraction.

Based on the VGQS, TEL and PEL indexes (Table 3a and 3b), the most affected Facies by contamination is found in the core section 154-104 cm (Facies 3), namely for As and Ni and a high level of contamination was found to Cr. Concentrations of Cu reveal regular level of contamination to very bad through the core, with exception of Facies 1.

Tab. 4. Values of D_{ms} , 1-D, density, richness, depth, and species of Testate amoebae. The sedimentary facies, were colored in order to emphasize changes in Testate amoebae community composition.

Facies	Depth	Richness (Nº of species)	Density (Nº Individuals/100 cc)	SIMPSON_1-D	MARGALEF	<i>A.c. angulosa</i>	<i>A. megastoma</i>	<i>A. gibbosa</i>	<i>C. aculeata discoides</i>	<i>C. constricta</i>	<i>C. ecornis</i>	<i>D. caprellata</i>	<i>D. chaquensis</i>	<i>D. corona</i>	<i>D. oblonga</i>	<i>D. cylindrus</i>	<i>D. elegans</i>	<i>D. litophila</i>	<i>D. gramen</i>	<i>L. globulosa</i>	<i>N. tuberculata</i>	<i>N.oviformis</i>	<i>N. wallei</i>	<i>P. c. ecornis</i>	<i>P. coroniformis</i>
8	3	7	0.45	1.03	0	0	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
16	3	7	0.45	1.03	0	0	0	0	5	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
18	2	3	0.44	0.91	0	0	0	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	
22	4	5	0.72	1.86	0	2	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	
26	2	2	0.50	1.44	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	
30	2	7	0.41	0.55	0	0	0	0	2	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	
34	3	3	0.67	1.82	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
42	3	16	0.62	0.72	0	0	0	0	5	0	3	0	0	8	0	0	0	0	0	0	0	0	0	0	
46	8	32	0.80	2.02	0	5	0	5	0	0	0	0	0	9	0	0	0	0	0	1	8	0	2	1	
50	6	16	0.73	1.80	0	2	0	7	0	1	0	0	0	2	0	0	0	0	0	3	0	0	0	1	
54	6	4	0.67	1.67	0	3	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	
62	6	22	0.79	1.62	0	4	0	7	0	0	0	0	0	5	0	0	0	0	0	2	0	1	0	3	
66	7	50	0.70	1.53	0	5	3	25	0	0	0	0	0	4	0	0	0	0	0	1	0	8	0	4	
70	4	21	0.69	0.99	0	9	0	3	0	0	0	0	0	6	0	0	0	0	0	0	3	0	0	0	
74	4	19	0.68	1.02	0	0	0	8	0	0	0	0	0	6	1	0	0	0	4	0	0	0	0	0	
82	5	43	0.65	1.06	0	0	0	13	0	0	0	0	0	5	0	0	2	0	21	0	0	2	0	0	
86	6	29	0.72	1.49	0	2	1	10	0	0	0	0	0	11	0	0	0	0	3	2	0	0	0	0	
90	5	45	0.74	1.05	0	11	6	18	0	0	0	0	0	4	0	0	0	0	0	6	0	0	0	0	
94	6	32	0.74	1.44	0	0	6	12	0	0	1	0	9	0	0	0	0	0	0	0	2	2	0	0	
114	2	5	0.48	0.62	0	0	2	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	
122	4	8	0.72	1.44	0	1	0	0	0	0	0	0	0	3	0	0	2	0	0	0	0	2	0	0	
126	3	6	0.61	1.12	0	3	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	
132	3	3	0.67	1.82	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
136	3	7	0.57	1.03	0	1	4	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
140	1	1	0.00	0.00	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
144	2	2	0.50	1.44	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	
148	4	7	0.69	1.54	1	0	3	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	
154	3	5	0.56	1.24	0	0	3	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	
158	4	11	0.71	1.25	0	0	1	0	0	0	0	0	0	4	0	0	0	0	3	0	0	0	0	3	
162	7	18	0.80	2.08	0	0	0	4	0	0	1	0	5	4	0	0	0	2	0	1	1	0	0	0	
167	3	10	0.58	0.87	0	0	5	0	0	0	0	0	0	4	0	0	0	0	1	0	0	0	0	0	
177	5	13	0.75	1.56	0	4	0	0	0	0	1	0	1	0	0	0	0	0	0	4	3	0	0	0	

Tab. 5. Pearson correlation between Testate amoebae. abundance and trace elements. Only the facies 3 and 4 where trace elements show the largest variations were used in the analysis. Fe and Mn are not shown since they are not considered pollutants by PEL and TEL Canadian indices.

Elements	Specimens
Zn	-0.49
Pb	-0.67
Ni	-0.56
Cu	-0.67
Cr	-0.68
As	-0.50

The core has no significant contamination by Zn and Pb according to parameters of TEL and PEL. For the elements Fe, Mn and Sc there is no reference criteria of TEL and PEL once these elements are considered naturally abundant, and Sc is not associated to anthropic activity.

Regarding the classification of species of Testate amoebae, it is important to note that the possible morphotype variations found in the RSG can be considered minimal, as no continuous variation was observed among the specimens found along the studied core. This greatly facilitated the taxonomic identification of the species, but allowed to detect significant variations of the population characteristics of Testate amoebae throughout the core (Figs. 7 and 8).

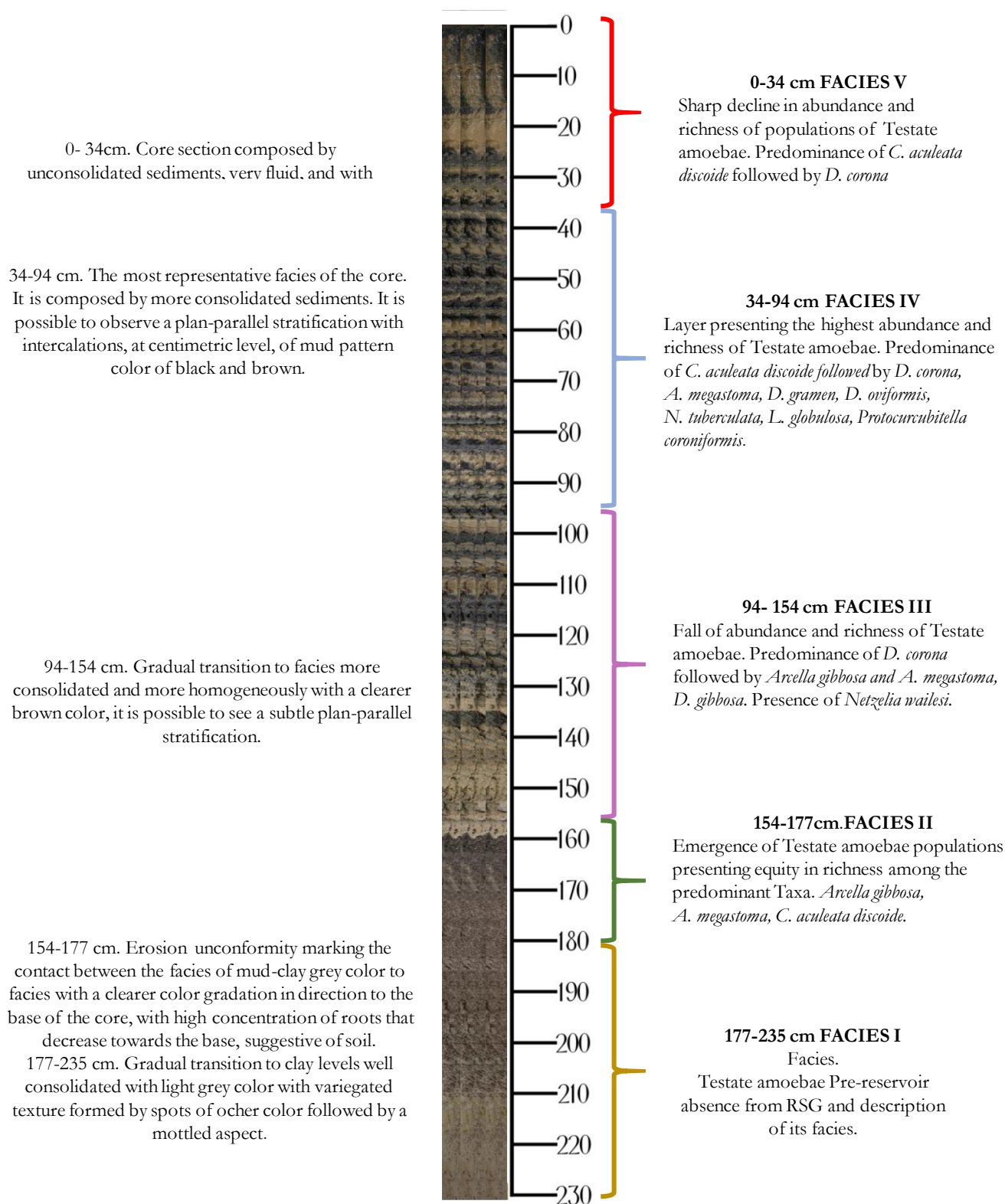


Fig. 6. Facies of the sedimentary core of the Salto Grande reservoir.

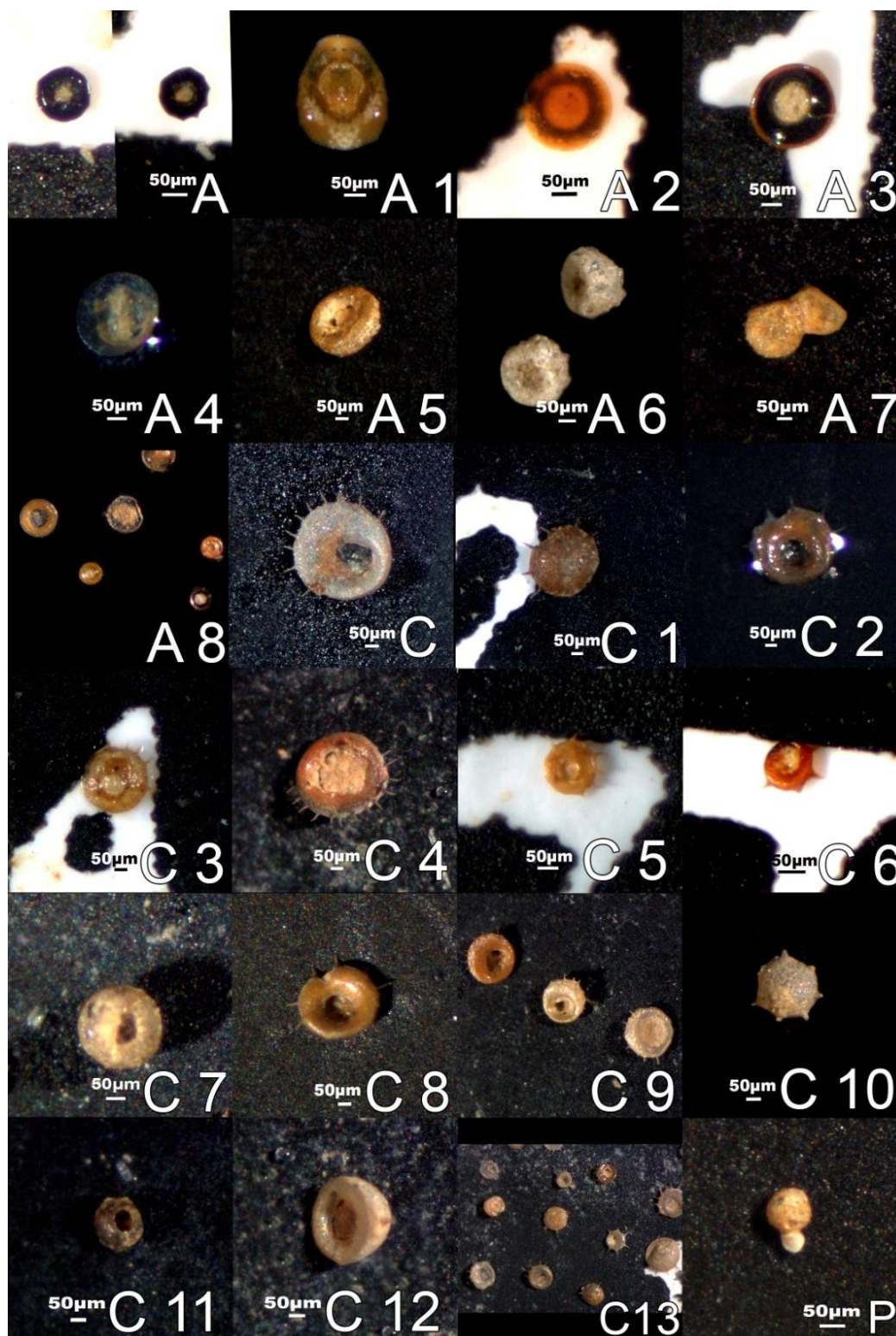


Fig. 7. Taxa of tectamias found in the sediments of the RSG. A - *Arcella costata angulosa* (Playfair, 1918); 109 µm. A1-A4 *Arcella megastoma* (Pénard, 1913); A1 - 262 µm, A2 - 208 µm, A3 - 219 µm and; A3 - 207 µm, respectively. A5, A6 - *Arcella gibbosa* (Pénard, 1890); 264 µm, 168 µm respectively. A7 - *Arcella* specimens in reproductive phase. A8 - *Arcella* specimens with different size and morphotypes. C to C9 - *Centropyxis aculeata discoide* (Ehrenberg, 1859); C - 347 µm, C1 - 309 µm, C2 - 317 µm, C3 - 259 µm, C4 - 365 µm, C5 - 130 µm, C6 - 91 µm, C7 - 221 µm, C8 - 302 µm, C10 - 210 µm, C11 - 188 µm and C12 - 269 µm. *Centropyxis ecoris* (Ehrenberg, 1841). C13 - *Centropyxis*; specimens with different size and species morphotypes. P - *Pontigula sp.* (Rhumbler, 1896); 106 µm.

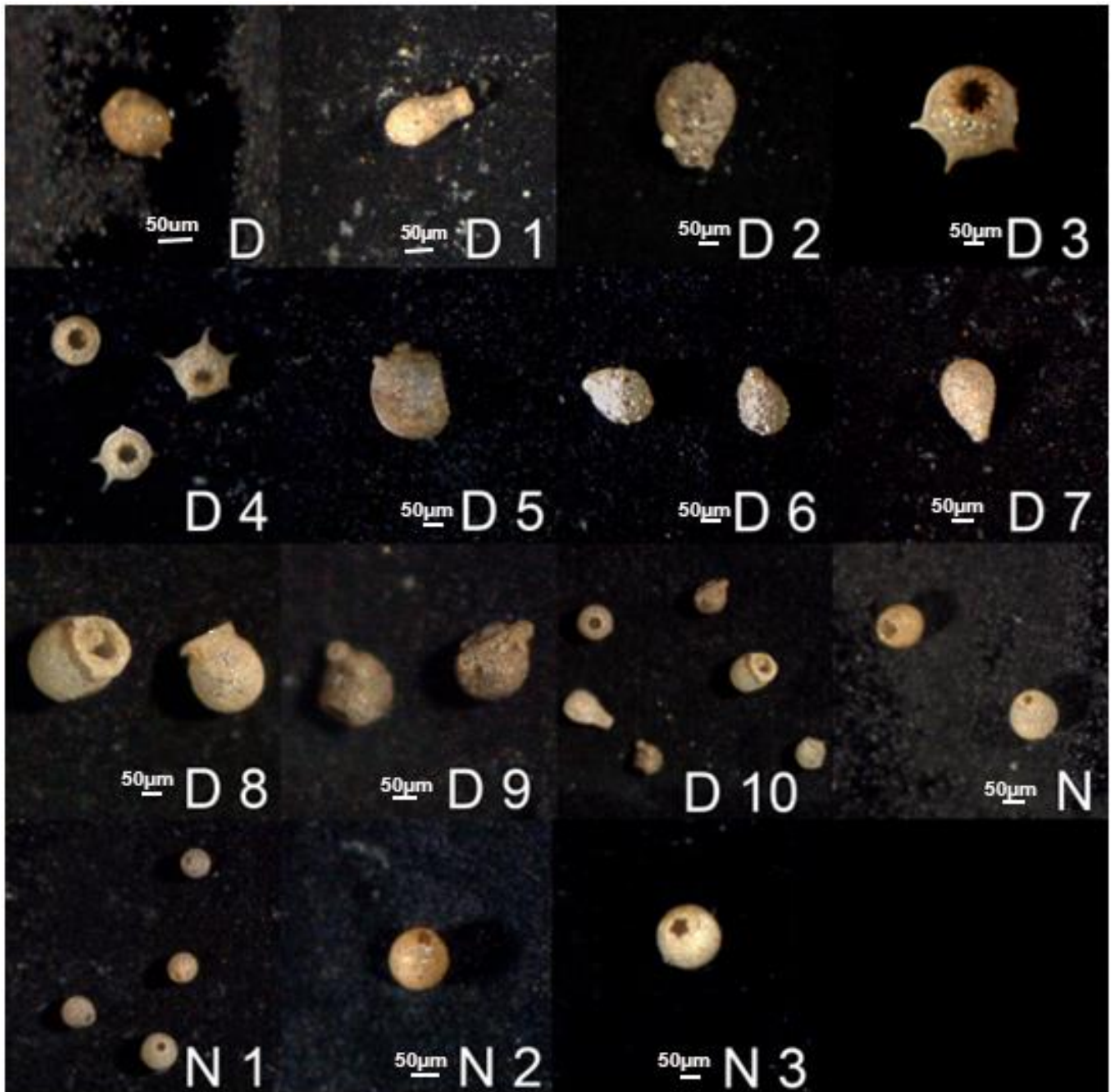


Fig. 8. D - *Diffflugia chaquensi* (Vucetich, 1989); 100 µm. D1 *Diffflugia capreolata* (Pénard 1902); 243 µm. D2 - *Diffflugia oblonga* (Ehrenberg, 1838); 307µm. D3, D4 - *Diffflugia corona* (Wallich, 1864); 221µm and 84-180 µm, respectively. D5- *Centropyxis ecornis* (Ehrenberg, 1841); 215 µm. D6 - *Lesquereusia globulosa* (Rhumbler, 1896); 214µm. D7 - *Diffflugia pyriformis* (Perty, 1849); 206 µm. D8 - *Diffflugia urceolata* (Pénard 1902); 212 µm. D9 - *Diffflugia oblonga capreolata* (Ehrenberg, 1838); 119 µm. D10 - *Diffflugia* spp. N, N1- *Netzelia oviformis* (Cash, 1909); 100 µm. N2, N3 - *Netzelia wailesi* (Ogden, 1980); 140 µm and 112 µm, respectively.

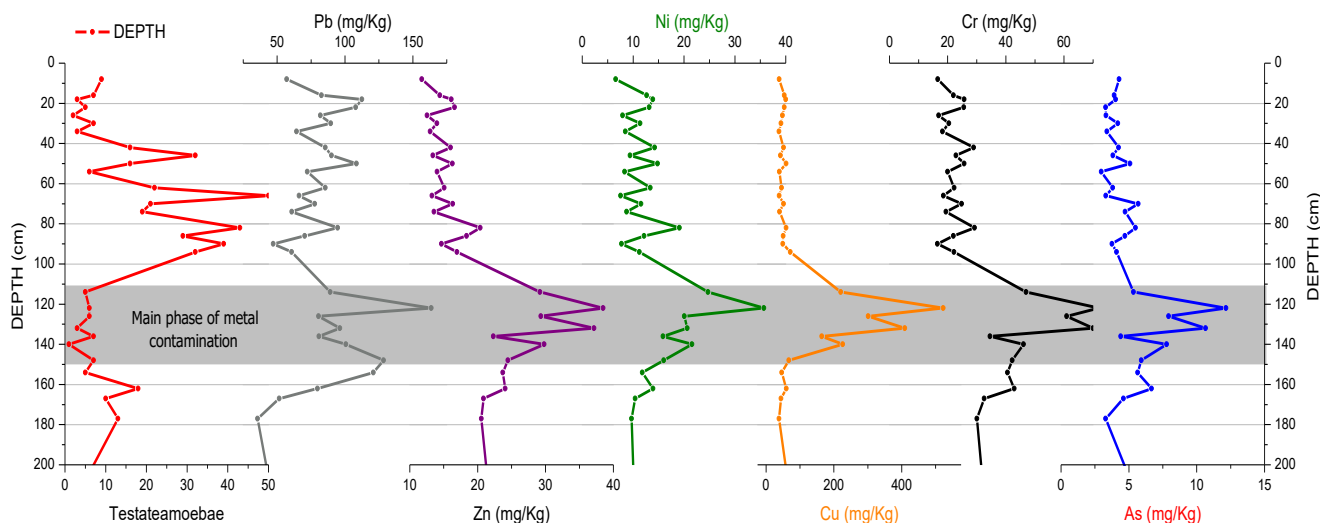


Fig. 9. Testate amoebae number of specimens plotted in the right axis, and most important trace elements pollutants plotted in left axis. The number of specimens is in red, Cu in yellow, Zn in deep blue, Cr in black, Pb in brown, Ni in green, and As in blue. X axis shows depth.

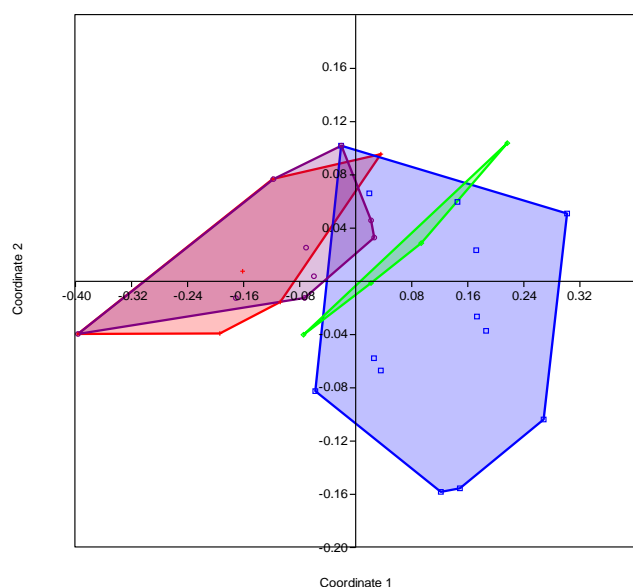


Fig. 10. NMDS Plot (Gower) diversity indices, as well as specimens and Taxa number, in function of depth. The two purple and red polygons located between the -0.4 and +2 points of coordinate 2 and -0.04 and +0.10 of coordinate 1 show the similarity of the two events of decline of abundance, richness and diversity of populations of Testate amoebae. The interpolating blue and green polygons between the points -0.04 and +0.29 of coordinate 2 and -0.16 to +0.10 of coordinate 1 show the two events that display abundance, wealth and diversity in the populations of Testate amoebae.

From the variations of the Testate amoebae populations, combined with the results of the abiotic data (nutrient

contents, trace elements), it was possible to identify sedimentary facies, representative of important moments in the environmental history of the RSG (Figs. 6 and 9).

These events were demonstrated through the statistical analysis NMMDS (Fig. 10) in which it was possible to observe two clusters with overlapping areas; a grouping that marks the intersection of Facies 3 and 5 that presented the events that showed a significant decrease in richness and abundance of the populations of Testate amoebae and a second group that overlaps the Facies 2 and 4 that marked the events of high richness and abundance of the Testate amoebae.

The predominant genera found in the RSG is shown in Figure 6 and Table 4, are *Centropyxis*, *Diffugia* and *Arvella*, genera that are considered opportunistic and tolerant to contaminated environments (Patterson and Kumar 2000; Farrooqui et al., 2012; Qin et al., 2013). Among these three genera, *Arvella* was shown to be the most tolerant and opportunistic, followed by *Diffugia* in RGS. *Centropyxis aculeata discoide* should be the most sensitive species to contamination by metals since its abundance deeply decline declines as trace metals concentrations increase. The same behavior was also observed by Farrooqui et al. (2012). The other RSG taxa were considered rare because they were represented by few individuals.

To assess the influence of anthropic activities, the use of bioindicators for the biomonitoring of water systems is less expensive than the use of physico-chemical indicators that require time and cost for purification, qualification and quantification.

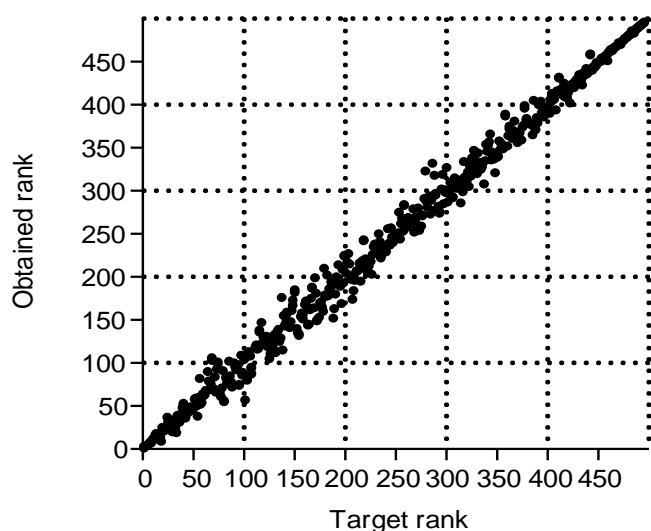


Fig. 11. Sheppard plot indicating the quality of results of the NMMDS graphic, almost all points are aligned in a single straight line.

The advantage that these thecamoebians organisms offer is to have quick responses to the introduction of pollutants, because they have a short life cycle, practically sampling in real time what happens in the environment, and being able to keep this information in the fossil record. They can indicate environmental changes such as climate, sea level oscillations, introduction of pollutants into the environment (Markert et al., 2003).

6. Conclusion

The beginning of the RSG dam filling was marked by a change in sediments grain size, which became quite fine. The sediment core witnessed the input of trace elements to the reservoir, as well as changes in populations of Testate amoebae. It is possible to infer that the rise of clay fraction in sediments played an important role in trace elements adsorption during 65 years, from the filling of the RSG until 2015.

Changes of Testate amoebae populations were conditioned by the input of trace elements. The results of this work evidence that these organisms are good bioindicators of pollution by metals. The most tolerant species to metals enrichment were *Arcella megastoma* and *Diffugia corona*. The most sensitive species were *Diffugia gramen*, *Lesquereusia globulosa* and *Netzelia tuberculata*.

It is observed that Testate amoebae communities are excellent bioindicators of pollutants in lake sediments and multiproxies analysis are required to better infer the ecosystem response to complex interaction between abiotic and biotic parameters.

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