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METAIS PESADOS E EFEITOS GENOTÓXICOS EM SEDIMENTOS DE RESERVATÓRIOS (LAGOS ARTIFICIAIS)

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METAIS PESADOS E EFEITOS GENOTÓXICOS EM SEDIMENTOS DE RESERVATÓRIOS (LAGOS ARTIFICIAIS)

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Orientador: Prof. Dr. Fábio Roland
Coorientador: Prof. Dr. Nathan Barros

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À todas as mulheres, tão fortes, que ainda sofrem tanto com o machismo e mantêm a cabeça erguida:

“This is for my girls all around the world
Who've come across a man who don't respect your worth
Thinking all women should be seen, not heard
So what do we do girls?
Shout out loud!
Letting them know we're gonna stand our ground
Lift your hands high and wave them proud
Take a deep breath and say it loud
Never can, never will, can't hold us down”
(Can't Hold Us Down – Christina Aguilera)

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“Alice: This is impossible!

The Mad Hatter: Only if you believe it is.”

Alice in Wonderland

ABSTRACT

Aquatic ecosystems provide many services to humanity, despite this; water pollution is one of the most serious environmental problems worldwide. Reservoirs, including ones used for water supply, receive from tributaries, or directly, several effluents from human activities that contain a pool of pollutants. Reservoir sediments work as sink or source for pollutants depending on environmental conditions. Therefore, pollutants present in these aquatic compartments can return to the water column or transfer through the food chain, where they can be toxic to biota and even to humans. Contaminants in the environment can cause mutagenic effects that can accumulate in DNA and harm a whole population. Then, genotoxicity tests, such as *Allium cepa* assay, may be used to evaluate the extent of environmental pollution, since synergistic effects might occur and physicochemical analysis just provide the presence of pollutants. Studies focusing on the presence of chemical compounds in reservoirs sediments and their toxicity are crucial. In this context, the study aimed to investigate contamination in sediments by metals in Brazilian reservoirs, as well as potential ecological risk and potential to cause cytogenotoxicity in *A. cepa*. Copper, chromium, cadmium, lead, zinc and iron were measured in sediment samples from six Brazilian reservoirs. Besides that, the surface layer of sediment was submitted to a resuspension simulation to the water column, and sediment solution was submitted to *Allium cepa* assay and metals analysis. Our findings showed that these reservoirs seem to be influenced by agricultural, industrial and domestical effluents because of the presence of copper, cadmium, and zinc above threshold effect levels in some samples and a potential ecological risk was observed. More than that decrease mitotic index and increase chromosomal aberrations in all treatments (except Furnas reservoir) showed cytogenotoxic effects. The presence of metals and other possibly inorganic and organic pollutants may be answerable for cytogenotoxic effects observed. In this sense, just chemical analyses were not enough to evaluate the extent of environmental pollution in sediments and its impact. We need to use more tools combined with chemical analysis to provide a better understand of pollution in aquatic ecosystems. It is important to implement law and regulations that are more efficient, intensive monitoring, sewage treatment and reforestation of riparian zones to protect these systems, including preventing public health problems.

Keywords: aquatic ecosystems; cytogenotoxicity; human health; metals; pollutants;

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GENERAL INTRODUCTION

Although they represent a small percentage of all world water, freshwater systems have an important role in nutrient cycling (Cole et al. 2007; Tranvik et al. 2009). Also, they provide many ecosystem services that are essential to humanity, which include climate regulation, waste assimilation, water and food supply, as well as cultural and recreational functions (Limburg 2009). Then, development and human health depend on availability and water quality. Nevertheless, despite all this obvious importance, humans are depreciating these ecosystems. One greatest challenge for humanity (it is already and will continue to be) will be water availability, not just because of volume, but also due irregular distribution on Earth, coupled to waste, pollution and degradation (Berbert 2003). Because of this, humans has created many reservoirs for multiple purposes, such as water supply, food production, hydroelectricity, water for irrigation and recreational reasons (Thornton et al. 1990; Tundisi 2005). However, reservoirs receive from affluent, or even directly, many types of contaminants from human activities.

Human development has been synonymous with environmental degradation, being that chemical pollution is one of the most obvious. Industrial development and uncontrolled urbanization release a variety of pollutants into the environment that causes damage to organisms (Förstner and Wittmann 1983; Filser 2008; Cavalcanti et al. 2014). All urbanization process usually results in land cover change, soil erosion and waste production, and these impacts lead a chemical load on surrounding water bodies, which alters the ecosystem and leaves a record of disturbance in sediments (Förstner and Wittmann 1983). Sediments are a compartment of aquatic ecosystems that work as a tool to investigate water quality. They reflect an accumulative contamination over time and pollutants adsorbed can be released to water column and uptake by biota, serving as a sink or a secondary source of pollutants depending on environmental conditions. Moreover, researches with sediments contribute to the decision-making process for establishing environmental control, mitigation and recovery programs, such as assessment of dredging process and navigation (Förstner and Witmman 1983; Salomons and Förstner 1984; Brandão et al. 2011).

Among pollutants that occur in the aquatic environment, the most worrying are those who are susceptible to remobilization and bioconcentration, bioaccumulation and biomagnification processes (Salomons et al. 1995). Potentially toxic elements are part of

this group, such as cadmium, chromium, zinc, iron, copper and lead (Förstner and Witmman 1983). More than that, they are not usually eliminated from aquatic ecosystems by natural processes and tend to accumulate in sediments where they are susceptible to release to water column by remobilization and may be transferred through the food chain, thereby reaching humans (Förstner and Witmman 1983). Then, this group of pollutants presents a direct risk to humans (Förstner and Wittmann 1983) and environmental impact caused by metals is a big environmental concern nowadays (Jesus et al. 2004).

The introduction of metals into freshwater ecosystems has various sources, including natural and anthropic ones. Metals are abundant elements in Earth's crust, and mineral weathering processes are responsible for the natural introduction of metals in aquatic environments (Salomons et al. 1995). On the other hand, anthropic sources include smelting processes and fuel combustion via atmospheric deposition, leaching of garbage and many waste discharges (Förstner and Witmman 1983; Salomons and Förstner 1984). Some industrial effluents and its metal content are presented in Table 1. The atmospheric input is of particular importance for lead (60%) and zinc (33%), for copper it contributes 13% and for cadmium 11%, being a secondary source for iron (Salomons and Förstner 1984).

In general, diffuse pollution is responsible for environmental contamination by contaminants, which hinders to detect the source conclusively (Förstner and Witmman 1983; Salomons et al. 1995). Deforestation and agricultural areas are significant contributors of nonpoint sources, leading to soil erosion and affect water quality, especially if soil is enriched by metals that frequently comes with fertilizers and pesticides (Förstner and Witmman 1983).

Table 1. Industrial effluents and its metal content (Brandão et al. 2011).

Industrial effluents	Copper	Chromium	Cadmium	Lead	Zinc	Iron
Car and batteries	X	X	X	X	X	X
Organic products	X	X	X	X	X	X
Textiles	X	X	X	X	X	X
Glass and ceramics	X	X	X	X	X	X
Electroplating	X	X	X	X	X	X
Waste incineration plant and sewage treatment plants	X	X	X	X	X	X
Electronic components	X	X	X	X		X
Petrochemical and refinery	X	X		X	X	X
Copper processing	X	X		X	X	X
Metallurgical and steel industry		X	X	X	X	X
Plastic and synthetic materials	X		X	X	X	
Cast Iron		X		X	X	X
Inorganic products		X	X	X		X
Aluminum processing	X	X			X	X
Fertilizers		X			X	X
Tanning and wooden furniture		X				

Aquatic and terrestrial systems are connected even in exchange of pollutants, where aquatic systems receive leaching from terrestrial systems and irrigation, dredging and biota flux can return pollutants to terrestrial systems (Förstner and Witmman 1983). Aquatic environment presents four abiotic compartments that strongly interact among themselves: suspended matter, sediments, surface waters and pore waters (Figure 1). Then, aquatic compartments, as well as groundwater and soil, influence metal uptake by biota (Salomons and Förstner 1984).

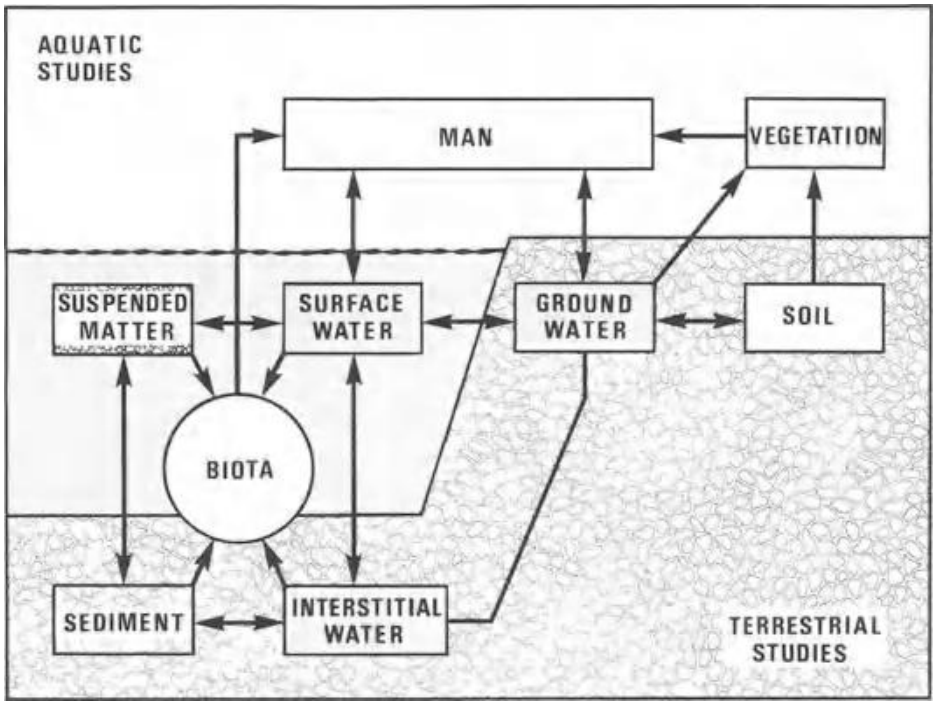

Figure 1. Scheme of metal reservoirs in an aquatic system and its interactions with the terrestrial environment (Salomons and Förstner 1984).

Sediment compartment act as a reservoir for metals but if the environmental conditions change, it is possible to observe a remobilization of the accumulated metals (Salomons and Förstner 1984). Remobilization of metals from sediments is dangerous to the aquatic system but also for drinking water supply (Förstner and Witmman 1983), being that contaminated sediments are an environmental problem, especially because contaminants in this compartment reach high levels (Salomons et al. 1995). Determination of bioavailable fraction that is the concentration in an abiotic compartment that can be transferred to biota represents a great study area in metal research (Salomons and Förstner 1984).

Most metals are essential to life, but all of them can be toxic if reach high levels in organisms. On the other hand, some metals did not have a known biologic function, and they are mentioned as nonessential (such as cadmium and lead) (Förstner and Witmman 1983; Mason 2013). Metals can cause lethal effects, but also sublethal/chronic effects, such as reduce growth and fecundity, change in species composition and decrease diversity and density of populations (Moore and Ramamoorthy 2012). Aquatic communities are continuously exposed to metals due continuously discharged into the environment, and humans are continually exposed through drinking water long-life. Several metals also can cause cytotoxicity and genotoxicity on organisms (Hadjiliadis 2012).

Humans influence metal cycling increasing their fluxes (Mason 2013) but also water and soil acidification, as well as eutrophication, affects metal behavior and change natural pathways from rivers to oceans (Salomons and Förstner 1984). Besides that, human activities release many other pollutants into the environment, include a pool of metals, and there is not much information about simultaneous effects of mixtures on organisms. Water quality criteria based on experiments with single pollutants probably not adequately protect organisms (Salomons and Förstner 1984).

Researchers developed many remediation technologies to mitigate problems with metals in aquatic systems, such as phytoremediation, advanced oxidation process, coagulation-flocculation, chemical and electrochemical precipitation, and also macrophytes has been shown to be useful for removing heavy metals from aquatic ecosystems (Salomons et al. 1995; Mishra and Tripathi 2008; Sharma 2015). It is necessary continuing invest on new technologies to remove metals from systems, but also implement pollution prevention. Pollution prevention reduces the generation of pollutants and wastes, prioritizing recycling and treatment, and minimize the use of harmful products (Chen 2012). Prevention also includes an implementation of more restrictive and effective laws and regulations and development of technologies of decontamination of metals (Chen 2012). In order to preserve the water quality in ecosystems, more than that need to be done, such as reforestation of riparian zones with native species, invest in sewage treatment, restraint and discipline of fertilizers and pesticides use in watersheds, control groundwater use and implement environmental education and participation of population (Tundisi 2005).

Environmental monitoring is also important to control metal pollution and provide basement to decision makers. Environmental samples from aquatic systems are complex,

they receive diffuse sources of pollution, and many chemicals are present. Then, with all synergetic effects that are possible to observe, the establishment of maximum values allowable in the environment for just one chemical isolated is such complicated. Then, is very well recommended use more than chemical analysis, such as toxicity approaches, whether these are theoretical or practical, representing a more effectiveness way to characterize the extent of pollution and its impacts (Bergman 1994; Bertoletti 2012). Besides, we need to improve our communication, especially among ecologists and ecotoxicologists (Filser 2008). This study showed how cooperation and partnerships are essential for risk assessment.

In this entire context, this study proposes to determine the concentration of metals in sediments of Brazilian reservoirs, exploring spatial and temporal variations and using a moderate acid extraction in order to access bioavailable fraction. Besides, also evaluate the potential of those sediments to cause cytogenotoxicity in *Allium cepa*, observing the extent of the environmental pollution.

CHAPTER 1

Temporal and spatial accumulation of metals in sediments of tropical reservoirs

Format according to Science of the Total Environment

Abstract: Water pollution has become one of the most serious environmental problems globally. Reservoirs receive effluents from human activities that contain a complex mixture of pollutants. Reservoir sediments work as either sink or source for pollutants depending on environmental conditions. Therefore, pollutants present in these aquatic compartments can be released to the water column and be transfered through the food chain, where they can be toxic to biota and even to humans. In this context, studies focusing on the presence of chemical compounds in sediments of reservoirs are crucial. In this study, the aim was to investigate contamination by heavy metals in Brazilian reservoirs, exploring spatial and temporal variations, as well as potential ecological risk. Copper, chromium, cadmium, lead, zinc and iron were measured in sediment samples from six different reservoirs with varying size and environmental characteristics. In general, metal concentrations maintained below the limits established by sediment quality guidelines. However, copper, cadmium, and zinc were found above threshold effect levels spread over all sediment depths, but especially in two reservoirs. Based on the mixture of the metals, a potential ecological risk was observed in all sediment depths and on the same reservoirs that the metal concentrations were highest. Our findings show how reservoirs are integrators of impacts by human activities. It is important to implement law and regulations that are more efficient, intensive monitoring, sewage treatment and reforestation of riparian zones to protect these systems, including preventing public health problems. Also, we need to use more tools combined with chemical analysis to provide a better understanding of aquatic ecosystems.

Keywords: aquatic ecosystems, heavy metals, mixture toxicity, reservoir sediments.

1. Introduction

Human health and development depend on water quality and availability, mainly via ecosystem services provided by freshwater systems, such as waste assimilation, provision of water and food and climate regulation (Naiman et al. 2002; Limburg 2009; Olorunfemi 2013). Despite the central role of clean water for humankind, uncontrolled urbanization and industrialization produce chemical waste, which puts water quality at risk. Consequently, a severe deterioration of aquatic ecosystems has been observed (Corvalan 2005; Barbério et al. 2008; Al-Shami et al. 2012). Anthropic effluents, such as those from industries, agriculture, and domestic activities, discharged into aquatic systems contain a complex mixture of inorganic and organic pollutants that can cause adverse effects on organisms (Hoshina and Marin-Morales 2009; Schwarzenbach et al. 2013; Cavalcanti et al. 2014).

Humans create reservoirs for specific purposes, such as water supply (Thornton et al. 1996; Suen and Eheart 2006). Hence, pollutants and chemical residues present in the reservoir may harm the ecosystem as well as humans (Helmer and Hespanhol 1997; Pereira et al. 2007). Therefore, studies aiming at understanding the distribution and risk caused by reservoir pollution is essential. On a global scale, sediments of aquatic ecosystems serve as a sink for both organic and inorganic pollutants. These pollutants can be released to the water column through many different pathways, such as resuspension of sediment particles by wind dredging and bioturbation, or via diffusive flux from sediment to water (Salomons and Förstner 1984; Zoumis et al. 2001; Eggleton and Thomas 2004; Nizzetto et al. 2010; Sobek et al. 2014; Remaili et al. 2016). Then, contaminated sediments may start acting as a secondary source of pollutants to water and aquatic organisms. Once in the sediment, pollutants can be toxic to benthic organisms, be transfered through the food chain, and reach even humans (Förstner and Wittmann 1983; Salomons et al. 1987; Siddique et al. 2009; Geras'kin et al. 2011). Humans are exposed to pollutants lifelong, especially by food tissues and drinking water (Lioy 1990; Pereira et al. 2007; Buschini et al. 2008; Castro-González and Méndez-Armenta 2008; Bhowmik et al. 2015; Alamdar et al. 2016; Saha and Paul 2016). Even if organic and inorganic pollutants are present in low concentrations, they can cause chronic effects and harm ecological functions (Scott and Sloman 2004; Matsumoto et al. 2006).

Among pollutants that may be present in reservoir sediments, heavy metals have been known and used by humans for a long time. Industries are built in every place all the time, and industrial effluents usually contain metals such as cadmium, lead, zinc, and

chromium (Chidambaram et al. 2009). Although they are known for their toxicity and persistence, anthropic activities continue to expose aquatic systems to these compounds (Asrari 2014; Crompton 2015). Many metals are essential to organisms, but in high concentrations, they can be toxic and offer risk to ecosystem balance, causing inclusive genotoxic effects (Förstner and Wittmann 1983; Barbosa et al. 2010). Metals also can accumulate in organisms tissues and reach human food (Loska and Wiechula 2003; Siddique et al. 2009; Geras'kin et al. 2011; Wang et al. 2012). Major anthropogenic sources of metals in aquatic environments are domestic, industrial, and agriculture effluents (Nikinmaa 2014). These anthropogenic effluents carry a diversity of pollutants, and environmental samples are characterized as complex mixtures (Lemos et al. 2009). Then, development of tools that try to assess ecological risks of mixtures of pollutants in the environment is also crucial.

This study aims to investigate contamination by metals in six different Brazilian reservoirs, exploring spatial and temporal variations, as well as potential ecological risk. Our findings show the need for more and systematic studies in these ecosystems and display the risk posed by pollution to both ecosystem and humans.

2. Material and methods

2.1. Study areas

The sediment samples were collected in six reservoirs located in southeastern Brazil: Furnas (FNS) and Chapéu D'Uvas (CDU) located in Minas Gerais State (MG), Funil (FUN) located in Rio de Janeiro State (RJ) and Monte Serrat, Bonfante and Santa Fé (MBS) are located between these states (MG / RJ). MBS reservoirs located in series in the same system: the Paraibuna River.

2.1.1. Furnas Reservoir (FNS)

FNS was created in 1963 on Grande River. It is 1327 km² with a maximum depth of 127m (Ometto et al. 2013). Sampling points were named as S1, S2 and S3 (Figure 1). FNS has a region that is one of Brazil's largest producers of potatoes and coffee (Alfenas, MG), and a heavy pesticide usage is applied there (IGAM 2012). The reservoir is necessary for surrounding municipalities that rely on agriculture (Paulino et al. 2014), and the quality of water is compromised because of this activity (Santos Neto and Siqueira 2005). The water also receives domestic and industrial effluents, despite this, FNS is not

used just for energy production and industrial supply, but also for irrigation, human and animal supply (IGAM 2012).

Figure 1. Sampling points of Furnas reservoir (Google Earth).

2.1.2. Chapéu D'Uvas Reservoir (CDU)

CDU was created in 1995 on Paraibuna River. It is 12 km² with a maximum depth of 41m (Machado 2012). Sampling points were named as S4, S5 and S6 (Figure 2). CDU is located 50 km from the spring and because of this, does not receive so much influence of anthropogenic activities. The water of CDU is vital for human drinking supply, especially to the city Juiz de Fora (Machado 2012; CESAMA - http://www.cesama.com.br/?pagina=chapeu-duvas), with about 500 000 inhabitants. The soil around CDU is vulnerable to erosion (CEIVAP 2006) and is a factor that causes water pollution, especially if the soils contain pollutants (Förstner and Wittmann 1983). Rural areas predominate the catchment of CDU (Machado 2012; IBGE 2010), where problems of basic sanitation can be observed. Then CDU is influenced by domestic effluents but also by livestock farming activities (Machado 2012).

Figure 2. Sampling points of Chapéu D'Uvas reservoir (Google Earth).

2.1.3. Monte Serrat, Bonfante and Santa Fé Reservoirs (MBS)

Monte Serrat, Bonfante and Santa Fé were created in series on Paraibuna River in 2008 and 2009. Sampling points were named as S7 for Monte Serrat, S8 for Bonfante (Figure 3) and S9 for Santa Fé (Figure 4). CDU These reservoirs are used for energy production (Brasil PCH - http://www.brasilpch.com.br/). They are small reservoirs with similar features, being that Santa Fé (2.05 km²) is bigger than Monte Serrat (0.34 km²) and Bonfante (0.2 km²). The dams have a height of 20, 10 and 6m for Monte Serrat, Bonfante, and Santa Fé, respectively. The Paraibuna River flows through the city Juiz de Fora upstream of MBS, which thus may influence the river with industrial and domestic effluents (Jordão et al. 1999; Brasil 2013; Araujo 2015).

Figure 3. Sampling points of Monte Serrat (S7) and Bonfante (S8) reservoirs (Google Earth).

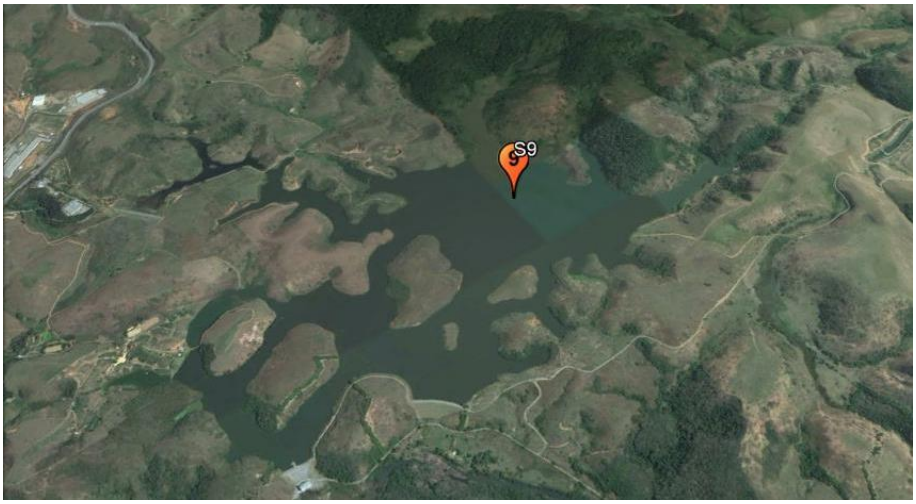

Figure 4. Sampling point of Santa Fé reservoir (S9) (Google Earth).

2.1.4. Funil Reservoir (FUN)

FUN was created in 1969 on Paraiba do Sul River. It is 26km^2 with a maximum depth of 79m (Ometto et al. 2013). Sampling points were named as S10, S11 and S12 (Figure 5). Funil reservoir is located in a densely populated and industrialized area (Soares et al. 2008; Ometto et al. 2013) and is well known because of blooms of toxic cyanobacteria (Soares et al. 2009; Ferrão-Filho et al. 2009). FUN is used for aquaculture, human and energy supply (Branco et al. 2002), and its water quality and availability is

considered to be threatened by industrial and domestic wastewater (Ferrão-Filho et al. 2009).

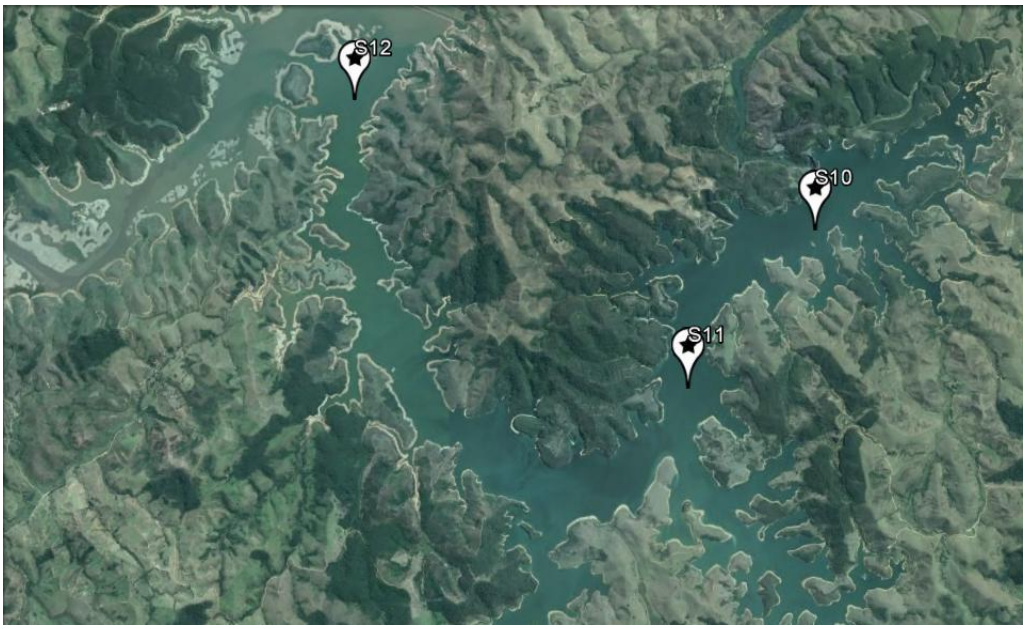

Figure 5. Sampling points of Funil reservoir (Google Earth).

2.2. Sediment sampling and samples treatment

Sampling was carried out during the dry season (September and October) in 2015. Brandão et al. (2011) previously demonstrated that one sampling during this period is sufficient for the diagnosis of the sediment quality. Three cores from each reservoir were taken in sites with highest sedimentation (between an affluent and the main river and near the dam) (Thornton et al. 1990; Brandão et al. 2011). For the MBS group, one core of each reservoir was taken. Sediment samples were taken by using a Kajak-Brinkhurst (K-B) gravity corer and was sectioned into 6-cm intervals (Mudroch and MacKnight 1994). After sampling, the samples were dried at 40°C and placed in airtight plastic bags until analysis.

2.3. Metal determination in sediment samples

A complete profile (all samples from one core) from each site was analyze, and the other two corers was analyze one sample representing surface, middle and bottom of the sediment. Metals are typically associated with clay and silt particles, and to access it the sediment needs to be sieved (Salomons and Förstner 1984). In this way, larger particles were macerated with a pistil of porcelain and sieved with a granulometric sieve

(72µm). Two grams of sediment was weighed and used for metal analysis. The sediment was extracted with hydrochloric acid (0.1M HCl) (Piper 1971; Fiszman et al. 1984; Barreto et al. 2004). This extraction method was selected since it provides information on the available compartment of the metals, which thus is relevant for environmental risk assessment (Adams et al. 1992; Barreto et al. 2004). Other studies also used the HCl extraction method for soils (Wear and Evans 1968; Lagerwerff and Specht 1970; Lagerwerff 1971) and sediments (Zonta et al. 1994; Silva et al. 2002). Accordingly, 20 mL of HCl (Merck, 37%) was added to samples for extraction, and after 24 hours, the samples were filtered using a cellulose filter with grade 42 (2.5µm, Whatman – slow filter paper). All the extractions were carried out in duplicate, including the analytical blanks, which were processed in parallel with the samples.

Metal analysis was performed by using an atomic absorption spectrometer (Varian AAS240FS, Santa Clara, United States), with deuterium background correction. Concentrations of copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn) and iron (Fe) were measured by the direct air-acetylene flame method with a flame atomic absorption spectrometry (FAAS). Standard solutions were prepared using dilutions of standard stock solutions (Merck – 1000 mg L^{-1}). Calibration curves developed for each metal were used to calculate the concentrations, using five points for Cu, Cr, Cd, Pb and Zn and six points for Fe on calibration curves. The limit of quantification (LOQ) was calculated, and recoveries were calculated by using standard addition method (Table 1), following Mitra (2013) and Soares (2016). All laboratory material was pretreated in neutral detergent (5% for 12h, Merck – Extran) and nitric acid (5% for 12h, Merck – HNO_3), and water was provided by a Milli-Q system (18.2Ωm – high purity deionized water). All procedures were used following protocols (APHA 1998; Csuros and Csuros 2002; Mitra 2003; Konieczka and Namiesnik 2009; Crompton 2015).

Table 1. Spectral lines used in emission measurements, limit of quantification (LOQ) and recovery by standard addition method for the elements measured by using FAAS.

Element	Wavelength (nm)	LOQ (µg g-¹, dry weight)	Recovery (%)
Cu	324.7	0.1724	76.47%
Cr	357.9	1.8551	88.55%
Cd	228.8	0.2203	90.56%
Pb	217.0	7.1726	83.77%
Zn	213.9	0.5079	81.18%
Fe	248.3	7.6852	79.98%

Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; LOQ: limit of quantification.

2.4. Geo-accumulation index and enrichment factor methods

Müller formulated the geo-accumulation index (I_{geo}) in 1969, and until today, it is widely used to evaluate changes in background metal concentrations caused by natural or anthropogenic sources. I_{geo} compares current and pre-industrial concentrations, providing the intensity of heavy metal contamination (Müller 1969; Förstner et al. 1990; Loska and Wiechuła 2003; Chai et al. 2016; Pourabadehei and Mulligan 2016; Zhang et al. 2016; Magesh et al. 2017). Background concentrations of heavy metals in the Earth's crust is used as reference values for pre-industrial levels. Loska and Wiechuła (2003), Hanif et al. (2016) and Djonlagic (2016) used background values from shales (Turekian and Wedepohl 1961). Here, concentration in Earth's sediments was used as background values (µg g-¹), provided by (Bowen 1979). The values are similar to Turekian and Wedepohl (1961), but in Bowen (1979) they are slightly lower. I_{geo} was calculated by the formula:

$$I_{geo} = \log_2 \frac{C_n}{1.5B_n}$$

where C_n is the metal concentration in bottom sediment (µg g-¹) and B_n is the metal background (µg g-¹). Factor 1.5 is used to minimize possible variation in background values due to lithogenic effects. The I_{geo} was divided into grades according to Zhang et al. (2016), as shown in Table 2.

Table 2. Categories used for the geo-accumulation index (I_{geo}) (Zhang et al. 2016).

I_{geo}	<0	0–1	1–2	2–3	3–4
Grade	0	1	2	3	4
Level	None	None–Moderate	Moderate	Moderate–Strong	Strong

The enrichment factor (EF) was also used to assess anthropogenic transformations on sediments (Djonlagic 2016). EF reflect the degree of enrichment in sediments by incorporating the concentrations of metals measured during a specified period (Loska and Wiechuła 2003). It is possible to calculated EF also using background values; however, here were used measurements on sites as defined by Zonta et al. (1994) and applied by Loska and Wiechuła (2003). EF was calculated by the equation:

$$EF\% = \frac{C - C_{min}}{C_{max} - C_{min}} \text{ x } 100$$

where C is the metal mean concentration in bottom sediment and C_{min} and C_{max} are the minimum and maximum concentrations determined. The EF was divided into grades according to Zhang et al. (2016), as shown in Table 3.

Table 3. Categories used for the enrichment factor (EF) (Zhang et al. 2016).

EF grades	**1–2**	**2–5**	**5–20**	**20–40**	**>40**
Level	Minimal	Moderate	Significant	High	Extremely high

2.5. Probable effect concentrations and potential ecological risk methods

Probable effect concentration quotient (PEC_Q) was calculated according to Ingersoll et al. (2001). This evaluation is important because environmental samples usually contain complex mixtures of contaminants, and sediment quality guidelines are likely to increase due to a combination of toxicity (Ingersoll et al. 2001). First, a PEC_Q was calculated for each chemical for each sample by dividing the concentration of a chemical by the normalized PEC. Normalized PECs were developed using sediment quality guidelines, and reliable PECs were considered if more than 75% of samples

exceeding the PEC present toxicity (Ingersoll et al. 2001). The reliable dry-weights normalized PECs used here are shown in Table 4. Then, a mean quotient was calculated for each sample by summing the PEC_Q for each chemical and dividing this sum by the number of PECs evaluated. The results were compared to thresholds developed based on the incidence of sediment toxicity for freshwater organisms (Ingersoll et al. 2001). Ingersoll et al. (2001) used reports with survival or growth of *Hyalella Azteca*, *Chironomus tentans* and *C. riparius* exposed to sediments (Ingersoll et al. 2001).

An ecological risk index (RI) was used to evaluate the ecological risk degree quantitatively in aquatic sediments by heavy metals and was originally described by (Håkanson 1980). This evaluation was done in previous studies such as Feng et al. (2016), Islam et al. (2016) and Zhang et al. (2016). Ecological risk index allows describing contamination in quantitative terms, considering more than just concentrations. It is an important diagnostic tool for water pollution and establishes ecological effects (Håkanson 1980). Håkanson calculated the potential ecological risk factor (E_r^i) by the equation:

$$\mathrm{E_r^i} = \mathrm{T_r^i} \times \frac{\mathrm{C^i}}{\mathrm{C_n^i}}$$

where C^i is the metal concentration in sediment, C_n^I is the background concentration and T_r^I is the toxicity factor for metal (Table 4) (Håkanson 1980). Then, RI is the sum of E_r^i for all metals in sediment. The E_r^i and RI were divided into grades according to Håkanson (1980) and Zhang et al. (2016), as shown in Table 5.

Table 4. Normalized PEC (µg g-¹) to calculate probable effect concentration quotient (PEC_Q) and toxicity factor for metal (T_r^I) to calculate potential ecological risk factor (E_r^i) (Håkanson 1980; Ingersoll et al. 2001).

Metals	**Cu**	**Cr**	**Cd**	**Pb**	**Zn**
PEC	149	111	4.98	128	459
$\mathbf{T_r^I}$	5	2	30	5	1

Table 5. Grades used for the potential ecological risks (Zhang et al. 2016).

Potential ecological risk factor (E_r^i)		**Potential ecological risk index (RI)**	
Low	<30	**Low**	<100
Moderate	30-60	**Moderate**	100-200
Considerable	60-120	**High**	200-400
High	120-240	**Very High**	>400
Very High	>240		

2.6. Statistical analysis

Simple statistical measures (mean, standard deviation) were calculated using Excel (Microsoft Office Home and Student 2014). Statistical analysis was made using one-way analysis of variance (ANOVA), followed by a Tukey test, considering p-values less than 0.05 as significant. Linear regression analysis (Pearson's correlation coefficient) was also applied to evaluate the correlation between metals. The statistical analysis was done using the open-source software "RStudio" (version 1.0.44). Graphs were elaborated using JMP® (version 13.0.0).

3. Results and Discussion

3.1. Trace metal concentrations in reservoir sediments

Brazilian resolution (CONAMA 344/2004) takes into account Canadian Environmental Quality Guidelines to decide their maximum permissible values for sediment quality. Because of this, the results was compared to Canadian Environmental Quality Guidelines for the Protection of Aquatic Life (2002), also to background values based on the elemental composition of Earth's sediments (Bowen 1979) and threshold effect concentrations (MacDonald et al. 2000) (Table 6).

Surface sediments provide information about current contamination. The highest surface mean for Cu was found in CDU (12.55 ± 0.7 µg g^{-1}) and lowest in FUN (9.51 ± 0.8 µg g^{-1}). In FNS, surface mean of Cu was 10.81 ± 0.77 µg g^{-1} and 10.08 ± 7.97 µg g^{-1} in MBS (Table 6). There was no significant spatial trend between the reservoirs ($F = 0.737$, $p = 0.549$), which means that sampling points have similar concentrations of Cu for all reservoirs in the surface layer and the overall mean supporting this small variation

(10.74 ± 3 µg g-¹). Considering the general temporal trend between layers and reservoirs was observed a significant difference ($F = 19.388$, $p < 0.001$) that can be observed graphically (Figure 6). It means that concentrations in different sediment layers were significantly different comparing the reservoirs. Cu concentration ranged from 2.54 to 22.29 µg g-¹ and presented a general mean of 10.95 ± 2.52 µg g-¹. Copper is an essential element to organisms but is needed in a small amount, and high levels are quite toxic (Csuros and Csuros 2002). It is used in electric wires, water pipes and boat paints as anti-fouling (Csuros and Csuros 2002; Nikinmaa 2014). Cu is also a residue from industries such as batteries, plastic and synthetic materials, and textiles (Brandão et al. 2011). Cu compounds are used in agricultural fertilizers and veterinary and medical products, being commonly found in the agricultural and domestic effluent (Eisler 2000).

Considering Cr concentrations, the highest surface mean was in MBS (4 ± 0.73 µg g-¹) and lowest in CDU (<LOQ). In FNS, a concentration of 2.54 µg g-¹ of Cr was found in S3, and FUN presented 2.16 ± 0.07 µg g-¹ as surface mean (Table 6). Cr concentration did not present significant difference spatially ($F = 162.48$, $p = 0.055$) and general mean support this small spatial variation (3 ± 0.89 µg g-¹). When the temporal pattern was investigated, it was possible to find a significant difference between the bottom and other layers ($F = 8.690$, $p < 0.001$), which can be observed because of a decrease in the concentrations over time (Figure 7). Cr concentrations ranged from 1.88 to 6.69 µg g-¹, and the overall mean was 3.44 ± 0.97µg g-¹. Chromium (Cr) compounds in the environment are commonly associated to domestic and many industrial effluents, and they have mutagenic properties (Cheng et al. 1998; Eisler 2000; Csuros and Csuros 2002; Chidambaram et al. 2009; Brandão et al. 2011; Eleftheriou et al. 2012).

Even using a moderate acid extraction, always when Cd concentration was higher than LOQ, values exceed at least one of comparison parameters (background and sediment guidelines). Cd concentration is usually found lower than other metals, and even these low levels may present high toxicity to organisms, inclusive can cause DNA damage (Eisler 2000; Lee et al. 2006; Ünyayar et al. 2006; Nikinmaa 2014). The highest mean of Cd in surface layers was in MBS (1.33 ± 0.794 µg g-¹) and lowest in CDU and FUN, that was below LOQ. In FNS, a surface mean of Cd was 0.31 ± 0.01 µg g-¹. The general surface mean of Cd was 0.92 ± 0.64 µg g-¹ (Table 6). The temporal pattern shows a significant difference ($F = 7.862$, $p = 0.007$) between depths, which is explained by higher concentrations found in MBS comparing to other reservoirs (Figure 8). The total mean of Cd was 0.82 ± 0.66 µg g-¹ and ranged from 0.24 to 2.51 µg g-¹. Cd compounds may be

associated with industrial discharges, phosphate fertilizers and household and municipal effluents (Csuros and Csuros 2002; Ünyayar et al. 2006).

For Pb, surface mean was possible to calculated just for FUN (8.47 ± 0.08 µg g-¹), since in the other reservoirs the majority of concentrations remained below LOQ (Table 6). There are many values below the LOQ that hinder the interpretation of statistical data, but it was not found any significant difference spatially ($F = 0.126$, $p = 0.8882$) and temporally ($F = 2.523$, $p = 0.125$) (Figure 9). Pb ranged from 6.94 to 12.1 µg g-¹. Lead was considered one of the most serious pollutants due to its compounds in fuel, but leaded fuel is not used in some countries anymore (Förstner and Witmman 1983; Nikinmaa 2014). In aquatic ecosystems, Pb may appear related to industrial effluents and urban runoff, especially due to the use of paints and batteries that contain lead (US Department of Health and Human Services 1999; Nikinmaa 2014). In freshwater systems, Pb is toxic to all organisms and can affect survival, growth, and reproduction (Eisler 2000).

The highest surface mean of Zn was in MBS (99.93 ± 17.91 µg g-¹) and lowest in FNS (6.65 ± 0.77 µg g-¹). In CDU, Zn surface mean was 10.49 ± 0.14 µg g-¹ and 22.31 ± 5.45 µg g-¹ in FUN (Table 6). Spatially, current concentrations of Zn show that MBS seems to be different from the others statistically ($F = 17.795$, $p = 0.019$) and a high value of standard deviation support this evaluation (34.84 ± 32.54 µg g-¹). Considering the general temporal trend, MBS also seems to be different from the others ($F = 57.92$, $p < 0.001$) (Figure 10). Zn ranged from 5.71 to 190.49 µg g-¹ with a total mean of 31.09 ± 27.86 µg g-¹. Zinc is essential to many enzymes in organisms but becomes toxic at high concentrations (Nikinmaa 2014). Zn pollution is related to domestic effluent, traffic activities, building waste, agriculture effluents and many industries discharges, is primarily used in metal alloys and galvanization (Hüffmeyer et al. 2009; Brandão et al. 2011; Nikinmaa 2014).

The highest surface mean of Fe was found in CDU (7,528.91 ± 194.97 µg g-¹) and lowest in MBS (6,236.1 ± 981.17 µg g-¹). In FNS, surface mean of Fe was 6,450.71 ± 453.82 µg g-¹ and 7,405.92 ± 481.08 µg g-¹ in FUN (Table 6). Spatially, current concentrations of Fe do not present different statistically ($F = 0.397$, $p = 0.703$) and the total mean of surface layers support this evaluation (6,905.41 ± 691.97 µg g-¹). Considering the general temporal trend, the concentrations of Fe was similar in all reservoirs, does not present significant difference temporally ($F = 1.772$, $p = 0.096$) (Figure 11). Fe concentrations range from 3,251.34 to 11,466.89 µg g-¹, and the total

mean was 7,579.51 ± 1,046.62 µg g^{-1}. Iron is the most abundant metal, and anthropic activities that can release iron (Fe) in the environment include industrial effluent, sewage and landfill leachate (Csuros and Csuros 2002; Nikinmaa 2014). Although it is essential for organisms in a significant amount (Nikinmaa 2014), in high concentration can accumulate in muscle and affects the nervous system (Saha and Paul 2016), besides can induce long-term genetic hazards (Rigaud et al. 2012). High concentrations of Fe can naturally occur in the environment; this happens in small boreal lakes that have a naturally high toxic iron level (Nikinmaa 2014). Along with geochemical characteristics, elevated levels of Fe can also be related to inadequate soil management (Bonai et al. 2009).

Table 6. Results of metals analyzed in sediment samples of Brazilian reservoirs presented as mean and standard deviation in sediment samples (µg g^{-1}, dry weight).

Reservoirs	Point	Depth (cm)	Cu (µg g^{-1})	Cr (µg g^{-1})	Cd (µg g^{-1})	Pb (µg g^{-1})	Zn (µg g^{-1})	Fe (µg g^{-1})
FNS	S1	0 to 6	11.3 ± 0.02	< 1.855	0.29 ± 0.03	< 7.173	6.42 ± 0.06	6,535.99 ± 21.71
	S1	45 to 51	12.19 ± 0.04	1.88 ± 0.26	0.31 ± 0.01	< 7.173	6.19 ± 0.2	8,480.97 ± 204.7
	S1	90 to 95	13.96 ± 0.06	3.4 ± 0.28	0.31 ± 0.04	8.53 ± 0.26	6.74 ± 0.0003	7,845.47 ± 129.89
	S2	0 to 6	11.47 ± 0.03	< 1.855	0.32 ± 0.01	< 7.173	5.71 ± 0.73	5,769.97 ± 76.77
	S2	15 to 21	13.18 ± 0.03	2.36 ± 0.28	0.33 ± 0.03	< 7.173	6.79 ± 1.29	7,553.16 ± 28.7
	S2	30 to 36	6.41 ± 0.1	< 1.855	0.43 ± 0.09	< 7.173	9.96 ± 0.18	5,116.17 ± 92.67
	S3	0 to 6	9.66 ± 0.11	2.54 ± 0.05	< 0.220	< 7.173	7.81 ± 0.1	7,046.16 ± 106.56
	S3	6 to 12	10.22 ± 0.07	2.12 ± 0.44	< 0.220	< 7.173	8.56 ± 0.12	7,937.81 ± 85.6
	S3	12 to 18	12.3 ± 0.04	2.2 ± 0.22	< 0.220	< 7.173	8.58 ± 0.03	7,700.66 ± 180.36
	S3	18 to 24	10.41 ± 0.11	2.37 ± 0.07	< 0.220	< 7.173	8.11 ± 0.1	7,552.87 ± 108.37
	S3	24 to 30	9.46 ± 0.08	2.5 ± 0.17	< 0.220	< 7.173	8.47 ± 0.04	7,711 ± 11.99
	S3	30 to 36	9.52 ± 0.06	2.55 ± 0.11	< 0.220	< 7.173	7.64 ± 0.11	7,768.86 ± 196.09
	S3	36 to 42	10.36 ± 0.01	1.94 ± 0.01	< 0.220	< 7.173	7.5 ± 0.08	8,111.32 ± 50.82
	S3	42 to 48	11.58 ± 0.6	2.75 ± 0.61	< 0.220	< 7.173	7.98 ± 0.38	8,226.49 ± 1157.71
	S3	48 to 54	11.57 ± 0.07	3.87 ± 0.32	< 0.220	< 7.173	8.65 ± 0.02	8,031.41 ± 122.02
	S3	54 to 60	10.02 ± 0.03	4.47 ± 0.16	< 0.220	< 7.173	8.5 ± 0.04	8,948.03 ± 96.07
	S3	60 to 66	10.3 ± 0.02	3.55 ± 0.08	< 0.220	< 7.173	8.15 ± 0.04	8,394.16 ± 152.3

	S3	66 to 72	9.99 ± 0.08	3.06 ± 0.14	< 0.220	< 7.173	8.39 ± 0.05	8,149.47 ± 144.23
	S3	72 to 78	9.91 ± 0.05	2.82 ± 0.04	< 0.220	< 7.173	8.29 ± 0.14	8,091.97 ± 36.63
	S3	78 to 84	10.18 ± 0.17	3.54 ± 0.32	< 0.220	< 7.173	8.45 ± 0.14	8,394.97 ± 199.26
	S3	84 to 90	10.04 ± 0.02	3.18 ± 0.001	< 0.220	< 7.173	8.25 ± 0.05	8,481.33 ± 55.73
	S3	90 to 96	10.4 ± 0.09	3.1 ± 0.04	< 0.220	< 7.173	8.43 ± 0.12	8,511.83 ± 224.69
CDU	S4	0 to 6	11.63 ± 0.34	< 1.855	< 0.220	< 7.173	10.28 ± 0.28	7,236.46 ± 215.95
	S4	12 to 18	8.26 ± 0.04	< 1.855	< 0.220	< 7.173	6.61 ± 0.14	7,596.74 ± 33.05
	S4	27 to 33	13 ± 0.01	< 1.855	< 0.220	< 7.173	9.1 ± 0.25	6,248.11 ± 5.78
	S5	0 to 6	13.6 ± 0.15	< 1.855	< 0.220	< 7.173	10.59 ± 0.06	7,589.39 ± 170.01
	S5	6 to 12	14.65 ± 0.06	< 1.855	< 0.220	< 7.173	10.33 ± 0.05	7,311.7 ± 129.04
	S5	12 to 18	13.74 ± 0.11	< 1.855	< 0.220	< 7.173	14.65 ± 0.07	3,251.34 ± 53.79
	S5	18 to 24	14.15 ± 0.002	< 1.855	< 0.220	7.74 ± 0.06	7.81 ± 0.15	3,957.36 ± 2.95
	S6	0 to 6	12.43 ± 0.08	< 1.855	< 0.220	< 7.173	10.59 ± 0.01	7,760.87 ± 41.23
	S6	12 to 18	14.21 ± 0.03	< 1.855	0.28 ± 0.02	< 7.173	11.18 ± 0.01	8,953.77 ± 76.53
	S6	24 to 30	12.87 ± 0.01	< 1.855	0.4 ± 0.01	< 7.173	13.4 ± 0.32	3,310.47 ± 50.8
MBS	S7	0 to 6	4.48 ± 0.29	2.9 ± 0.17	2.51 ± 0.01	< 7.173	126.79 ± 0.69	4,764.34 ± 2366.38
	S7	15 to 21	5.07 ± 1.75	2.91 ± 0.1	1.81 ± 0.04	< 7.173	94.08 ± 0.28	5,777.04 ± 71.06
	S7	30 to 36	22.29 ± 5.16	4.02 ± 0.04	2.38 ± 0.03	< 7.173	190.49 ± 0.92	5,456.37 ± 3846.01
	S8	0 to 6	22.03 ± 0.09	4.27 ± 0.22	0.67 ± 0.06	< 7.173	95.2 ± 0.45	7,390.53 ± 1052.39
	S8	15 to 21	18.08 ± 9.15	4.2 ± 0.11	2.13 ± 0.03	< 7.173	180.29 ± 0.46	7,091.94 ± 152.52

	S8	30 to 36	6.47 ± 0.43	4.67 ± 0.14	2.22 ± 0.01	< 7.173	139.24 ± 66.46	8,514.63 ± 112.64
	S9	0 to 6	3.73 ± 0.32	4.84 ± 0.19	0.8 ± 0.01	< 7.173	77.81 ± 24.21	6,553.43 ± 3050.6
	S9	6 to 12	4.67 ± 1.27	5.32 ± 0.06	0.25 ± 0.04	< 7.173	80.93 ± 0.24	9,951.42 ± 77.98
	S9	12 to 18	12.42 ± 5.72	5.91 ± 0.17	0.24 ± 0.05	< 7.173	62.5 ± 0.12	9,930.91 ± 73.29
	S9	18 to 24	19.69 ± 0.01	6.09 ± 0.25	0.4 ± 0.02	< 7.173	59.04 ± 0.2	11,466.89 ± 501.24
	S9	24 to 30	13.3 ± 8.55	6.45 ± 0.06	0.59 ± 0.09	< 7.173	56.87 ± 1.24	9,778.05 ± 790.84
	S9	30 to 36	3.95 ± 0.1	6.69 ± 0.05	0.32 ± 0.08	< 7.173	31.71 ± 18.28	10,851.26 ± 200.48
	S9	36 to 42	2.54 ± 0.15	5.85 ± 0.05	0.31 ± 0.02	< 7.173	22.28 ± 3.53	6,874.9 ± 37.3
FUN	S10	0 to 6	9.1 ± 0.02	2.12 ± 0.02	< 0.220	8.39 ± 0.37	17.62 ± 0.03	7,338.72 ± 37.6
	S10	27 to 33	10.05 ± 0.2	2.35 ± 0.07	< 0.220	7.91 ± 0.59	20.91 ± 0.35	8,254.98 ± 1180.89
	S10	54 to 60	9.49 ± 0.05	2.95 ± 0.03	< 0.220	9.74 ± 0.08	26.21 ± 0.19	7,643.81 ± 53.6
	S11	0 to 6	8.73 ± 0.13	2.09 ± 0.18	< 0.220	8.55 ± 0.14	18.83 ± 0.14	6,751.5 ± 181.09
	S11	27 to 33	10.37 ± 0.1	3.46 ± 0.05	< 0.220	9.45 ± 0.08	34.08 ± 0.49	7,406.12 ± 615.15
	S11	54 to 60	12.02 ± 0.03	4 ± 0.0003	< 0.220	12.1 ± 0.36	41.97 ± 0.37	7,713.09 ± 96.79
	S12	0 to 6	10.71 ± 0.04	2.27 ± 0.05	< 0.220	< 7.173	30.48 ± 0.31	8,127.55 ± 66.37
	S12	6 to 12	10.4 ± 0.09	2.26 ± 0.05	< 0.220	7.2 ± 0.2	26.62 ± 0.01	7,266.15 ± 11.25
	S12	12 to 18	10.28 ± 0.02	2.44 ± 0.03	< 0.220	8.39 ± 0.21	23.93 ± 0.17	7,461.04 ± 125.11
	S12	18 to 24	11.55 ± 0.26	3.39 ± 0.36	< 0.220	6.94 ± 0.27	30.76 ± 0.32	8,323.36 ± 455.58
	S12	24 to 30	11.17 ± 0.03	3.3 ± 0.02	< 0.220	8.39 ± 0.41	23.13 ± 0.04	7,067.42 ± 124.05
	S12	30 to 36	10.5 ± 0.09	3.17 ± 0.09	< 0.220	8.92 ± 0.7	18.31 ± 0.06	7,855.14 ± 197.39

S12	36 to 42	10.22 ± 0.05	3.12 ± 0.02	< 0.220	7.98 ± 0.3	19.14 ± 0.1	8,949.33 ± 44.26
S12	42 to 48	10.61 ± 0.04	3.28 ± 0.12	< 0.220	8.5 ± 0.13	20.72 ± 0.05	7,991.32 ± 99.61
S12	48 to 54	10 ± 0.09	3.23 ± 0.27	< 0.220	9.76 ± 0.3	17.4 ± 0.2	8,643.22 ± 15.74
BCG	-	33	72	0.17	19	95	4.1%
TEC	-	31.6	43.4	0.99	35.8	121	-
TEL	-	18.7	52.3	0.7	30.2	124	-
PEL	-	197	90	3.5	91.3	315	-

FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; <LOQ = below the limit of quantification; BCG = Background value (Bowen 1979); TEC = Threshold Effect Concentrations (MacDonald et al. 2000); TEL = Threshold Effect Levels (Canadian Environmental Quality Guidelines 2002). Underlined values were higher than at least one of these references (background and/or sediment guidelines).

Table 7. Comparison of the metal concentrations (µg g^{-1}, dry weight) in the six investigated Brazilian reservoirs data from international and national studies.

Place	Cu	Cr	Cd	Pb	Zn	Fe	Reference
Three Gorges Reservoir, China	54.2	NA	0.878	51	174	NA	Bing et al. 2016
Simly Lake, Pakistan	23.4	41	1.55	41	132	6,122	Iqbal et al. 2016
Itá Reservoir, South of Brazil	176	119.5	6.6	17	245.6	95,663.00	Bonai et al. 2009
Macela Reservoir, Northeast of Brazil	34.9	58.1	NA	25.9	75.4	NA	Santos et al. 2013
Paiva Castro Reservoir, Southeast of Brazil (SP)	24.27	26.52	NA	26.75	69.5	46,810.52	Cardoso-Silva et al. 2016
Rasgão Reservoir, Southeast of Brazil (SP)	179	NA	3	92	982	NA	Araújo et al. 2006
Billings Reservoir, Southeast of Brazil (SP)[a]	240	40	2	70	400	70,000	Silva et al. 2002
Furnas Reservoir, Southeast of Brazil (MG)[b]	10.81	2.54	0.31	<LOQ	6.65	6,450.71	This study
Chapéu D'Uvas Reservoir, Southeast of Brazil (MG)[b]	12.55	<LOQ	<LOQ	<LOQ	10.49	7,528.91	This study
Monte Serrat, Bonfante and Santa Fé, Southeast of Brazil (MG / RJ)[b]	10.08	4	1.33	<LOQ	99.93	6,236.10	This study
Funil Reservoir, Southeast of Brazil (RJ)[b]	9.51	2.16	<LOQ	8.47	22.31	7,405.92	This study

Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; NA = not analyzed; <LOQ = below the limit of quantification; SP = São Paulo State; MG = Minas Gerais State; RJ = Rio de Janeiro State; a = graph approach; b = mean values.

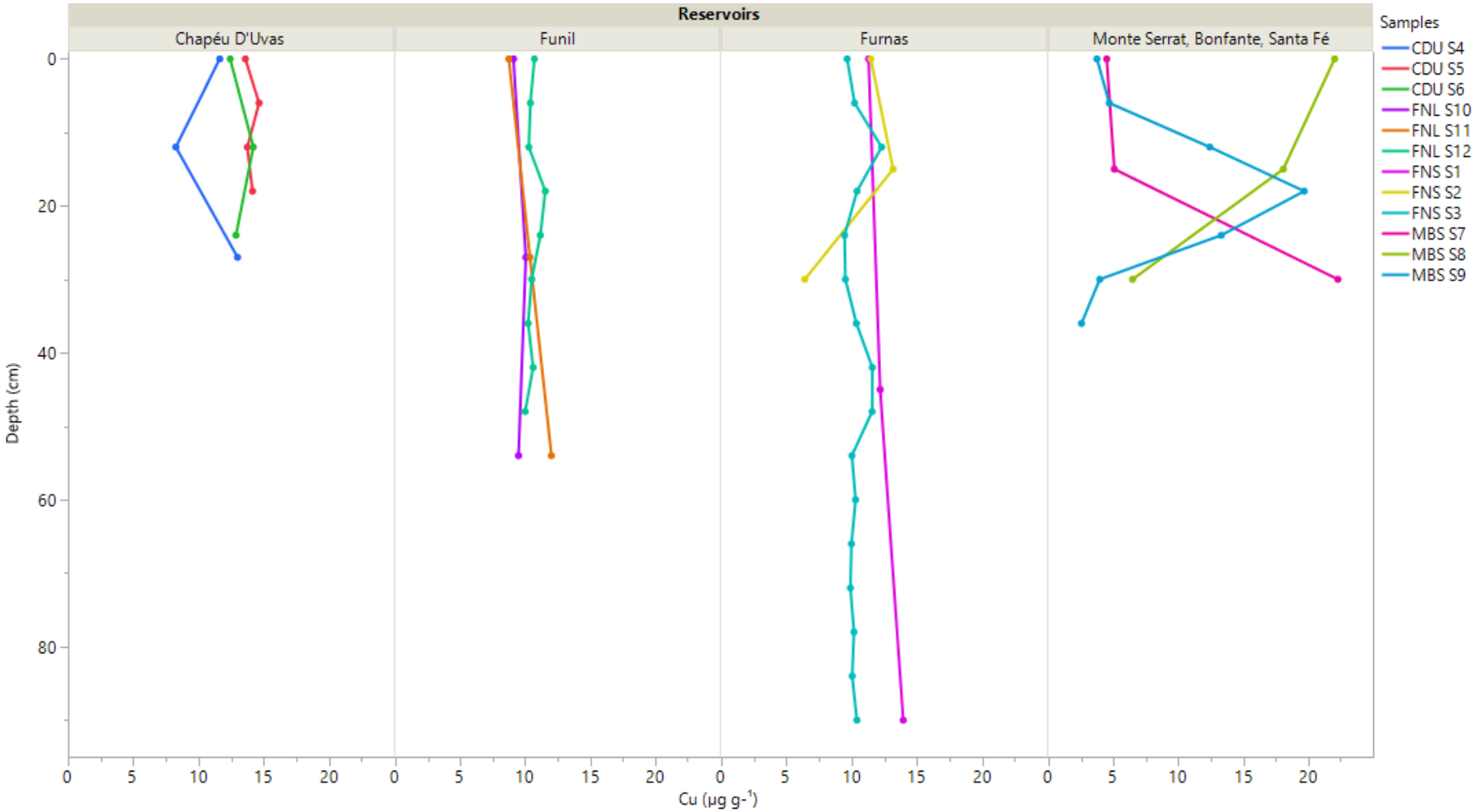

Figure 6. Copper concentration in sediment samples (µg g-¹, dry weight), considering spatial and temporal distribution. FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Cu = copper.

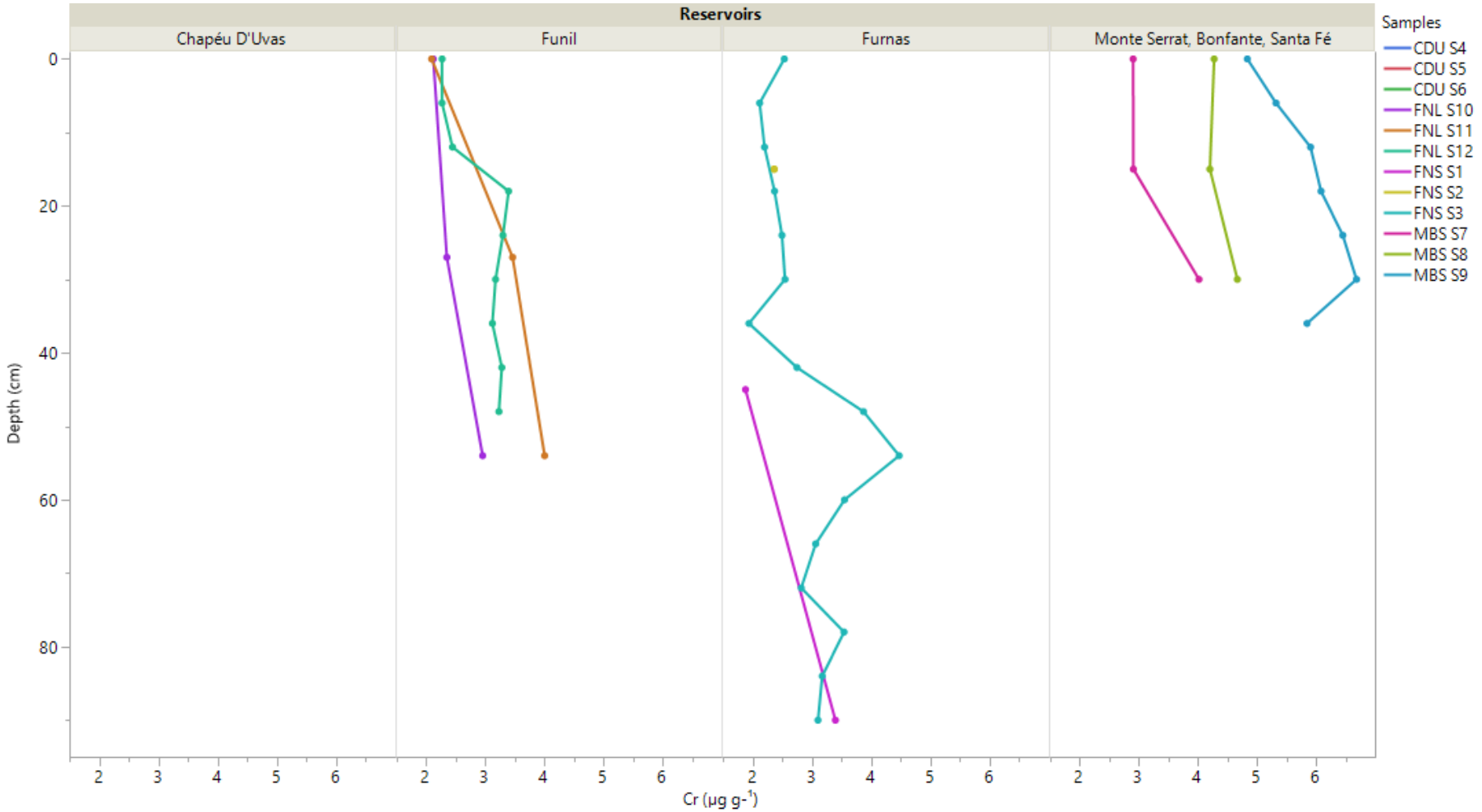

Figure 7. Chromium concentration in sediment samples (µg g-¹, dry weight), considering spatial and temporal distribution. FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Cr = chromium.

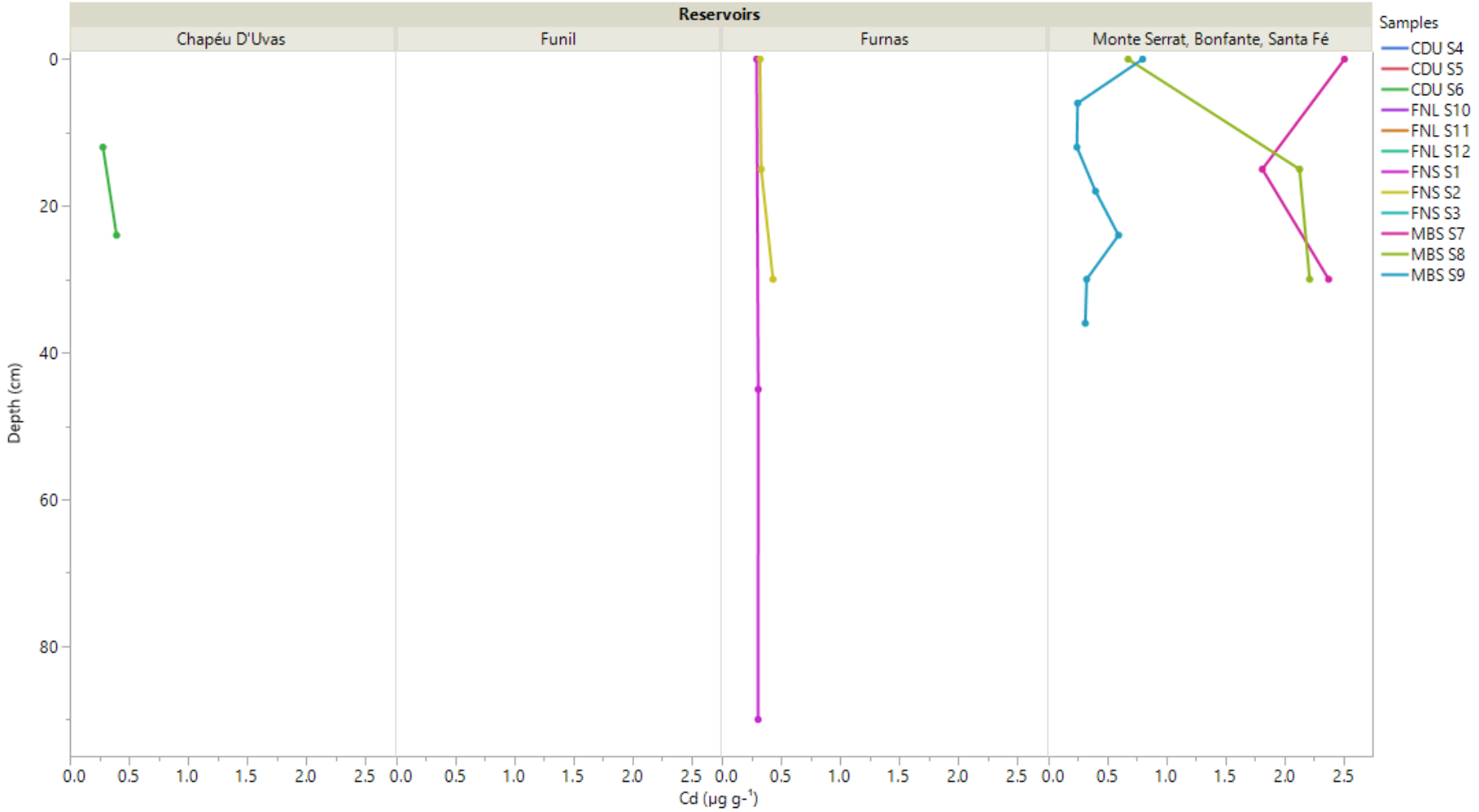

Figure 8. Cadmium concentration in sediment samples (µg g-¹, dry weight), considering spatial and temporal distribution. FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Cd = cadmium.

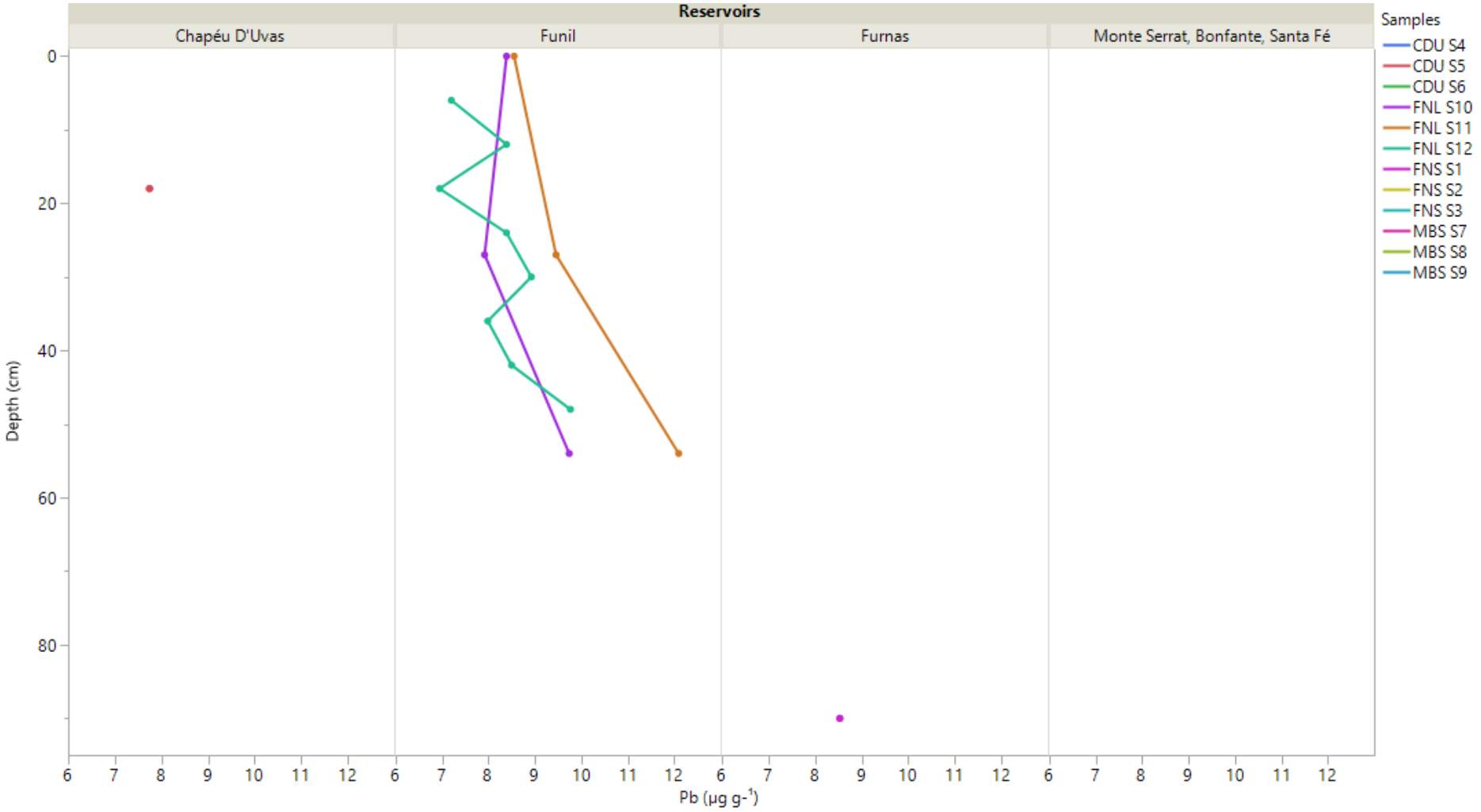

Figure 9. Lead concentration in sediment samples (µg g-¹, dry weight), considering spatial and temporal distribution. FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Pb = lead.

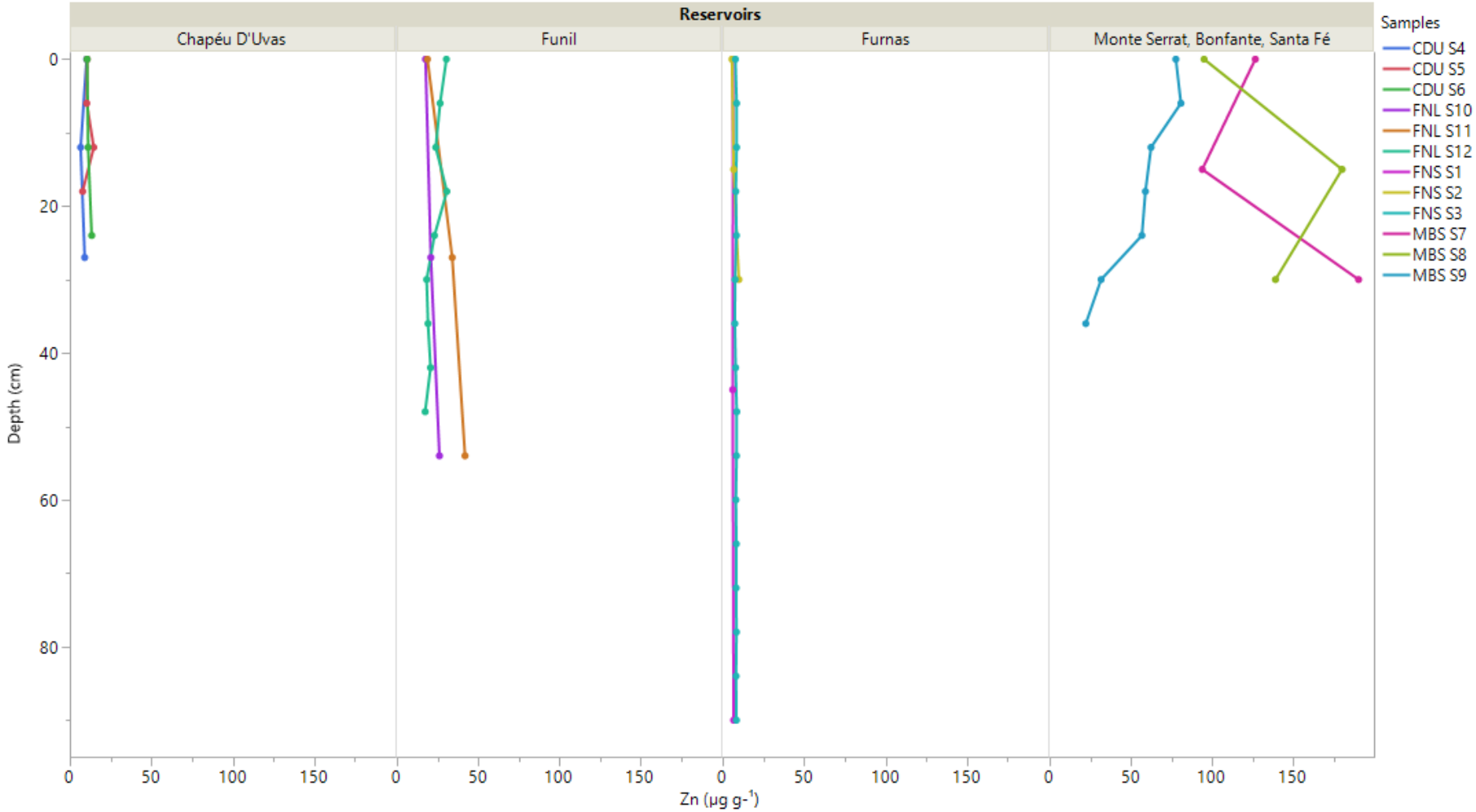

Figure 10. Zinc concentration in sediment samples (µg g-¹, dry weight), considering spatial and temporal distribution. FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Zn = zinc.

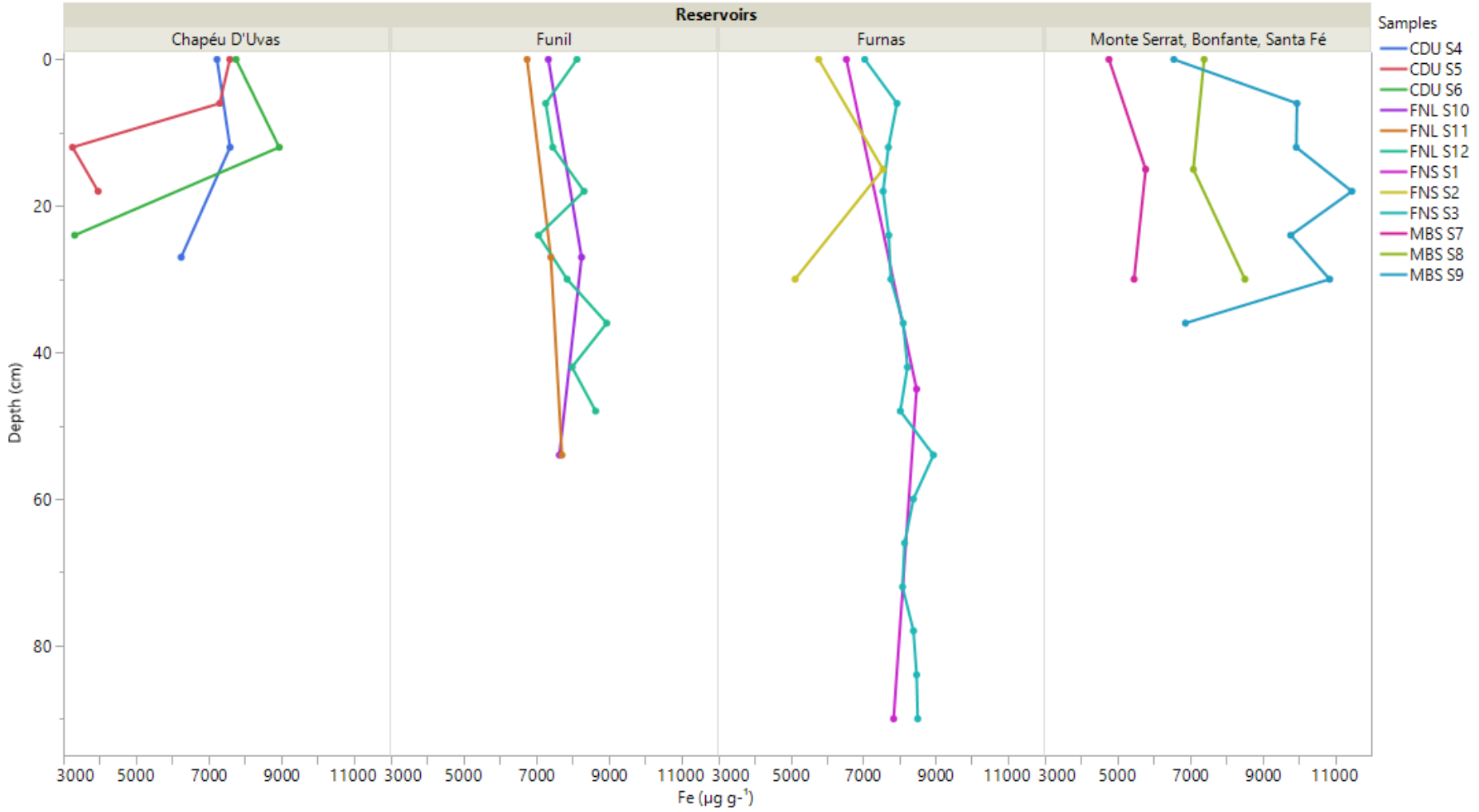

Figure 11. Iron concentration in sediment samples (µg g-¹, dry weight), considering spatial and temporal distribution. FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Fe = iron.

3.1.1. Furnas Reservoir (FNS)

Copper concentration in FNS has a maximum value of 13.96 µg g-¹ represented by S1 and minimum by S2 (6.41 µg g-¹), both in bottom sediments. The total mean found of Cu was 10.66 ± 1.12 µg g-¹. Comparing to bottom sediments, S1 and S3 seems to remain Cu concentration over time, and S2 seems to increase over time. Chromium concentration had a maximum of 4.47 µg g-¹ represented by S3 and minimum by S1 (1.88 µg g-¹), both in the middle layer. Minimum concentration was the lowest value for Cr in all reservoirs. The total mean found for Cr was 2.85 ± 0.56 µg g-¹. Comparing to bottom sediments, S1 and S2 seems to decrease Cr concentration over time, and S3 vary a lot during the time. Cadmium concentration had a maximum value in FNS of 0.43 µg g-¹ represented by bottom sediment in S2. The minimum of 0.29 µg g-¹ of Cd was observed at the surface in S1. The total mean found for Cd was 0.33 ± 0.03 µg g-¹. Comparing to bottom sediments, S1 and S2 of FNS seems remained Cd concentration over time, and all values were below LOQ in S3. Lead was found just in the bottom sediment of S1 (8.53 µg g-¹) in FNS and seems to decrease the concentration over time. Zinc concentration had a maximum value of 9.96 µg g-¹ represented by bottom sediment in S2. The minimum of 5.71 µg g-¹ of Zn was observed at the surface of S2 and was the lowest value found in all reservoirs. The total mean of Zn was 7.89 ± 0.76 µg g-¹. Comparing to bottom sediments, S1 and S3 seems to remained concentration of Zn over time and S2 seems to decrease over time. Iron concentration had a maximum value found in FNS of 8,948.03 µg g-¹ represented by the middle of S3. The minimum of 5,116.17 µg g-¹ of Fe was observed at the bottom of S2. The total mean found of Fe was 7,743.64 ± 633.01µg g-¹. Comparing to bottom sediments, S1 and S3 seems to decrease Fe concentration over time, and S2 seems to increase, being that all samples have a peak in middle layers.

3.1.2. Chapéu D'Uvas Reservoir (CDU)

Cu concentration in CDU had the maximum value of 14.65 µg g-¹ represented by S4 and minimum of 8.26 µg g-¹ by S5, both at middle sediments. The total mean of Cu was 12.85 ± 1.25 µg g-¹. Comparing to bottom sediments, S4 and S6 seems to decrease the Cu concentration over time, and S5 seems to remain over time; was observed a peak in the middle of S6 probably associated with urbanization around the reservoir. Cr

concentration was below LOQ in CDU, that in a good indicator of less anthropogenic influence in this reservoir. Maximum value for Cd found in CDU was 0.4 µg g^{-1} represented by bottom S6 and minimum 0.28 µg g^{-1} was observed at middle S6. The total mean found of Cd in CDU was 0.34 ± 0.06 µg g^{-1}. Comparing to bottom sediments, S6 seems to decrease Cd concentration over time, and S4 and S5 were below LOQ. Lead concentration was found just in bottom sediment of S5 (7.74 µg g^{-1}) in CDU and seems to decrease over time. The maximum value of Zn found in CDU was 14.65 µg g^{-1} represented by the bottom of S5 and the minimum of 6.61 µg g^{-1} was observed at the middle of S4. The total mean found of Zn in CDU was 10.45 ± 1.63 µg g^{-1}. Comparing to bottom sediments, S5 and S6 seems to decrease Zn concentration over time, and S4 seems to increase. The maximum value of Fe found in CDU was 8,953.77 µg g^{-1} represented by middle S6. The minimum of 3,251.34 µg g^{-1} of Fe was observed at bottom S5 and was the lowest seen in all reservoirs. The total mean found of Fe in CDU was 6,321.62 ± 1,703.84 µg g^{-1}. Comparing to bottom sediments, all samples seems to increase Fe concentration over time, being that high values are presented in the middle of S5 and S6, being almost two times greater than bottom sediments.

3.1.3. Monte Serrat, Bonfante and Santa Fé Reservoirs (MBS)

The higher value found in MBS for Cu was 22.29 µg g^{-1} represented by bottom sediment in S7 (Bonfante Reservoir) and was the higher value found for Cu in all reservoirs. The minimum of 2.54 µg g^{-1} of Cu was observed at the bottom of S9 and was the lowest value found by Cu in all reservoirs. The total mean found of Cu in MBS was 10.67 ± 6.74 µg g^{-1}. Comparing to bottom sediments, S7 and S9 seems to decrease Cu concentration over time, being that S9 presented a peak in the middle and S8 increase over time. Cr concentration had a maximum of 6.69 µg g^{-1} in MBS represented by bottom sediment in S9 (Santa Fé Reservoir) and was the highest value found by Cr in all reservoirs. The minimum of 2.9 µg g^{-1} of Cr was observed at the surface of S7. The total mean of Cr found in MBS was 4.93 ± 1.03 µg g^{-1}. Comparing to bottom sediments, Cr concentration in S7 seems to decrease over time, and S8 and S9 remained over time. The maximum value found for Cd in MBS was 2.51 µg g^{-1} represented by surface S7 (Monte Serrat Reservoir) and was the highest value found by Cd in all reservoirs. The minimum 0.24 µg g^{-1} was observed at the middle of S9 and was the lowest value found by Cd in all reservoirs. The total mean of Cd found in MBS was 1.13 ± 0.83µg g^{-1}. Comparing to

bottom sediments, S7 remained Cd concentration over time, S8 decrease over time and S9 of MBS seems to increase over time. For Lead, all samples remained below LOQ in MBS. The maximum value found for Zn was 190.49 µg g-¹ represented by bottom sediment in S7 (Bonfante Reservoir) and was the higher value found by Zn in all reservoirs. The minimum of 22.28 µg g-¹ of Zn was observed at the bottom of S9. The total mean found for Zn in MBS was 93.63 ± 40.66 µg g-¹. Comparing to bottom sediments, S7 and S8 seems to decrease Zn concentration over time, being that in S8 a peak occurs in the middle of the core, and S9 seems to increase. The maximum value found for Fe in MBS was 11,466.89 µg g-¹ represented by middle in S9 (Santa Fé Reservoir) and was the higher value found by Fe in all reservoirs. The minimum of 4,764.34 µg g-¹ of Fe was observed at the surface in S7. The total mean of Fe was 8,030.90 ± 1,893.50 µg g-¹. Comparing to bottom sediments, S7 and S8 seems to decrease Fe concentration over time, and S9 concentration of Fe remained over time, but a peak occurs in the middle of the core.

3.1.4. Funil Reservoir (FUN)

The maximum value found for Cu in FUN was 12.02 µg g-¹ and the minimum 8.73 µg g-¹, both represented by S11, bottom and surface, respectively. The total mean found for Cu in FUN was 10.35 ± 0.61 µg g-¹. Comparing to bottom sediments, S10 and S12 seems to remain Cu concentration over time and S11 decrease over time. The maximum value found for Cr in FUN was 4 µg g-¹ and the minimum 2.09 µg g-¹, both represented by S11, bottom and surface, respectively. The total mean found for Cr in FUN was 2.90 ± 0.51 µg g-¹. Comparing to bottom sediments, all samples for Cr seems to decrease over time, being that in S12 appears a peak in the middle. For Cd, all values were below LOQ. For Pb, a maximum of 12.1 µg g-¹ was found in the bottom layer of S11 and minimum of 6.94 µg g-¹ in middle S12. The total mean of Pb was 8.73 ± 0.90 µg g-¹. Concentrations of Pb seems to decrease over time in all samples. The maximum value found for Zn in FUN was 41.97 µg g-¹ and the minimum 17.4 µg g-¹ represented by bottom layers, S11 and S12, respectively. The total mean of Zn found was 24.67 ± 5.61 µg g-¹. Comparing to bottom sediments, S10 and S11 seems to decrease Zn concentration, and S12 appears to increase. The maximum value found for Fe in FUN was 8,949.33 µg g-¹ represented by middle of S12 and the minimum 6,751.50 µg g-¹ represented by the surface of S11. The total mean found for Fe in FUN was 7,786.18 ± 485.55 µg g-¹.

Comparing to bottom sediments, Fe concentration in S11 seems to decrease over time and S10, and S12 remained over time.

3.2. Exploring specific sources and risk discussion by isolated metals

For Cr, Pb, and Fe, all samples remained lower than background and threshold values, that means values rarely associated with biological effects, and isolated in the environment seems not to be a problem in reservoirs studied. For Cu, Cd and Zn it was observed some exceptions that exceeded the references used, especially in MBS group. Values found for Cd exceeded background values in FNS and CDU, but probably just not represent a problem to biota. Nevertheless, we always need to consider the effects of the mixture, because any contaminant is isolated in the environment. However, in MBS group, current contamination can be observed for Cu, Cd, and Zn. In Monte Serrat, current contamination for Cd and Zn since concentrations exceed TEC that means that adverse effects occasionally occur. In Bonfante, Cu exceeds TEL while Cd and Zn just the background value. In Santa Fé, Cd also exceeds TEL.

None geo-accumulation was observed in all reservoir for Cu, Cr, Pb and Zn (Table 8). For Cd, FUN presented none geo-accumulation, while FNS presented grade 1 in S1 and S2, CDU showed grade 1 in S6, and moderate geo-accumulation was observed in MBS. In Monte Serrat, surface layer exhibits a strong geo-accumulation (Table 9). In FNS, an extremely high enrichment factor was found for Cu, Cr, Cd, Zn, and Fe. Non-detection of Pb in FNS is an indication that this metal is not a problem. In CDU, an extremely high enrichment factor was found for Cu, Cd, Zn, and Fe. Non-detection of Cr in CDU is a good indication that human influences are not evident there since Cr is associated with many industries effluents (Brandão et al. 2011). In FUN, an extremely high enrichment factor was found for Cu, Cr, Pb, Zn, and Fe. Non-detection of Cd in FUN is an indication that this metal is not a problem. In MBS, an extremely high enrichment factor was found for Cu, Cr, Cd, Zn, and Fe (Table 9).

Table 8. Geo-accumulation index (I_{geo}), enrichment factor (EF), probable effect concentration quotient (PEC_Q) and potential ecological risk index (RI) of sediment samples by reservoirs.

		FNS	CDU	MBS	FUN
I_{geo}	**Cu**	-2.23	-1.96	-2.60	-2.26
	Cr	NA	NA	-4.50	-5.25
	Cd	NA	NA	1.61	NA
	Pb	NA	NA	NA	NA
	Zn	-4.19	-3.80	-0.84	-2.58
EF	**Cu**	51.04	79.46	39.97	53.80
	Cr	52.83	NA	51.34	68.24
	Cd	55.03	100.00	61.39	NA
	Pb	NA	NA	NA	69.64
	Zn	62.80	43.45	56.51	45.28
	Fe	53.28	21.99	32.59	56.81
Mixture	**PEC_Q**	0.04	0.06	0.14	0.05
	RI	15.44	14.18	201.48	4.05

FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; NA = not analyzed due many samples below the limit of quantification.

Table 9. Geo-accumulation index (I_{geo}), probable effect concentration quotient (PEC_Q) and potential ecological risk index (RI) of each sediment sample.

Reservoirs	Point	Depth (cm)	I_{geo} Cu	I_{geo} Cr	I_{geo} Cd	I_{geo} Pb	I_{geo} Zn	RI	PEC_Q
FNS	S1	0 to 6	-2.13	NA	0.20	NA	-4.47	0.00	0.05
	S1	45 to 51	-2.02	-5.84	0.27	NA	-4.52	56.26	0.04
	S1	90 to 95	-1.83	-4.99	0.27	-1.74	-4.40	58.61	0.05
	S2	0 to 6	-2.11	NA	0.34	NA	-4.64	58.88	0.05
	S2	15 to 21	-1.91	-5.51	0.38	NA	-4.39	60.87	0.05
	S2	30 to 36	-2.95	NA	0.76	NA	-3.84	77.35	0.05
	S3	0 to 6	-2.36	-5.41	NA	NA	-4.19	1.62	0.03
	S3	6 to 12	-2.28	-5.67	NA	NA	-4.06	1.70	0.04
	S3	12 to 18	-2.01	-5.62	NA	NA	-4.05	2.02	0.04
	S3	18 to 24	-2.25	-5.51	NA	NA	-4.14	1.73	0.04
	S3	24 to 30	-2.39	-5.44	NA	NA	-4.07	1.59	0.03
	S3	30 to 36	-2.38	-5.41	NA	NA	-4.22	1.59	0.03
	S3	36 to 42	-2.26	-5.80	NA	NA	-4.25	1.70	0.03

	S3	42 to 48	-2.10	-5.30	NA	NA	-4.16	1.92	0.04
	S3	48 to 54	-2.10	-4.80	NA	NA	-4.04	1.95	0.04
	S3	54 to 60	-2.31	-4.59	NA	NA	-4.07	1.73	0.04
	S3	60 to 66	-2.26	-4.93	NA	NA	-4.13	1.75	0.04
	S3	66 to 72	-2.31	-5.14	NA	NA	-4.09	1.69	0.04
	S3	72 to 78	-2.32	-5.26	NA	NA	-4.10	1.67	0.04
	S3	78 to 84	-2.28	-4.93	NA	NA	-4.08	1.73	0.04
	S3	84 to 90	-2.30	-5.09	NA	NA	-4.11	1.70	0.04
	S3	90 to 96	-2.25	-5.12	NA	NA	-4.08	1.75	0.04
CDU	S4	0 to 6	-2.09	NA	NA	NA	-3.79	1.87	0.05
	S4	12 to 18	-2.58	NA	NA	NA	-4.43	1.32	0.03
	S4	27 to 33	-1.93	NA	NA	NA	-3.97	2.06	0.05
	S5	0 to 6	-1.86	NA	NA	NA	-3.75	2.17	0.06
	S5	6 to 12	-1.76	NA	NA	NA	-3.79	2.33	0.06
	S5	12 to 18	-1.85	NA	NA	NA	-3.28	2.24	0.06
	S5	18 to 24	-1.81	NA	NA	-1.88	-4.19	4.26	0.06
	S6	0 to 6	-1.99	NA	NA	NA	-3.75	1.99	0.05
	S6	12 to 18	-1.80	NA	0.14	NA	-3.67	51.69	0.06
	S6	24 to 30	-1.94	NA	0.63	NA	-3.41	71.87	0.06
MBS	S7	0 to 6	-3.47	-5.22	3.30	NA	-0.17	445.44	0.21

	S7	15 to 21	-3.29	-5.22	2.83	NA	-0.60	322.03	0.16
	S7	30 to 36	-1.15	-4.75	3.22	NA	0.42	425.09	0.27
	S8	0 to 6	-1.17	-4.66	1.40	NA	-0.58	123.51	0.13
	S8	15 to 21	-1.45	-4.68	3.06	NA	0.34	380.78	0.24
	S8	30 to 36	-2.94	-4.53	3.12	NA	-0.03	393.70	0.21
	S9	0 to 6	-3.73	-4.48	1.65	NA	-0.87	142.42	0.10
	S9	6 to 12	-3.40	-4.34	-0.05	NA	-0.82	45.27	0.08
	S9	12 to 18	-1.99	-4.19	-0.08	NA	-1.19	45.19	0.08
	S9	18 to 24	-1.33	-4.15	0.64	NA	-1.27	74.11	0.10
	S9	24 to 30	-1.90	-4.06	1.22	NA	-1.33	107.68	0.10
	S9	30 to 36	-3.65	-4.01	0.34	NA	-2.17	58.22	0.06
	S9	36 to 42	-4.29	-4.21	0.29	NA	-2.68	55.80	0.05
FUN	S10	0 to 6	-2.44	-5.67	NA	-1.76	-3.02	3.83	0.05
	S10	27 to 33	-2.30	-5.52	NA	-1.85	-2.77	3.89	0.05
	S10	54 to 60	-2.38	-5.19	NA	-1.55	-2.44	4.36	0.06
	S11	0 to 6	-2.50	-5.69	NA	-1.74	-2.92	3.83	0.05
	S11	27 to 33	-2.26	-4.96	NA	-1.59	-2.06	4.51	0.06
	S11	54 to 60	-2.04	-4.75	NA	-1.24	-1.76	5.56	0.08
	S12	0 to 6	-2.21	-5.57	NA	NA	-2.22	2.01	0.05
	S12	6 to 12	-2.25	-5.58	NA	-1.99	-2.42	3.81	0.05

S12	12 to 18	-2.27	-5.47	NA	-1.76	-2.57	4.08	0.05
S12	18 to 24	-2.10	-4.99	NA	-2.04	-2.21	3.99	0.06
S12	24 to 30	-2.15	-5.03	NA	-1.77	-2.62	4.23	0.06
S12	30 to 36	-2.24	-5.09	NA	-1.68	-2.96	4.22	0.05
S12	36 to 42	-2.28	-5.11	NA	-1.84	-2.90	3.94	0.05
S12	42 to 48	-2.22	-5.04	NA	-1.75	-2.78	4.15	0.05
S12	48 to 54	-2.31	-5.06	NA	-1.55	-3.03	4.36	0.05

FNS = Furnas Reservoir; CDU = Chapéu D'Uvas Reservoir; MBS = Monte Serrat, Bonfante and Santa Fé Reservoirs; FUN = Funil Reservoirs; Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; NA = not analyzed due many samples below the limit of quantification.

These results show that an anthropic influence in all reservoirs exists, presenting similar sources of pollution, and point sources of metals are not evident. It is important to note that I_{geo} takes into account the background value from sediments of Earth, while EF considers just our findings on sites, and because of this different results were obtained from these indexes. There are several sources of metals in the environment, which includes natural and anthropogenic sources (Barbosa et al. 2010; Pohren et al. 2013; Saha and Paul 2016). Among natural sources, the main ones are atmospheric deposition and weathering of soils and rocks (Saha and Paul 2016; Subha et al. 2016). On the other hand, anthropogenic sources are associated with urbanization and industrialization (Saha and Paul 2016), consisting in agricultural, municipal, housing or industrial effluents (Förstner and Wittmann 1983; Salomons et al. 1995; Geras'kin et al. 2011). Then, the presence of metals in study areas may be related to anthropogenic activities around the reservoirs. However, in general, the concentrations found in all samples were lower than background value for sediments (Bowen 1979), and threshold levels by MacDonald et al. (2000) and Canadian Environmental Quality Guidelines (2002).

Current contamination indicates higher means of Cu and Fe in CDU, Cr, Cd and Zn in MBS, and Pb in FUN, when comparing to other reservoirs studied. In FNS, metals concentration, in general, seems to decrease or remained the same over time, being that increases were found in S2 for Cu and Fe. In CDU, many samples were below LOQ, being that increases were found in S4 for Zn and Fe and S5 for Fe, but majority decrease over time. In MBS, increases were observed in S8 for Cu and in S9 for Cd and Zn. In FUN, an increase occurred just in S12 for Zn, and all the rest remained or decrease over time.

In FNS, current contamination, enrichment factor, geoaccumulation and increases of metal concentrations over time can be associated to steel, tannery, fertilizer, textile, and wastewater treatment plant effluents from affluent of the reservoirs (Cavalcanti et al. 2014). Besides the industrial effluents, sewage inflow in several places also can increase metals input on the reservoir, and tourism plays an important role on this (Nogueira et al. 2009; Santos et al. 2011). However, the most significant threatening factor of environmental balance is agriculture in FNS, which covers over 32% of the total area. Riparian forests in FNS were largely replaced by agricultural activities, mainly coffee and pasture. Pastures on the reservoir cover 28% of the total area (Santos et al. 2011). Similar concentrations of metals found between sampling points and over time is associated to the predominance of certain cultures on the reservoir (Sadauskas-Henrique et al. 2011).

According to Alvim et al. (2011), textile effluents from a tributary of FNS presented cytogenotoxicity, indicating that water quality can be compromised. Another study in FNS found compounds of organophosphorus pesticides in 10% of samples that can be associated with pesticides use in coffee crops around the reservoir (Santos Neto and Siqueira 2005). Then, although our findings did not present high values of metals in FNS sediments, studies should be continued there, mainly because Cd was detected greater than background in two sampling points.

In CDU, current contamination, enrichment factor, geoaccumulation and increases of metals concentrations over time can be associated with deforestation and predatory agriculture. These activities lead to erosion of soil, and geological formation in CDU presents metal such as Cu, Pb, and Zn that are carried to the reservoir (Ribeiro 2012; Ribeiro and Leal 2012). Pastures in this reservoir cover 64.63% of the total cachtment area, forests 28.43%, silviculture 3.19% and exposed Soil 1.38% (Machado 2012). Around the reservoir, there is a predominance of rural areas, with small properties that do not have an adequate sanitary system, and because of this, domestic effluents also may impair the water quality of the reservoir (Machado 2012; Ribeiro 2012; Ribeiro and Leal 2012). According to Machado (2012), the area near S6 concentrates most of the basin population (43.2%) and probably affected the geo-accumulation of Cd in the system.

In MBS group, current contamination, enrichment factor, geoaccumulation and increases of metals concentrations over time are mainly associated to effluents from Juiz de Fora city. Approximately 70% of Paraibuna River pass by Juiz de Fora city and receiving domestic and industrial effluents, including a textile and tannery effluents, which is an important sector of the city (Jordão et al. 1999; Brasil 2013; Araujo 2015). However, it is important to confirm if the problem is associated with Juiz de Fora, being an issue for the river after pass through there. Different from presented by Sendacz et al. (2005), the cascade reservoirs studied does not exhibit a decrease of pollutants throughout its spatial sequence. The first of them (Monte Serrat) it is not working as chemostat, may be because they are quite small and river current carries metals.

In FUN, current contamination, enrichment factor and increases of metals concentrations over time are associated with population growth and industrial and agricultural effluents, following by deforestation (Vidal 2012; Souza Lima et al. 2016). Domestic effluents are discharged into the reservoir via various points, even by rural area, which does not have an adequate basic sanitation (Souza Lima et al. 2016). Pastures dominated the land use in the reservoir, covering 62% of the total area, followed by forest

(26.85%), silviculture (4.8%), urban areas (2.27%), agriculture (1.15%) and exposed soil and mining (0.78%) (Souza Lima et al. 2016). The reservoir is located in a highly populated and industrialized area, also with the agriculture of irrigated rice and the use of fertilizers and pesticides are threatening the water quality of the reservoir (Souza Lima et al. 2016). Matos et al. (2014) found toxicity in water and sediment samples from Funil Reservoir, and they associated it to present of cyanobacteria blooms, surfactants, and metals, especially copper. In FUN, previous studies found toxic cyanobacteria (Panosso et al. 2003) and an excess of nutrients (Souza Lima et al. 2016). Then, monitoring programs should continue there due to its importance for human supply and fishery production, and possibly synergistic effects can occur due to various pollutants.

Although using a moderate acid extraction, samples were collected during a dry period, when the water column present a decrease and pollutants are more concentrated, and interaction between sediment and water is more evident (Thornton 1990; Brandão et al. 2011). Comparing with other studies it is possible to observe that our results are lower than others for Cu, Cr, Pb, and Fe, including ones that use similar extraction methodology (Silva et al. 2002; Araújo et al. 2006) (Table 7). MBS group shows Cd concentrations similar to Billings reservoir in deeper layers. Billings is located in Sao Paulo state and is known as a polluted environment (Araújo et al. 2006). Including other comparisons show that the cadmium values in MBS can be worrisome since the values in other studies are not higher even using much stronger extractions. Concern about cadmium increases because can be a problem inclusive in areas that concentrations is not considered dangerous. Cd seems to accumulate in organisms reaching measurable amounts even in these places (Eisler 2000). The global mean for Zn in MBS was much higher than other studied reservoirs (Table 7) and higher inclusive comparing to studies that used an acid extraction stronger than did in this study. Effects of mixtures are known for zinc, such as the combination of copper that is more-than-additive in toxicity to various aquatic organisms (Eisler 2000; Csuros and Csuros 2002). Then, although it is the second most abundant element in organisms, it is essential to control zinc sources in aquatic systems.

FNS, MBS, and FUN are reservoirs used to produce energy, and although this use does not change water quality, it is crucial to supervise the degradation around the reservoirs to protect aquatic resources. Around reservoirs usually occur an increase of urbanization as consequence of economic development and domestic, industrial and agricultural effluents are discharged into water bodies and affect the ecosystem balance (Paulino et al. 2014). Although CDU is not used for energy production, the concern

cannot be less than others; otherwise, this reservoir is widely used for human supply and must be continuously monitored, as well as implement measures to control sources of pollution and preservation of surrounding area. Generally, it is hard to define a source of metal pollution in sediments (Förstner and Witmman 1983). In this study, anthropogenic sources of metals in the sediments are mainly related to agricultural, domestic and industrial effluents, as well as atmospheric deposition. Cu, Cd, and Zn are metals associated with fertilizers and pesticides, then, high concentrations of them in the environment are usually related to farming activities (Förstner and Witmman 1983; Bonai et al. 2009). In this study, these metals are found in higher concentration than others, and because of this, it is possible to observe an influence of agricultural activities on studied reservoirs. As a whole, Cd is a priority concerned metal in the sediments of reservoirs (excluding FUN), especially if the reservoir is used for human supply or fishery production. However, a long-term and more sites monitoring of metals in sediments is recommended to reveal the effects on the aquatic ecosystems comprehensively. Continued monitoring of metals in these reservoirs is crucial to preserve resources and reduce emission sources is necessary. Metal pollution can cause lethal effects in aquatic organisms, but how they are continuously discharged into the environment, they also lead chronic effects, such as for disturb on growth and reproduction (Zhou et al. 2008).

3.3. Ecological risk assessment

In general, PEC_Q evaluation showed that samples presented values lower than 0.1, that means the lowest incidence of toxicity to *Hyalella Azteca* and *Chironomus* spp. as showed by Ingersoll et al. (2001). Monte Serrat and Bonfante reservoirs showed a PEC_Q evaluation between 0.1 and 0.5, that present increased incidence of toxicity on organisms comparing to the first value (Ingersoll et al. 2001).

Potential ecological risk factor (E_r^i) evaluation showed that for Cu, Cr, Pb, and Zn was low, but for Cd goes to moderate until very high. The only reservoir that do not present high E_r^i was FUN. In S1 of FNS, moderate E_r^i was observed for Cd, while S2 present considerable E_r^i in bottom sediment and moderate in surface and medium layers. In S6 of CDU, considerable E_r^i was observed at bottom sediment and moderate E_r^i in the medium layer, being that surface present low E_r^i. In MBS, very high E_r^i was observed in all samples from S7 and medium and bottom layers from S8, while surface layer from S8

presents considerable E_r^i. In S9 of MBS, surface layer presents high E_r^i, while others vary from moderate to considerable (Table 9).

Potential ecological risk index (RI) was low in FNS, CDU, and FUN, while MBS seems to be susceptible to ecological risks by metal contamination. The general mean of MBS showed a moderate ecological risk, but in some samples reach very high risk. In S7 from MBS, a very high risk is observed in the surface layer, as well as bottom sediment, while in the middle layer a high risk was observed. In S8, a moderate risk was found in the surface layer and middle and bottom present high risk. In S9, moderate risk is also found in the surface layer, while others vary from low to moderate (Table 9).

Metals in environmental samples never are isolated (Chu and Chow 2002), and the possibility of synergy effects always exist, especially in sediments that usually have a complex mixture of pollutants. As showed by Chu and Chow (2002), cadmium, copper, and chromium increased mortality rate in combination; lead and cadmium, as well as lead and copper, presented synergistic effect on lethality assay with *Caenorhabditis elegans*. Fleeger et al. (2007) showed that a mixture of metals and polynuclear aromatic hydrocarbon are greater-than-additive (synergistic) toxicity in tests with benthic copepods. Because of this evidences, it is crucial to continue with research about potential ecological risk and mixtures toxicity.

Our findings point out the need for further research and monitoring chemicals in sediments of these reservoirs to protect aquatic organisms and human health, once humans use these systems as a source of water and food. Also, it is important to combine methods with chemical analysis to find out ecological risk by a combination of chemicals. These results indicate a requirement to control human impacts on basins, decreasing sources of pollution as well as deforestation and intensive farming, besides investments in the sanitation sector.

3.4. Exploring the biogeochemistry

A significant Pearson's correlation coefficient was found between Cr and Pb ($t = 2.208$, $p = 0.04582$), Cr and Zn ($t = 2.849$, $p = 0.006$), Cr and Fe ($t = 3.76$, $p < 0.001$), and, Cd and Zn ($t = 7.794$, $p < 0.001$) (Figure 12). Based on literature, cadmium and zinc are usually associated in the environment (Förstner and Witmman 1983; Nikinmaa 2014), which corroborates with our data. Nadeem-ul-Haq et al. (2009) also found a significant and positive correlation between Cr and Pb in ground water in Pakistan. These

correlations show that probably similar sources of metals are influencing the reservoirs studied and sources are based on agricultural, domestic and industrial effluents, as well as atmospheric deposition.

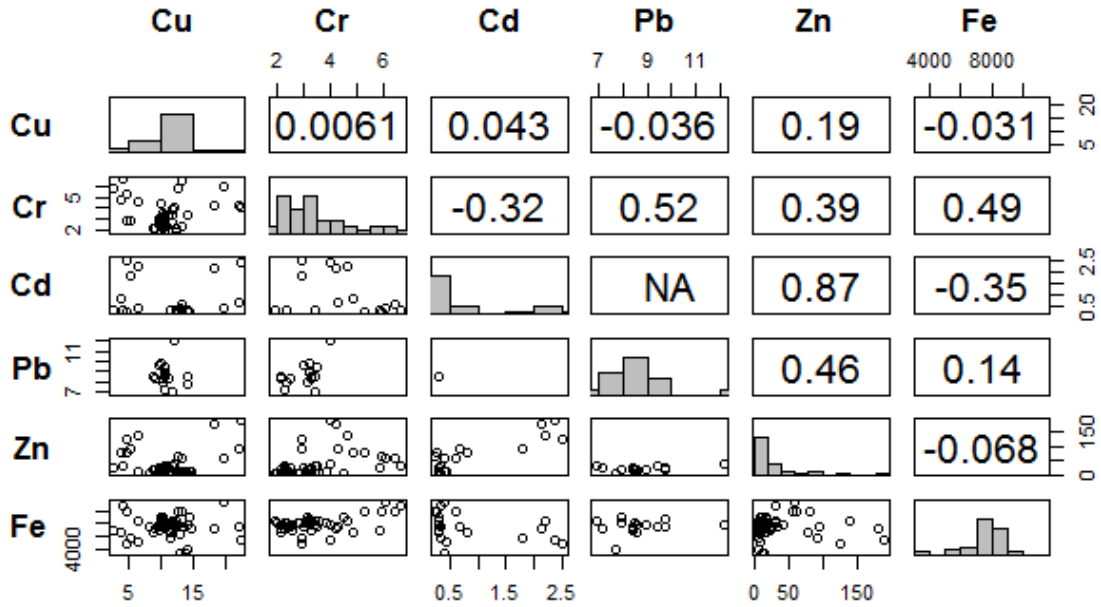

Figure 12. Graphical presentation of Pearson's correlation coefficient with the correlation of metals in all sediment samples. Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; NA = not analyzed due to many samples below the limit of quantification.

4. Conclusions

Almost all sediment samples were higher than LOQ for metals analyzed (Cu, Cr, Cd, Pb, Zn and Fe) and lower than background and threshold values. Current contamination was observed in FNS for Cd and in MBS for Cu, Cd, and Zn. The high values were seen in MBS probably relate to effluents from Juiz de Fora city. In general, geo-accumulation was considerable just for Cd in FNS and MBS; however, enrichment factor was high in almost all reservoirs for all metals, with an exception for Cr in CDU, Cd in FUN and Pb in FNS, CDU, and MBS. Exceptions observed in CDU is a good indication for less anthropogenic activities on this reservoir. Differences in these factors were found because I_{geo} considers background value from sediments on Earth while EF uses the values found on this study. Anthropogenic sources of metals in the sediments on studied reservoirs are mainly related to agricultural, domestic and industrial effluents, as well as atmospheric deposition. Farming actives are a great influence on reservoirs, once

Cu, Cd, and Zn are associated with fertilizers and pesticides, and Pearson's correlation coefficient shows a positive and significant correlation between Zn and Cd.

Even using a moderate acid extraction, always when Cd concentration was higher than LOQ, values exceed at least one of comparison parameters. Then, as a whole, Cd is a priority concerned metal in the sediments of reservoirs, especially in reservoirs used for human supply or fishery production. A long-term and more sites monitoring of metals in sediments is recommended to reveal the effects on aquatic ecosystems. Metals in environmental samples never are isolated, and the possibility of synergy effects always exist, especially in sediments that usually have a complex mixture of pollutants. MBS group present concentrations that are a problem to biota according to PEC_Q evaluation. Potential ecological risk factor (E_r^i) evaluation corroborate that Cd is a priority concerned and potential ecological risk index (RI) shows that MBS seems to be susceptible to ecological risks by metal contamination.

It is recommended further research and monitoring chemicals in these reservoirs to preserve aquatic resources. Also, it is important to combine methods with chemical analysis to understand ecological problems by a combination of chemicals. Our findings indicate a need to control anthropogenic effluents to decrease sources of pollution as well as deforestation and intensive farming. Moreover, investments in sanitation sector are crucial, especially in Juiz de Fora. Therefore, develop strategies to control pollution is vital for the maintenance of water resources.

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CHAPTER 2

Allium cepa assay as a tool to understand the extent of environmental pollution: applicability to sediment of tropical reservoirs

Format according to Chemosphere

Abstract: Freshwater systems provide many ecosystems services, but humankind insists on not giving the deserved attention to them, and water pollution became a worldwide problem. Contaminants in the environment can cause mutagenic effects that can accumulate in DNA and harm a whole population. In this context, genotoxicity tests, such as *Allium cepa* assay, may be used to evaluate the extent of pollution, since synergistic effects might occur and physicochemical analysis just provide the presence of pollutants. Studies focusing in sediments toxicity of reservoirs are crucial, especially because reservoirs are used for water supply, and residues can reach humans. Therefore, this study aims to evaluate the potential of sediments from four Brazilian reservoirs to cause cytogenotoxicity in *A. cepa*. These reservoirs seem to be influenced by agricultural, industrial and domestical effluents. After sampling, the surface layer of sediment was submitted to a resuspension simulation to the water column, and supernatant was submitted to *Allium cepa* assay and metals analysis. Decreased of the mitotic index and a significant increase in chromosomal aberrations was observed in all treatments, showing cytogenotoxic effects. The presence of metals and other inorganic and organic pollutants may be responsible for the cytogenotoxic effects observed. Metals may be increasing genotoxic properties of other compounds. In this sense, just chemical analyses were not enough to evaluate the extent of pollution in sediments and its impact. Our findings reinforce that *Allium cepa* assay is a good test for environmental monitoring and genotoxicity bioassays should be used in environmental monitoring. This study demonstrates a need to combine methods, and for this, we should improve our communication between researchers.

Keywords: aquatic ecosystems; cytogenotoxicity; human health; metals; mitotic index; pollutants.

1. Introduction

Freshwater systems are crucial for humankind, providing many services such as waste assimilation, provision of water and food and climate regulation (Naiman et al. 2002; Limburg 2009). Therefore, human development, human health, and well-being depend on quality and availability of water (Naiman et al. 2002; Olorunfemi 2013). Although all services provide by aquatic ecosystems, humanity insists on not giving the deserved attention to them. A severe deterioration of freshwater resources has been observed due to uncontrolled urbanization (Corvalan 2005) and industrialization to meet demands of humankind (Barbério et al. 2008; Al-Shami et al. 2012). In this context, we are producing much more waste that we are capable of managing and water bodies tend to be the final destination of these (Van der Oost et al. 2003; Hoshina and Marin-Morales 2009). Anthropic activities produce solid residues, wastewater, agricultural and industrial effluents that may carry a pool of pollutants (Hoshina and Marin-Morales 2009; Cavalcanti et al. 2014). Therefore, chemicals are introducing on aquatic ecosystems continuously, and water pollution became a worldwide problem, which affects environmental and human health (Schwarzenbach et al. 2010; Al-Shami et al. 2012; Cavalcanti et al. 2014).

Due to water availability issues, humans has been created reservoirs for energy production but also for water and food supply. Therefore, studies focusing on water quality of reservoirs are crucial, once residues present can reach humans through drinking water and food tissues (Thornton et al. 1996; Helmer and Hespanhol 1997; Suen and Eheart 2006; Pereira et al. 2007). Reservoirs sediments can be a major sink for contaminants, particularly heavy metals, and they can return to water column via ressuspention due to wind, storms, dredging, fishing and bioturbation (Zoumis et al. 2001; Eggleton and Thomas 2004; Araújo et al. 2006). Then, these contaminants can be toxic to biota and transfered through the food chain (Förstner and Wittmann 1983; Salomons et al. 1987; Siddique et al. 2009; Geras'kin et al. 2011). In this context, it is crucial evaluate sediment toxicity, because they integrate pollution over time and reflects actual situation of contamination (Geras'kin et al. 2011).

Water bodies, inclusive reservoirs, receive diffuse sources of pollution that contain a complex mixture of contaminants (Lemos et al. 2009). Some pollutants are found in low concentrations in the environment, but even these are worrisome because can cause chronic effects and harm ecological functions (Scott and Sloman 2004; Matsumoto et al. 2006). For example, contaminants can cause mutagenic effects, causing

damage in genetic material, fertility problems and cancer (Mortelmans and Zeiger 2000). At aquatic organisms, DNA damage can be associated with reduced growth, abnormal development and decreased survival of embryos, larvae, and adults (Lee and Steinert 2003). Beyond all problems to aquatic ecosystems, adverse effects on human health can also be observed (Pereira et al. 2007). Humans are exposed to pollutants, such as heavy metals, especially via drinking water, and they are susceptible to exposure over lifelong (Lioy 1990; Florea and Busselberg 2006; Buschini et al. 2008; Castro-González and Méndez-Armenta 2008; Saha and Paul 2016).

Regarding this, genotoxicity tests may be used to evaluate the extent of pollution (Caritá and Marin-Morales 2008) and can be associated with chemical analyses. To evaluate a complex pollution, it is crucial to do biological tests, since synergistic effects might occur by the high input of chemicals in the environment and physicochemical analysis just provide concentrations of these pollutants (Smaka-Kincl et al. 1996; Geras'kin et al. 2011). Moreover, pollutant concentrations could be too low to be analytically determined giving false negative results (Kungolos et al. 2006; Žegura et al. 2009). On the other hand, bioassays provide toxic or genotoxic potential of environmental samples, but without detailed chemical composition (Ohe et al. 2004; Kungolos et al. 2006).

In order to assess genotoxicity of environmental samples, including from aquatic ecosystems, there are some tools available, and *Allium cepa* assay has been used for this purpose (Majer et al. 2005; Hoshina and Marin-Morales 2009; Radić et al. 2010; Athanásio et al. 2014). *A. cepa* has good chromosomal conditions, occupy a small space and is an efficient test organism due to high sensitivity and good correlation with other organisms, prokaryotic and eukaryotic, that includes mammalians (Grant 1982; Fiskesjö 1988; Leme and Marin-Morales 2009; Palmieri et al. 2016). Because of these reasons, *A. cepa* has been used to evaluate chromosomal and cell cycle alterations caused by chemical pollutants, what is called cytogenotoxic effects (Leme and Marin-Morales 2009).

Among all contaminants that may be present in reservoirs sediments, trace metals raise a problem because they are not usually removed from aquatic ecosystems by natural processes (Beyersmann and Hartwig 2008) and they are susceptible to resuspension processes as mention before (Förstner and Wittmann 1983). Several metals can cause cytotoxicity and genotoxicity depending on its form and concentration (Beyersmann and Hartwig 2008; Hadjiliadis 2012).

Therefore, this study aims to evaluate the potential of sediments from Brazilian reservoirs to cause cytogenotoxicity in *A. cepa*, observing the extent of environmental pollution, causing effects that are not visible and using just chemical analyses. This study shows the need for more research in these ecosystems and worries about water resource and organisms that utilizing it, including humans.

2. Material and methods

2.1. Field site and sampling

Sediments sampling was carried out in four Brazilian reservoirs located in southeastern of country: Furnas (S 21°13'54.84" W 45°57'19.02") and Chapéu D'Uvas (S 21°35'15.52"" W 43°33'10.68") in Minas Gerais state, Santa Fé (S 22° 3'58.46" W 43° 9'54.40") and Funil (S 22°31'10.89" W 44°37'30.04") in Rio de Janeiro state. A sample of soil in a coffee agriculture was also collected on margins of Furnas reservoir (S 21°10'21.57" W 45°52'53.16") to compare with sediment.

Furnas reservoir (FNS) was created in 1963 and has 1327 km² (Ometto et al. 2013). The FNS region is known because of coffee and potatoes production, and water quality is threatened due to an intensive use of pesticides and fertilizers by the farmers (Santos Neto and Siqueira 2005; IGAM 2012; Paulino et al. 2014). FNS also receive both, domestic and industrial effluents and is used for energy production, irrigation, human, animal and industrial supply (IGAM 2012).

Chapéu D'Uvas reservoir (CDU) was created in 1995 and has 12 km². CDU is critical for water supply, mainly to Juiz de Fora city (Machado 2012; CESAMA - http://www.cesama.com.br/?pagina=chapeu-duvas). Juiz de Fora is also located in Minas Gerais state and has more than 500 thousand inhabitants (IBGE - http://www.cidades.ibge.gov.br/xtras/perfil.php?lang=&codmun=313670). The region of CDU is vulnerable to soil erosion, and degraded pastures predominate 80% (CEIVAP 2006). Soil erosion is a factor that causes water pollution and, carries to the water contaminants presents (Förstner and Wittmann 1983). Then, coupled with domestic and livestock farming effluents, erosion can be a problem to CDU (Machado 2012).

Santa Fé (SNF) was created in 2008 and has 2.05 km². SNF is a small reservoir located in Paraibuna River and is used for energy production (Brasil PCH - http://www.brasilpch.com.br/12_santafe.htm). Paraibuna River pass by Juiz de Fora

before SNF and received industrial and domestic effluents (Jordão et al. 1999; Brasil 2013; Araujo 2015).

Funil reservoir (FUN) was created in 1969 and has 26 km². FUN is located in a densely populated and industrialized area (Soares et al. 2008; Ometto et al. 2013). FUN is very well known because of cases of algae blooms (Soares et al. 2009; Ferrão-Filho et al. 2009) and is used for aquaculture; human and energy supply (Branco et al. 2002). FUN has quality and water availability threatened due to industrial pollution and domestic wastewater (Ferrão-Filho et al. 2009).

According to Brandão et al. (2011), one sampling during the dry season is proper for diagnosis of sediment quality, and then sediment sampling was carried out during this period (September and October) in 2015. Samples of sediment were performed using core samplers, valuable tools to investigate historical contamination in aquatic ecosystems (Mudroch and MacKnight 1994). Sediment samples were collected near the dam, where a high sedimentation usually occur and consequently a greater accumulation of contaminants (Brandão et al. 2011).

2.2. Treatment of samples

After sampling, the surface layer of sediment (20cm) was submitted to a resuspension simulation to water column according to Messias (2008) and Magdaleno et al. (2008) with some modifications. For this, distilled water was added to sediment respecting a proportion of 4:1 and samples were shacked manually for one minute. 24 hours later, the supernatant was collected for *Allium cepa* assay and quantification of trace metals. The same was done with distilled water only to obtain the negative control. The entire methodology was thought and adapted trying to get closer to the real environmental condition. In total, six treatments were done and named as NC, FNS, CFE, CDU, SNF and FUN (negative control, Furnas, coffee soil, Chapéu D'Uvas, Santa Fé and Funil, respectively).

2.3. Chemical analysis

After the treatment, copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn), and iron (Fe) were measured. Water samples were submitted to vacuum filtration using glass apparatus and 0.45µm filter; therefore, results are presented as soluble metals. Metals were analyzed using a direct air-acetylene flame method by a flame atomic

absorption spectrometry (FAAS), model Varian (AAS240FS, Santa Clara, United States), equipped with deuterium background correction (APHA 1998; Mitra 2003). All laboratory material was pretreated in neutral detergent (5% for 12h, Merck – Extran) and nitric acid (5% for 12h, Merck – HNO_3), and water was provided by a Milli-Q system (18.2Ωm – high purity deionized water). Standard elements solutions and reagents were provided by Merck. Instrumental detection limit (LD) was calculated according to Konieczka and Namiesnik (2009) (Table 1).

Table 1. Spectral lines and the instrumental detection limit for the elements measured by using FAAS.

Element	Wavelength (nm)	Instrumental detection limit (mg L^{-1})
Cu	324.7	0.0172
Cr	357.9	0.1855
Cd	228.8	0.0220
Pb	217.0	0.7173
Zn	213.9	0.0508
Fe	248.3	0.7685

Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron.

2.4. Allium cepa assay

Allium cepa seeds (Baia Periform variety) were placed in Petri dishes covered with filter paper soaked in distilled water to stimulate root emergence. The treatments were arranged in a completely random design with three replications (three meristems analyzed per replicate). For this, six pre-germinated seeds were placed in Petri dishes with 3mL of sediment solution. Petri dishes were sealed and rested for 24 hours in a BOD camera. After this period, the roots were washed in tap water, and were collected and fixed in a cold solution of ethanol:acetic acid (3:1 v/v) for 24 hours. The fixed roots were washed in distilled water and slides were set up by using squashing technique. The roots were hydrolyzed in 5N HCl for 30 minutes, and then, the meristematic region was crushed between slide and coverslip. The cover glass was removed with liquid nitrogen and material was stained with Giemsa 5% for 2 minutes. The slides were evaluated under optical microscope at 400x magnification and was quantified: (1) mitotic index (M_I),

corresponding the ratio between the number of dividing cells and the total number of cells observed; (2) phases indices, corresponding the ration between the number of each phase division (prophases, metaphase, anaphases and telophases) and the total number of cells observed in division; (3) chromosomal and cellular abnormalities (frequency of different abnormalities).

2.5. Statistical analysis

The data are showed as the mean + standard deviation (SD). Statistical evaluation of the data was performed by one-way analysis of variance (ANOVA) followed by the Dunnett's test ($p>0.05$).

3. Results

3.1. Trace metals results

Brazilian resolution (CONAMA 357/2005) does not have a maximum value allowed for all dissolved metals, and then, the results were compared to water quality criteria from US EPA and Canadian resolution, both aim to protect freshwater aquatic organisms. Metal analysis showed concentrations higher than resolutions in all samples, except for chromium (Table 2).

Table 2. Results of trace metals analyzed after resuspension simulation of sediment (mg L^{-1}).

Treatments	Cu	Cr	Cd	Pb	Zn	Fe
NC	< 0.017	< 0.186	< 0.022	0.28	0.308	0.379
FNS	< 0.017	< 0.186	< 0.022	0	0.294	75.547
CFE	0.114	< 0.186	< 0.022	0.405	0.635	1.395
CDU	0.025	< 0.186	< 0.022	0.109	0.61	15.098
SNF	< 0.017	< 0.186	0.017	0.084	2.408	51.141
FUN	0.041	< 0.186	< 0.022	0.108	0.727	58.706
EPA Fresh Acute Criteria	-	-	0.001	0.065	0.12	-
EPA Fresh Chronic Criteria	-	-	0.0007	0.002	0.12	0.1
Canadian Short Term	-	-	0.001	-	-	-
Canadian Long Term	0.002	-	0.00009	0.001	0.03	0.3
Brazilian Resolution	0.009	-	-	-	-	0.3

Cu = copper; Cr = chromium; Cd = cadmium; Pb = lead; Zn = zinc; Fe = iron; NC = negative control; FNS = Furnas; CFE = soil of coffee agriculture; CDU = Chapéu D' Uvas; SNF = Santa Fé; FUN = Funil.

Chromium remained below the limit of detection in all samples, that means concentrations are too low to be detected accurately. The highest concentration of copper was found in CFE, on the other hand, in SNF and FNS was below LD. Cadmium was found just in SNF. Zinc was higher than allowed in all samples, being that the highest concentration was found in SNF and lowest in FNS. Lead also was higher than allowed in all samples except in FNS, and the highest concentration was found in CFE. Iron was greater than resolutions, and the highest concentration was found in FNS and the lowest in CFE. All the values that were greater than LD were also higher than resolution, showing that there is metal contamination in all reservoirs.

3.2. Cytotoxic effects

Decreased of the mitotic index was observed in all treatments when comparing to NC, except in FNS treatment (Dunnett, $p<0.05$). Largest inhibition of cells in division were observed in CFE treatment, representing a decrease of 60.21% compared to NC. On the other hand, CDU presented the lowest inhibition (32.05%) (Table 3). Prophase index increases 20.61% in CFE, 17.79% in FUN and 7.68% in CDU, while in SNF, metaphase index increases 20.72% comparing to NC. All treatments had a significant increase in the percentage of chromosomal aberrations, that means cytogenotoxic effects (Dunnett, $p<0.05$), except FNS (Table 4). Samples obtained from CFE and FNS, represented, respectively, the highest and lowest increases in the total percentage of aberrations (13.48X and 7.45X) (Table 4). Fragments and chromosomal bridges were found predominantly in CDU, CFE and FUN sediments. On average for all these treatments, the percentage of fragments and chromosomal bridges was approximately 3.2X higher than negative control (Table 4). Aneugenic alterations were predominantly found in SNF and FUN. Only SNF demonstrated a significant result for c-metaphase comparing to NC, with an increase of 1.94X (Table 4). Adherent chromosomes and condensed nuclei were the most apparent changes in results. SNF showed the highest percentage of adherent chromosomes (2.49X higher than NC) and CFE the highest percentage of condensed nuclei (2.60X higher than NC). Micronuclei percentage also increased in all treatments (Dunnett, $p <0.05$), except FNS (Figure 1). Representative images of alterations are shown in Figure 2.

Table 3. Mitotic and phases indexes in meristematic cells of *Allium cepa* after exposure to different treatments.

Treatments	M_i (%)	Pro_i	Met_i	Ana_i	Tel_i
NC	7.99	41.37	30.93	16.40	11.30
FNS	7.01	42.78	30.11	15.67	11.44
CFE	3.18*	49.90*	27.84	12.45*	9.81
CDU	5.43*	44.55*	28.90	17.30	9.30
SNF	4.63*	38.39*	37.34*	11.56*	12.71
FUN	3.85*	48.73*	27.45	11.34*	12.48

*Differ significantly from negative control according to Dunnet test (5%). M_i = mitotic index; Pro_i = prophase index; Met_i = metaphase index; Ana_i = anaphase index; Tel_i = telophase index; NC = negative control; FNS = Furnas; CFE = soil of coffee agriculture; CDU = Chapéu D' Uvas; SNF = Santa Fé; FUN = Funil.

Table 4. Chromosomal/cellular aberrations in meristematic cells of *Allium cepa* after exposure to different treatments.

Chromosomal/cellular alterations		Treatments (negative control and sediments)					
		NC	**FNS**	**CFE**	**CDU**	**SNF**	**FUN**
Clastogenic effects	**Fragments**	0.86	0.96	2.37*	1.85*	1.03	2.57*
	Bridges	1.49	1.60	6.91*	4.81*	4.70*	3.96*
Aneugenic effects	**C-metaphase**	2.34	1.73	2.96	2.18	4.54*	2.74
	Chromosome loss	1.11	1.38	1.10	0.96	0.90	1.35
	Multipolarity	1.03	1.03	1.09	1.32	1.51*	1.52*
	Later segregation	1.38	1.38	1.46	1.66*	2.02*	1.52*
Toxic effects	**Sticky chromosomes**	4.98	6.92*	9.62*	7.52*	12.39*	10.98*
	Condensed nuclei	4.32	5.23*	11.23*	8.90*	9.39*	10.34*
-	**Total percentage of abnormalities**	6.55	7.45	13.48*	10.71*	12.71*	12.59*

*Differ significantly from negative control according to Dunnet test (5%). NC = negative control; FNS = Furnas; CFE = soil of coffee agriculture; CDU = Chapéu D' Uvas; SNF = Santa Fé; FUN = Funil.

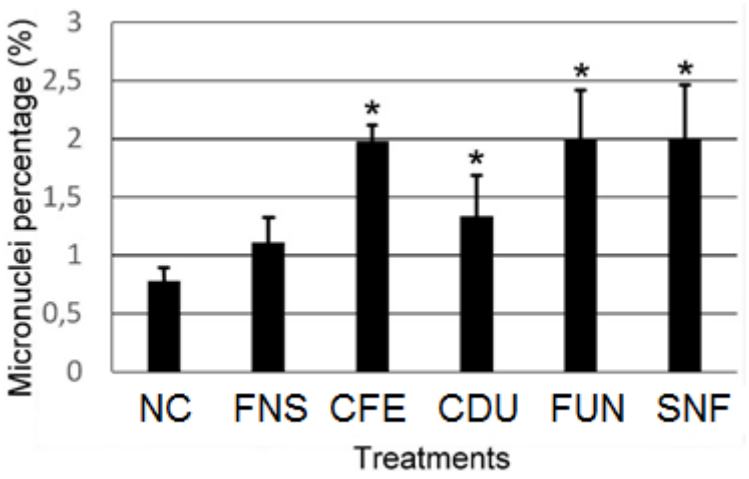

Figure 1. Micronuclei percentage (%) of meristematic cells of *Allium cepa* after exposure to different treatments. *Differ significantly from negative control according to Dunnet test (5%). NC = negative control; FNS = Furnas; CFE = soil of coffee agriculture; CDU = Chapéu D' Uvas; FUN = Funil; SNF = Santa Fé;

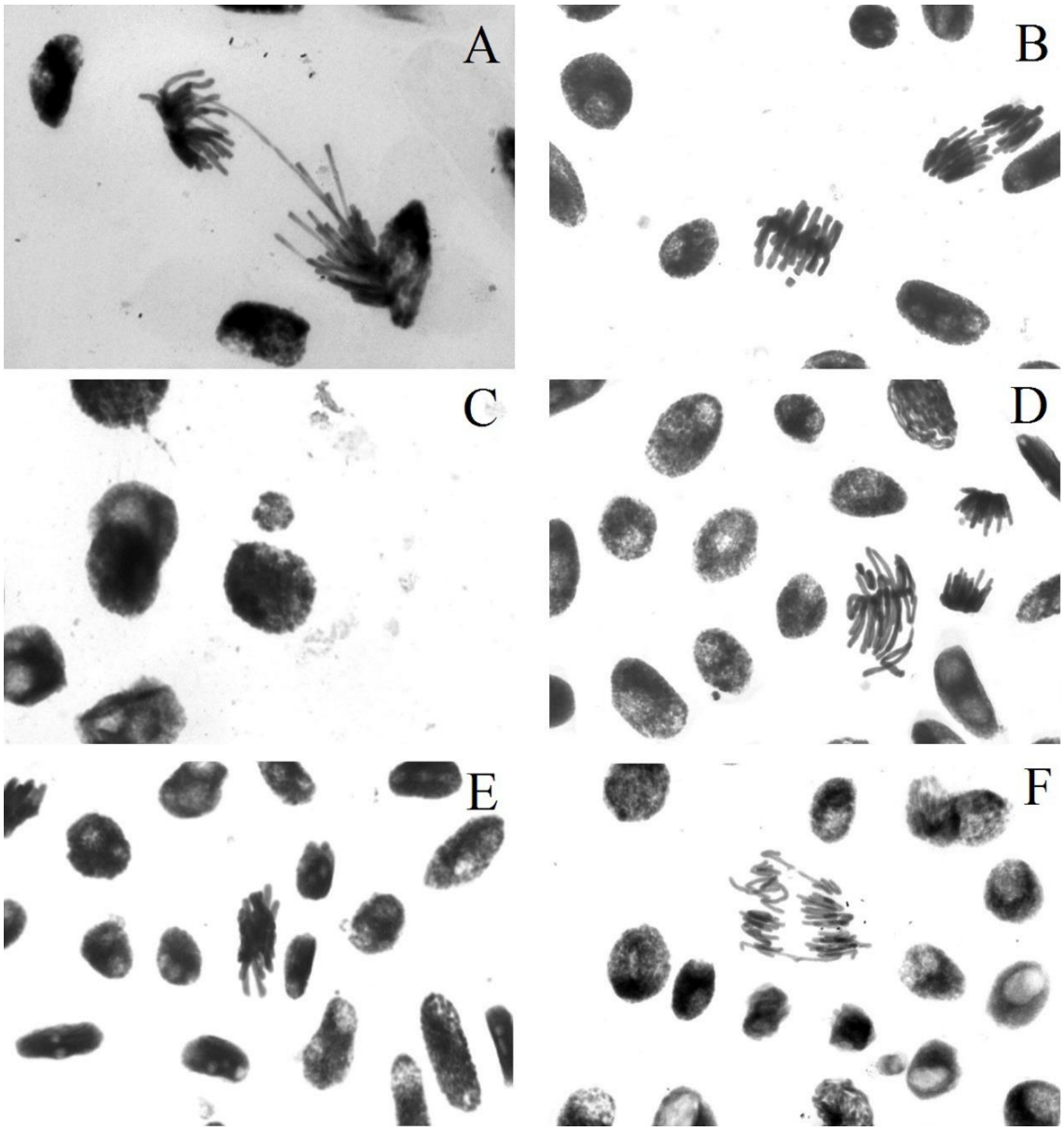

Figure 2. Examples of chromosomal aberrations observed in *Allium cepa* meristematic cells exposed to sediment treatments. A = Bridge; B = fragment; C and D = micronucleus with different size; E = chromosome adherence; F = abnormal segregation.

4. Discussion

Metal pollution in aquatic ecosystems is a major environmental problem nowadays that is a reflection of the increasing number of industries. Industrial wastewaters commonly contain metals such as cadmium, lead, zinc, and chromium (Chidambaram et al. 2009). Some metals are essential to living organisms, but all of them are toxic in high concentrations (Förstner and Wittmann 1983; Barbosa et al. 2010). Metals offer risk to ecosystem balance, mainly because they can accumulate in organisms

tissues so that metals can also reach human food (Loska and Wiechula 2003; Siddique et al. 2009; Geras'kin et al. 2011; Wang et al. 2012). Metal pollution can expose aquatic organisms to harmful effects, but continuous discharges also lead to chronic effects, such as affect growth and reproduction (Zhou et al. 2008). There are natural and anthropogenic sources of metals in the environment (Barbosa et al. 2010; Pohren et al. 2013; Saha and Paul 2016). The main natural sources are atmospheric deposition and weathering of soils and rocks (Saha and Paul 2016; Subha et al. 2016). Anthropogenic sources are associated with fast urbanization and industrialization (Saha and Paul 2016) and consist of agricultural, municipal, residential or industrial effluents (Förstner and Wittmann 1983; Salomons et al. 1995; Geras'kin et al. 2011). The presence of metals in study areas may be related to anthropogenic effluents coming from drainage basins.

Sources of chromium (Cr) in the environment are associated especially with industries (Cheng et al. 1998; Chidambaram et al. 2009; Eleftheriou et al. 2012). Many kinds of industries have Cr compounds in effluents, such as electronic components, batteries, sewage treatment plants, textiles, fertilizers, concrete and cement, metallurgical, petrochemical and refinery, steel industry and textiles (Brandão et al. 2011). Cr compounds are associated with adverse effects in freshwater species in ppb concentrations (Eisler 2000). However, in this study we do not find contamination by Cr in any reservoir.

Copper (Cu) is widely used in electric wires, water pipes, also as anti-fouling in boat paints (Csuros and Csuros 2002; Nikinmaa 2014). Industrial effluents also contain Cu, such as industries of electronic components, plastic and synthetic materials, and textiles (Brandão et al. 2011). Besides, Cu is a fertilizer compound and appears in agricultural effluents (Eisler 2000). Cu is essential to organisms and because of this, authorities does not give so much attention to control it (Fiskesjö 1998). However, Cu is required in small amounts and can be toxic at high levels (Csuros and Csuros 2002). For example, Karouna-Renier and Zehr (2003) showed that *Chironomus tentans* larvae exposed to Cu concentration above 0.25 mg L^{-1} showed decreased growth and increased mortality. In this study, the value of Cu was below than showed by Karouna-Renier and Zehr (2003) but was higher than Brazilian and Canadian resolution and can be associated with long-term effects on aquatic organisms. Malm et al. (1988) found 3.6 µg L^{-1} of Cu in Paraiba do Sul River (Rio de Janeiro State) and comparing to international studies, our values were also higher than some freshwater systems in India (Wasim Aktar et al. 2010) and Turkey (Varol 2013).

Cadmium (Cd) sources in freshwater systems include industrial discharge, phosphate fertilizers and household and municipal disposal (Ünyayar et al. 2006). Cadmium (Cd) even at low concentration is highly toxic and carcinogenic (Lee et al. 2006). It is estimated that more than 3 μg L^{-1} in freshwater systems are associated with adverse effects on organisms; 10 μg L^{-1} is enough to cause mortality, reduced growth and inhibited reproduction (Eisler 2000). Cadmium can accumulate in freshwater and marine organisms even in places which Cd concentrations was not considered warning previously (Eisler 2000). Then, SNF organisms may be susceptible to adverse effects by Cd, and probably concentrations are associated with industrial and domestic discharges in Juiz de Fora city.

Zinc (Zn) pollution is associated with domestic effluent, traffic activities, building waste, agriculture, and industries discharge (Hüffmeyer et al. 2009). Water concentrations between 10 and 25 μg Zn L^{-1} can cause effects on growth, reproduction, and survival of aquatic organisms (Eisler 2000). Besides, a combination of Zn and Cu can be more toxic to aquatic biota than these isolated (Eisler 2000). Then, Zn seems to be a problem in reservoirs studied, since all values were higher than resolutions in all samples, also higher than values cited above, including a possibility to synergetic effects of Cu and Zn in CFE, CDU, and FUN. Lima et al. (2015) found 0.139 mg L^{-1} of Zn in Cassiporé River (Amapá State) and Malm et al. (1988) found 13 μg L^{-1} of Zn in Paraiba do Sul River (Rio de Janeiro State). Therefore, our results were higher than these others in Brazil.

In aquatic ecosystems, lead (Pb) has sources such as industries effluents and urban runoff (US Department of Health and Human Services 1999). Pb increased tumor incidences in animal experiments and at low concentrations is possible to observe genotoxicity (Beyersmann and Hartwig 2008). Pb in concentrations of 1 to 5 μg L^{-1} can cause adverse effects on aquatic biota, such as reduced survival, reproduction, and growth (Eisler 2000). In this study, Pb was higher than resolutions in all samples, exceeding FNS, and can be associated with adverse effects on organisms, especially comparing to data from Eisler (2000). Lima et al. (2015) found a higher concentration of Pb (1.18 mg L^{-1}) in Cassiporé River (Amapá State) and Malm et al. (1988) found 1.7 μg L^{-1} of Pb in Paraiba do Sul River (Rio de Janeiro State).

Sources of iron (Fe) include industrial effluent, sewage, and landfill leachate. Although it is essential for organisms, in high concentration can accumulate in muscle and affects the nervous system (Saha and Paul 2016), besides can induce long-term genetic hazards (Rigaud et al. 2012). Brazilian soils are rich in iron, and poor conservation

and deforestation of riparian zones cause an intensification of erosion (Guerra et al. 2014; Bucci et al. 2015), that seems to be a problem for aquatic systems, including the reservoirs studied. All concentrations found to Fe was higher than resolutions what can be associated to erosion and industrial and sewage effluents, which may be a problem for aquatic ecosystems.

Our results reported the presence of Cu, Cd, Zn, Pb and Fe at levels higher than those accepted by freshwater guidelines, that means concentrations relate to acute and chronic effects on aquatic organisms. As reported by Rodgher et al. (2005), metals concentrations found in reservoirs in the Tietê River (São Paulo, Brazil) was lower than our findings for Cu, Cr, Cd, and Zn. Tietê river had polluted areas and impacted affluent (Rodgher et al. 2005; Araújo et al. 2006). Then, it is possible to infer the contamination by metals in studied sites, also that the methodology of resuspension worked in releasing pollutants from sediments to water.

In general, cytogenotoxic effects was observed in all treatments that is support by interferences observed in the cell cycle: decreases in mitotic index and increases in the percentage of chromosomal/cellular aberrations. The mitotic index indicates the frequency of cell division and is an important parameter to evaluate if cell proliferation is affected (Qian 2004; Zou et al. 2006). A significant increase in percentages of prophase cells was observed in CFE, CDU, and FUN after treatment with sediment solution. As reported by Scolnick and Halazonetis (2000) this effect may be a consequence of a block at the checkpoint between prophase and metaphase. Additionally, the SNF sediment induced an increase in the percentage of cells in metaphase, probably due to a cell cycle block at this point. SNF treatment also induced a significant increase in the percentage of C-metaphases, which probably corroborates with the observed effect. This effect occurs when we have agents causing interference on the mitotic spindle, disrupting the proper functioning of microtubules, which disturb the normal chromosomes segregation in anaphase (Fiskesjö 1985; Fiskesjö and Levan 1993; Ray et al. 2013). All these alterations mention above are called aneugenic, as well as multipolarity and later segregation that were also observed in our results (Leme and Marin-Morales 2009).

Clastogenic chromosomal alterations were the most frequent after exposure to the sediments reservoirs, such as chromosomal bridges and fragments, and are related to DNA breaks (Leme and Marin-Morales 2009). Bridges can be formed through the chromosomal rearrangements with a dicentric chromosomes formation or by terminal breaks, leading to the chromatids fusion, which also explains the presence of fragments

(Singh 2003). Toxic effects were also observed in treatments, such as adherent chromosomes and condensed nuclei that were frequently found. Adherent chromosomes appear as a consequence of toxic effect on the chromatin organization, more precisely on the proteinaceous matrix, and are often an irreversible effect that leads to cell death (Fiskesjö and Levan 1993; Marcano and Del Campo 1995). Also, the micronuclei formation in treatments corroborates to the cytotoxic effect hitherto reported.

The toxic effect of copper has been well reported in the literature in several works with plants (Lequeux et al. 2010; Bes et al. 2013; Muccifora and Bellani 2013; Bellani et al. 2014). Yıldız et al. (2009) and Bellani et al. (2014) reported influence on the mitotic index of *Allium cepa* and *Vicia faba*, respectively. Bellani et al. (2014) show that after exposure of *Vicia faba* to copper bromide ($CuBr_2$) there was a significant presence of micronuclei, c-metaphases, bridges, and fragments. Then, the presence of copper may be contributing to explain cytotoxic effects reported for the sediments collected in Chapéu D'Uvas and Funil reservoirs, in addition to the soil sample collected in the coffee agriculture in Furnas.

The effects of cadmium on plants are well documented in the literature. Several studies have demonstrated that this heavy metal can interfere with the mitotic index and induce the formation of chromosomal alterations (Zhang et al. 2009; Wang et al. 2014). Zhang et al. (2009) and Wang et al. (2014) reported the induction of c-metaphases, chromosomal bridges, adherent chromosomes, late segregation, chromosomal fragments, and micronuclei. In this study, SNF treatment showed a high concentration of cadmium.

Borboa and Torre (1996) reported chromosomal bridges formation after exposure to Zn (II), and Steinkellner et al. (1998) demonstrated a moderate effect of Zinc in inducing the micronuclei formation in plants. Although zinc has been found in almost all treatments, it has been considered a moderately genotoxic element.

Previous studies have reported cytotoxic effects of lead in plants (Lamhamdi et al. 2011; Malar et al. 2014; Venkatachalam et al. 2017). Venkatachalam et al. (2017) used the RAPD technique to evidence the genotoxic effect of lead. Additionally, lead induces oxidative stress due to an increase in the synthesis of reactive oxygen species (ROS). These ROS are often toxic and can induce damage to macromolecules, leading to a programmed cell death (Venkatachalam et al. 2017). The sediments collected in Chapéu D`Uvas, Santa Fé and Funil, besides the coffee soil sample in Furnas, showed high levels of lead.

Santa Fé and Funil were treatments with the greatest genotoxic effects observed, and both sites seem to be contaminated with the largest number of heavy metals. In Santa Fé, metal pollution can be associated with effluents from Juiz de Fora city because the reservoir passes through the city and receive domestic and industrial effluents, including a textile and tannery effluents, which are associated with heavy metals (Jordão et al. 1999; Brasil 2013; Araujo 2015). The area around Funil reservoir is highly populated and industrialized, then pollution observed can be associated with domestic effluents, industrial and agricultural effluents (Vidal 2012; Souza Lima et al. 2016).

Then, our findings indicated the presence of genotoxic substances in the environment. This hypothesis is based on literature that shows cytotoxicity effects in *Allium cepa* promoted by many pollutants, such as heavy metals (Steinkellner et al. 1998; Fatima and Ahmad 2005), polycyclic hydrocarbons (Leme et al. 2008), pesticides (Marcano et al. 2004) and other emerging contaminants (Herrero et al. 2012). These genotoxic substances may offered risks to aquatic organisms and for all other organisms that depend on aquatic ecosystems for water and food supply. DNA is affected when genotoxicity occurs, and this is a common structure to all living organisms; then, all organisms that use these ecosystems may be threatened. Damages on the genetic structure can persist to subsequent generations of cells replicating errors that may affect a whole organism or even all-local community where effluents are discharged (Caritá and Marin-Morales 2008).

The presence of Cu, Cd, Pb, Fe and Zn and other possibly inorganic and organic pollutants (while not analyzed in this study) in different concentrations may be responsible for inhibition of cell division and an increase of chromosomal aberrations observed in *A. cepa* meristems cells. Then, this complex mixture of pollutants in sediments caused genotoxic effects (Vargas et al. 2001). In this context, metals may be increasing genotoxic properties of other compounds, which also was found by Magdaleno et al. (2008). Furthermore, interactions between metals also have synergetic activity (Olorunfemi 2013), such as Fe, Cr and Zn (Godet et al. 1993). The differences found in treatments can be related to land use. In this sense, just chemical analyses were not enough to evaluate the extent of environmental pollution in sediments and its impact (Magdaleno et al. 2008).

Our results show that the methodology of resuspension worked in releasing pollutants from sediments to water and was useful to evaluate genotoxicity of sediments using *Allium cepa* assay. As showed years ago and researchers continuing to confirm that

both, seeds and bulbs of *A. cepa,* are a good assay to evaluate the capability of environmental pollutants to cause cytogenotoxicity. Our results were not different from that: *Allium cepa* respond quickly and is highly sensitive to genotoxic agents. Besides, it is possible to exposure directly to complex mixtures without prior treatment (Fiskesjö 1985; Matsumoto and Marin-Morales 2004; Barbério et al. 2008; Leme and Marin-Morales 2009). *A. cepa* test provides a screening of environmental pollution, and these results work as a warning to both human health and other organisms of ecosystems (Leme and Marin-Morales 2009; Geras'kin et al. 2011). *Allium cepa* is sensitive to many environmental pollutants, including heavy metals (Panda et al. 1996; Palacio et al. 2005), and is suitable to investigate potential synergistic effects of mixtures of pollutants (Barbério et al. 2008). Sediments are a complex mixture of pollutants, and because of this, it is hard to point the real cause of toxicity (Araújo et al. 2006; Magdaleno et al. 2008), and it is the case of this study. Cytogenotoxicity results probably are related to this complex mixture of pollutants in sediments from reservoirs. For example, was presented by Santos Neto and Siqueira (2005) that FNS have contamination by organophosphorus pesticides. In FUN toxic cyanobacteria was found before (Panosso et al. 2003), an also excess of nutrients (Souza Lima et al. 2016) and sediment toxicity perhaps explained the due presence of metals (Matos et al. 2014). CDU is located near the Spring of Paraibuna River and probably is not very affected by human activity, but SNF received a considerable amount of domestic and industrial effluents from Juiz de Fora (Jordão et al. 1999; Brasil 2013; Araujo 2015). CFE presents the highest cytogenotoxicity effects probably by pesticides and fertilizers.

Standard chemical analyses are not capable of showing us toxicity and genotoxicity capability of a complex mixture that is found in ecosystems. Moreover, unfortunately, it is hard to do all chemical analyses required to understand real impacts of contamination, and because of this, genotoxic assays are crucial to determining consequences of sediment pollution and contribute to decision-makers. These findings have important implications for Brazilian monitoring programs, and control pollution sources are urgent. Also, it is important to identify causes of sediment toxicity to support Brazilian government to create new regulations and decrease sources of pollution. Our findings show that if only based on physicochemical analysis we may be underestimating impacts of contamination. They do not provide biological effects of micropollutants that occur in low concentrations, which sometimes our equipment is not capable of detecting, also not show us synergetic effects (Kungolos et al. 2006; Radic et al. 2010). Therefore,

it is crucial to put together both methods, to evaluate the presence of genotoxic substances and try to find out the agents to reverse it (Geras'kin et al. 2011). To achieve this combination of methods nothing better that open up the doors of our labs to do collaborations. It is the best way to do science, after all, we have the same goal: understand ecosystems process to protect them, including human health. Therefore, it is important to improve communication between ecologists and ecotoxicologists; they need to work hand in hand for risk assessment (Filser 2008). Accordingly, we need to improve our communication and collaborate much more than we are doing so far.

5. Conclusions

Our findings reinforce that *Allium cepa* assay is a good test for environmental monitoring, especially if the aim is to evaluate the extent of pollution and impacts of a complex environmental mixture. *Allium cepa* presented high sensitivity, minimum facility requirements, simplicity and low cost. This study also demonstrated that toxicity/genotoxicity bioassays should be used in environmental monitoring, because they provide useful data in risk assessment, serving as a tool for warning about the presence of mutagenic pollutants in aquatic ecosystems, including their sediments.

All treatments caused cytogenotoxicity effects on meristematic cells of *A. cepa*, because we observed an interference on the mitotic index, which is indicative of toxicity or blocking cell cycle, and increase the percentage of chromosomal aberrations. This effect probably has relation with the presence of metals, but also mixture effects of pollutants coming from anthropogenic effluents. Only with results of the chemical analysis is not possible to infer about genotoxicity capability of micropollutants, which demonstrates a need to combine methods, such as physicochemical analysis and cytogenetic test to better understand the extent of environmental pollution and toxicity of chemical pollutants.

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SUMMARY AND FUTURE PERSPECTIVES

In any reservoir studied was found major problems related to contamination by chromium, copper, cadmium, lead, zinc and iron. Some concentrations have exceeded values compared, but mostly in deeper concentrations, which will only cause problems if there are sudden changes in environmental conditions and a deeper layer remobilization. However, potential ecological risks were found due to a mixture of metals. Besides that cytotoxicity test with *Allium cepa* was observed and demonstrated genotoxic effects of sediments.

Thus, this study confirms how sensitive is *Allium cepa* to mutagenic compounds in the environment and demonstrates the need to implement this type of test in environmental monitoring. Not necessarily the concentrations of pollutants will be chemically detectable, but may already be in enough level to cause adverse effects on aquatic organisms and even in humans. Another important point confirmed by this work is the complexity of environmental samples and how many pollutants may be present, even in systems with less direct human influence, since indirectly pollutants can be received by the atmosphere and deposited in water bodies. The compounds are not isolated in the environment and may exhibit synergistic effects, which may be one of the reasons to explain adverse effects caused by the sediment to *Allium cepa* since the concentrations of metals analyzed were not so high. Due to the impossibility of carrying out all possible chemical analysis, it is useful apply toxicity tests to evaluate the real extent of environmental pollution and its consequences. In addition, chemical analysis is not able to detect synergistic effects that may be occurring in the environment.

Therefore, it is crucial to combine methods to understand what is happening in ecosystems. The best way to combine more methods is to implement partnerships. The development of this work was only possible due to partnerships between experienced laboratories in the area of detection of environmental pollutants and environmental mutagenesis. Thus, we will be able to assist decision makers, develop new technologies for pollution control and remediation, and continue with environmental monitoring by implementing the use of toxicity tests for this purpose.

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