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Toxicological Monitoring of a Water Supply Reservoir by Acute Assays with *Daphnia Pulex*

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Daphnia pulex assays (48 hours) were performed to determine acute toxicity of water samples from La Fe reservoir, Antioquia, Colombia. Eight sampling campaigns were done between March 2010 and June 2012, a period that included rainfall episodes. Samples were taken from the water column at three depths and from lotic areas. Physicochemical properties such as pH, electrical conductivity, temperature and dissolved oxygen were measured both in field and laboratory, and concentrations of dissolved iron (Fe), manganese (Mn), aluminium (Al), lead (Pb), cadmium (Cd) and chromium (Cr) were assessed. The overall results for the physicochemical properties remained at expected levels for natural waters with slightly higher conductivities in samples collected from bottom and some tributaries. The results of the toxicity tests showed significant mortalities in only 28% of assays. A higher mortality of *Daphnia pulex* was detected for tests performed during periods of higher precipitation (August 2010, February and July 2011) mainly for samples taken near the confluence of natural tributaries. No significant relationships between physicochemical variables and mortality of *Daphnia pulex* were found.
Keywords: *acute toxicity, Daphnia pulex, reservoir, La Fe.*

1 Introduction

Water quality of reservoirs is affected by human activities in the basins. The main environmental problems include nutrient enrichment and income of emerging pollutants, pesticides and metals. The transport of these substances depends on the basin topography and rainfall, which can determine leaching processes according to the nature and use of the land. The characteristics of the substances and the physicochemical properties of the aquatic receiving environment are also important.

The operating regime of a reservoir also interferes with the system. Thus, the retention times and uptake volumes can determine environmental conditions, mainly regulating mixing processes in the water column and transfer of substances from the sediments (Dong, Sun, & Zhao, 2007; Zhang et al., 2005).

The increasing pollution of aquatic environments threatens the sustainability of water resources, limits their use and affects their quality as habitats. High concentrations of heavy metals in water reservoirs have been associated with carcinogenic, cytotoxic; genotoxic effects; developmental delays and increased frequency of DNA strand breaks by occupational exposures (Hengstler et al., 2003) (Babich, Devanas, & Stotzky, 1985) (Chakravarty & Srivastava, 1992) (Steinkellner et al., 1998; Vargas et al., 2001) (Iannacone & Alvariño, 2005).

Water quality of freshwater reservoirs is assessed through routine physicochemical measurements which are compared to maximum permissible levels according to its use. However, physical and chemical analyses do not allow identifying the bioavailability of toxic substances and the potential risk to biota. Thus, toxicity tests have become a complementary tool in monitoring programs of drinking water supply systems.

Toxicity tests using the cladoceran Daphnia are widely accepted in studies aimed to assess the potential risk posed by the presence of toxicants in surface waters. Because of their sensitivity, *D. pulex*, *D. similis*, *D. magna*, and *D. ambigua* have been employed to determine risks by organic substances such as pesticides, cyanotoxins and metals (Carlos Barata et al., 2007; Barata, Solayan, & Porte, 2004; Frear & Boyd, 1967; Govers, Aiking, & Ruepert, 1984; Martin & Hartman, 1984) (Anderson, Glibert, & Burkholder, 2002; Ferrão-Filho, Soares, de Magalhães, & Azevedo, 2010; Sierosławska et al., 2010) (Carvalho, Zanardi, Buratini, Conde, & Coimbra, 1998; Winner, 1984)

The American Environmental Protection Agency (EPA) and the European Union have used these tests to characterise natural water sources and wastewaters (Organization for Economic Co-operation and Development (OECD), 1992; United States Environmental Protection Agency (USEPA), 2002).

This ecotoxicological monitoring study aims to determine how some physicochemical properties of the water, changes in operation and periods of rainfall can affect the toxicological status of a reservoir used as water supply.

2 Materials and methods

2.1 Study area

La Fe Reservoir is located in the municipality of El Retiro (Antioquia, Colombia) between coordinates 75º30'10–75º30'13 'W and 6º06'47,5'–6º06'46,5 'N. The reservoir covers an area of 173 km^2 with altitudes ranging between 2,175 and 3,000 m.a.s.l. and has an approximate storage volume of 12 Mm^3 of water, maximum length of 0.65 km, average width of 2.21 km, maximum depth of 28 m and the average depth of 9.79 m. Agricultural activities and a remarkable process of urban planning take place in the watersheds of its tributaries (Empresas Públicas de Medellín (EPM) and Universidad de Antioquia (UdeA), 2007). The reservoir is divided into two basins: the North sector has been operating since 1973 and the South sector since 1987, both are separated by Los Salados old dam (immersed), which introduces a discontinuity in the hydrodynamics and heat transport (UdeA & Universidad Nacional de Colombia-Medellin (UNALMED), 2011). An average annual rainfall of 1,782 mm, two dry periods from December to February and July to August and two rainy periods from April to May and September to November are recorded. The reservoir is filled naturally from four streams (Palmas, Espiritu Santo, San Luis, and Boquerón) and by means of a pumping system from three rivers (Pantanillo, Buey, and Piedras) (Figure 1).

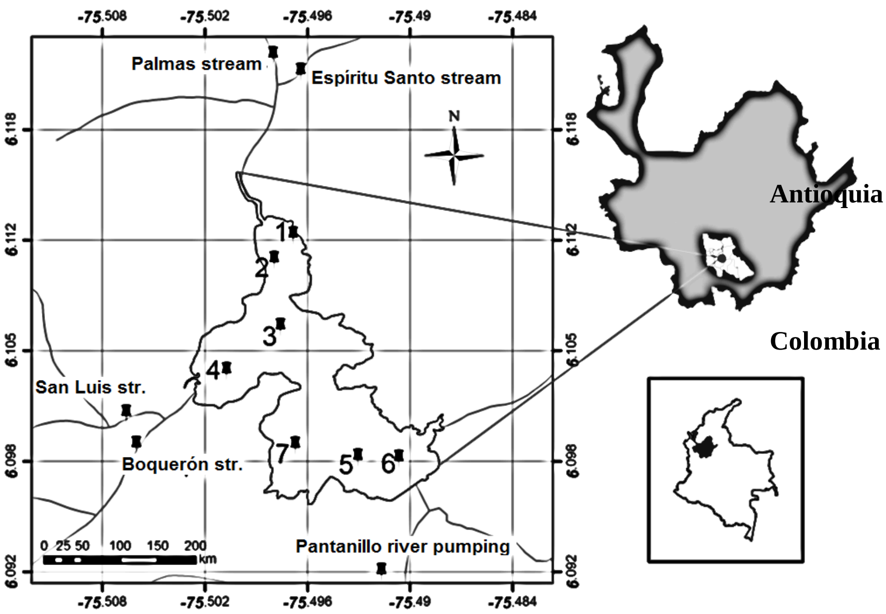

Figure 1. Sampling sites location in La Fe Reservoir.

Eight samplings were carried out between March 2010 and June 2012 in seven sites of the reservoir. Sites 1 to 4 were located in the North sector, which is strongly influenced by its natural tributaries, and sites 5 to 7 were located in the South sector, which is characterised by its greater depth (ca. 24 m) and proximity to the dam (Figure 1 and Table 1). Two litres of water samples were taken in each site at subsurface, limit of photic zone and bottom using a Van-dorn sampler. Samples were stored in amber glass bottles and kept under cooled conditions (4 °C) until their analysis according to the APHA (2012) recommendations.

Table 1. Sampling sites location in La Fe Reservoir.

Site	Latitude (N)	Longitude (W)	Location
1	6°06'42.97"	75°29'48.59"	After the convergence of Las Palmas and Espíritu Santo streams
2	6°06'37.69"	75°29'52.66"	Reservoir, northern sector
3	6°06'24.41"	75°29'52.55"	Draw-off tower
4	6°06'13.95"	75°30'02.85"	Reservoir, after the convergence of the Boquerón and San Luis streams
5	6°05'54.76"	75°29'35.25"	Reservoir, near to submerged pumping output from Pantanillo river
6	6°05'55.04"	75°29'25.40"	Reservoir, south-eastern sector
7	6°05'57.97"	75°29'48.12"	Reservoir, south-western sector

2.2 Laboratory tests

Water samples were passed through a 0.45-um filter and acidified to pH 2.0 (HNO_3; 10% v/v) for the determination of Fe, Mn, Al, Cd, Cr and Pb by flame ionization atomic absorption spectrophotometry (SM-3111B). *Daphnia pulex* assays were performed according to the guidelines established by the American Environmental Protection Agency (USEPA, 2002). Conditions for the toxicity tests are summarised in Table 2.

D. pulex strain was obtained from the La Fe Reservoir, acclimated to laboratory culture conditions and its sensitivity controlled by periodic tests with potassium dichromate. Water samples for the toxicity tests were filtered and pH (SM-4500H-B), electrical conductivity (SM-2510B), dissolved oxygen (SM-4500O-G), temperature (SM 2550B) and total hardness (SM-2340C) were determined according to the Standard Methods (APHA, 2012). Oxygen saturation equal to or greater than 60% was ensured in all assays.

As a criterion for decision, organisms were simultaneously exposed to the negative control which consists of semi-hard reconstituted water with sodium, potassium, magnesium and calcium salts, prepared according to EPA 2021, which constitutes the medium in which organisms are cultivated at the laboratory and mortality values less or equal to 10% are expected for the validity of the test.

Table 2. Laboratory conditions for Daphnia sp. toxicity tests, according to EPA 2021 (USEPA, 2002).

Variable	**Condition**
Temperature (°C)	20 ± 2
Light quality	Ambient laboratory illumination
Photoperiod	16 h light, 8 h darkness
Test chamber size	30 ml
Test solution volume	25 ml
Age of test organisms	24 hours
Neonates per chamber	10
Replicates per dilution	Four
Feeding regime	none
Test solution aeration	No
Dilution water	Moderately hard
End point	Immobility
Exposure time	48 hours
Acceptability criteria	10% control mortality

2.3 Statistical treatment of the data

Descriptive analysis of the variables was performed using Microsoft Excel®, statistical comparisons of controls and samples were performed by multiple range and T-student tests. Spatio-temporal analysis was performed meanwhile the analysis of Kruskal Wallis and relationships among variables were established using Spearman coefficient and principal component analysis (PCA) using the program Statgraphics Centurion XVIII®.

3 Results and discussion

3.1 Reservoir dynamics and operation

In March 2010, the low-flow of the tributaries (below $5m^3\ s^{-1}$) was compensated by active pumping from the Pantanillo River. In May 2010, the onset of rains brought about a transition period, during which inflow of natural streams increased and pumping compensation was reduced. By August 2010, La Niña weather phenomenon was well established, so natural inputs increased and pumping was stopped. A short dry period occurred in early February 2011, from then on most of the samplings were performed during transition and rainy periods (Román, 2011).

Data shown in Figure 2 were obtained by reading the current meter of natural tributaries during the days of sampling, operation data of the reservoir in terms of the inputs from the Pantanillo River by pumping were provided by Empresas Públicas de Medellin (EPM), additionally the mortality percentages for each station and depth in the eight samplings are shown.

The highest rainfalls were recorded during the samplings of October 2011, June 2012 and January 2012, with daily averages of 40.9, 35.6 and 25.4 mm/day, respectively. For its part, the greatest average flow rates of Las Palmas, Espíritu Santo, Boquerón and San Luis streams were found in samplings of July and October 2011 during the La Niña weather phenomenon (Figure 2). The effluent flows were just dependent on the uptake for the purification process. The surface pumping from the Pantanillo River worked in all sampling campaigns, except in August 2010 and October 2011. The submerged pumping was only stopped in October 2011 (Figure 2).

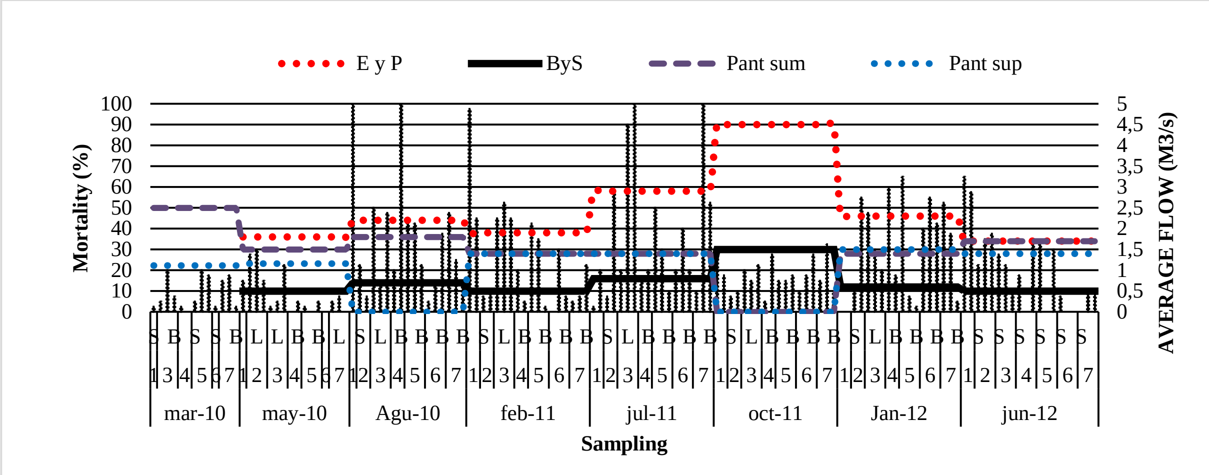

Figure 1. *Relationship between D. pulex mortality for each site (1-7) and depth (S, L, B) and tributaries flow (daily averages) for eight different samplings. Read: E y P: Espiritu Santo and Palmas streams, B y S: Boqueron and San Luis streams. Pant sum: Pantanillo submerged and Pant sup: Pantanillo surface.*

3.2 Physicochemical variables

The highest mean concentration of Fe (4.49 mg/l) was observed in the sampling of October 2011, with values reaching up to 18.38 mg/l in the bottom sample from site 3. No significant differences were evident among the sites. In the water column, a higher mean (2.72 mg/l) was found for the bottom samples.

Higher concentrations of Mn were detected in October 2011 (mean: 0.16 mg/l) and the bottom samples had a significantly higher concentration (mean: 0.14 mg/l). Samples from site 6 had a higher average relative concentration of Mn (0.16 mg/l), possibly due to the greater depth of this sampling site.

Concentrations of Al did not vary significantly among sites and showed slightly higher values for the bottom samples than for the surface and the limit of photic zone. As for samplings, the highest average (2.75 mg/l) was recorded in March 2010.

The higher concentrations of Fe, Mn and Al in the bottom water samples are due to reducing conditions in the bottom water that increase the availability of these metals in soluble form. Also, the forming of insoluble oxides or sweep precipitation by suspended material sedimentation processes from the surface. Additionally, the basin composition allows the eventual release of these metals from the sediment (mineral based enrichment dilution) (Calmano, Hong, & Foerstner, 1993).

No significant differences between Fe and Al concentrations were found in water samples from the north and south sectors of the reservoir. In the south sector, however, Mn concentrations were slightly higher (mean: 0.09 mg/l).

The concentrations of Pb, Cd and Cr were generally below 0.01 mg/l, making a more rigorous statistical comparative analysis not possible. These metals are usually found in very low concentrations in soluble form.

The pH levels remained near neutral with an average value of 7.54 and there were no significant differences for pH values among sites. However, significantly higher pH values (average 8.0 units approximately) were recorded for March and May 2010 samplings. Also, relatively high pH values were found for samples of the surface where a high photosynthetic rate takes place.

Electrical conductivity (EC) values showed no significant variations among depths in the water column, which may indicate a good vertical mixing facilitated by the shallowness of the water body. However, there were statistically significant differences for the EC among sites, with higher values (57–63 uS/cm) in the sites closer to the natural tributaries, evidencing a dilution effect in the limnetic zones.

The results of total hardness (TH) showed significant differences among samplings, with higher mean values in March 2010 (24.6 mg $CaCO_3$/l). No spatial differences among depths, sites or north and south sectors were found.

3.3 Acute toxicity assays

Neonate mortality of *D. pulex* exposed to water samples ranged between 0.0 and 100% (Table 3). In 28% of cases (39 assays), mortalities were significantly different from the control. Among these, 24 samples (62%) were from the north sector and 15 (38%) from the south sector. There were significant differences between the two sectors particularly in May 2010 and June 2012, possibly due to the higher contribution of the natural tributaries during those months (Figure 3).

According to the Kruskal Wallis test results, there were significant differences ($\alpha = 0.05$) in the mortality of *D. pulex* neonates among samplings. Most of the mortalities significantly different from the control occurred with samples collected in August 2010 (23%), February (18%) and July 2011 (18%) (Figure 4). These results coincided with an increased rainfall and the consequent higher flow of the tributaries. No statistically significant differences in the mortality of *D. pulex* were found among the samples from surface, limit of photic zone and bottom. Samples of August 2010, July 2011 and January 2012 showed mortalities higher than 50% (Figure 2).

In general, mortality of *D. pulex* neonates exposed to water from the confluence of Las Palmas and Espiritu Santo streams (site 1) was higher than that from the other sites (Figure 5). It is worth noting that in the onset of the rainy season in August 2010, the highest mortalities were observed with samples from sites 1 and 4 that are under the direct influence of the tributaries inputs (Las Animas, and San Luis and Boquerón, respectively).

These findings let us conclude that the acute toxicity of the La Fe Reservoir water on *D. pulex* is mainly affected by the period of sampling and does not appear to be related to the depth in the water column.

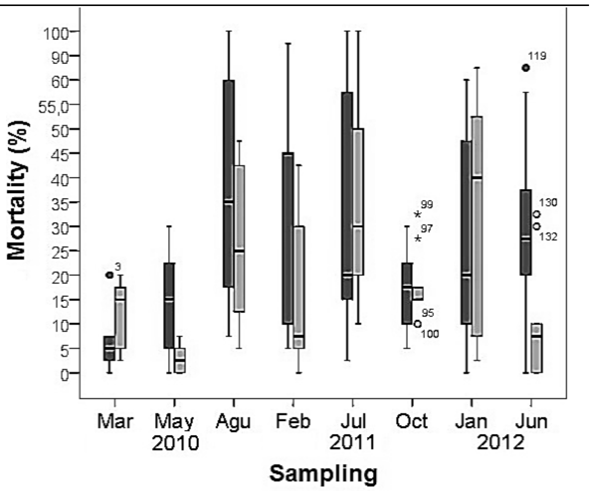

Figure 3. Mortality of D. pulex exposed to water samples from the north sector (■) (sites 1-4) and the south sector (■) (sites 5–7) for eight samplings.

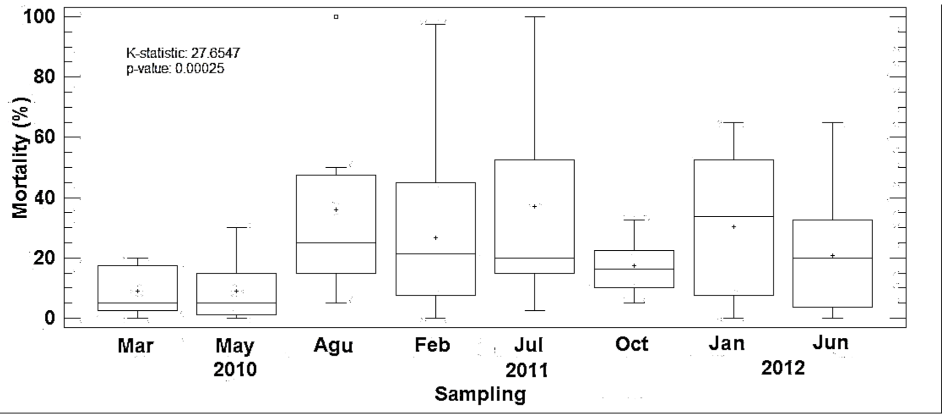

Figure 4. Variation in mortality of D. pulex among samplings in La Fe Reservoir.

There were significant differences in the mortality results between samples from the north and south sectors in May 2010 and June 2012. Treatments with water from the north sector showed slightly higher mortalities, possibly due to the onset of the rains, which may cause washing of the streambeds in the north basin. It is important to note that in May 2010 and June 2012 the surface pumping system from the Pantanillo River was in operation, which can give different characteristics to the water in the south sector (Figure 2). Mortality results had significant differences among different depths in March 2010 and July 2011, showing lower values in samples from subsurface (Figure 6).

Table 3. Mortality of D. pulex neonates exposed to water samples collected between March 2010 and June 2012.

Site	Depth	Mortality (%)							
		March 2010	May 2010	August 2010	February 2011	July 2011	October 2011	January 2012	June 2012
1	**Surface**	35.0*	15.0	100.0*	97.5*	2.5	30.0	30.0*	65.0*
	Bottom	NR	NR	NR	45.0*	20.0	17.5*	17.5*	57.5*
2	**Surface**	NR	27.5*	22.5	7.5	7.5	7.5	7.5	22.5*
	LPZ	NR	30.0	NR	NR	NR	NR	NR	35.0*
	Bottom	NR	15.0	7.5	10.0	57.5*	10.0	10.0	37.5*
3	**Surface**	5.0	2.5	50.0*	45.0*	20.0	20.0	20.0*	27.5*
	LPZ	20.0*	5.0	15.0	52.5	90.0*	15.0	15.0	22.5
	Bottom	7.5	22.5	47.5*	45.0	100.0*	22.5*	22.5	10.0
4	**Surface**	2.5	0.0	20.0	20.0	15.0	5.0	5.0	17.5
	LPZ	NR.	NR.	NR.	NR.	NR	NR	NR	0.0
	Bottom	0.0	5.0	100.0*	5.0	20.0	27.5	27.5	32.5
5	**Surface**	5.0	2.5	42.5*	42.5*	50.0*	15.0	15.0	32.5
	LPZ	20.0	0.0	42.5*	35.0*	30.0	15.0	15.0	0.0
	Bottom	17.5	5.0	22.5	22.5	10.0	17.5	17.5	30.0

Site	Depth	Mortality (%)							
		March 2010	May 2010	August 2010	February 2011	July 2011	October 2011	January 2012	June 2012
6	Surface	2.5	0.0	5.0	2.5	20.0	10.0	10.0	7.5
	LPZ	NR	NR	12.5	0.0	40.0*	17.5	17.5	0.0
	Bottom	NR	NR	37.5*	30.0	20.0	27.5	27.5*	0.0
7	Surface	15.0	5.0	47.5*	5.0	10.0	15.0	15.0	0.0
	LPZ	17.5	7.5	25.0*	7.5	100.0*	32.5	32.5	10.0
	Bottom	0.0	0.0	12.5	22.5	52.5	10.0	10.0	10.0

LPZ: Limit of the photic zone; (*) Mortality significantly different (α = 0.05) from the negative control; NR: assays not carried out.

3.4 Correlations between physicochemical variables and mortality of D. pulex

The Spearman test showed no significant correlations between *D. pulex* mortality and the physicochemical characteristics of the water used in the toxicity tests ($r < 0.5$).

The principal component analysis (PCA) allows associating the variables (mortality, TH, EC, DO, SO, pH, Al, Fe, Mn, T) to obtain a small number of linear combinations, which can explain the greater variability in the data. In this case, four components were extracted, which account for 65.8663% of the variability in the original data (Table 4 and Figure 7). The principal component analysis groups the metals Al, Fe and Mn in the same component (component 2), which shows the similar environmental behaviour that these metals have in the water body possibly linked to their lithogenic origin.

Furthermore, the mortality was within the same component directly associated with the conductivity and inversely associated (negative sign) with total hardness because of their highest weight in component 4 (Table 4). Thus, an increase in conductivity (dissolved salts) and a decrease in hardness would favour an increase in mortality as already reported in other studies (Miller & Mackay, 1980, Winner 1986, Winner & Gauss, 1986, Schuytema, Nebeker, & Stutzman, 1997).

In contrast, the results of principal component analysis showed that the mortality of *D. pulex* (%) was directly related to the concentration of Fe and inversely related to the pH (Table 4 and Figure 7).

According to Randall (1998), the mortality of *D. longispina* exposed to water of a reservoir treated with Fe salts was not significant ($p > 0.5$) and the response decreased with increasing particulated Fe. Iron is completely soluble at pH between 2 and 3 (Mance & Campbell, 1988). Since the reservoir has a predominantly alkaline pH, the main chemical species are expected to be the ferric oxide hydrate, which precipitates in a few days at pH greater than 8 (Randall, Harper, & Brierley, 1999). The concentrations of this metal in samples of filtered water from the La Fe Reservoir did not exceed the value of the LC50-48h (3.61 mg/l) reported in the literature for *D. pulex* (Milam & Farris, 1998).

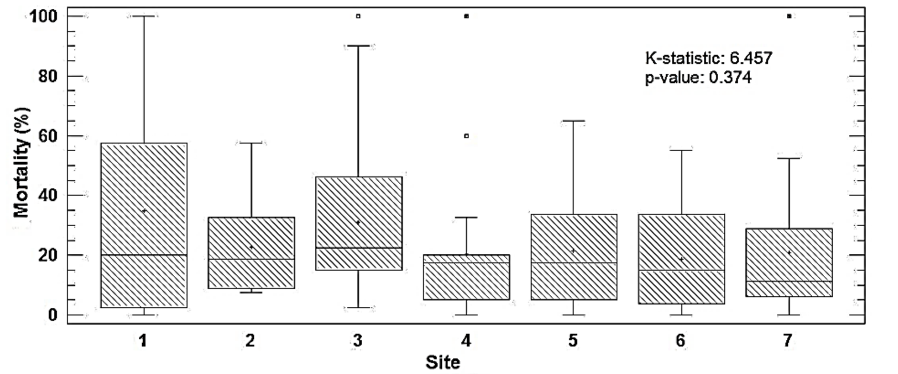

Figure 5 Mortality of D. pulex neonates exposed to filtered water samples from seven sites in La Fe Reservoir.

Table 4. Weight of components.

	Component 1	Component 2	Component 3	Component 4
Mortality (%)	0.190352	-0.181174	0.458385	0.590408
pH	-0.155825	0.47121	-0.293039	-0.0555624
EC (µS/cm)	0.285103	0.0499647	-0.273864	0.489644
DO (mg/l)	-0.570477	0.160231	0.242891	0.126647
OS (%)	-0.534098	0.0686887	0.398007	0.0307117
T (°C)	0.182305	-0.370809	0.422722	-0.241819
TH (ppm CaCO3)	0.0856904	-0.303117	0.0257073	-0.447083
Fe (mg/l)	-0.309977	-0.438211	-0.282423	0.15003
Mn (mg/l)	-0.240696	-0.397435	-0.257271	0.305362
Al (mg/l)	-0.229995	-0.364536	-0.293767	-0.132224

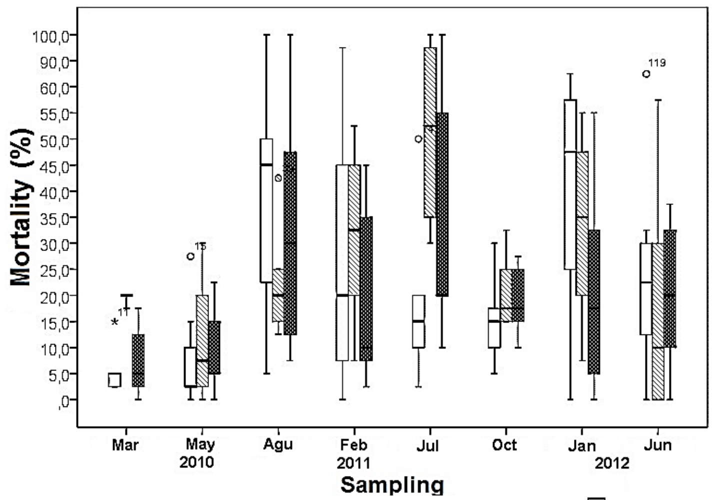

Figure 2. *Mortality of D. pulex exposed to water samples from three depths: surface (□), limit of photic zone (▧) and bottom (▩) for eight samplings.*

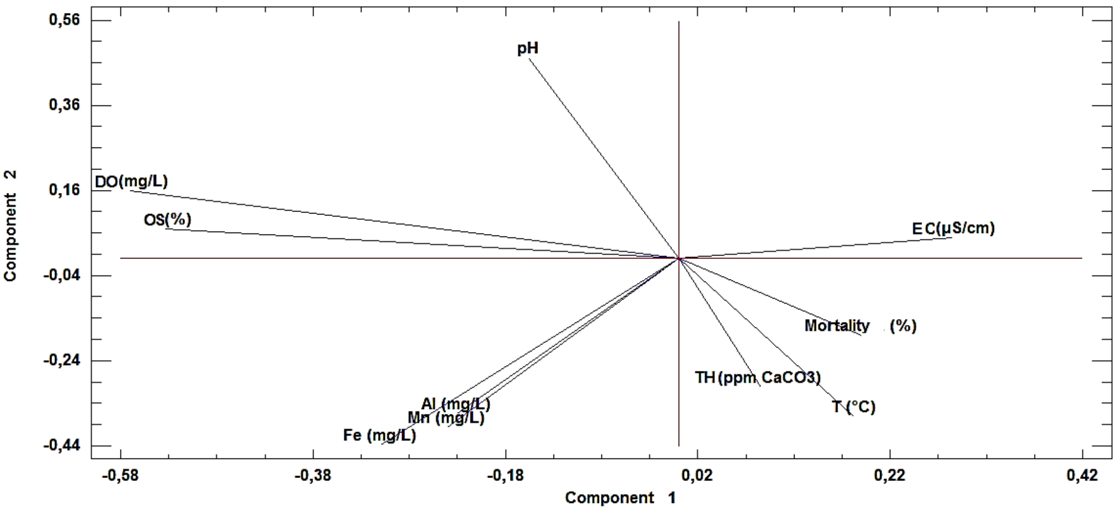

Figure 3. *Principal component analysis (PCA) for the variables evaluated.*

4 Conclusions

The reservoir dynamics ruled mainly by variation in rainfall, which is a factor that appears directly, influences the composition of the mixture in the reservoir and thus affects responses in bioassays at spatial and temporal level. The low relative depth of the reservoir could explain small differences in mortality of *D. pulex* neonates exposed to samples from different levels in the water column.

The species used in the tests showed to be sensitive to the sample acute toxicity analysis, although spatiotemporal variations in the body's response were not significant for most cases; therefore, performing of chronic tests is recommended.

No relationship between mortality percentage, physicochemical variables and metals was evidenced in small correlation coefficients, which could be related to the low concentrations of metals of higher toxicity (Pb, Cd, Cr) and possible antagonistic effects in the mixture. Therefore, none of the analysed variables could explain the behaviour of the mortality of *D. pulex* exposed to water from La Fe Reservoir. Simple associations were confirmed with electrical conductivity and total hardness, as previously described in the literature.

In general, periods of raining onset can increase the biomarker response due to eventual washing of basins, which was observed directly in the stations nearby tributaries' inputs, showing a possible enrichment of toxic substances that reach the reservoir. Therefore, the operation of the reservoir should take into account these critical conditions for the management of environmental risks.

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Toksikologinis vandens tiekimo rezervuaro monitoringas taikant ūmaus toksiškumo testus su *Daphnia pulex*

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Siekiant nustatyti ūmų vandens mėginių iš *La Fe* baseino (Antiokija, Kolumbija) toksiškumą buvo atliekami *Daphnia pulex* testai (48 val.). Mėginiai buvo imami 8 kartus, nuo 2010 m. kovo mėn. iki 2012 m. birželio mėn., – laikotarpis, kurio metu buvo liūčių. Mėginiai buvo imami iš vandens stulpelio trijuose skirtinguose gyliuose judančio gėlo vandens vietovėse. Mėginių paėmimo vietoje ir laboratorijoje buvo matuojamos tokios fizikocheminės savybės, kaip pH, elektrinis laidumas, temperatūra ir ištirpusio deguonies kiekis, ir buvo nustatyti ištirpusios geležies (Fe), mangano (Mn), aliuminio (Al), švino (Pb), kadmio (Cd) ir chromo (Cr) kiekiai.

Bendros gamtinių vandenų fizikocheminės savybės buvo tokio lygio, kaip tikėtasi; nustatytas šiek tiek didesnis iš dugno ir iš kai kurių intakų paimtų mėginių laidumas. Toksiškumo testų rezultatai parodė didelį mirtingumą tik 28 % mėginių. Didesnis *Daphnia pulex* mirtingumo lygis nustatytas atliekant testus, kai kritulių kiekis buvo didesnis (2010 m. rugpjūčio mėn., 2010 m. vasario ir liepos mėn.), ir daugiausia mėginiuose, paimtuose netoli vietų, kur suteka natūralūs intakai. Jokių reikšmingų sąryšių tarp fizikocheminių kintamųjų ir *Daphnia pulex* mirtingumo nenustatyta.

Raktiniai žodžiai: ūmus toksiškumas, Daphnia pulex, baseinas, La Fe.