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RESEARCH / INVESTIGACIÓN

Heavy metal analysis at the Cerrillos de Ponce Reservoir, Puerto Rico

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Abstract: *The Cerrillos reservoir is located in the city of Ponce, is one of the most important reservoirs in the southern area of Puerto Rico. The purpose of this research was to analyze the presence and concentration of heavy metals in Cerrillos de Ponce Reservoir as part of a monitoring of water quality. For this investigation a simple sampling was made in threepoints of the Reservoir Cerrillos de Ponce for a period of nine months that were identified like zones A, B and C. These samples were analyzed by an Optical Spectroscopy Emission Coupled Plasma Inductor (ICP-OES 3300 XL). In the results obtained it was demonstrated that the elements Silver (Ag), Arsenic (As), Chromium (Cr), Lead (Pb), Vanadium (V), Cadmium (Cd) and Zinc: World Health Organization and the Agency for Toxic Substances and Disease Registry. The Kruskal Wallis test was performed to test whether there is no significant difference in the number of elements detected and the sampling points at Cerrillos de Ponce Reservoir. The results of the test were not significant,* $\chi 2$ *(N = 26) = .467, p = .792. However, the linear regression analysis confirmed that the water level in Cerrillos de Ponce Reservoir in Puerto Rico affects the heavy metals that reached the limits established by the World Health Organization and the Agency for Toxic Substances and Disease Registry.*

Keywords: *Cerrillos reservoir, heavy metals, linear regression.*

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INTRODUCTION

Water is a primary resource for life. A total of 97.24% of water on the planet is in the oceans, whereas 2.76% is distributed in rivers, glaciers, atmosphere, subterranean waters and lakes (U.S. Geological Survey, 1984). Lake and reservoir ecosystems support complex and important interactions of food chains and provide necessary habitats to support numerous endangered species (EPA, 2013). The Cerrillos Reservoir, located in the city of Ponce is one of the most important reservoirs in the southern area of Puerto Rico. Its construction was determined in 1992 by the U.S Army Corp of Engineers and it is even the largest reservoir in the Caribbean. The main purposes of the Cerrillos de Ponce Reservoir, Puerto Rico are to control floods, recreation and supply water to the inhabitants (U.S Geological Survey, 2008).

Changes in climate and pollution threaten lakes and rivers; the sources of water that are used for daily living when in contact with pollutants are leading the planet to a water crisis (Natural Resources Defense Council, s.f). Water pollution is defined as the incorporation of substances into a body of water that deteriorates its quality, so that it is no longer suitable for the use that was designated (JCA, 2003). The main causes of unnatural contamination in bodies of water in Puerto Rico are: domestic discharges, industrial discharges, agriculture waste, sedimentation and erosion (JCA, 2003).

One of the main water pollutants corresponds to heavy metals (JCA, 2003). These are naturally found on earth and become toxic pollutants if their distribution in the environment is altered through anthropogenic activities such as the use of products for agriculture (Instituto Nacional de Ecología y Cambio Climático, 2009). Heavy metals, unlike organic pollutants are persistent in nature and therefore, they tend to accumulate in different niches of ecosystems (Madera-Parra et. al. 2014).

It is necessary that water be in optimal conditions for the good of sake of communities. On the other hand, heavy metals become toxic to people and environment (Instituto Nacional de Ecología y Cambio Climático, 2009), it is even possible to develop diseases by being in contact with water contaminated by heavy metals in high quantities (Instituto Nacional de Ecología y Cambio Climático, 2009). Under these circumstances, there is an absence of research related to the presence of heavy metals in the Cerrillos de Ponce Reservoir, Puerto Rico. This research was aimed to analyze the presence and concentration of heavy metals in the Cerrillos de Ponce Reservoir, as part of a water quality monitoring.

LITERATURE REVIEW

In a study conducted by Olgunoğlu et al. (2015) northeast of the Mediterranean Sea, the levels of heavy metals in muscle and gills of 4 different species of fish were studied, to then compare both quantities found. To carry out this study, samples of Merluccius merluccius, Lophius budegassa, Helicolenus dactylopterus and Chlorophtalnus agassizi, were collected at a depth between 459 and 582 m. After collecting the samples, they were rinsed with clean sea water, separated by species, stored in containers, preserved in ice and transferred to the laboratory, where both weight and size were measured. Likewise, tissues and gills were separated.

Samples were placed in polyethylene bags and stored at -20°C until the process of analyzing the presence of metals. In the study, 16 samples of each species were collected in the sampling area. When analyzing the presence of heavy metals, a greater amount of heavy metals (Fe>Zn>Cu>Pb>Cd) was found in the gills than in muscles. The quantities varied between species due to their different eating and living habits, although they were in the same area. Olgunoğlu, et al. (2015) concluded that the levels of heavy metals found in the fish, despite being high, were within the limits allowed by food regulations by determining that the consumption of the analyzed fish are not a risk to humans.

However, Abubakar et al. (2015) conducted a study in Kaduna, Nigeria about the presence of heavy metals on the water surface. In order to carry out this study, they performed a study of recognition, collection of samples and laboratory analysis. This was carried out to know the sources of pollutants throughout the southern area of the Kaduna River. A total of 5 samples were obtained around the Kaduna River and the Kakuri-Makera drains, in which the first samples were obtained from the drain of Kakuri-Makera, being called point A. They also collected three samples in different areas of point A at 50 m intervals, and the same procedure was followed with samples B, C, D and E obtained in the area of point B, at 100 m intervals. The samples were preserved with nitric acid and the placed in the refrigerator before taking them for analysis in the laboratory, where they prepared the samples to analyze them on the XRF-X ray and quantify the presence of heavy metals.

The results of this study indicated the presence of Cr, Fe, Cu, Zn, As and Pb and it was also found that Cr and Pb exceeded the limit of accepted quantity in samples A and B; Fe exceeded the limit of accepted quantity in samples A and B; Cu exceeded the limit of accepted quantity in sample A; Zn was below the accepted level and As exceeded the limit in samples A and B. These authors concluded that there was a contamination due to the high presence of heavy metals as a result of the urban, municipal and industrial discharges.

In another work carried out by Madera-Parra et al. (2014) for 60 days in the micro-station biology research of del Valle University, the effect of the concentration of heavy metals on the physiological response and accumulation capacity of three tropical plant species used in phytoremediation of leachates from landfills. The three ropical species selected were: Gynerium sagittatum, Colocasia esculenta and Heliconia psittacorum, obtained from a local nursery.

The experimental design considered by Madera-Parra et al. (2014) included two factors: species of plants and concentration of heavy metals in the leachate. The reactors were fed with synthetic leaching, condition used in other studies aimed to minimize potential inhibitory effects on phytoremediation. Chlorophyll, water potential and concentration of heavy metals were measured in plant tissues, whereas water potential presented no changes between species and the distribution of heavy metals in plant tissues decreased in the following order: root, leaf and stem. The plants evaluated demonstrated potential for phytoremediation of leachate and all of them can be classified as accumulators of heavy metals.

In a study carried out by Lychagin, et al (2015) in the mouth of the Volga River in Russia, the presence of heavy metals was studied in water, plants and sediment. This study was aimed to obtain information about the characteristics of the terrestrial and aquatic geochemical structure of the delta. In that study, 150 samples of water, 160 samples of suspended matter and more than 100 samples of sediment and plants were collected in the larger delta branches. The contamination of the aquatic system was manifested in the excess of heavy metals in the suspended matter, more notable in the flood period. The macrophyte content varied depending on the physiological characteristics of the species. Thus, these authors concluded that the conditions of the Volga River delta remain relatively safe, especially when compared to other estuaries of large rivers.

Monikh, et al. (2015) in the northeast of the Persian Gulf studied the concentrations of heavy metals in sediment, shrimp and two species of fish. In order to achieve this, three locations for sampling were chosen, including the mouth of the Arvand River, the Meleh estuary and the Musa estuary. A total of 60 fish, 50 shrimp and 15 sediments were the samples chosen in each location in the summer of 2010. The samples were placed on ice and taken directly to the laboratory to be frozen until their use. Fish samples were separated in muscles, gills and liver, whereas shrimp were separated in muscles and hepatopancreas. Each part was submerged separately in nitric acid and perchloric acid until they were completely dissolved.

Sediment samples of each station were dried in the oven until they reached constant weight. Then, they were digested in HNO3 and hydrochloric acid to the dilute them in distilled and filtered water. The concentrations of heavy metals were determined with an atomic absorption spectrophotometer, and the sediments and organisms from the mouth of the Arvand River presented higher concentration of metals than the other areas. The researchers demonstrated the result of the anthropogenic influence in the presence of metals. Finally, the levels of heavy metals in fish liver and shrimp hepatopancreas was significantly higher than the levels of heavy metals in the tissues of muscles and gills.

Another study related to the presence of metals in the water was carried out by Haiyan Li et al. (2013) in Beijing, China. These authors analyzed the effects of pH, temperature, dissolved oxygen and flow rate on the release of heavy metals in the sediments in sewer sediments. The sediment samples were collected in a sewer located in North Lishi Road, Beijing and then, they were transported to the laboratory to be studied. Each sample was prepared to detect each of the heavy metals contained in the sediment samples (Pb, Cu, Zn, Cr, Cd) and they were classified according to their characteristics and concentrations.

Results indicated that the effect of pH on release of heavy metals increased in acidic and alkaline conditions, but the release was higher with a low pH. Regarding temperature, the release of heavy metals in the sediment increased as the temperature increased, the performance of the release of the metals with the influence of the dissolved oxygen was similar between the same metals. The release increased rapidly under aerobic conditions and the absorption occurred under anaerobic conditions and increased even more when the concentration of dissolved oxygen was low. In the same way, the flow rate indicated that a high speed releases heavy metals very effectively, but at the same time, it changes the conditions of pH and dissolved oxygen. Haiyan Li et al. (2013) concluded that the release of heavy metals is different in different flow rates.

However, Voica et al (2013) investigated heavy metals in the Aries River in Romania by using the instrument or technique of mass spectrometry with inductively coupled plasma (ICP-MS). These authors decided to start this study due to the concern of possible contamination of heavy metals by a nearby mine, according to previous studies, the work carried out in the mines causes contamination of heavy metals in the nearby areas. The samples were taken in a rainy period in 9 localities of the Aries River. Subsequently, the samples were filtered to be then analyzed by ICP-MS.

The concentration of heavy metals showed a common pattern in all their concentrations for all localities, except in the Aries Bay. There, the concentrations of Pb, As, Fe, Se, Cu and Zn exceeded in comparison to the other localities. Aries Bay used to be a gold mine until 2004, although a small fraction of heavy metal contamination was found in the water, it is obvious that the old mine in the area led to the contamination of the waters with heavy metals.

However, Ajami & Fataei (2015) determined the levels of heavy metals in the waters for agricultural use in the Meshkinshahr River, located in northeastern Iran. The water samples were taken in the high rainfall and low rainfall seasons and each sample was sterilized and then subjected in the atomic absorption device in order to measure the levels of heavy metals. Then, the results were compared with standard values from the Environmental Protection Agency (EPA) and the World Health Organization (WHO) about the allowed limit of heavy metals. During the high precipitation season, values of lead and chromium were found below the EPA and WHO limits were higher than the limits established by the EPA and WHO. On the other hand, during the low rainfall season, lead and chromium values exceeded the limits of EPA and WHO.

Lannacone & Salazar (2007) studied the toxicological effect of water samples on the invertebrate organism Chirohmus calligraphus in the Junin Lake, located in Peru and that research was aimed to determine the degree of toxicity by heavy metals in samples of surface waters, using C. calligraphus as an ecotoxicological tool. The egg masses of C. calligraphus were extracted to then be transferred in plastic containers. In the laboratory, the egg masses were separated from the substrate to which they were attached and those eggs were incubated to analyze the larvae. This stage is the most susceptible to heavy metals in toxicity tests.

Egg masses were collected every month in the lagoon in order to determine the mean lethal concentration for all water samples from the three study points of the Junin Lake. In total, three dilutions were used: 6.25%; 12.5%; 25%; 50% and 100%. The larvae of C. calligraphus were exposed during 48 hours to the same laboratory conditions in which the eggs were incubated and four repetitions were considered, in total 240 first instar larvae of C. calligraphus. These larvae were not fed during the test and all bioassays were carried out in parallel to assays with copper sulfate as a reference toxic to evaluate the sensitivity of C, calligraphus and thus, to ensure the health of the organisms.

When considering the chemical analyses in the waters, the concentrations of lead and chromium were above the maximum water allowed quality limits of Peru. The metals Fe, Mn and Zn caused the ecotoxicological effects of mortality in terms of the mean lethal concentration observed in bioassays with C. calligraphus with samples from the three locations. However, the decrease in toxicity occurred by a decrease in pH and with an in crease in Fe. From the three localities were water was collected, Pari, Upamayo Dam and Upamayo Bridge, Pari had the highest concentration value of dissolved oxygen with a 7.17; it was observed that the less amount of dissolved oxygen, the less amount of metals were found. Lannacone et al. (2007) concluded that the use of the C. calligraphus organism confirms that samples from Lake Junin showed toxicological effects.

METHODOLOGY

Sample collection

Water samples were collected in three areas of the Cerrillos de Ponce Reservoir, Puerto Rico. Polyethylene bottles will be used to collect the samples. This prevents the samples from adhering to the walls of the bottle and losing part of the sample. At the time of sampling, two observations will be considered to avoid sample contamination: do not touch the mouth of the bottle and use gloves without dust. Bottles were immersed in water and after obtaining the sample; bottles were quickly closed and stored in a cooler with ice. For proper preservation, the sample will not be filtered. After 16 hours, its pH will be checked and if the pH is less than 2, the sample wil be stored for a period of 6 months before its analysis.

Location of sampling areas

For this study, a simple sampling was performed in three areas of the Cerrillos de Ponce Reservoir for a period of nine months. These areas were identified as A, B and C. Area A corresponded to the entry of the main river that provides water to the Reservoir. Area B was located at a midpoint of the reservoir, where the berth of fishing boats is located. Finally, area C is the deepest part and the one with the highest water stirage of the Reservoir (Figure 1). The reason of the simple sampling was because it is considered that the characteristics of the reservoir are homogeneous, that is, when the body of water presents no alterations in its spatio-temporal composition (Mezquida, 2012).

Figure 1: Sampling points in the Cerrillos de Ponce Reservoir: Image taken from Google Earth.

Analytical procedure

Samples were analyzed in the inductively coupled plasma optical Emission Spectroscope (ICP-OES 3300 XL). The method 200.7 of the Environmental Protection Agency (EPA) was used. At the time of analyzing the samples, 50 ml of the previously preserved sample were transferred to a 250 ml flask. A total of 1 ml of HNO3 (1+1) and 0.5 ml of HCl (1+1) were added to break chemical bonds and eliminate non-metallic products. The flask was placed on a hot plate, previously set at a temperature not exceeding 85°C. The flask was covered with a watch glass in order to prevent contamination of the sample. The volume was reduced to 10 ml. To reduce further evaporation, it was reheated for other 15 minutes. After reheating, the sample was allowed to cool to room temperature; the solution was transferred to a 50 ml volumetric flask and brought to the calibration mark with deionized water.

The instrument was calibrated using a calibration blank composed of HCl and HNO3. The metals to be analyzed were Be, Cd, Co, Cr, Se, Si, Ag, Zn, As, Fe, Mn, Ni, Cu, Ca, Mg, V, Ba, Na, Pb and Al. For this analysis, two multielemental standards of the PelkinElmer Company were used. One of the standards had 1,000 ug/mL of K, 500 ug/mL of Si, 100 ug/mL of Al, B, Ba, Na and 50 ug/mL of Ag; whereas the other had 100 ug/mL of As, Be, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, V and Zn. A 3-point calibration curve was performed. To make this curve, 3 concentrations were prepared at 0, 3 and 5 ppm of each metal to be analyzed.

RESULTADOS

Table 1: Heavy metals found in the Cerrillos de Ponce Reservoir, November 2015.

Metal	November 2015		
	A	B	C
Ag (mg/L)	1.26	0.8	1.17
As (mg/L)	0.46	9.8	8.3
Ca (mg/L)	45.3	35	40.4
Cr (mg/L)	0.41	0.5	0.44
Cu (mg/L)	0.2	---	0.28
Fe (mg/L)	0.02	---	---
Mg (mg/L)	5.68	3.6	4.43
Pb (mg/L)	---	---	0.33
Si (mg/L)	61.7	49	50.9
V (mg/L)	0.91	1.1	0.87

Table 2: Heavy metals found in the Cerrillos de Ponce Reservoir in 2016.

Metal (mg/L)	January			February			March		April			September			October			December		
	A	B	C	A	B	C	A	B	A	B	C	A	B	C	A	B	C	A	B	C
Ag	0.372	1.85	0.744	1.47	1.84	0.619	1.91	0.49	0.914	1.54	1.25	1.81	1.33	0.34	1.65	1.24	1.2	---	---	---
As	12.8	11.7	11.4	---	14.4	1.03	7.71	2.85	5.01	9.63	4.35	9.2	16.2	12.4	10.5	14.2	8.35	7.06	16	13
Ba	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	0.06	0.1	0.08
Be	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ca	45.2	37.7	35.6	38.9	32.9	34.1	34.3	33.7	36	34.5	33.4	36.6	32.5	31.4	0.012	0.021	0.02	54.2	53	60.6
Cd	---	---	---	---	---	---	---	---	---	---	---	---	---	---	0.122	0.1	0.1	---	---	---
Co	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Cr	0.305	0.372	0.362	0.403	0.345	0.368	0.358	0.377	0.375	0.362	0.37	0.37	0.373	0.49	0.655	0.623	0.68	---	---	---
Cu	0.009	---	---	---	---	---	---	---	---	---	---	---	---	---	0.026	0.07	0.04	---	---	---
Fe	0.012	---	0.027	0.056	0.032	0.047	---	---	0.082	---	---	0.01	0.071	---	---	0.031	---	---	---	---
Mg	4.94	3.9	3.52	5.1	3.67	4.09	4.92	4.42	4.23	4.17	4.28	4.38	3.85	4.36	0.532	0.864	0.77	3.1	3.1	3.2
Mn	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Na	---	---	---	---	---	---	---	---	---	---	---	---	---	---	49.6	57.2	44.7	59.6	72	59.8
Ni	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Pb	---	---	0.414	---	0.29	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Se	---	---	---	---	--	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Si	66	59	54.5	40.1	34	35.2	39.7	38.4	40.6	37.2	42.1	39.6	40.4	39.4	0.543	1.03	0.33	73.8	69	66.4
V	1.07	1.08	1.15	1.05	1.23	1.06	0.925	1.16	0.805	1.29	0.978	0.91	0.878	1.19	0.883	0.812	0.52	---	---	---
Zn	---	---	---	4.94	---	---	---	---	---	---	---	---	---	---	0.229	0.203	0.2	---	---	---

Table 3: Heavy metals found in the Cerrillos de Ponce Reservoir, in 2017.

Metal	**January 2017**		
	A	B	C
As (mg/L)	15.7	17.3	8.02
Ba (mg/L)	0.055	0.071	0.09
Ca (mg/L)	33.4	47.5	51.9
Fe (mg/L)	0.018	---	---
Mg (mg/L)	---	2.56	2.64
Na (mg/L)	56.9	62.7	67.9
Si (mg/L)	38.1	53.3	56.9

Table 4: Water level in the Cerrillos de Ponce Reservoir in the months the samples were analyzed. (U.S Geological Survey).

Year	**Months**	**Average (feet)**
2015	November	558.02
2016	January	555.42
	February	551.89
	March	551.39
	April	550.49
	September	555.40
	October	569.22
	December	573.35
2017	January	572.44

Table 5: Heavy metals that reached the limits established by WHO and the Agency of Toxic substances and Disease Registry * in November 2015, in 2016 and 2017.

Mes/Muestra		Ag (mg/L)	As (mg/L)	Cr (mg/L)	Cd (mg/L)	Pb (mg/L)	V (mg/L)	Zn (mg/L)
November (2015)	A	1.26	0.46	0.409	----	----	0.913	----
	B	0.785	9.78	0.459	----	----	1.11	----
	C	1.17	8.3	0.443	----	0.33	0.869	----
January (2016)	A	0.372	12.8	0.305	----	----	1.07	----
	B	1.85	11.7	0.372	----	----	1.08	----
	C	0.744	11.4	0.362	----	0.414	1.15	----
February (2016)	A	1.47	----	0.403	----	----	1.05	4.94
	B	1.84	14.4	0.345	----	0.29	1.23	----
	C	0.619	1.03	0.368	----	----	1.06	----
March (2016)	A	1.91	7.71	0.358	----	----	0.925	----
	B	0.49	2.85	0.377	----	----	1.16	----
April (2016)	A	0.914	5.01	0.375	----	----	0.805	----
	B	1.54	9.63	0.362	----	----	1.29	----
	C	1.25	4.35	0.37	----	----	0.978	----
September (2016)	A	1.81	9.2	0.368	----	----	0.905	----
	B	1.33	16.2	0.373	----	----	0.878	----
	C	0.343	12.4	0.49	----	----	1.19	----
October (2016)	A	1.65	10.5	0.655	0.122	----	0.883	----
	B	1.24	14.2	0.623	0.1	----	0.812	----
	C	1.2	8.35	0.678	0.096	----	0.52	----
December (2016)	A	----	7.06	----	----	----	----	----
	B	----	16.4	----	----	----	----	----
	C	----	13	----	----	----	----	----
January (2017)	A	----	15.7	----	----	----	----	----
	B	----	17.3	----	----	----	----	----
	C	----	8.02	----	----	----	----	----
Estándar		0.01 mg/L	0.01 mg/L	0.05 mg/L	0.003 mg/L	0.01 mg/L	0.22 mg/L*	3 mg/L

Table 6: Kruskal-Wallis test

	Elements detected
Chi-squared	.467
gl	2
Sig.	.792

a. Kruskal-Wallis test
b. Grouping variable: sampling points

Table 7: ANOVA test in linear regression

ANOVA[a]

Model		Sum of squares	gl	Square average	F	Sig.
1	Regression	12.776	1	12.776	16.422	.005b
	Residual	5.446	7	.778		
	Total	18.222	8			

a. Dependent variable: metals detected that exceeded the limit
b. Predictor: (Constant), water level at the reservoir.

Tabla Nº 8. Summary of the linear regression model.

Model summary

Model	R	R square	R adjusted square	Estimated standard error
1	.837a	.701	.658	.882

a. Predictor: (Constant), water level at the reservoir.

Tabla Nº 9. Linear regression test

Coefficients

Model		B	Standard error	Beta	t	Sig.
1	(Constant)	80.007	18.895		4.234	.004
	Water level at the reservoir	-.137	.034	-.837	-4.052	.005

a. Dependent variable: metals detected that exceeded the limit.

DISCUSSION

According to the World Health Organization and the Agency of Toxic Substances and Disease Registry, the metals that exceeded the limit in November 2015 were Silver (Ag), arsenic (As), chromium (Cr), Llead (Pb) and vanadium (V). On the other hand, the metals that exceeded the limit in January, February, March, April, September, October and December 2016 were silver (Ag), arsenic (As), chromium (Cr), lead (Pb), vanadium (V) and zinc (Zn). Finally, the only element that exceeded the limit in January 2017 was arsenic (As). These results are observed in Table 5.

To discuss the research question: How does the amount of the elements detected at the different sampling points of the Cerrillos de Ponce Reservoir differ? A non-parametric test was carried out to test if there is no significant difference in the amount of detected elements and sampling points at the Cerrillos de Ponce Reservoir. A non-parametric test was used because the assumption of normality was not fulfilled (Shapiro-Wilk = $p < 0.05$) in the three sampling points for comparison (Field, 2005). The test results were not significant, ÷2 (N= 26) = .467, p= .792. This p value, when compared to a significance of 0.05 ($p > 0.05$) indicates the null hypothesis is not rejected. Therefore, there is no significant difference in the amount of detected elements and sampling points at Cerillos de Ponce (Table 6). This indicates that there is no specific point that represents a greater amount of detected elements as a source of contamination.

Silver tends to accumulate and cause toxicity in aquatic organisms, specifically in low trophic levels (Ratte et al., 1999), though also affects higher level organisms such as fish, through the transfer of silver in aquatic trophic chains (Luoma et al., 1999). In this study, high concentrations of Ag were found in each sampling point and the averages of the abovementioned concentrations mentioned in Table 5 are indicated as follows: point A (1.34 mg/L), point B (1.29 mg/L) and point C (0.887 mg/L). According to the World Health Organization, the concentration of silver should not exceed 0.01 mg/L.

arsenic is very toxic and one of the 10 substances that WHO considers more worrying for public health. According to Jabeen G. et al. (2011), the significant enrichment of As in fish organs has a direct dependence on the toxicity of As in the water, by presenting bioaccumulation of the toxic, affecting the food chains of ecosystems. The averages of the mentioned concentrations that can be observed in Table 5 are indicated as follows: point A (8.5 mg/L), point B (12.49 mg/L) and point C (10.95 mg/L). According to the World Health Organization, the concentration of arsenic should not exceed 0.001 mg/L.

According to Song et al. (2016), chromium is one of the most dangerous heavy metals. Its toxic effects on growth and development of plants include alterations in the germination process, as well as in the growth of roots, stems and leaves that affect production and yield (Shanker et. al 2005). Both averages and concentrations mentioned in Table 5 are indicated as follows: point A (0.410 mg/L), point B (12.416 mg/L) and point C (0.452 mg/L). According to the World Health Organization, the concentration of chromium should not exceed 0.005 mg/L.

Another of the elements found that exceeded the concentration of the World Health Organization was cadmium. This element accumulates and causes morphological, physiological, biochemical and structural changes by including problems in growth and development (Benavides et al., 2005). The concentrations mentioned in Table 5 found in October are the following: point A (0.122 mg/L), point B (0.1 mg/L) and point C (0.096 mg/L).

Regarding lead, high levels in blood not only cause disorders of the central nervous system, but also affect kidneys, blood, and central nervous system as children grow and develop. Health problems derived from environmental contamination by lead are a serious public health problem in many developing countries. According to Radulescu et al. (2014) Pb has a strong toxicity for the aquatic biota when modifying the properties. The averages of concentration mentioned in Table 5 are the following: point A (0 mg/L), point B (0.29 mg/L) and point C (0.372 mg/L). According to the World Health Organization, the concentration of lead should not exceed 0.001 mg/L.

A linear regression was carried out to discuss the research question: How does the level of water at Cerrillos de Ponce Reservoir affect the heavy metals that reached the limits established by the World Health Organization and the Agency of Toxic Substances and Disease Registry? The linear regression was performed to prove that the water in the Cerrillos de Ponce Reservoir is not affecting the heavy metals established by these organizations. It should be noted that for such linear regression analysis, the assumptions of the variable measurement scale, normality (Shapiro-Wilk = $p > 0.05$) and linearity (Field, 2015) were fulfilled.

When analyzing the ANOVA test of linear regression (Table 7) it can be established that the model of detected metals that exceeded the limit and water level at the reservoir was significant ($p < 0.005$). This shows that the water level at the reservoir has a significant impact on the metals detected that exceed the contamination limit. In addition to the fact that both variables are linearly related, in Table 8 it can be observed that the adjusted coefficient of determination (R2) explains that the level of water at the reservoir ha affects 65.8% of the results of metals detected that exceed the limit. However, in order to analyze the coefficients in the prediction of the model, it is interpreted that the value of the regression model slope (-.137) indicates how much the dependent variable changes (heavy detected metals that exceeded the limit), given a change in the independent variable (water level at the reservoir). According to this, the regression is as follows: forecast for heavy metals that exceed the limit = 80.007 - .137 (water level of the reservoir in feet).

During December 2016 and January 2017 the elevation of the water in the Cerrillos de Ponce Reservoir reached 573.35 and 572.44 feet, respectively. These months recorded the highest amount of water during the period studied at the reservoir. According to Table 5, the concentrations of heavy metals that exceeded the limit were lower during December 2016 and January 2017 (Table 5). This indicates that as long there is a high the amount of water at the reservoir, the lower concentration of heavy metals will be found in it. This finding is consistent with the research carried out by Ajami & Fataei (2015), where in low rainfall, the values of lead and chromium exceeded the limits established by the Environmental Protection Agency and the World Health Organization.

CONCLUSIONS

Based on the results of this research, it is proved that the elements Silver (Ag), arsenic (As), chromium (Cr), Llead (Pb), vanadium (V), cadmium (Cd) and zinc (Zn) exceeded the limit allowed by the World Health Organization and the Agency of Toxic Substances and Disease Registry. In addition, it can be concluded that during the months in which the reservoir was analyzed, there is no significant difference in the amount of detected elements and the sampling points in the Cerrillos de Ponce Reservoir. Therefore, in throughout the Cerrillos de Ponce Reservoir, Puerto Rico there were the same amount of elements detected in the months of the study. Finally, a prediction model was established at the Cerrillos de Ponce Reservoir for the detection of heavy metals that exceed the limit by regulatory agencies such as the WHO and the US Agency of Toxic substances and Disease Registry.

Forecast in heavy metals that exceed the limit = 80.007 - .137 (water level of the reservoir in feet).

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