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ECOTOXICOLOGICAL ASSESSMENT WITH *PSEUDOKIRCHNERIELLA SUBCAPITATA* (CHLOROPHYTA) AND PHYSICOCHEMICAL PARAMETERS IN THE UPPER-MIDDLE PART OF CAMANA-MAJES-COLCA BASIN (AREQUIPA-PERU)

EVALUACIÓN ECOTOXICOLÓGICA CON *PSEUDOKIRCHNERIELLA SUBCAPITATA* (CHLOROPHYTA) Y PARÁMETROS FISICOQUÍMICOS EN LA PARTE MEDIA ALTA DE LA CUENCA CAMANA-MAJES-COLCA (AREQUIPA, PERÚ

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ABSTRACT

The ecotoxicological evaluation of the upper-middle part of Camana-Majes-Colca Basin, Peru was carried out in three sampling points: Capiza, Colca and Majes compared with $CuSO_4$ (1 mg·L⁻¹), which is a toxic standard for the bioindicator microalga *Pseudokirchneriella subcapitata*. In each sampling point, pH, temperature, conductivity, dissolved oxygen, total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), sulfides, ammoniacal nitrogen, cyanide WAD, free cyanide, nitrates, total phosphorus, total nitrogen, thermo-tolerant coliforms, oils grease and heavy metals were measured. The lowest rate of *P. subcapitata* growth was in Capiza's sampling Point (0.008) where values of pH, manganese and iron exceeded Peruvian EQS (Environment Quality Standard) for water in category 3. These results show a correlation with high level of significance (P<0.01) between the physicochemical parameters pH, Mn, TSS and the growth inhibition percentage of *P. subcapitata* explained by the Pearson coefficient (r=0.98).

Keywords: bioindicator, ecotoxicological, EQS, Pseudokirchneriella, rate of growth.

RESUMEN

La evaluación ecotoxicológica de la parte media alta de la cuenca Camaná-Majes-Colca, Perú se realizó en tres puntos de muestreo: Capiza, Colca y Majes en comparación a un tóxico de referencia CuSO₄ (1 mg·L⁻¹) mediante la microalga *Pseudokirchneriella subcapitata* como bioindicador. En cada punto de muestreo se midió pH, Temperatura, conductividad, oxígeno disuelto, (STS) sólidos totales suspendidos, Demanda Bioquímica de Oxígeno (DBO₅), Demanda Química de Oxigeno (DQO), Sulfuros, Nitrógeno amoniacal, Cianuro WAD, Cianuro libre, nitratos, fósforo total, nitrógeno total, coliformes termotolerantes, aceites y/o grasas y metales pesados. La menor tasa de crecimiento de *P. subcapitata* fue en el punto de muestreo Capiza (0,008) observándose valores de pH, Manganeso y Fierro que superan los ECA Peruanos (Estándares Nacionales de calidad ambiental) para aguas categoría 3. Estos resultados demuestran una correlación con alto nivel de significación (P<0,01) entre los parámetros fisicoquímicos pH, Mn, STS y el porcentaje de inhibición de crecimiento de *P. subcapitata* explicado por el coeficiente de Pearson (r=0,98).

Palabras clave: bioindicador, evaluación ecotoxicológica, ECA, Pseudokirchneriella, tasa de crecimiento.

INTRODUCTION

Pseudokirchneriella subcapitata (Hindak, 1990) (ex Selenastrum capricornutum) is a unicellular Green microalgae (Chlorophyta) with semi-circle form, and with a volume between 40 and 60 µm³; can be found in eutrophic or oligotrophic epicontinental aquatic systems. The assay with this algae can be used as a useful tool for the potential phytotoxicity estimation of superficial freshwater, underground water, waste water and other forms of liquid samples, like eluated and leachate Interstitial water from sediment or any pure soluble compound of water. This test is especially useful to be practiced in laboratories with basic equipment, being a simple assay of low cost (Ramirez & Mendoza 2008).

A variety of methods and tests are available for the determination of phytotoxic effects of chemical products, effluents and sediments in *P. subcapitata*, which is the most common used algae from sweet water. Laboratories of toxicity studies in microalgaes focused in the measurement of final points as a result of chronic expositions, generally between three and four days; tests of toxicity in the growth inhibition are generally used to determinate the presence of toxic substances in the environment (Pardos et al. 1998). The algae are the most important primary producers in aquatic ecosystems, and are potent environment indicators of water quality. Various standard protocols have been developed as part of test batteries for ecotoxicologic assessment of chemical products and wastewater (Nyholm & Kallqvist 1989) as well as the experimental factors of form. Media composition is also known for its significant effect in the toxicity of some chemical products (Kamaya et al. 2004).

Pseudokichneriella subcapitata, has been highly recommended by different environment

regulatory agencies due to its elevated and constant toxic sensibility, high availability, genetic stability, population uniformity, representative of trophic level and its facility for culture (OECD 1984, ISO 1989, EPA 1994, APHA 1998).

The ecotoxicology is a multidisciplinary science that studies the effect of the chemical substances of anthropogenic source in the ecosystems; its fundamental study problem is pollution in biotic systems in toxic form, alteration of species, productivity reduction, etc. Due that not always a pollutant has a behavior as toxic agent, we only can suppose the generation of undesirable levels in a determined ecosystem. In the field of investigation referent to the ecotoxicology; the biologic changes expressed as organism, populations or communities are useful as signals of possible alteration that suffers an ecosystem due to the anthropogenic activities (Espinas & Vanegas 2005, Orrego et al. 2005, Capó 2007).

The Basin of Camaná-Majes-Colca River is located in the south of Peru; it's area of influence is principally covered by Arequipa department; also includes a part of Cusco Department and West of Puno. Including the Angostura Sub-Basin, has a total extension of 18454237 km², formed by Colca river, which originates in the Occidental Mountain range, born at 4886 masl with a length of 388 Km; take different names: Pacco Pacco, Chilamayo, Colca, Majes and Camana; and to Molloco river which has the origin in Shila Mountain range which flows in Colca; In cojuction with Andamayo river and other smaller form Majes river, which flows in Pacific Ocean with the name of Camana river. In the case of Capiza river; perpetual snow of Coropuna snow-capped mountain and the influence area, are important and considerable; other snow-capped mountains that contribute to the basin are the Pabellon, Huajrahuire, Accomasto, Chinchon, Chila; they flow into

the Misapuqui, Hancarama, Pisaca, Pasque, Andagua river and whose related settlement are the localities of Orcopampa and Andagua (AUTODEMA 2009, Chira *et al.* 2011, ANA 2012).

Our purpose was to make an ecotoxicologic assessment of the sampling points from the upper-middle part of the Camana – Majes - Colca basin, using the growth inhibition of *P. subcapitata* compared to physicochemical parameters.

MATERIALS AND METHODS

Study Areas

The three water sample points were (1) Capiza river before the confluence with Colca river, localized between the UTM coordinates 773746 Est. and UTM 8246070 North, 897 m.a.s.w; (2) Colca river before the confluence with Capiza river that is located between the UTM coordinates 773809 Est. and UTM 8246113 North, 909 m.a.s.w; (3) Majes river around Huancarqui bridge located between the UTM coordinates 769422 Est. and UTM 8220939 North, 618 m.a.s.w. (Figure 1)

Pseudokirchneriella subcapitata (Chlorophyta) was provided by CENMA (National environment center) University of Chile, Metropolitan region, Santiago, Chile.

Culture and maintenance of *P. subcapitata*

The culture and maintenance took place in the Laboratory of Aquatic Biology from the Academic Department of Biology, National University of Saint Augustine (UNSA), Arequipa-Peru, between November and December of 2013. The culture media 18X was used, and consisted in five solutions, the first contained micronutrients and the other four contained macronutrients (Castillo 2004) completed with 500 mL of distilled water each one. For each liter of culture media, 1 mL of each of the five solutions was added to distilled

water respectively. The *P. subcapitata* culture was make within a lighting chamber in flasks of 50 mL, using an Aquarius air pomp with continuum illuminance of 2000 lux; temperature ($T^{\circ}=24 - 25^{\circ}C$) and pH = 7.5 + 0.1. The pH was calibrated using NaOH or HCl 1N (Figure 2.a); In the same way, *P. subcapitata* culture was made in solid media, and consisted in 100 g of agar-agar in 200 mL of 18X culture media. Subsequently, various tubes were middle filled with the culture media: were sealed and sterilized in autoclave to 120°C at 15 pounds of pressure for 20 min. After that period, the tubes were left inclined until the media was solid, then the microalgae were transferred on the surface of culture media using a platinum wire loop.

The inoculation began in the deeper zone of the tube and continued until the exterior part of the media; once inoculated the new tubes were incubated in a lighting camera to $24 \pm 2^{\circ}$ C, with an illuminance of 2000 lux for 72 h; then, the tubes were removed from the chamber and were store in a refrigerator between 2 and 4 °C thus, the algae continue in low growing rates (Figure 2.b). Finally, an inoculum of 2.5×10^6 cell·mL⁻¹ was obtained, counting with Neubauer chamber Cell Counting (Figure 2.c and d), and 2mL of the inoculum were extracted and diluted in 18 mL of culture media with the purpose of obtain in each probe vial for ecotoxicologic bioassays with an initial concentration of 10000 cell·mL⁻¹ (Castillo 2004).

Ecotoxicological bioassays

An aqueous sample was obtained preparing a probe buffer solution (NaHCO₃ 15 mg·L⁻¹) for the different concentrations of the bioassay.

A toxicity probe was done to 72 h of exposition, using as positive control and reference a Toxic substance $CuSO_4$ (1 mg·L⁻¹) in four concentrations: 50%; 25%; 12.5%; 6.25% a positive control of 100% and a negative control with aqueous sample.

Ecotoxicological Bioassays were made using the solutions $CuSO_4$ (1 mg·L⁻¹) and the water samples from Capiza, Colca and Majes river; using an experimental design of static type as follow: four concentrations for each bioassay and three repetitions for each concentration with the exposition of 10000 cell·mL⁻¹ in each sample unit.

The concentrations of the water of river were expressed in 50; 25; 12.5 and 6.25 %. One positive control of pure problem sample water and a negative buffer control solution of NaHCO₃ (15 mg·L⁻¹). The sample units were assay tubes with 2.5 mL of the solution with same concentrations, expressed in volume percentages of water. The growing and the inhibition of cell growth were evaluated too.

The cellular density (N) was determined by microscopy, using a Neubauer shining light chamber for counting after 72 h of exposition. The growing rate was also calculated with the division number formula (μ).

 $\mu = (\underline{\ln N} - \underline{\ln No})/\underline{\ddot{A}}t$

N = Cellular density at the end of the bioassay. No =Initial nominal Cellular density.

t = Interval of the time considered in the assay.

The data analysis was carried out using ANOVA (Analysis of variance) for testing the growing of *P. subcapitata* under effect of different ecotoxicological bioassays and different concentration after 72 h of exposition; also, the multiple contrast probe of Tukey was used to evaluate the difference between the growing groups to the bioassays and concentrations using the statistical program SPSS ver. 20.0.

Physicochemical parameters

The sample collect was done using the "national protocol for quality monitoring of natural shallow water bodies" (ANA 2011). For the quality evaluation of shallow water

from sample stations, we used the EQS (Environment quality standard) established in the supreme decree N°022-2008-MINAM; with the objective of establish the concentration level or the element grade of substances and physical, chemical and biological parameters present in water in its conditions of receptor body, and basic component of the aquatic ecosystems that does no't present risks for people health and for the environment.

The parameters measured in situ were pH, Dissolved Oxygen (DO), temperature and electric conductivity, using a multi-parameter of brand Odeon, according to the National Protocol of Quality Water Monitoring of Natural Shallow Water Bodies; the parameters measured in laboratory were oil and grease (EPA-821-R-10-001 method); Biochemical oxygen demand (BOD₅) (SM 5210 5-Day BOD Test); Chemical oxygen demand (COD) (SM 5220. Closed reflux, colorimetric method); cyanide WAD (SM 4500 - CN - I,E. Cyanide. Weak Acid Dissociable Cyanide/Colorimetric Method.); free cyanide (SM 4500-CN- J,E. Cyanide. Cyanogen Chloride. Colorimetric Method); sulfide (SM 4500 S2- D. Sulfide. Methylene Blue Method); phosphates (SM 4500-P E. Phosphorus. Ascorbic Acid Method); Nitrates according $(SM 4500-NO_3 - E. Nitrogen; ammoniacal)$ nitrogen (SM 4500-NH₃ - D. Nitrogen. Ammonia - Selective Electrode Method); Total Nitrogen (SM 4500-Norg-B.). Macro-Kjeldahl Method); total suspended solids (TSS)(SM 2540 D. Solids. Total Suspended Solids Dried at 103-105°C); fecal coliforms (SM 9221 E. Multiple-Tube Fermentation. Technique for Members of the Coliform Group); Mercury (SAG-120201- Method validated); Metals and trace elements (Aluminium; Antimony; Arsenic; Barium; Boron; Beryllium; Cadmium; Calcium; Cerium; Cromium; Cobalt; Cupper; Iron; Lead; Lithium, Magnesium; Manganese; Molybdenum; Nickel; Phosphorous;

Potassium; Selenium; Silice (SiO₂); Silver; Sodium; Strontium; Talium; Tin; Titanium; Vanadium; Zinc) (EPA Method 2007, Rev.4.4. EMMC Version).

To correlate the growing inhibition of *P. subcapitata* and physico-chemical parameters of the sample points of Capiza, Colca and Majes; ANOVA, Lineal regression and the multiple Pearson correlation were done using the statistical software SPSS ver. 20.0.

RESULTS AND DISCUSSION

The growth inhibition of *P. subcapitata* exposed to different concentrations of CuSO₄ $(1 \text{ mg} \cdot \text{L}^{-1})$ and Majes, Capiza and Colca river water samples, showed that the greatest inhibition of growth was in the samples from Capiza river, followed by Colca, Majes and CuSO₄ during the exposition period of 72 h (Figure 3). In the sample points from Majes and Colca river; the physicochemical parameters values were elevated, but didn't surpass the EQS for water; on the other hand, the sample points from Capiza river shown that pH, concentration of manganese, Iron and suspended total solids were above the EQS; the pH value was 8.62; being 1.4% above the 8.4 value of the EQS; The Mn value was 0.298; compared to the 0.2 mg·L⁻¹ value of the EQS; The iron value was $3.72 \text{ mg} \cdot \text{L}^{-1}$; being 272% higher than the value of 1 mg/L of EQS. All those comparisons were for the third water category of irrigation of vegetables of tall and short stemmed plants. This monitoring was done in November of 2013 and the values were compared to the monitoring done in November 2012 were very high values were observed in termotolerant coliform (fecal) from Majes river, which overtook the EQS with more than 3000 NPM; also, Arsenic values were very high overtaking the EQS in the three points (Majes, Capiza and Colca); compared with the bioassays in the evaluation of the water quality

from Estero Limache (Chile central), were the inhibition of the growing rate of *P subcapitata* was observed in two sample points, being 30 and 29% higher than the control respectively (Córdova *et al.* 2009). A growth probe with *P. subcapitata* using leachate water from a warm pad of PCM-Plovdiv had a significant inhibition of the growth after 24 h until the 72 h of exposition (Ivanova & Groudeva 2006).

In a bioassay in situ for freshwater environments with *P. subcapitata*, a reduction was observed in algae growth in the impacted sites, confirming the sensitivity of the assay; clearly the nearest site to the discharge of the effluent was more impacted, and the distant site down the river was moderately impacted (Moreira et al. 2004). This ecotoxicological assay was fast and simple to estimate the effect of Simazine (CAT) or 3.5 dichlorophenol (3.5-DCP) in the growth of *P. subcapitata* using the results of inhibition probes of standard growing with 72 h of exposition (Katsumata et al. 2006). Other assay in growth inhibition due to the effects of antibacterial agents and their mixture on P. subcapitata to low concentrations in aquatic media, shown that antibacterial mixtures potentially affect the growth of the microalgaes in freshwater systems due to their mixture action (Yang et al. 2008). In previous inhibition studies of growth in P. subcapitata; the cupper shown an activator effect when was incubated with an enzyme (Jonsson & Aoyama 2007).

The values of the "variance analysis of two factors" had high significant differences in the growth of *P. subcapitata* due to the effect of the different concentrations (F =38.81; P <0.01) and bioassays (F = 13.98; P<0.01); with a confidence level of 99%. Compared with the inhibition bioassays of growth with *P. subcapitata* at the same time in MOPS (3-*N* morpholino propane sulfonic acid) and NaHCO₃ buffered with the probes media. In the microalgaes; significant differences were

observed in the toxicity of the metal between MOPS and HCl buffered (Schamphelaere et al. 2004). For the comparison between the growth dependence of the ecotoxicological bioassays, the contrast probe of Tukey presents three homogenous groups (a, b, c), noting that the samples bioassays of water from Majes river -(a) had the major growth of *P. subcapitata* until the 72 h of exposition, then Colca water -(b) and the lowest growth is shown for the bioassays of Capiza water samples -(c). For the comparison between the growth dependence of the concentrations; Tukey test had four homogenous groups (100 %a, 50 %b, 25 %c and 12.5 %c, and finally 6.25 %d), showing that the concentration of 6.25 % present the major growth of *P. subcapitata* until the 72 h of exposition.

In reference to the bioassays; it was observed

that a greatest inhibition of growth rate of P. subcapitata was in the concentration of 100%; being the bioassay of Capiza river followed for Colca and CuSO₄ $(1 \text{ mg} \cdot \text{L}^{-1})$ those which had a growing rate below of cero (Figure 4). These results suggest the presence of inhibitory elements in the middle sector which could be associated to high concentrations of manganese and iron, whose values exceeded the EQS of category 3. However, growth rate under cero was not observed in bioassays with water samples from Majes river that could be associated to the flux of the ravine among the river that dilute the toxicity of the inhibitory upriver, compared to the positive agents effects in the growth of *P. subcapitata* observed in lowest concentrations (Anastácio et al. 2000). The pure effluents are very toxic for the probe of P. subcapitata; however, satisfactory growth of microalgaes was seen in



Figure 1. Sampling points Map. 134RCapi1=Capiza river; 134Col4=Colca river; 134RMaje1= Majes river.

the effluents of 0.01 % (diluted). The effluents of the posterior stages of treatment required very lower dilution to eliminate the toxicity (Gaur & Kumar 1986). The results show that the effects of concentrations of surfactants and shampoos, had a result in the reduction of 50% in the growth of sweet water planktonic algaes, compared with the controls without probing the substances (Pavli *et al.* 2005). Hardening to long term of *P. subcapitata* to different concentration of cupper had significant diminution in the biomass of the microalgaes hardened to 0.5 and 100 µg Cu·L⁻¹ (Bossuyt & Janssen 2004).

The greatest growth of inhibition rate of *P.* subcapitata was present in the bioassay of the sample point from Capiza, seeing that the physico-chemical parameters pH, TSS (total suspended solids), Manganese and Iron surpass the EQS (Peruvian National standards of environment) for water (Table 1), with the regression and correlation analysis, a relation highly significant (F= 65.562; P < 0.01) was determined between the physicochemical parameters pH, Mn, TSS and the growth of inhibition percentage of *P. subcapitata*. The relation between the variables is high, explained by the Pearson correlation coefficient (r=0.98).



Figure 2. (a) *Pseudokirchneriella subcapitata* culture in liquid media; (b) *P. subcapitata* culture in solid media; (c) microscopic view of *P. subcapitata* (40X); (d) *P. subcapitata* count in Neubauer chamber(10X).



Figure 3. Growth inhibition of *Pseudokichneriella subcapitata* in bioassays with $CuSO_4(1 \text{ mg} \cdot L^{-1})$, Majes, Capiza and Colca river samples to 72 h of exposition to different concentrations.



Figure 4. Growth rate (μ) of *P. subcapitata* 72 h after Ecotoxicological Bioassay (CuSO₄, Majes, Capiza y Colca) to different concentrations.

						Catego	ıry 3*	Category 4*
Parameter	Analysis	Unit	Capiza	Colca	Majes	Irrigation of tall and short stemmed plants	Drinking water for animals	Conservation of aquatic environment
-Flow Tomocreturo	in situ	m ³ .5 ⁻¹	3.28	25.6 20	25.8 24			
- remperature -DH	in situ) ·	20.2 8.62	8.13 8.13	24 8.72	6.5 - 8.5	6.5 - 8.4	6.5 - 8.5
-Conductivity	in situ	µS·cm ⁻¹	851	587.5	629.5	<2000	5000	-
-Disolved Oxygen	in situ	mg-L ⁻¹	6.25	6.95	8.02	4	> 5	4
-N-NO ³	Laboratory	mg·L ⁻¹	0.138	0.204	0.062	10	50	10
-PO4 ³⁻	Laboratory	mg-L ⁻¹	<0.030	0.167	0.12	-		0.5
-N-NH ₃	Laboratory		<0.02	<0.02	<0.02			0.02
-DBO	Laboratory	mg·L_	<2.00	<2.00	<2.00	15	15	<10
-DQO	Laboratory	mg·L_	<10.0	<10.0	<10.0	40	40	ı
-TC	Laboratory	mg·L ⁻¹	110	2	0	1000	1000	2000
-Oils and grase	Laboratory	mg-L ¹	<1.00	<1.00	<1.00	-	-	ı
-Total Nitrogen	Laboratory	mg-L ⁻¹	<1.00	<1.00	<1.00	•		1.6
-Sulfide	Laboratory	mg-L ⁻¹	<0.002	<0.002	<0.002	0.05	0.05	0.002
-Cyanide Wad	Laboratory	mg·L ⁻¹	<0.006	<0.006	<0.006	0.1	0.1	I
-P	Laboratory	mg·L ¹	0.5495	0.68305	0.67643	0.5 - 6	5	ı
-Na	Laboratory	mg-L ⁻¹	40.55583	46.71383	47.25183	200		ı
-AI	Laboratory	mg·L	3.8352	0.07429	0.30859	5	5	ı
I-	Laboratory	mg·L ⁻¹	0.0564	0.00113	0.00888	·	ı	·
>-	Laboratory	mg·L ¹	0.01254	0.03679	0.03414	ı		ı
ċ	Laboratory	mg·L ⁻¹	0.00127	<0.0004	<0.0004	·		ı
-Mn	Laboratory	mg-L ⁻¹	0.29811	0.01268	0.0198	0.2	0.2	I
-Cu	Laboratory	mg-L ⁻¹	0.00915	<0.0004	<0.0004	0.2	0.5	0.02
-Zn	Laboratory	mg·L ⁻¹	0.0213	0.01125	0.00748	0	24	0.03
-As	Laboratory	mg·L ¹	0.00727	0.02054	0.01866	0.05	0.1	0.05
-Se	Laboratory	mg·L	<0.003	<0.003	<0.003	0.05	0.05	I
-Ag	Laboratory	mg-L ¹	<0.0005	<0.0005	<0.0005	0.05	0.05	ı
-Cd	Laboratory	mg-L ⁻¹	<0.0004	<0.0004	<0.0004	0.005	0.01	0.004
-Sn	Laboratory	mg·L ⁻¹	<0.001	<0.001	<0.001		ı	
Continua Tabla 1								

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Continua Tabla 1								
-Sb	Laboratory	mg·L ⁻¹	<0.002	<0.002	<0.002	ı	·	ı
-Ba total	Laboratory	mg·L ⁻¹	0.10282	0.01927	0.0251	0.7	ı	0.7
-Hg	Laboratory	mg-L ⁻¹	<0.0001	<0.0001	<0.0001	0.001	0.001	0.001
-Pō	Laboratory	mg·L ⁻¹	0.02104	0.0077	0.00599	0.05	0.05	0.001
-Fe	Laboratory	mg·L ⁻¹	3.72415	0.08819	0.24231	-	-	
-P total	Laboratory	mg·L ⁻¹	0.25721	0.0548	0.05279		·	
-Be total	Laboratory	mg·L ⁻¹	<0.0002	<0.0002	<0.0002		0.1	
-Ca total	Laboratory	mg-L ⁻¹	104.70696	43.89196	50.12096	200	·	
-Ce	Laboratory	mg-L ⁻¹	0.01416	0.00405	0.00207	·		
-Co total	Laboratory	mg·L ⁻¹	0.00354	<0.0003	<0.0003	0.05	-	
-K total	Laboratory	mg·L ⁻¹	8.2312	5.0211	5.498			
-Li Total	Laboratory	mg·L ⁻¹	0.02644	0.12266	0.11921	2.5	2.5	
-Mg total	Laboratory	mg·L ⁻¹	>20	10.5097	12.0587	150	150	•
-Mo total	Laboratory	mg-L ⁻¹	<0.002	0.0041	0.00298			
-Ni total	Laboratory	mg·L'	0.00273	<0.0004	<0.0004	0.2	0.2	0.025
-TSS	Laboratory	mg·L ⁻¹	439	4.54	18.18	ı		25 - 100
-Free Cyanide	Laboratory	mg∙L ⁻¹	<0.004	<0.004	<0.004	·		0.022
TSS (Total suspended solids);	CT (Termotolerant coliforms)	; *Peruvian N	ational Environment quali	ity standards for water	(EQS).			

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