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Tesis

Influence of the Microalgae Chlorella Vulgaris on the Adsorption of Zinc and Iron from the Effluents of the Chungar Mine, Yauli - Peru

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Influence of the microalgae *Chlorella vulgaris* on the adsorption of Zinc and Iron from the effluents of the Chungar mine, Yauli – Perú

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Abstract. The mining industry in central Peru is the most important activity and one of the main contributors to the country's economy, however, it usually causes harmful impacts to the environment through mining tailings, the release of inorganic compounds, discharges of acid effluents to water sources that alter the structure of the ecosystem. Therefore, the objective is to evaluate the influence of the *Chlorella vulgaris* microalgae on the adsorption of Zn and Fe metals from effluents from the Chungar mine, Yauli. The design of the research was experimental with a duration of 14 days, where we worked with samples of the mining effluent and microalgae strains at the laboratory level, using a completely random block design with factorial arrangement of 2 x 4 with 3 replications, being a total of 12 experimental units including the control, where a treatment was applied with doses of 50 ml, 100 ml and 200 ml of *Chlorella vulgaris*. During the experimentation, the results show that the optimal dose of the microalgae is 200 ml, since the concentrations of the metals were considerably reduced compared to their initial amount, the microalgae strains were exposed to environmental conditions that facilitated their growth, being their capacity of adsorption of Fe (62.16%) greater than Zn (26.98%). Concluding, that strains of *Chlorella vulgaris* microalgae significantly influence the adsorption of metals Zn and Fe.

Keywords: Industrial Effluents, Microalgae, Heavy Metals, Wastewater Treatment.

1 Introduction

While it is true, metals are naturally present in the earth's crust and are part of ecosystem interactions to maintain a dynamic environment. Some of them are essential and vital that act as nutrients for plants, animals, and human health, these are called trace elements and other metals are the soul of the production that moves the economy worldwide. In Latin America, Peru is considered one of the main producers of Au, Cu, Ag, Fe, Zn, Pb minerals, among others, which are exported to China, USA, generating large revenues for economic recovery after the pandemic hit. Mining in Peru is a major contributor to the national economy more than other industries, but it is also the one that generates the most socio-environmental conflicts due to contamination of natural resources and other environmental liabilities. Generally during the process of extraction and processing of metals, toxic waste is generated, inorganic compounds that if they exceed a density greater than 6 g/cm^3 are called heavy metals [1], These are released into the environment without prior treatment, so they accumulate in receiving bodies with high concentrations exceeding environmental quality standards. Repeated practices have a significant risk of bioaccumulating and remaining for years in the environment affecting all trophic levels [2].

Mining activities in the Carhuacayán district are of great interest for research, as it is considered the fourth district with the most unremediated environmental liabilities in Peru. Precisely for this reason, it was chosen as a study area to look for sustainable alternatives to change the water conditions in the area, since there is a subway mine water drainage product of the exploitation of minerals such as Zn and Fe, which runs in the form of a stream that flows into the San Pedro river turning it into acidic water due to the inorganic compounds of sulfides of Pb, Zn, Cu and the presence of pyrite that react quickly depending on numerous factors [3] [4].

However, the acidic water reaches the area where the inhabitants of Carhuacayán live, affecting them directly, since a pilot test determined that this effluent contains Zn and Fe in greater quantities than Ar, Pb and Cu. Therefore, it was proposed to treat the wastewater with the *Chlorella vulgaris* to reduce the high concentration of Zn and Fe, starting with the study of the area, sampling, laboratory analysis and data processing. Taking into account that the *Chlorella vulgaris* microalgae is a spherical unicellular microorganism that acts as a bioremediation agent without involving high production costs, but naturally removes heavy metals through bioadsorption processes, complex ion formation and ion exchanges [5].

2 Materials and Methods

2.1 Location and description of the place

The "Vichaycocha Tres" mining concession is owned by Compañía Minera Chungar S.A.C. located in the district of Santa Bárbara de Carhuacayán, which is located in the central Andes of Peru with an altitude variation of 4200 to 4600 m.s.n.m. with a distance of 176.2 km from the city of Lima, capital of Peru (see Fig. 1). According to the Geological and Mining Cadastral Information System (GEOCATMIN), said concession with code 08017193X01 of the Rule of Law has an area of 87, 86 hectares [6]. The sampling point was georeferenced with the coordinates 11º11'16.30" S; 76º18'56.40" W.

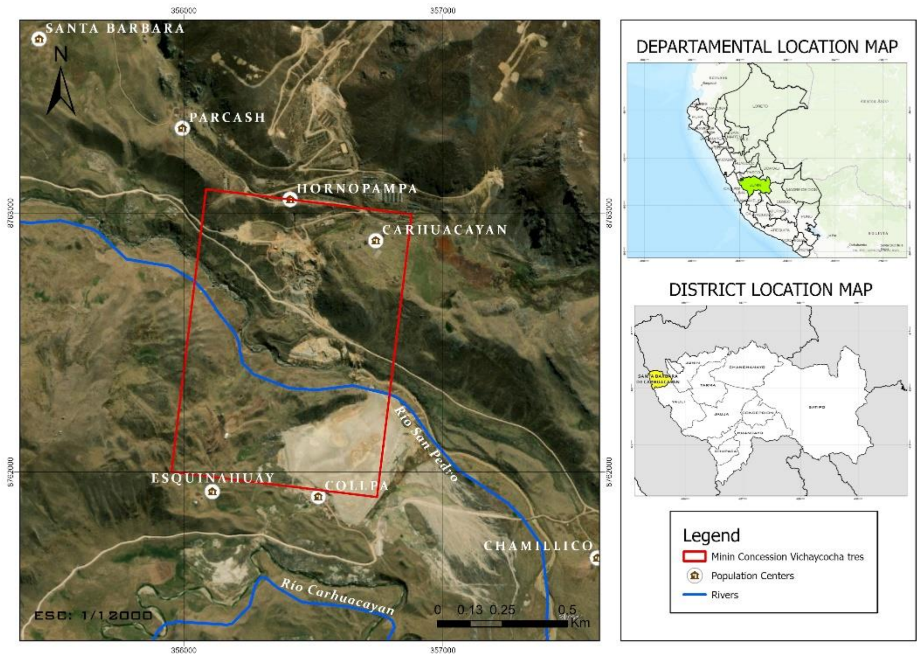

Fig. 1. Location map of the study area: Mining Concession "Vichaycocha Tres", province of Yauli – Junín.

2.2 Sampling Method

The collection of samples of the effluent from the Chungar mine was taken in August during the winter season with a maximum temperature of 21°C and a minimum rainfall of 12.3 mm [7]. The sampling was carried out in a lotic body of water with the protocol of the National Water Authority - 2017. At 20 m downstream of the discharge, 12 liters of sample were taken in 4 3-liter containers, previously sterilized and rinsed three times with the same water. The pH and temperature parameters were measured in situ with a portable multiparameter [8], for the transfer of the wastewater sample as well as the strains of the *Chlorella vulgaris* microalgae that were obtained from the laboratory strain of the Ricardo Palma-Peru University, it was carried out following a cold chain.

Table 1. Factorial treatment design.

Heavy metals	A_1= Zn y Fe			
Chlorella vulgaris dosage	Sample=T_1	B_1= 50ml	B_2= 100ml	B_3= 200ml
Number of repetitions	$A_1 * T_1$= 1	$A_1 * B_1$= 4	$A_1 * B_2$= 7	$A_1 * B_3$= 10
	$A_1 * T_1$= 2	$A_1 * B_1$= 5	$A_1 * B_2$= 8	$A_1 * B_3$= 11
	$A_1 * T_1$= 3	$A_1 * B_1$= 6	$A_1 * B_2$= 9	$A_1 * B_3$= 12
	Total experimental mining wastewater units			12

The level of research is explanatory with a completely randomized block design, having residual water with heavy metals Zn, Fe as variable A and microalgae doses as variable B. Three treatments were proposed with a 2x4 factorial design with 3 repetitions making a total of 12 experimental units including the control. 2 liters of residual water were poured into each container previously sterilized and rinsed with the water to be treated, 3 of them were the controls and 9 were experimental units with concentrations of 50 ml, 100 ml and 200 ml of *Chlorella vulgaris* that were controlled for 14 days as shown in Table 1.

The following formula was used to determine the adsorption percentage:

$$PA = ((C_o - C_f)/C_o) \times 100$$

Where PA is percentage adsorption, C_o is the initial concentration and C_f is the final concentration at a given time [9].
The method used to determine the concentration of Zn and Fe was atomic absorption spectrophotometry, which consists in the fact that atoms absorb electromagnetic radiation at a specific wavelength, taking into account that the transition energies are unique for the element [10], according to other authors, the metal atoms must be in a ground state to measure the amount of energy absorbed, the result is directly proportional to the concentration of that element in the sample analyzed, this is a complex and efficient method for heavy metal quantification [11] [12].

3 Results

Table 2. Characterization of the initial wastewater.

Physical Parameters	pH	Temperature °C	Zn (mg/l)	Fe (mg/l)
Wastewater	3.2	21	285.09	97.18

The data obtained from the physical parameters of the residual water from the Chungar mine were recorded in situ after 3 repetitions in the measurements, while the values of the metals Zn and Fe were obtained after being analyzed in a laboratory. These results will serve as a starting point to start the treatment and later compare them with the final results.

Table 3. Control of Experimental Zn Units.

CONTROL DAY	Day 1	Day 7	Day 11	Day 14
Chlorella vulgaris Dosage	Initial sample	First check (mg/l)	Second check (mg/l)	Third check (mg/l)
Sample	285.09	285.03	283.05	282.80

50 ml		272.57	261.93	252.46
100 ml		267.77	248.49	230.36
200 ml		258.37	226.09	208.17

Table 4. Control of Experimental Fe Units.

CONTROL DAY	Day 1	Day 7	Day 11	Day 14
Chlorella vulgaris Dosage	Initial sample	Firs check (mg/l)	Second check (mg/l)	Third check (mg/l)
Sample	97.18	97.12	97.03	96.98
50 ml		71.08	56.28	48.44
100 ml		63.57	46.41	42.45
200 ml		74.54	46.56	36.77

Tables 3 and 4 show the average results of the 3 repetitions of each treatment applied with *Chlorella vulgaris* to the experimental units, 3 controls were carried out during the 14 days after the microalgae had adapted to the medium. During the treatment phase there was a slight decrease in the initial residual water in each container, but it was compensated with distilled water to maintain the 2 liters of water.

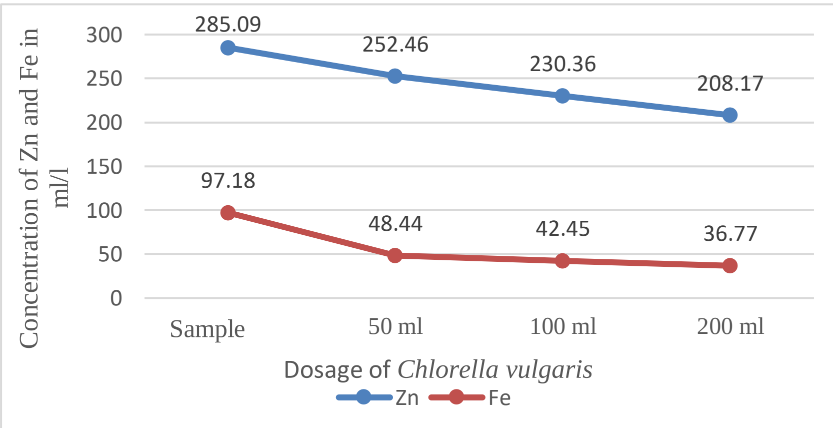

Fig. 2. Final Zn and Fe concentration after treatment.

(see Fig 2) The graph shows the variations in the final concentration of the metals evaluated in each treatment, with respect to Fe there was a rapid decrease in its concentration compared to Zn which showed a slight decrease.

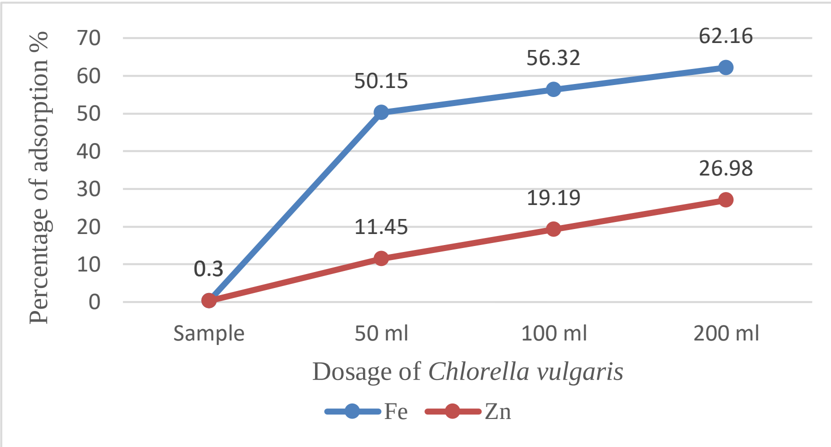

Fig. 3. Percentage of adsorption with respect to the dose of *Chlorella vulgaris*.

4 Discussion of results

The results of the initial characterization shown in Table 2, were compared with the Maximum Permissible Limits for effluent discharges from mining activities specified in Table 5 [13]. The pH data is 3.2, a value that exceeds the established range of 6 - 9, which indicates that the wastewater is extremely acidic.

Table 5. Maximum permissible limits for miners' effluents.

Parameters	Unit of measurement	Límit at any time
pH		6 - 9
Fe	mg/l	2
Zn	mg/l	1,5

Temperature is a physical parameter that greatly influences the speed of chemical reactions, such as accelerating the oxidation of pyrite (FeS_2) that is extracted in the "Vichaycocha Tres" mining concession and produces the acidity of the water faster. On the other hand, in acidic water, metal ions are more soluble, which due to the greater presence of protons tend to deactivate the adsorption capacity of the absorbent [14] [15]. That is, the *Chlorella vulgaris* microalgae acts as an absorbent in a medium only when it is in optimal conditions for its development, such as a medium pH, temperature, light intensity, dissolved oxygen, and cell density of the culture.

For this reason, a pH adjustment was made to alkalize them before beginning the treatments of the experimental units, since the microalgae cannot develop in acidic waters. The optimal conditions for the adaptation of the microalgae are shown in Table 6.

Table 6. Environmental conditions for the *Chlorella vulgaris* microalgae.

Parámetros físicos	pH	Temperatura	Oxígeno disuelto	Índice de UV
Microalga *Chlorella vulgaris*	6.5 -7.5	16 – 28	1.16 – 1.30	3

The treatment began after the adaptation of the microalgae to the residual water that initially contained 285.09 mg/l and 97.18 mg/l of Zn and Fe respectively, being a very high value compared to the values established by the norm of Maximun Permissible Limits for Zn 1.5 mg/l and for Fe 2 mg/l as observed in Table 5.

It can be observed that there is a high content of dissolved metal ions in the water that exceed the established limit, thus failing to comply with the environmental quality standard in Peru. Therefore, it can cause harmful effects in aquatic ecosystems, especially in organisms that are sensitive to acidification [16]. According to geological studies, the high concentration of Zn and Fe in the Carhuacayán district may be due to the presence of magnetite, which is the most abundant mineral, followed by iron-rich pyrrhotite [17].

After 14 days of treatment, a remarkable result was seen as shown in Figure 2, the adsorption percentage of Fe was 62.16% more than Zn, which was 26.98% in the last control on day 14 with the dose of 200 ml of the microalgae as shown in Figure 3. The influence of *Chlorella Vulgaris* on Fe was more than 50% absorption, this is because it helps with the proper functioning of the metabolism and plays an important role in the transport of electrons, reduces nitrates, fixes molecular nitrogen in the microalgae [18]. Regarding Zn, the result is similar to the results obtained in other referenced investigations, the adsorption varies between 25 to 26%; by ionic exchange and by the cell wall of the microalga, which is the main uptake mechanism [19]. On the other hand, it is important to mention that the microalgae as an absorbent can develop and absorb heavy metals when it is in an optimal pH range, this parameter is determinant for adaptation in a contaminated environment.

Given that Fe had a better result, in the Vichaycocha Tres mining concession where mining operations are carried out, this treatment could be incorporated into a wastewater treatment plant, specifically it could be applied in the biological treatment stage with the use of a photobioreactor with the necessary conditions for the cultivation of the *Chlorella vulgaris* microalgae on large scales. The mining industry should apply in its treatment systems, this being an investigation into a sustainable and economic mechanism to minimize heavy metals.

5 Conclusion

The *Chlorella vulgaris* microalgae have the ability to adapt and multiply in waters contaminated with high concentrations of heavy metals such as Zn and Fe that exceed the Maximum Permissible Limits evidencing a tremendous difference. It was also possible to demonstrate that the microalgae has a significant influence on the adsorption of heavy metals from Zn and Fe, since all the doses applied in the treatment were able to reduce in different scales in the different controls. But the most efficient dose was the 200 ml that absorbed 26.98% of Zn and up to 62.12% of Fe, a higher dose of the microalga is needed to reach the ranges established in the Maximum Permissible Limits, since it is a directly proportional relationship, the higher the concentration of heavy metals and the higher the dose of *Chlorella Vulgaris,* the higher the percentage adsorption will be. On the other hand, the efficiency of metal adsorption by the microalga will depend on various environmental factors such as exposure to solar radiation, light intensity, aeration and other physical parameters that must be taken into account.

6 Gratitude

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