

Bioadsorption Potential of Microalgae *Chlorella vulgaris* for Heavy Metal Removal: A Case Study of the Chacote River, Junin, Peru

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Abstract—Heavy metal pollution is a latent problem, and even more so because of the expansion of mining industrialization in recent years. As Peru is a country from which a great variety of minerals are extracted, the problem has increased, and with it the sustainable search to restore the different environmental components affected, one of these is the physico-chemical alterations caused by the dumping of mining effluents into water bodies, causing serious damage to aquatic ecosystems. The present study aims to investigate and determine the percentage of removal of heavy metals found in the Chacote River, located in the Chongos Alto District, Huancayo Province and Junin Region. The analysis was carried out at the laboratory level with samples collected from the river, and those with higher concentrations (arsenic, copper, chromium, lead, and zinc), the induction of the microalga *Chlorella vulgaris* was used as a method of bioadsorption of heavy metals for a period of 15 days at different applied doses (D1, D2, D3) and specific treatments for each. The final results showed to be an effective technique for removing heavy metals at higher concentrations, obtaining that Dose 3 achieving greater removal of heavy metals: Zinc at 97.07%, Arsenic at 62.49%, Lead at 65.70%, Copper at 61.64%, and finally Chromium with 43.81%. These findings play a key role for mitigating the environmental impacts caused by heavy metals in aquatic ecosystems. Therefore, further research is recommended to evaluate and expand current knowledge regarding the potential of microalgae as a bioremediation mechanism.

Keywords—treatment, *Chlorella vulgaris*, microalgae, heavy metals, adsorption

I. INTRODUCTION

Pollution by heavy metals in the natural environment has become increasingly severe and has caused great damage to the ecological environment and public health since the Industrial Revolution of the eighteenth century, by incorporating new technologies that fostered the rapid development of the world economy but also generated increasing contact between Heavy Metals (HMs) and humans through waste effluents from residual sources generated by anthropogenic activities, and especially by the mining industries [1].

In Peru, by the end of 2021, authorized untreated industrial waste water discharges amounted to 1,000,810,000 m³, causing significant impacts on the environment [2]. But these, in turn, are costly, and even more so if they involve effluents and drainage from mines, which are mainly responsible for the degradation of aquatic ecosystems due to their high toxicity and persistence in the water environment, where there has been a greater impact by effect of mining

effluents [3]. Prolonged exposure of water, soil, and air to mining effluents leads over time to bioaccumulation of Heavy Metal and metalloids throughout the food chain (plants, animals, and humans) [4]. Heavy Metal, like other metals and metalloids, by their recalcitrant nature, are resistant to degradation and do not undergo self-purification, causing socio-environmental and socio-economic problems in Peru [1].

Junin, Huancavelica, and Lima are regions of Peru that have a common problem, which is the pollution of Rio Chacote, since its constant source comes to unify with its related tributaries that are used by the 32 peasant communities belonging to the federation of indigenous peoples in the southern zone of Huancayo, north of Huancavelica and Lima (Atcas, Palaco, Valle del Canipaco, Huasicancha, among others.) [5]. The continuous discharges of mining effluents from the detoxification plant and different water treatment systems of the Corihuarmi Mine Production Unit of Minera IRL S.A. [6], authorized by the National Water Authority (ANA) through a directive resolution, with full knowledge that this resource is used for agriculture, livestock, and mainly for the consumption of peasant communities [7]. The use of resources is maximized by the communities since their location is far from the province and they do not have drinking water, generating problems for public health; this is due to heavy metals that alter changes in the Deoxyribonucleic Acid (DNA) (mutagenicity), among other related variants [8]. They also cause serious environmental problems such as soil degradation, reduction of grassland and vegetation cover, causing death in their livestock, as evidenced in the ANA Water Resources Management Plan-Mantaro 2023 [9–11].

Thus, different researches, especially those that are aligned in biological treatments, have proposed the method of phytoremediation, that means the use of microalgae for HM removal [12]. Microalgae are a large and diverse group of phototrophic and heterotrophic unicellular organisms; for their growth, they use solar radiation, water, and atmospheric CO₂, transforming it into chemical energy with an efficiency four times higher than that of plants. These are mostly composed of 9.5%–42% lipids, 17%–57% carbohydrates, and 20%–50% proteins; in addition to that about 100,000 species have been identified in the world, but only 35,000 species have been characterized, of which almost the majority have been used for various scientific research [13]. This is how microalgae have had a global impact in recent years for its rapid adaptability to different media but especially for its

ability to remediate effluents and treat waste water with high HM loads; being one of them the microalga “*Chlorella vulgaris*”, which has been studied since before the beginning of the 21st century to present an ideal biological system for different lines of research and also presents a high efficiency by its easy adaptation in laboratory conditions, with greater adaptability to the environment in which it reproduces, and best of all at low cost, since its source of power is nitrites and phosphates.

It was discovered in 1980 by M.D. Beijerinck and classified taxonomically [14]. The microalga “*Chlorella vulgaris*” is a single-celled green spherical alga, its cells have a spherical shape and measure about 2 to 10µm in diameter, have no flagella, and can accumulate pigments such as chlorophyll a and b, β-carotene and xanthols. They have a cell wall made up of polysaccharides, whose function is to protect and structure the cell through a protective layer called membrane contained by proteins and lipids, also have a membrane of RER where they assimilate proteins and store the calcium ion (Ca^{2+}) and is coated by ribosomes; on the other hand REL membrane lacks ribosomes, but it is where the lipids are synthesized; and finally, the chloroplast which is where photosynthesis takes place, contains the pigments chlorophylls and carotenoids to be able to absorb the energy of light [13, 15]. It is a species of microalgae that reproduces rapidly through photosynthesis in autotrophic, heterotrophic, and mixotrophic media. It has been shown to absorb large quantities of metals, mainly Cr^{4+} , Fe^{2+} , Cu^{2+} , Zn^{2+} , Pb^{2+} , and Hg^{2+} [15, 16].

The microalgae *Chlorella vulgaris* can reduce the toxicity of metals through a number of processes: a) bonding metals on the cell surface; b) development of a compound during deposition involving insoluble metals; c) eliminate metabolites that combine with hazardous metals to create compounds; d) use cell inlet pumps to move hazardous metals from the environment into the cytoplasm of the cell; e) alter the state of oxidation of metals to transform them into less toxic materials; f) transform metals into volatile forms that are rapidly expelled from the microalgae cell; g) bind metals in the cytoplasm of proteins or polysaccharides to limit their toxicity; h) metal ions are methylated by enzymes to prevent them from reacting with the -SH groups of the cell [17].

HMs react with groups on the surface of algal cells or binding enzymes on the surface of the plasma membrane of algal cells to form cellular complexes, crystals, or precipitates, which are then transferred to the interior of the algal cells via active transport and finally bind to intracellular proteins and peptides. This enrichment step mainly refers to the rapid bioaccumulation and biotransformation of various HM in the cell body after entering the cell membrane and is closely related to the normal activity of cellular metabolism, the same which is slow but irreversible [16]. Demonstrating its ability to remove HM in the biological treatment of industrial wastewater by using biological material, applying a new potential and environmentally friendly technology [18].

Therefore, the main objective of this research is to determine the bioadsorption efficiency of heavy metals (As, Cu, Cr, Pb, and Zn) from water samples obtained from the Chacote River using strains of the microalgae *Chlorella vulgaris*, carried out in a laboratory controlled and certified

according to Peruvian regulations. Proving to be an innovative and sustainable solution for the recovery of the Chacote River, in order to mitigate socio-environmental conflicts and protect communities that depend on its waters.

II. MATERIALS AND METHODS

A. Study Area

The chosen area was the Chacote River, located in the department of Junin Peru (see Fig. 1); where industrial mine wastewater is discharged from the different processes for obtaining minerals.

The study points were taken at two points along the entire length of the river, especially in areas of interest close to mining and peasant communities.



Fig. 1. Geographic location of the chosen study area—Chacote River, Junin region, Peru (2024)—sampling coordinates (UTM Zone 18S, WGS 84) P1 [439547 mE: 8613243 mN] and P2 [439275 mE: 8613623 mN].

B. Method

The research is of an experimental nature, taking as a variable the microalgae *Chlorella vulgaris*, which will determine the “adsorption capacity” of heavy metals such as As, Cu, Cr, Pb, and Zn, since this microalga has the ability to remove heavy metals from water sources. Therefore, the study started with the application of environmental parameters and regulations related to “water resource management approved in Peru” [3, 19], called Environmental Quality Standards (ECS) for water [20]. According to the sampling protocol, “Physico-Chemical-inorganic parameters” for heavy metals, it details that plastic bottles must be used that have a wide mouth and a hermetic seal, with a capacity of 1 liter, must be taken into consideration; for the collection of the effluent, it must be submerged about 20 cm below the surface, labelled and preserved in protective plastic boxes at approximately 4°C, keeping in mind that the samples collected must be delivered to the laboratory in less than 24 h after obtaining the samples. Likewise, the protocol of parameters carried out by the ANA details that there are 3 categories for sampling bodies of water from mining activities, for the control of effluent in order to determine the impact caused by wastewater; among them are categories 1, 3, and 4. According to the National Water Authority, the category that belongs to the Chacote River is category 3, but for this experimental design, category 4 is being used because it is the most rigorous and demanding, for the conservation of the aquatic environment and testing the microalgae *Chlorella*

vulgaris in its adsorption process according to the strictest parameters.

C. Sample Collection

In accordance with the protocol processes for the sampling of surface waters of the National Water Authority of Peru, 2 reference points were determined for the sampling of the Chacote River, labeled as Rio-1 and Rio-2, which refer to samples 1 and 2, both with a capacity of 2.5 L, for which it was decided to carry out the experiment with the analysis sample called Rio-2, considering that the collection section is close to peasant communities. Subsequently, one (01) liter of the sample from Rio-2 was sent to the Société Générale de Surveillance (SGS) Sociedad Anónima Cerrada (S.A.C.) laboratory in Lima, Peru, certified by the National Institute of Quality, to determine the existing heavy metals [19].

For the experimental phase, liquid biomass will be employed. This biomass was produced at the laboratory scale via the cultivation of live *Chlorella vulgaris* microalgae. The resulting microalgal suspension will serve as the principal agent driving metal removal.

D. Microalga's Cell Density

Cell density of the microalga *Chlorella vulgaris* was determined by direct counting using a Neubauer chamber (hemocytometer) under optical microscopy, following the standard equation Eq. (1) for estimating cell concentration per unit volume [21, 22]:

$$D = \frac{N \times F_d \times 10^4}{n} \quad (1)$$

where: D : Cell Density, F_d : Dilution Factor, 10^4 : conversion factor accounting for the defined volume of the Neubauer counting chamber, n : Number of counted grids.

The results indicated a cell density of $2 \times 10^6 \pm 2.1$ cells/mL during the application of the microalgal culture in the bioadsorption process.

E. Experimental Phase

The experimentation was carried out in previously sterilized 900 mL glass containers, they were applied in series with 3 repetitions (called T1, T2, T3) in a total of 4 doses including the control sample that will be in the containers (labeled with D0) with 250 mL of contaminated water; for the second dose, 250 mL of contaminated water with 40 mL of liquid biomass (labeled with D1) was added, for the next 250 mL of water with 80 mL of liquid biomass (labeled with D2), and finally for the last, 250 mL of river water with 120 mL of biomass (labeled with D3), then the agitation of 100 rpm was carried out for approximately 2 min, this process was repeated daily for the 15 days in which the experimentation was carried out; for the following days, a control of the environmental conditions was carried out [23, 24]. All samples were exposed to indirect sunlight to optimize the adsorption process by the microalgae strains, and at temperatures no less than 14°C.

After the time of two weeks, all the samples were sent the next day to the SGS S.A.C. laboratory in Lima, Peru, according to the company's protocol and together with staff for greater effectiveness in obtaining the results. It should be noted that the analysis carried out by said laboratory to obtain

the results for this type of test is through the determination of trace elements in water and waste by mass spectrometry with inductively coupled plasma, validated according to "EPA Method 200.8 Rev. 5.4, 1994". After the results were sent by the laboratory, the procedure for finding the adsorption capacity of metal ions was calculated using Eq. (2). Determining the adsorption of the metal was determined as a percentage (%) [16, 19].

$$PA = \left[\frac{C_i - C_e}{C_i} \right] \times 100\% \quad (2)$$

where C_i : Initial Concentration, C_e : Final Concentration, PA : Adsorption or removal percentage (%).

F. Data Analysis

For the present study, the statistical software IBM SPSS Statistics 27 (2022) was employed. The dataset was analyzed across its three dosages and treatments. A one-way Analysis of Variance (ANOVA) was conducted, and Tukey's multiple range test ($p < 0.05$) was applied to determine differences among treatments, yielding a significance value of 0.004.

III. RESULT AND DISCUSSION

The physicochemical characterization of the initial water sample from the Chacote River reflects the overall quality and environmental condition of the aquatic system. The relatively low values of pH, Electrical Conductivity (EC), Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD₅), compared to typical surface water standards, indicate an altered condition of the river prior to the application of the biosorption treatment. These results provide an important baseline for evaluating the efficiency of the proposed treatment, as presented in Table 1.

Table 1. Initial physicochemical parameters of Chacote river water sample

Physicochemical parameters	Unit	Value
pH	-	4.68
T	°C	10
DO	mg/L	6.5
EC	µS/cm	1180
BOD ₅	mg/L	<1.1
COD	mg/L	<8

Table 2 shows the first results of the analyses obtained from the laboratory without the application of *Chlorella vulgaris*, compared with the 2 categories, detailing that category 3, according to the ANA, corresponds to the Chacote River, and category 4 is the comparison in which the research will be carried out because it has more demanding parameters in heavy metals that produce greater impacts on humans and the ecosystem.

Table 2. Preliminary laboratory analyses without the application of *Chlorella vulgaris*, compared to the categories

Heavy Metal	As (mg/L)	Cu (mg/L)	Cr (mg/L)	Pb (mg/L)	Zn (mg/L)
Category 3-ECS	0.1	0.2	0.1	0.05	2
Category 4-ECS	0.15	0.1	0.011	0.0025	0.12
Chacote R.	0.1415	0.26471	0.0111	0.0034	0.3173

The values of the concentration of heavy metals obtained from the spectrophotometric scanning analysis by the laboratory (mg/L) after treatments are presented in Table 2, with favorable average values compared to the initial value, since the removal percentages exceed 50% for arsenic,

copper, and lead in doses 2 and 3; while for zinc, the values exceed 60% in the 3 doses applied. It should be noted that, although a percentage of more than 50% was not obtained for

chromium metal, the adsorption trend for all metals is favorable the more the proportions of microalgal liquid biomass are used (see Table 3 and Fig. 2 (b)).

Table 3. Results after experiment by dose and treatment applied

Metal	Dose	Initial value (mg/L)	T1 (mg/L)	T2 (mg/L)	T3 (mg/L)	Average (mg/L)	Removal
Arsenic	D1	0.1415	0.0780	0.0756	0.0785	0.0774	45.32%
	D2	0.1415	0.0639	0.0646	0.0644	0.0643	54.57%
	D3	0.1415	0.0535	0.0531	0.0527	0.0531	62.49%
Copper	D1	0.2647	0.1400	0.1442	0.1438	0.1427	46.10%
	D2	0.2647	0.1207	0.1205	0.1199	0.1204	54.53%
	D3	0.2647	0.1013	0.1020	0.1014	0.1015	61.64%
Chromium	D1	0.0111	0.0096	0.0091	0.0094	0.0094	15.65%
	D2	0.0111	0.0073	0.0071	0.0077	0.0074	33.59%
	D3	0.0111	0.0063	0.0060	0.0064	0.0062	43.81%
Lead	D1	0.0034	0.0019	0.0020	0.0018	0.0019	44.10%
	D2	0.0034	0.0016	0.0015	0.0014	0.0015	55.98%
	D3	0.0034	0.0012	0.0013	0.0010	0.0012	65.70%
Zinc	D1	0.3173	0.1229	0.1122	0.1242	0.1198	62.25%
	D2	0.3173	0.1110	0.1112	0.1010	0.1077	66.06%
	D3	0.3173	0.0093	0.0090	0.0095	0.0093	97.07%

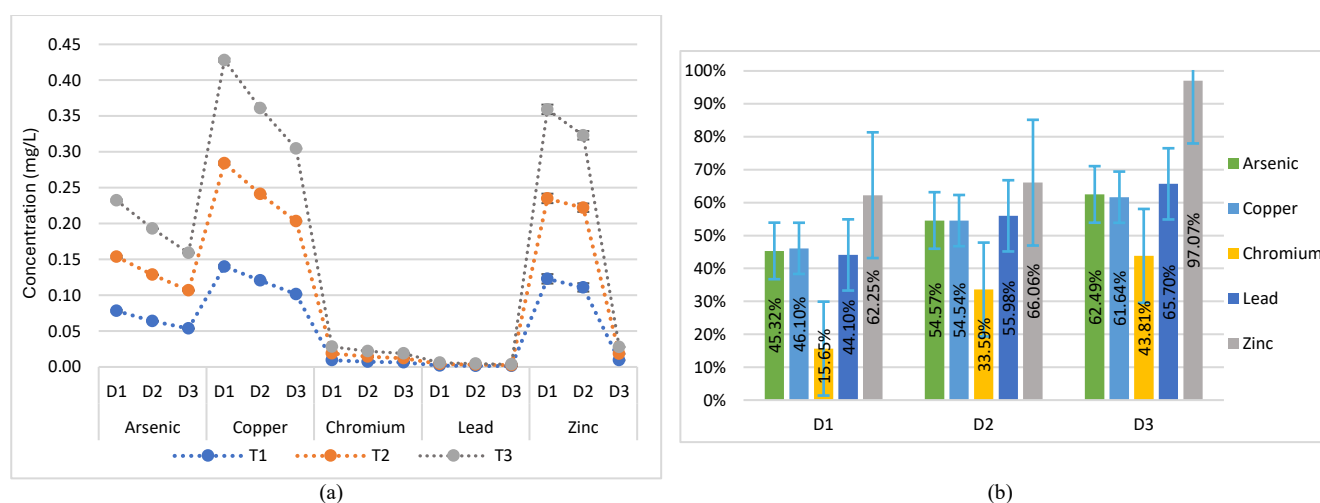


Fig. 2. Results according to treatment and dosage applied: (a) Concentrations in mg/L of *Chlorella vulgaris* in relation to the 3 doses used and treatments; (b) Percentage values of heavy metal removal according to dosage.

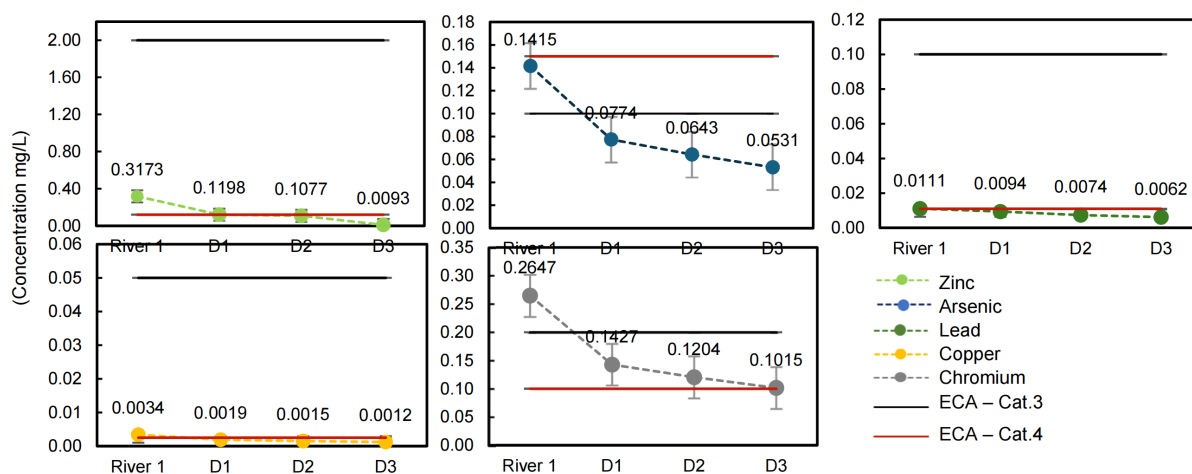


Fig. 3. Concentration of *Chlorella vulgaris* in relation to ECS water parameters (Cat. 3 and 4).

In the positional changes of the removal, we have the following order for the first dosage: $Zn > Cu > Ar > Pb > Cr$ while for the second (D2) and third (D3) the following position $Zn > Pb > Ar > Cu > Cr$ is maintained, observing a positional change only between the metals lead and copper; that is, the values are directly proportional to the dosages with minute variations. The metals zinc, lead, arsenic, and copper are the most adsorptive; therefore, it can be determined that the microalgae present accelerated adhesion within their

molecular structure to these metals.

The results of the average analyses presented are compared in relation to the primary analyses sent to the laboratory by applying the equation Eq. (2). The highest average adsorption percentages were obtained in D3: zinc at 97.07%, arsenic at 62.49%, lead at 65.70%, copper at 61.64%, and finally chromium at 43.81%, thus showing the removal efficiency of heavy metals with the microalgae.

As shown in Fig. 3, each scheme shows a constant decrease

according to the concentration of doses applied, obtaining for zinc the highest value of bioadsorption during the experimentation carried out; however, it has to be said that for chromium, a metal that has almost not been investigated in previous scientific experiments related to the microalgae *Chlorella vulgaris*, it has been determined that the greater the inoculated microalgal solution, the greater the adsorption of the metal. Through this process, we can affirm that microalgae are an effective adsorbent with values that are within the parameters established by the regulations.

Previous studies have documented the bioadsorption capacity of *Chlorella vulgaris* over a 20-day period, using a biomass concentration of 0.25 g/L based on Total Volatile Suspended Solids (TVSS), in 200 mL of contaminated effluent collected from the Dhaleswari River (Bangladesh). The results showed removal efficiency of 71% for zinc (Zn) and 91% for chromium (Cr). Both studies demonstrate a significant removal of heavy metals, highlighting the strong adaptability and adsorption potential of *Chlorella vulgaris*. However, a key distinction lies in the form of biomass employed. In the present study, *Chlorella vulgaris* was inoculated as liquid biomass, while the referenced study used dry biomass. This difference suggests that zinc adsorption may occur more rapidly when liquid biomass is applied, while chromium removal appears to be more efficient with dry biomass [8].

In the case of zinc, we present a removal of 97%, compared to the 53.72% reported for a study on day 15, with a concentration of 20 ppm of zinc and use of active biomass from BBM cultivation [22]. As compared with the biosorption process, bioaccumulation is an active process where living *Chlorella vulgaris* cells transport metals into organelles and cytoplasm by binding them to intracellular proteins such as metallothioneins or storing them in vacuoles; this could be an important difference in the behavior of the front under different conditions, since there is competition to access the functional groups in the cell wall of algae when the culture is with water samples containing more than one metal. Zinc can be used as a food source for microalgae and, in an environment without competition, by binding to functional groups, and at not-so-high concentrations could lead to lower bioadsorption, which would indicate why a lower removal value was obtained for the study mentioned earlier in mention than with the first study and the present one [25].

Additionally, previous studies have reported copper (Cu) removal efficiencies of up to 95% using immobilized microalgae, where *Chlorella vulgaris* biomass was chemically processed into alginate beads (adsorptive biopolymers). These systems were evaluated at concentrations of 1, 3, and 5 ppm in 250 mL of contaminated effluent over an 8-day period, achieving the highest desorption capacity at the highest concentration tested. The present study achieved a Cu removal efficiency of 61.6% using liquid biomass. This can be attributed to the enhanced adsorption performance of alginate-immobilized systems, which provide greater surface area and structural stability, thereby increasing adsorption rates per unit time. However, these advantages are offset by higher production costs and the need for chemical processing. In contrast, the use of liquid biomass in this study represents a more cost-effective and sustainable approach, although requiring longer contact times

and no additional chemical modification or inoculation [26].

Similarly, other studies have reported Copper removal efficiencies exceeding 89% using *Chlorella vulgaris* cultivated in Bold's Basal Medium (BBM), at concentrations of 1, 5, and 10 ppm, under both multi-metal and single-metal systems over a 7-day period. These results can be explained by biosorption mechanisms involving the interaction of metal ions with functional groups such as hydroxyl, phosphate, and carboxyl moieties through cation exchange, chelation, and complexation processes. Such interactions enhance metal stabilization via coordination bonding with amino groups present on the cell surface. What underpins the increase in removal for the other investigations compared to the result we obtained; let us also consider experimental conditions, including metal concentrations, culture media, and operational parameters [25].

Regarding lead (Pb), previous research has reported removal efficiencies of up to 92.45% using dry microalgal biomass at inoculation concentrations of 4, 5, and 6 mg/L in synthetic heavy metal solutions for a period of 24 days for the immobilization of the microalgae and 24 h for the biosorption process. In contrast, the present study applied a single inoculation of liquid biomass into the effluent, followed by exposure to natural light at ambient temperature (15–20°C) for 15 days, resulting in a Lead removal efficiency of 65%, approximately 30% lower than previously reported values [27]. This difference may be influenced by pH conditions, as Pb removal is known to increase at pH values around or above 6. The pH of the initial sample river water used in this study was relatively low (4.68), which may have limited metal binding and reduced biosorption efficiency. However, over time, photosynthetic activity likely contributed to an increase in pH, thereby enhancing the affinity of Pb ions for available adsorption sites [25].

A study conducted in 2022 on the removal of arsenic by the microalga *Chlorella sp.* obtained, a removal efficiency of 63% on average. Inoculated living biomass was used in controlled environments, and an experimental design composed of three treatment systems and three replicates was applied. The cultures were exposed to arsenic concentrations of 0.4, 1, and 2 mg/L over three, seven, and twelve days of evaluation for the removal trials, with temperatures recorded ranging from 14.91°C to 16.08°C. The conditions are similar, hence also the behavior of the metal, without significant variations in both experiments, so we could infer a significantly good competition regardless of whether it is developed under an individual crop or with the presence of other metals, affiliation by the functional groups is most determinative at 7 days, with a higher peak of removal according to the previous research and with a removal of more than 89%; which gives us insights into revalidating the behavior of each metal according to the concentrations and the time at which they are exposed, since some remain adsorbed on the wall on the surface of the algae during the first phases and others are incorporated afterwards [28].

Future research should focus on exploring the ability of microalgae to remove other metals, deepening biosorption and bioaccumulation processes at the microscopic level by adjusting and extending biomass concentrations and contact time to further improve the removal efficiency of heavy metals. A detailed analysis could be decisive in obtaining

better results and applicability to real cases and more complex scenarios. Finally, studies could assess biomass reuse or regeneration potential post-experiment.

IV. CONCLUSION

In the present research work, the following results were obtained: the metals Zinc, Arsenic, and Chromium maintain their position in the 3 dosages, unlike Copper and Lead, since the order of adsorption changes as shown in Fig. 2. In the first (D1), $Zn > Cu > Ar > Pb > Cr$, while for the second (D2) and third (D3), the following position is maintained: $Zn > Pb > Ar > Cu > Cr$. The metals zinc, lead and arsenic are the highest adsorption with values of 97.07%, 66.06%, and 62.49%, respectively; thus demonstrating the removal of heavy metals in its different contracts inoculated with *Chlorella vulgaris*.

On the other hand, the comparison with the categories according to the environmental quality standards of the Peruvian regulations, based on all the analyses obtained from the adsorption of heavy metals using the microalgae *Chlorella vulgaris*, was promising; therefore, the reduction of the HM on a large scale according to concentration resulting in being excellent for the treatment of mine wastewater, since it allows us to be within the parameters of the quality standards established for the conservation of natural water sources due to its high capacity as a biosorbent in the removal of heavy metals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Geraldine A. García: supervision, methodological conceptualization, data analysis, research, writing-review and editing of the article. Jhon H. Gutarra: research and publishing-elaboration of the article's thematic map. Dayanne P. Orozco: research, writing. Steve D. Camargo: Advisory, Oversight, and Review. All authors had approved the final version.

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