International Journal of Environmental Science and Development, Vol. 15, No. 2, 2024

Influence of Chlorella Vulgaris Microalgae on the Adsorption of Pb and Zn in Leachates at El Porvenir Open-Air Dump—“El Tambo”

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Manuscript received April 7, 2023; revised on June 6, 2023; accepted October 26, 2023, published April 26, 2024

***Abstract*—The application of *Chlorella vulgaris* microalgae in the adsorption of heavy metals is an economical and very efficient method. Being negatively charged, the heavy metals have a high affinity with polyvalent metal ions. The adsorption capacity of Zinc (Zn) and Lead (Pb) found in the leachate was studied taking into account the biomass production indices of the microalgae. In order to evaluate its adaptability in the environment, a pilot test was carried out with 2 samples: MP01 consisting of 3 L of leachate + 100 mL of Chlorella vulgaris; MP02 consisting of 3L of leachate + 200 mL of *Chlorella vulgaris*. The results of the pilot test showed that the leachate concentration was unfavorable for its growth; for this reason, it was decided to carry out a second experimental test with a dilution of 1:3, thus creating the necessary conditions for its growth. M01 made up of 3 L of dilution + 250 mL of *Chlorella vulgaris* and M02 made up of 3 L of dilution + 500 mL of *Chlorella vulgaris*. The results indicated that M02 presented better adaptability in the dilution; Regarding the adsorption of Pb, M01 presented an absorption of 72.09% and M02 reached an adsorption of 53.40%. For Zn adsorption, M01 had an adsorption of 88.98% and M02 reached an adsorption of 94.13%. It is concluded that the *Chlorella vulgaris* microalga can adapt to the leachate in a 1:3 dilution and with an optimal concentration of 500 mL, it is also significantly effective in the adsorption of Zn and Pb.**

***Keywords*—adsorption, leachate, heavy metals, chlorella vulgaris microalgae**

I. INTRODUCTION

Solid waste production is influenced by various factors such as the migration of people to urban areas, population growth, industrialization and quality of life [1], bringing the uncontrolled increase of this waste. Although the most developed countries have designed programs that control the production of solid waste, developing countries generally continue to use simple and unfriendly methods for the environment. In much of Latin America, the most common method for final disposal of solid waste is the open-air dump; Such is the case of the district municipality of El Tambo, where waste is disposed of in “El Porvenir” open-air dump. The Regional Health Authority (DIRESA) confirmed that daily confinement of 180 tons of solid waste is carried out [2].

When the waste is disposed of in a landfill, it has a certain humidity, when that humidity exceeds the field capacity, leaching occurs as a result of the percolation of liquids through the waste. Leachate is mainly composed of organic matter, mineral salts, nitrogen, phosphorus, diluted chemical substances and heavy metals. The latter is the most polluting [3] since dissolved organic matter influences the toxicity of heavy metals due to the fact that the interactions of soluble organic compounds and metals are pH dependent, so heavy metals tend to separate from solids when the pH is low. When the pH increases, they are absorbed or precipitated, but only up to a certain point, then they are solubilized again [4].

The use of microalgae to treat wastewater dates back to about 1940, when the first investigations on the possibility of mass cultivation of microalgae for the treatment of industrial wastewater emerged. Among these investigations, a group of microalgae for biological treatment was analyzed. From them: *Scenedesmus sp.*, *spirulina sp.*, *Chlorella sp.*, *Tetraselmis sp.* and *Pseudochlorella sp.* are the most important ones because of their high efficiency in the removal of heavy metals and other impurities, where the genera *Chlorella sp.* and *Pseudochlorella sp.* were the microalgae that presented a 100% removal of phosphates and sulfates, followed by *Spirulina sp.*, *Scenedesmus sp.* and *Tetraselmis sp.* (>95%) [5].

Recent research confirms the use of microalgae as effective bioadsorbents because in addition to chlorophyll, they contain significant amounts of intracellular proteins, carbohydrates, lipids and vitamin C, which are often used in the preparation of food supplements and for the detoxification of heavy metals in wastewater [6]. Among other reasons of their effectiveness, we have their diversity, abundance, availability of species, and their affinity for polyvalent metals such as Pb and Zn [7]; In addition, they have different biochemical mechanisms for the absorption of heavy metals and the neutralization of toxicity. However, microalgae growth is limited by factors such as pollution levels, nutrient availability, salinity, pH, light and CO_2 [8].

Treatment with *Chlorella vulgaris* is beneficial to minimize the number of pathogenic bacteria and heavy metals [9]; In addition, it is worth mentioning that *Chlorella vulgaris* has a high growth rate, which can vary according to the growing conditions [10].

The microalgae capacity to remove organic and inorganic components has been evaluated mainly in wastewater and soils. There are still many fields of study to explore and among them are leachates, which have a pollutant load much higher than any effluent. So far, these are treated in more than one stage that include equalization, sedimentation, aeration, ultrafiltration and chlorination, and in few cases reverse osmosis is used, which indeed is the most expensive technology to implement. The research addresses this field of study with the objective of evaluating the effectiveness of the treatment of the adsorption of heavy metals found in the leachate through the cultivation of the microalgae *Chlorella*

doi: 10.18178/ijesd.2024.15.2.1474

vulgaris, so that the treatment of leachate becomes more accessible and easy to implement in Huancayo city, which has scarce economic resources in environmental matters, thus contributing to the recovery of bodies of water and soil, in addition to promoting research in this field of study and generating bibliographic material for future research.

II. MATERIALS AND METHODS

Fig. 1 shows the development of the stages carried out in the research. In the first stage, the sampling and characterization of the sample were carried out in order to obtain measurements of the parameters prior to the treatment. In the next stage the culture and the search for the adaptation of the microalgae to the conditions presented by their new environment (leachate) were developed. And as a last stage, it was possible to determine the efficiency of each dose in the bioremediation of the leachate.

Fig. 1. Research stages.

A. Location Analysis

Fig. 2 shows the location of “El Porvenir” open-air dump with UTM coordinates N 8669600.0 and E 473400.0 at an altitude of 3230.00 m.a.s.l. at the district of El Tambo in the province of Huancayo - Junín Region, with an area of 57434.67 m^2 and a perimeter of 939.97 m [11]. The water table of the sector has an average depth of 1.50 meters [12] approximately; it has 2 pools of concrete leachate, which, by their characteristics, allow the evaporation of water and leachate concentration for recirculation. The samples were taken from pool 01 geo-referentially located at a latitude of −120353472, longitude of −752439952 and an altitude of 3198.6893746 m.a.s.l.

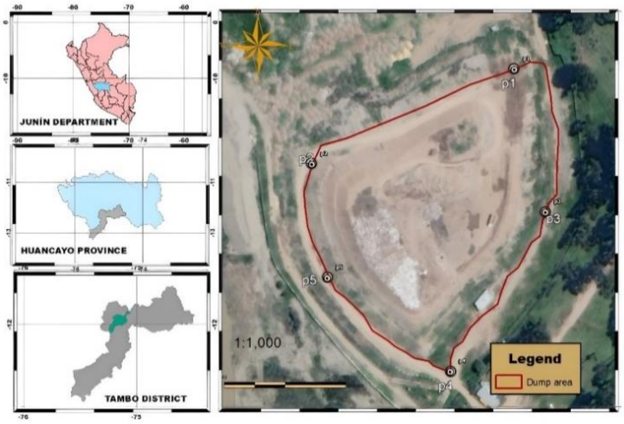

Fig. 2. Location map of the place of study - “El Porvenir” open-air dump - El Tambo.

B. Leachate Sampling

Four monitoring points were chosen from the leachate pool of the “El Provenir” sanitary landfill to homogenize the sample, as indicated in the water quality monitoring protocol of the Peruvian Ministry of Energy and Mines (MINEM). Three 3 L polyethylene bottles were used to collect the samples and three representative samples were obtained and labeled respectively.

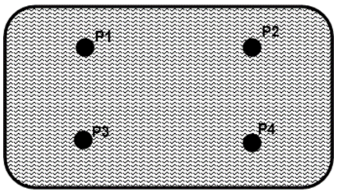

Fig. 3. Sampling points from Poza 01 of the “El Porvenir” dump.

C. Characterization of the Leachate

The heavy metals were evaluated in the chemical laboratory RCJ Labs Universal in Huancayo by atomic absorption spectrometry (AAS). This technique is based on the measurement of energy absorbed by the chemical element to be evaluated after affecting a sample containing that element with a specific monochromatic light radiation [13]. The physical parameters of the sample were evaluated in the microbiology laboratory at Continental University. The measured parameters and their respective methods are shown in Table 1.

Table 1. Physicochemical parameters of the leachate

Parameter	U	Method or Equipment
Hydrogen Potential (pH)	pH	Extech digital potentiometer *exPH100*
Temperature (T °)	°C	HI 991301 Multiparameter
Turbidity (yT)	ntu	HI 991301 Multiparameter
Electric conductivity (CE)	more	HI 991301 Multiparameter
Lead (Pb)	mg/L	Atomic Absorption Spectroscopy-AAS
Zinc(Zn)	mg/L	Atomic Absorption Spectroscopy-AAS

The pH, temperature and EC were evaluated in order to identify the initial physical conditions and then compare them once the microalgae was applied [14], which may be an important indicator for *Chlorella vulgaris* biomass growth.

D. Characterization of Chlorella Vulgaris

The strains were imported from the Algae Research Supply laboratory in California to later be cultivated at Ricardo Palma University and subsequently transported to the biotechnology laboratory of Continental University, where the initial culture conditions were analyzed for their conservation. For their growth, a longitudinal experimental study was carried out for 4 weeks, consisting of the following: For the pilot test, 2 sessions were carried out during the first week, and for the second experimental test, 3 sessions per week were carried out; during this time, the physicochemical parameters of the samples contained in previously labeled glass containers were evaluated.

E. Chlorella Vulgaris Count

To evaluate the growth of the biomass of the microalga, the Neubauer’s chamber was used using the diagonal square counting methodology as shown in Fig. 4, where there are 9 quadrants and each one is divided into 16 small 4×4 squares. In this case, for the count, only Quadrant 1 and 9 were considered, taking into account two criteria, the order and the limits of each box. In addition, the existing convention of this method was taken into account, which indicates the following: If the cell touches the lower or right limit, the count is not considered; however, if it touches the upper or left limit, it is considered [15].

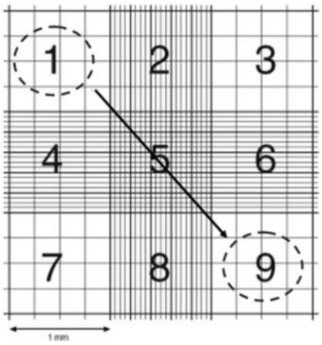

Fig. 4. Neubauer’s chamber.

Eq. (1) was used to calculate the cell count in the Neubauer’s chamber.

$$C\left(\frac{cel}{ml}\right)=\frac{\text{Number of cells counted}*10^4}{Number\ of\ boxes\ counted}*dilution \qquad (1)$$

where:

C: Concentration

Number of cells counted: sum of cells in the two quadrants considered

Number of boxes counted: quadrant 1 and quadrant 9= 2

dilution: ⅓

III. RESULTS AND DISCUSSION

A. Concentration of Heavy Metals and Physicochemical Parameters of the Characterization of the Leachate from the “El Porvenir” Open-Air Dump

The heavy metals with the highest concentration that are present in the leachate were determined. Heavy metals are part of the contaminants found in the leachate, which due to its toxicity causes severe damage to the environment. Table 2 shows the initial concentrations of heavy metals and the various physicochemical parameters found in leachates from the “El Porvenir” dump.

Table 2. Initial Parameters of Leachate from the “El Porvenir” dump

Inorganic Parameters		**Physico-chemical parameters**	
Pb	0.086 mg/L	pH	8.05
		Turbidity	211 ntu
Zn	0.699 mg/L	T°	16.22 °C
		CE	20 µS/cm

According to the environmental quality standards (ECA) established by the Ministry of the Environment in Peru (MINAM) in the Supreme Decree No. 004-2017-MINAM-PERU for the conservation of the aquatic environment, values that exceed 0.0025, 0.12 of Pb and Zn respectively can present significant risks to the ecosystem. The same happens for the pH with a range of 6.5 to 9.0, and a conductivity of 1000 (µS/cm) [16].

Compounds within the inorganic parameters are considered “trace” elements, which means that in high concentrations they are toxic and accumulative to different degrees, varying according to their degree of solubility and chemical nature; [17] In addition, each of these elements can easily be mobilized through groundwater and generate cumulative, synergistic and significant negative impacts on the environment and indirectly on human health.

Table 3 shows that all heavy metals have significantly high values to be poured into the body of water, such as Mantaro River. In case of Pb, it exceeds at 0.0835 mg/L, and Zn it exceeds at 0.579 mg/L.

Table 3. Physicochemical parameters of the experimental tests

Paraameter	**MP00**	**MP01**		**MP02**	
	Session 1	Session 1	Session 2	Session 1	Session 2
pH	8.05	8.84	8.72	8.83	8.62
Turbidity	211	226	245	226.67	195.33
T°	16.22	13.83	15.53	13.87	15.50
CE	20	17.92	18.03	17.90	18.40

B. Pilot Test

Microalgae can grow in various environmental conditions due to their adaptability, they manage to expand and develop even in contaminated environments such as wastewater [18], and use some of their components as a substrate for their growth. A preliminary study was carried out using two experimental samples (named MP01 and MP02 respectively) with the objective of determining the adequate concentration of *Chlorella vulgaris* for the adaptation and growth of the biomass of the microalgae. MP01 included a 100% concentration of leachate with 100 mg/L from *Chlorella vulgaris*. MP02 included a 100% concentration of leachate with 200 mg/L of *Chlorella vulgaris*. The use of microalgae in the treatment of contaminated water has the objective of increasing the concentration of oxygen in the effluent, decreasing the concentration of solids, and reducing nitrogen in all its chemical forms and phosphorus [19]. Table 3 shows the data collected from the physicochemical parameters, both samples were compared with the control sample (MP00) to see the variation of the data.

The growth of microalgae is influenced by various physical and chemical factors, the most significant ones are pH, CE and the intensity of the light [18]; adding a greater amount of leachate means providing a greater amount of nutrients in the environment, which allows the microalgae to reach higher biomass concentrations [10]; however, the data obtained show that the initial conditions were not favorable for their adaptation because there was an oversaturation of components in the leachate, obtaining as a result that the mortality rate is higher than the rate of new cell generation.

C. Second Experimental Test

Taking into account the results obtained in the pilot test, the following experimental test was carried out with three new samples, using a dilution value of 1:3 adding distilled water, assuming the improvement of culture conditions. The first sample without treatment used as a control sample, hereinafter called M00, the second with a treatment of 250 mL of *Chlorella vulgaris* called hereinafter M01, and the last one with a treatment of 500 mL of *Chlorella vulgaris*, hereinafter M02.

As evidenced in Table 4, the 1:3 dilution of the leachate altered the initial conditions of the experimental sample, significantly decreasing the concentration of salts and turbidity, the pH became slightly alkaline. These characteristics positively influenced the adaptation and growth of the microalgae, extending its life cycle.

Table 4. Results of the physicochemical parameters of the experimental test

Parameter	**Sample leachate + 250 mL**								**Sample leachate + 500 mL**							
	0	1	2	3	4	5	6	7	0	1	2	3	4	5	6	7
Ph	8.21	9.62	8.52	8.86	8.88	9.09	9.98	9.44	8.21	9.86	8.59	8.84	9.03	9.16	9.27	9.14
Turbidity	106.67	103.00	62.97	39.20	64.10	31.87	99.43	295.00	106.67	107.67	72.63	38.37	51.87	52.60	34.20	67.50
T°	14.17	14.50	20.77	11.67	20.43	12.53	12.03	13.90	14.17	14.20	19.40	10.57	19.17	12.30	11.10	13.20
C.E	9.43	2.85	7.13	6.75	5.51	5.02	2.35	4.66	9.43	2.55	6.15	5.86	5.13	4.62	2.29	4.70

D. Analysis of Biomass Growth (Microalgae Metabolism) and Adaptation of Chlorella Vulgaris

The adaptability of *Chlorella vulgaris* in the leachate was analyzed based on the 4 phases that are plotted on a growth curve and are called: lag phase, exponential phase or logarithmic phase, stationary phase, and death phase [20]. Table 5 shows the microalgae count during 8 sessions

Fig. 5 shows the adaptation process of *Chlorella vulgaris* in its different phases of growth. In the latency phase, a population growth of 70000 cel./mL was obtained in M01 in Session 5, unlike M02 which shows a population growth of 97167 cel./mL in Session 7. During this stage, nutrients are absorbed for its growth, taking into account that two of its main characteristics are: its resistance to tolerate extreme environments and its easy adaptation [21].

Table 5. Chlorella vulgaris count in the Neubauer's chamber

Count	**N° of Chlorella.vulgaris (cel./mL)**	
	Chlorella Count M02	Chlorella Count M01
Session 1	50,500	471,667
Session 2	99,333	328,333
Session 3	97,167	70,000
Session 4	55,333	648,333
Session 5	59,833	295,000
Session 6	15,000	5845,000
Session 7	3,000	243,333
Session 8	158,833	425,000

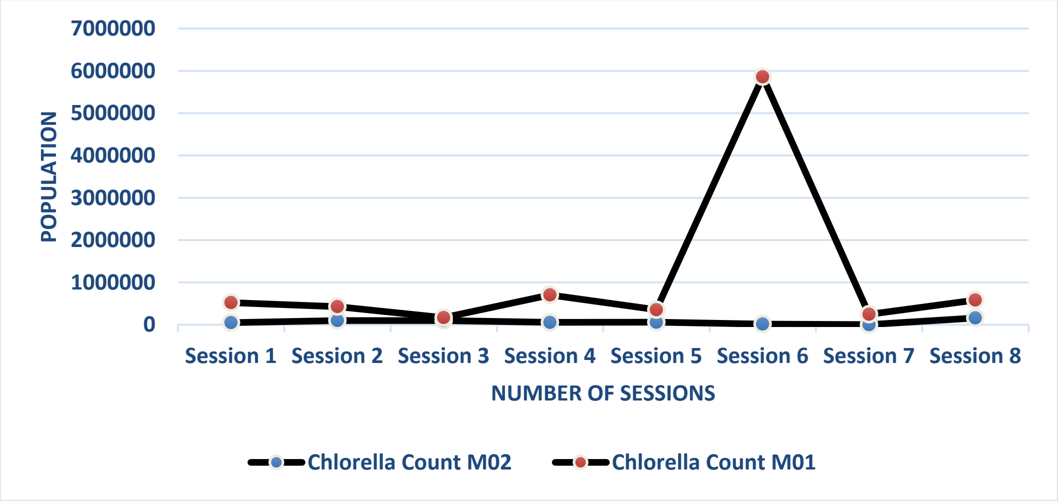

Fig. 5. Chlorella vulgaris growth kinetics graph.

For M01, it was possible to identify session 6 as an exponential phase, during this phase a massive growth of 5,845,000 cel./mL was observed, a reproduction 5 times greater. For M02, it was possible to identify session 8 as exponential phase with a massive growth of 158,833 cel./mL. This is because the metabolism and reproduction of this species is accelerated, with a high capacity for adaptability and photosynthetic efficiency, which makes them capable of converting solar energy into biomass with an efficiency of two to five times [22] . Finally, it was observed that M01 had a population decline in Session 7, however, in Session 8 a population growth of 425000 cel./ml was obtained, this is attributed to the fact that the microalgae had an initial cycle of habituation and will have a new growth cycle. For M02 the decline could not be identified, indicating that their habituation period was longer. The adaptation of the microalgae *Chlorella vulgaris* suggests that leachate is a rich source of nitrogen and phosphorus, which are fundamental micronutrients for its growth and reproduction [23].

E. Removal of Heavy Metals with Chlorella Vulgaris Microalgae

The main objective of using *Chlorella vulgaris* for leachate treatment is to biologically use and convert nutrients into biomass, these microalgae store important metals intracellularly. The *Chlorella vulgaris* microalgae is capable of absorbing and storing large amounts of metal ions, a process that normally takes place in two steps: adsorption or biosorption and bioaccumulation [24].

F. Calculation for the Removal of Heavy Metals

To evaluate the effectiveness of *Chlorella vulgaris* in the removal of lead and zinc, the values established in ECA - Category 4, specified in Table 6, were compared with the values obtained in the experimental test.

Based on the data obtained through laboratory analysis, the removal percentage for each metal was calculated using Eq. (2).

$$\%R = \frac{V_o - V_f}{V_o} * 100 \qquad (2)$$

where:

%R: Percentage removal

V_o: Initial value

V_f: Final value

Table 6. Water ECA—Category 4

Category 4: Conservation of the aquatic environment		
E2 RIVERS		
inorganic parameters	Unit of measurement	value
Lead	mg/L	0.0025
Zinc	mg/L	0.12

Source: Supreme Decree Nº 004-2017-MINAM

G. Lead Removal

Table 7 specifies the behavior of the strain in both treatments. Taking into account the values of the ECA, the initial value (V_o) and the final value (V_f) obtained from the analysis of the treatments, it is evident that M01 results with a significant removal of 72% and M02 with an average removal of 53%; However, none of them manages to reduce the concentrations of the metal to be within the value established in the standard. Lead is considered one of the most toxic metals, it is found in the environment in batteries, paints, insecticides and pipes; once it is deposited on the ground, it contaminates directly. The addition of organic acids can increase its solubility and increase the transport capacity of Pb, thus reaching groundwater. In previous research about comparing microalgae species Scenedesmus obliquus and *Chlorella vulgaris* in metal removal, it was identified that *Chlorella vulgaris* had the highest average absorbance value with 92.45% of lead. This suggests that this species of microalgae has a higher affinity for these heavy metals compared to Scenedesmus obliquus [8].

Table 7. Calculation of Pb removal in the treatments

Code	**Lead (Pb)**				
	Initial Value	Final Value	Δ (V_o-V_f)	% removal	ECA value
M01	0.086	0.024	0.062	72.09%	0.0025
M02	0.086	0.04	0.046	53.49%	0.0025

H. Zinc Removal

Table 8 shows the behavior of the strain in both treatments. Taking into account the values of ECA, the initial sample (V_o) and the final sample (V_f), it is evident that both treatments (M01 and M02) present optimal results since Zn in both cases manages to decrease its concentration until it is found within the values established in ECA. This is due to the influence of factors such as light intensity (due to light/dark change), temperature (since it was not kept constant) and aeration. The latter factor is important as a provider of carbon source (CO_2) for nutrient distribution and to avoid sedimentation since the aeration mechanism was present in the sessions, thus allowing to remove the Zn concentration effectively [24].

Table 8. Calculation of Zn removal in the treatments

Code	Zinc (Zn)				
	Initial value	Final value	Δ (V_o-V_f)	ECA value	% removal
M01	0.699	0.077	0.622	0.12	88.98%
M02	0.699	0.041	0.658	0.12	94.13%

IV. CONCLUSIONS

According to the results obtained during the research, the microalga *Chlorella vulgaris* has a great capacity to adapt to different contaminated environments; however, in the pilot test it is evident that the pure leachate contains compounds that are unfavorable for biomass growth, so it quickly reached the death phase. Consequently, in the experimental test it was observed that when diluting the leachate in 1:3, the conditions of the environment were favorable for the growth of the biomass. This species of microalgae has a great affinity for the adsorption of metals, which is why it has a high percentage of adsorption efficiency in Pb and Zn, adsorbing from 53.49% to 72.09% in Pb and from 88.98% to 94.13% in Zn, which is reflected in the increase in cell density, demonstrated through the growth curve in each sample that was carried out. This adsorption depends on different environmental factors such as: light intensity, concentration, pH and components that harm the composition of the biomass. The good adaptation of the biomass allows the removal of Pb and Zn, resulting in concentrations below the corresponding values in the norm. This capacity that *Chlorella vulgaris* possesses makes it an effective alternative for the treatment of leachates.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors participated jointly in the research process from sample collection, analysis and writing of this article.

FUNDING

This research was funded by the same researchers.

ACKNOWLEDGMENT

F. A. our sincere thanks to the Algae Research Supply laboratory in California. Ricardo Palma University, for the imported strains and subsequent cultivation of the *Chlorella vulgaris* microalgae. Continental University for providing us with the use of their equipment to carry out the research, and to the chemical laboratory RCJ Labs Universal in Huancayo for the evaluation of heavy metals.

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