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Efficiency of the microalgae *Spirulina platensis* in the bioremediation of leachate from the San Jeronimo De Tunan solid waste treatment plant, Junin, Peru

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ABSTRACT

The purpose of this work was to evaluate the efficacy of the microalgae *Spirulina platensis* in the bioremediation of leachate from the solid waste treatment plant of San Jerónimo de Tunán, Junín, Peru. Initially, a pilot test was carried out using undiluted leachate (100%) in which no microalgal growth was evidenced, in response to this, a second experimental phase was designed using a 40% dilution with distilled water complemented with essential mineral salts to optimize the culture medium. Treatments were established with different volumes of microalgae: N-01 and N-02 with 550 mL, and M-01 and M-02 with 750 mL. The experimental tests were carried out over seven days for N-01 and M-01, and fourteen days for N-02 and M-02. During the experimental period, physico-chemical parameters (pH, temperature, electrical conductivity, turbidity, BOD and COD) were monitored, as well as the removal capacity of heavy metals (lead, zinc, iron, chromium, arsenic and cadmium). The results showed a decrease in turbidity, with final values of 42.56 and 35.9 NTU in the one-week treatments (N-01 and M-01) and in the two-week treatments (N-02 and M-02) values of 45.3 and 49.43 NTU were recorded respectively. BOD and COD values, especially in longer duration treatments, BOD was reduced between 15.98% and 19.86% in one week, reaching up to 34.31% and 37.09% in two weeks. On the other hand, COD was reduced by 24.57% and 26.62% in one week, and up to 42.08% and 45.23% in two weeks. Regarding heavy metals, variable removals were obtained: lead (69.33–80.67%), zinc (44.34–50.91%), iron (70.73–75.72%), chromium (64.73–75.84%), arsenic (61.49–64.87%) and cadmium (55.55–73.33%). The findings support the potential of *Spirulina platensis* as an effective and promising alternative for leachate bioremediation.

Keywords: leachate, bioremediation, microalgae *Spirulina platensis*.

INTRODUCTION

Solid waste represents a persistent challenge for global sustainability, given that it is a by-product generated by human activities (Sánchez, 2019). The disposal of this waste remains a critical and widespread problem in both urban and rural areas in many countries, regardless of their level of development, because solutions to this challenge must be sustainable, considering financial factors, practical feasibility, legal compliance and their compatibility with environmental protection (Abdel-Shafy and Mansour, 2018).

In Peru, approximately 8,450,715 tons of municipal solid waste are generated annually (Ministry of the Environment [MINAM], 2024), which has motivated the implementation of measures at the national level to address this problem. Various solutions are being applied for the treatment and proper management of this waste, such as the use of sanitary landfills and solid waste treatment plants, which are part of a comprehensive strategy to improve municipal solid waste management (MINAM, 2015). These techniques employ engineering principles to confine waste in a compactly bounded area, covering it with layers of soil; however, during the decomposition of solid waste, a dark and foul-smelling liquid known as leachate is produced (Jaramillo, 2002), the variability in its composition may be due to various factors, including the age of the waste, the morphological composition of the waste, the water content, the temperature, the methods of operation, the among others (Podlasek et al., 2023). It is mainly formed from percolated rainwater, water generated by the biodegradation of wastes and the water inherent to them, and contains large amounts of dissolved organic matter (DOM), salts, heavy metal ions and other organic compounds (Teng et al. 2021), thus leachate requires effective treatment due to the risk they represent, in order to mitigate health and environmental hazards (Ilmasari et al., 2022).

According to the District Municipality of San Jerónimo de Tunán (2024), 170.85 kg/m³ of municipal solid waste is produced in the district, with a per capita generation of 0.46 kg per inhabitant per day, which corresponds to 2280.08 t/year of household solid waste and 215.28 t/year of non-household solid waste, this district has a solid waste treatment plant, which includes a composting plant, a nursery and a sanitary landfill from which leachate is generated, which is later discharged to a well for storage. The technique used for the treatment of the leachate generated is recirculation, considered a viable alternative due to its low cost.

Over time, various technologies have been developed for the treatment of leachate generated from solid waste, including chemical treatments such as the Fenton process and chemical precipitation, physical-chemical treatments that encompass adsorption and membrane processes and finally biological treatments such as activated sludge systems and fluidized bed reactors (Teng et al., 2021). The constant generation of solid waste has motivated the search for more sustainable alternatives, including bioremediation and phytoremediation, which is why the use of microalgae has gained great interest in recent years, due to its ability to capture carbon dioxide (CO_2) and remove pollutants simultaneously (Pérez, 2020). Microalgae, due to their simple cellular structure, have rapid growth and high biomass productivity; this characteristic, together with their ability to take advantage of light as a source of energy and CO_2 as a source of carbon, allows them to be classified as photoautotrophic organisms (Benavente et al., 2012). The microalgae *Spirulina platensis* is a filamentous cyanobacterium widely studied for its multiple applications in various fields of research, due to its biotechnological properties. This microorganism can survive in extreme conditions of high alkalinity, temperatures and salt concentrations. Likewise, it can not only tolerate environments with high levels of heavy metals and persistent organic compounds but is also capable of generating a considerable amount of biomass under these conditions (Cepoi and Zinicovscaia, 2020).

Different bioremediation studies were carried out using the microalgae *Spirulina platensis* such as the study carried out by Al-Homaidan et al. (2014), in which its potential as a copper biosorbent in aqueous solutions was evaluated. In this research, the microalgae were exposed to different concentrations of copper, where the adsorption efficiency was analyzed under different experimental conditions, considering variables such as pH, contact time, temperature, adsorbate concentration and amount of dry biomass used. The results demonstrated a high efficiency in the removal of copper ions with a maximum adsorption of 90.61% in a solution with 100 mg/L of copper, at a pH of 7, using 0.050 g of dry biomass, at 37 °C, for a contact time of 90 minutes. Another research carried out by Emalya et al. (2023), evaluated the capacity to reduce pollutants in leachate through the use of a mixed culture of microalgae using a phytobioreactor, including species such as *Spirulina* sp., *Synedra acus*, *Euglena* sp., *Trichocerca* sp., *Paramecium* sp., and *Closteriopsis longissima*, promising results were obtained because the study reported an elimination of 75.48% of BOD, 76.26% of COD, 74.86% of nitrates and 73.52% of nitrites, concluding that microalgae can grow favorably in leachate, while significantly reducing contaminants.

Based on the above, the present study seeks to evaluate the adsorption of contaminants present in the leachate of the solid waste treatment plant of San Jerónimo de Tunán through the cultivation of *Spirulina platensis*, considering various dose and time conditions. This approach could be beneficial for the environment and represent a low-cost alternative.

MATERIALS AND METHODS

Study area

The solid waste treatment plant is in the district of San Jerónimo de Tunán, in the province of Huancayo, within the department of Junín, with UTM coordinates: 469080 E and 8679838 N. This plant is located at an approximate altitude of 3411 meters above sea level, in the Mantaro Valley, the facility occupies an area of 47,311.00 m² and has been designed to process various types of municipal solid waste, excluding those classified as hazardous, it also has two wells for the management and storage of leachate, although only one of them is currently in operation (Figure 1).

Sampling and characterization of leachate

The sampling was carried out randomly in the operational well of the solid waste treatment plant of San Jerónimo de Tunán, this procedure was intended to analyze the concentration of heavy metals and initial physicochemical parameters of the leachate, to establish the starting conditions for subsequent analyses. The leachate samples were collected using six sterile polyethylene bottles with a capacity of one liter each, which were appropriately labeled to ensure their correct identification during the analysis process, and the samples were homogenized to form a representative sample. Subsequently, the physicochemical parameters and heavy metals found in Table 1 and 2 respectively were analyzed, it is also important to mention that the parameters of pH, temperature, electrical conductivity and turbidity were evaluated within the microbiology laboratory of the Continental University Huancayo campus, on the other hand the analysis of BOD, COD and heavy metals were carried out by the testing laboratory Ambiental Laboratorios S.A.C.

Pilot test

To evaluate the behavior of the microalgae with the leachate, a pilot test was carried out, carrying out two experimental tests, where PI-01 and PI-02 contained 250 ml of leachate (100%) incorporating 100 ml and 200 ml of *Spirulina platensis* microalgae respectively. Under both conditions, after microscopic observation carried out with the Neubauer chambers, the presence of microalgal cells or signs of growth was not evidenced, suggesting a total inhibition possibly associated with the high toxicity of the leachate, which suggests that the culture media used do not provide adequate conditions for the proliferation of new microorganisms since they have a higher mortality

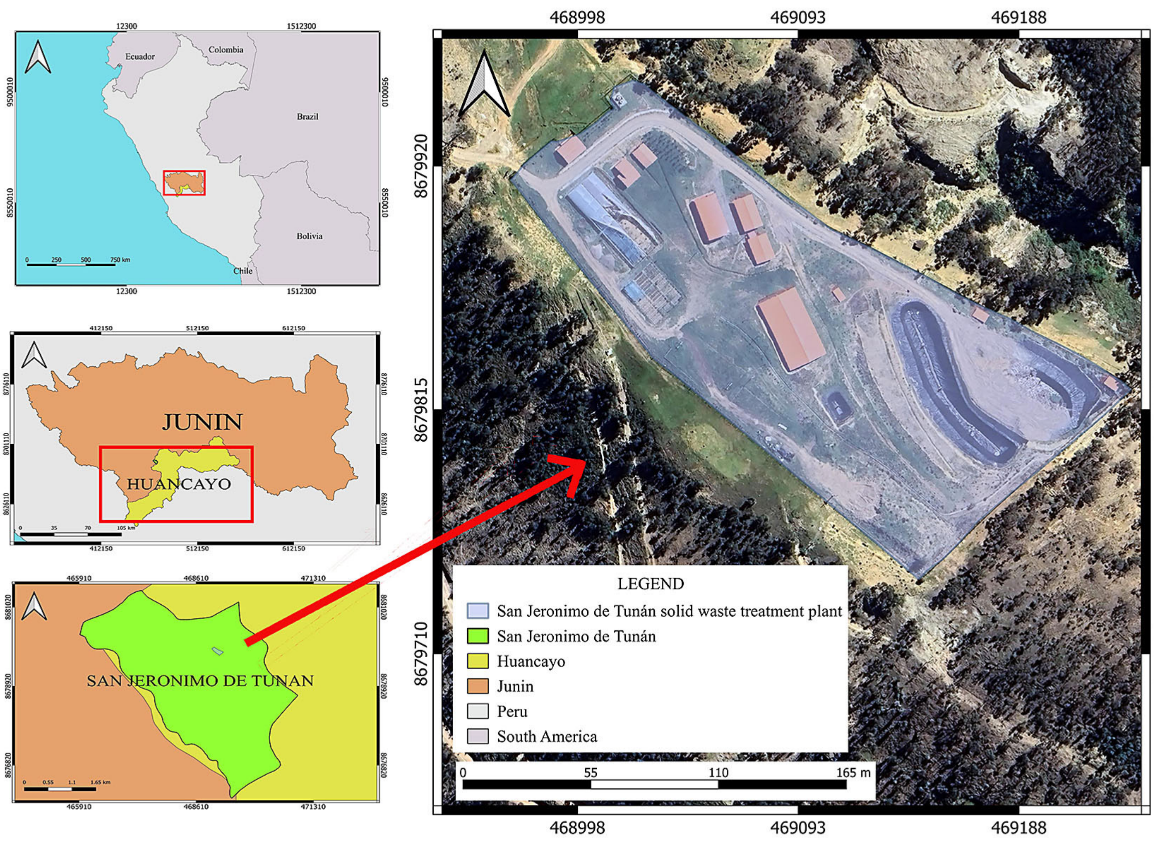

Figure 1. Map of the location of the solid waste treatment plant in San Jerónimo de Tunán, Huancayo, Junín

Table 1. Physicochemical parameters analyzed

Parameters physicochemical	Unit	Equipment or method
Hydrogen potential (pH)	pH	Hi9829 Multiparameter Meter
Temperature (T°)	°C	
Electrical conductivity (EC)	mS/cm	
Turbidity	NTU	TB 211 IR Turbidity Meter
Biochemical oxygen demand (BOD)	mg/L	SMEWW-APHA-AWWA-WEF Part 5210 B, 24th Ed. 2023
Chemical oxygen demand (COD)	O_2 mg/L	SMEWW-APHA-AWWA-WEF Part 5220 D, 24th Ed. 2023

Table 2. Heavy metals tested

Heavy metals	Unit	Method
Lead (Pb)	mg/L	EPA Method 200.8
Zinc (Zn)		
Iron (Fe)		
Chromium (Cr)		
Arsenic (As)		
Cadmium (Cd)		

rate. The use of leachate as a culture medium for microalgae represents a significant challenge due to the variability and complexity of its composition. These effluents contain high concentrations of toxic compounds, which can negatively affect the growth and development of microalgae, limiting their viability in bioremediation processes (Ortiz et al., 2023).

Preparing the culture medium

Initial escalation phase

An active strain of *Spirulina platensis* (1 liter) was acquired from the company Algatex Biotechnology, specializing in the biotechnological cultivation of microalgae and cyanobacteria. Along with the strain, a culture medium formulated with seven essential chemical components was provided: sodium bicarbonate (10 g/L), sodium chloride (5 g/L), potassium nitrate (2 g/L), potassium sulfate (0.5 g/L), monoammonium phosphate (0.1 g/L), crystalline ferrous sulfate (0.008 g/L), and micronutrients (1 mL/L). This medium was prepared with distilled water following the supplier's instructions; to start the culture, 400 mL of active biomass was added to 2 liters of the previously prepared culture medium. Subsequently, the volume was progressively increased to reach a total of 10 liters of active biomass, necessary for the experimental phase of the study.

Experimental phase

The second experimental test was developed based on the results obtained in the preliminary pilot test. Based on this, it was decided to carry out the main experimental tests using larger volumes of *Spirulina platensis* biomass and using a leachate previously diluted to 40% with distilled water, with the aim of reducing its initial toxicity. Each experimental sample had a total volume of 1.25 liters, composed of 500 mL of leachate and 750 mL of distilled water. This dilution represented a considerable decrease in the concentration of essential nutrients for microalgal development, so it was decided to supplement the medium with the addition of 7.5 g of sodium bicarbonate ($NaHCO_3$), 3.75 g of sodium chloride (NaCl) and 1.5 g of potassium nitrate (KNO_3). During the preparation of the medium, a pH below 9 was detected, which further justified the need to supplement with baking soda. This compound plays an essential role as a source of inorganic carbon, favoring the increase of microalgal biomass (Mokashi et al., 2016), in addition to contributing to the increase of the alkalinity of the medium, adjusting the pH towards optimal values for the growth of *Spirulina* (Pérez and Torres, 2008). Likewise, sodium chloride was incorporated with the aim of maintaining the osmotic pressure and ionic balance of the medium, considering that *Spirulina* requires certain minerals, such as sodium and chlorine, for its proper development and biomass production (Silos, 2021). Potassium nitrate was added as a source of nitrogen, a key element in the synthesis of proteins and pigments. In addition, potassium plays a fundamental role in the osmotic regulation and cellular stability of the microalgae, directly contributing to its growth (Avalos, 2017).

Prior to inoculation, the mixture was carefully homogenized to ensure an even distribution of nutrients across all treatments. The main purpose of this strategy was to reduce the initial toxicity of the medium, while optimizing the culture conditions to favor both the survival and the metabolic activity of the microalgae. It is worth mentioning that the medium used in the treatments did not correspond to standard formulation but was a partial adaptation of the medium provided by Algatex Biotechnology, adjusted to the specific conditions of the present study.

Spirulina platensis culture conditions

For the cultivation of the microalgae, both during the initial scaling and in the experimental phase, various factors were considered, such as the availability of essential nutrients, temperature, light intensity, appropriate aeration and a high pH to develop properly (Soni et al., 2019). The culture media were exposed to a photoperiod of approximately 12 hours of indirect natural light and 12 hours of darkness (12:12), a simple condition to apply and effective for the growth of *Spirulina platensis*. This cycle has been shown to promote a good accumulation of biomass, outperforming other lighting regimes. In addition, it has been reported that it is also optimal for other nearby microalgae and cyanobacteria, such as Arthrospira maxima, which supports its use in this study (Sánchez-Bayo et al., 2020; Uganu, 2007). During the experiment, the samples were kept in a pH range between 9 and 10, considered suitable for the growth of *Spirulina*, since this microalga preferentially develops in alkaline environments, with a pH between 9 and 11, and in media rich in mineral salts (AlFadhly et al., 2022).

During the experiment, the microalgae was kept at room temperature, between 15 °C and 20 °C. Although this range is below that considered optimal (25–35 °C) (Fagiri et al., 2013), it has been documented that *Spirulina* can develop adequately in more moderate thermal conditions, showing good tolerance between 15 and 30 °C (Rehman et al., 2025). In this case, the strain showed favorable adaptation and satisfactory growth.

Aeration was maintained constantly by a pump that pushed ambient air through an air stone, allowing the formation of small, uniform bubbles with an airflow of 2 L/min. With a smaller bubble size, it significantly improves the microalgal growth rate, by favoring a homogeneous mixture of the culture and optimizing the supply of carbon dioxide necessary for photosynthesis and the synthesis of compounds such as lipids, essential for cell development (Yang et al., 2018).

Experimental design

Microalgae have several mechanisms to remove pollutants in water, including biosorption, bioaccumulation, and biodegradation (Abdelfattah et al., 2023). To evaluate the efficiency of *Spirulina platensis* in the bioremediation of leachate, an experiment was designed consisting of a control group and two experimental groups, which was differentiated by the number of microalgae used and by the treatment time. The first experimental group was made up of samples N-01 and N-02, to which 550 mL of microalgae were added and treated for a week. The second experimental group included samples M-01 and M-02, with 750 mL of microalgae each, and were left in treatment for a longer period of two weeks.

During the process, the most relevant physicochemical parameters were monitored: pH, electrical conductivity, temperature, and turbidity, as well as microalgae counts. For samples N-01 and M-01, treated for one week, measurements were taken on days 1, 3, and 5, and at the end of 168 hours of treatment (calendar day 8, corresponding to day 7 of incubation). For samples N-02 and M-02, treated for two weeks, measurements were taken on days 1, 3, 5, 8, 10, and 12, and at the end of 336 hours of treatment (calendar day 15, corresponding to day 14 of incubation). This monitoring allowed for more detailed observation of the evolution of leachate conditions during the microalgae treatment. In addition to the monitoring of the physical-chemical parameters, the biochemical oxygen demand (BOD), the chemical oxygen demand (COD) and the presence of heavy metals (Pb, Zn, Fe, Cr, As and Cd) were also analyzed. These analyses were performed at the end of each treatment stage: first on the samples of the first week (N-01 and M-01) and then, at the end of the second week, on the remaining samples (N-02 and M-02). Likewise, the results obtained were compared with the values of the initial leachate without treatment, which allowed establishing a baseline. In this way, it was possible to know the evolution of the bioremediation process and compare the effects according to the duration and concentration of the treatment applied. It should be noted that the experimental treatments were carried out at different times and under similar conditions, but no simultaneous replicates were performed. No formal statistical analysis was performed, but the results show significant trends in the effectiveness of *Spirulina platensis* in leachate bioremediation.

Determination of cell density

To calculate cell density, Neubauer chambers were used, an instrument that is composed of a series of horizontal and vertical lines that cross each other forming a set of squares and rectangles that serve as a reference for counting (Barbedo, 2013). This chamber features nine main divisions each with an area of 1 mm^2. Before each measurement, the Neubauer chambers were disinfected, to prevent any type of contamination, after which a sample of the culture was taken and loaded into the chamber to perform the count under the microscope. The counting of the cells was systematically performed under the microscope in grids 1, 3, 7 and 9 according to Figure 2. This procedure was carried out during the pilot test and during the experimentation, performing the count four times consecutively, in order to guarantee the accuracy and consistency of the results obtained. Subsequently, a general average was calculated, which allowed a single representative value to be determined through the following equation (Arredondo and Voltolina, 2007).

$$Cell\ concentation \left(\frac{cell}{mL}\right) = N \times 10^4 \times dil \quad (1)$$

where: N – average of cells present in 1 mm^2 (0.1 µL) dil – dilution factor, 10^4 – conversion factor from 0.1 µL to 1 mL.

Contaminant reduction analysis

To determine the efficiency of the treatment applied with *Spirulina platensis* in the reduction of contaminants present in the leachate, a comparison of the results obtained with the Environmental Quality Standards for Water in category 4: Conservation of the aquatic environment (MINAM, 2017) was carried out, due to the fact that currently in Peruvian environmental regulations a specific maximum permissible limit (LMP) for leachates from municipal solid waste treatment plants, it should also be noted that not all the parameters studied have reference values established in the ECA-Water, so the comparison with said regulation was made only for those parameters that are contemplated in the ECA-Water.

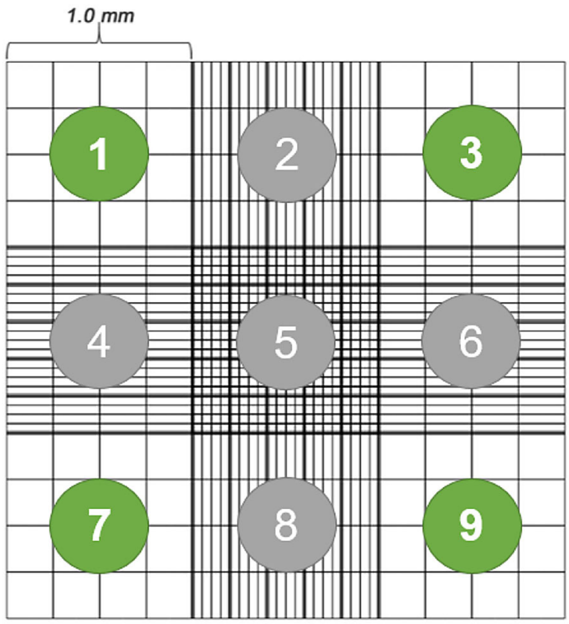

Figure 2. Neubauer chambers

Likewise, the percentage removal formula was used, which allows quantifying the relative decrease of each parameter analyzed by comparing its initial and final concentration, according to the following formula:

$$PR = \left(\frac{Vi - Vf}{Vi}\right) \times 100 \quad (2)$$

where: PR – percentage reduction, Vi – initial value, V_f – final value.

RESULTS AND DISCUSSIONS

Heavy metal concentration and initial physicochemical parameters

To evaluate the quality of the leachate generated in the solid waste treatment plant of San Jerónimo de Tunán before the application of the microalgae *Spirulina platensis*, various physicochemical parameters and heavy metals present in the initial sample were analyzed as shown in Table 3 to determine the level of contamination of the leachate. The results revealed a pH of 7.89, indicating that the leachate is slightly alkaline, and a temperature of 18.30 °C. The BOD reached a high value of 431.10 mg/L, while the COD was 857.00 mg/L. It should be noted that BOD exceeds the limit allowed by the ECA-Water, suggesting a strong presence of decomposing organic matter and a high pollutant load (Carbonel, 2024). Electrical conductivity

Table 3. Initial concentration of metals and physicochemical parameters of the leachate

Physicochemical parameters		ECA-Water	Heavy metals		ECA-Water
pH	7.89	6.5 to 9	Lead	0.03 mg/L	0.0025 mg/L
Temperature	18.3 °C	Δ 3	Zinc	0.3196 mg/L	0.12 mg/L
Conductivity electric	28.2 mS/cm	1 000 µS/cm	Iron	13.7065 mg/L	-
Turbidity	95.1 NTU	-	Chromium	0.7894 mg/L	-
BOD	431.1 mg/L	10 mg/L	Arsenic	0.20131 mg/L	0.15 mg/L
COD	857 O_2 mg/L	-	Cadmium	0.00135 mg/L	0.00025 mg/L

was recorded at 28.20 mS/cm, a value that is above the ECA and the turbidity reached 95.10 NTU, which represents a significant concentration of suspended particles. In relation to heavy metals, an initial concentration of lead (Pb) of 0.0300 mg/L was observed, higher than the limit established by the ECA of 0.0025 mg/L. Zinc (Zn) registered a concentration of 0.3196 mg/L, which exceeds the permitted value of 0.12 mg/L. Likewise, arsenic (As) registered 0.20131 mg/L and cadmium (Cd) 0.00135 mg/L, both above the permissible values according to the ECA. On the other hand, iron (Fe) reached a considerable concentration of 13.7065 mg/L and chromium (Cr) 0.7894 mg/L; however, for these two metals the regulations do not establish a specific reference value.

Microalgae cell count

Spirulina platensis cell count was performed using Neubauer chambers under an optical microscope at 40X. During the development of the experiment, progressive growth of *Spirulina platensis* was observed in all treatments, with notable increases in cell density from the first day to the seventh day of incubation (168 hours). In the one-week treatments (N-01 and M-01), the density increased from 8.750 and 16,250 cells per milliliter, respectively, reaching 46,250 and 48,750 cells/mL at the end of the treatment. In the case of the two-week treatments (N-02 and M-02), a significant increase was also noted, going from 8, 750 and 16,875 cells/mL to 43,750 and 45,625 cells/mL in the same initial period (Table 4) (Figure 3). This behavior suggests adequate adaptation and proliferation of the microalgae in the medium diluted with leachate; however, on the tenth day of incubation, microscopic observation revealed the formation of cell groups, preventing the visualization and individual quantification of the cells under the microscope with Neubauer chambers.

Auto flocculation is observed in certain microalgae when they have culture media with conditions such as high pH, specific metal ions, and high salinity (Huang et al., 2023). It should be noted that, during the experimentation, the pH of the medium remained within the optimal range for the growth of *Spirulina platensis* (between 9 and 11) (AlFadhly et al., 2022) and can grow in media with high salinity (Hadiyanto et al., 2021). This suggests that other factors present in the environment, such as the presence of heavy metals or the interaction with polluting compounds, would have been mainly responsible for cell aggregation. Flocculation in microalgae is a process that occurs when cells clump together and sediment, which can be induced by the presence of heavy metals in the environment, these contaminants interact with the functional groups present

Table 4. Cell density of *Spirulina platensis* during the treatment of experimental groups

Monitoring sessions	Cell density of Spirulina platensis (cell/mL)			
	N-01	M-01	N-02	M-02
Day-1	8 750	16 250	8 750	16 875
Day-3	15 000	23 750	14 375	23 750
Day-5	35 000	43 125	32 500	41 250
Day-7	46 250	48 750	43 750	45 625

Note: N-01: Treatment with 550 mL of microalgae and M-01: Treatment with 750 mL of microalgae (duration of 1 week); N-02: Treatment with 550 mL of microalgae and M-02: Treatment with 750 mL of microalgae (duration of 2 weeks). “Day 7” corresponds to the sampling carried out after 168 hours of treatment (1 week).

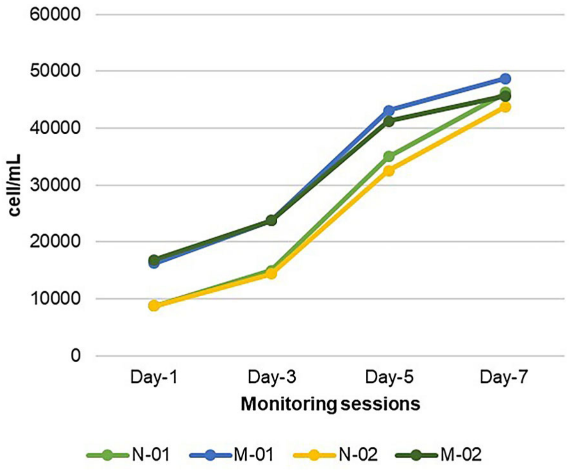

Figure 3. Cell growth of *Spirulina platensis* (cell/mL) in treatments N-01, M-01, N-02 and M-02 during the incubation period, measured on days 1, 3, 5 and 7 (168 h)

in the cell wall of microalgae, such as carboxyls, hydroxyls and sulfates, which causes a reduction in the surface charge of the cells and decreases electrostatic repulsion between them. facilitating their adhesion and the formation of flocs. In addition, in response to this type of environmental stress, microalgae increase the production of extracellular polymeric substances (EPS), compounds that not only enhance the capture of heavy metals but also strengthen cell aggregation. It has been observed that, in low concentrations, metals can stimulate the metabolic activity of microalgae, which promotes an increase in the secretion of EPS, thus favoring the adsorption capacity and flocculation, also the microalgal cell surface can form complexes with specific contaminants of water, which further enhances the aggregation of particles and contributes to the reduction of suspended and dissolved solids, Together, these mechanisms make heavy metal-induced flocculation a key strategy in the bioremediation of polluted waters (Abdelfattah et al., 2023).

Analysis of physicochemical parameters

According to the results presented in Table 5 and 6 of treatments N-01 and M-01 (one week) and N-02 and M-02 (two weeks), there is evidence of a consistent trend in the efficacy of *Spirulina platensis* in improving the physicochemical parameters of contaminated water. In all treatments, a progressive increase in pH was observed, reaching maximum values close to 9.98, in terms of temperature it showed a moderate downward trend, possibly influenced by environmental factors, on the other hand the electrical conductivity (EC) remained relatively stable in the four treatments with slight increases, which suggests that the microalgae does not generate significant alterations in the ionic charge of the medium, however, the most prominent parameter was turbidity. In the last monitoring session of both groups, a decrease was observed, reaching final values of 42.56 and 35.90 NTU for (N-01 and M-01) while in (N-02 and M-02) values of 45.30 and 49.43 NTU were obtained respectively, it should be noted that physicochemical parameters such as pH, temperature and electrical conductivity were not considered in the comparison with the ECA-Water. because they were adjusted as part of the experimentation to favor the growth of *Spirulina platensis* during treatment.

"Day 7" corresponds to the sampling carried out after 168 hours of treatment (1 week). BOD is an indicator of the level of water contamination by biodegradable organic matter (Wei et al., 2023). This allows estimating the amount of oxygen needed by microorganisms for the oxidation

Table 5. Physicochemical parameters of treatments N-01 and M-01 for one week

Code	Monitoring sessions	pH	T°	CE	Turbidity
N-01	Day-1	9.2	17.02	21.58	129.33
	Day-3	9.89	18.56	22.66	109
	Day-5	9.79	17.17	22.23	56.76
	Day-7	9.92	16.37	22.43	42.56
M-01	Day-1	9.1	17.43	21.02	137.33
	Day-3	9.77	18.97	23.13	98.32
	Day-5	9.75	17.21	22.24	71.8
	Day-7	9.89	15.57	22.16	35.9

Note: N-01: Treatment with 550 mL of microalgae and M-01: Treatment with 750 mL of microalgae.

Table 6. Physicochemical parameters of the N-02 and M-02 treatments for two weeks

Code	Monitoring sessions	pH	T°	CE	Turbidity
N-02	Day-1	9.17	16.77	21.32	124.66
	Day-3	9.86	18.44	22.44	106.67
	Day-5	9.83	17.02	21.82	67.13
	Day-7	9.87	16.31	22	50.07
	Day-10	9.94	15.73	22.05	40.72
	Day-12	9.93	15.34	22.17	42.53
	Day-14	9.89	15.43	22.19	45.3
M-02	Day-1	9.07	16.38	21.01	135.33
	Day-3	9.72	19.39	22.55	107.43
	Day-5	9.78	17.39	22.47	65.9
	Day-7	9.87	16.66	22.21	34.07
	Day-10	9.97	15.37	22.23	41.6
	Day-12	9.97	15.34	22.2	44.92
	Day-14	9.94	15.67	22.24	49.43

Note: N-02: Treatment with 550 mL of microalgae and M-02: Treatment with 750 mL of microalgae. "Day 7" corresponds to the sampling carried out after 168 hours of treatment (1 week), and "Day 14" after 336 hours (2 weeks).

of said matter present in a water sample, because of the activity of aerobic oxidation processes (Lecca and Lizama, 2014). On the other hand, the COD represents an approximate estimate of the biodegradable and non-biodegradable organic matter content of a water sample (Romero-Aguilar et al., 2009).

According to Table 7, it is observed that in the one-week treatments (N-01 and M-01), the reduction in BOD was 15.98% and 19.86%, while in two weeks (N-02 and M-02) it reached 34.31% and 37.09%, respectively, similarly, COD decreased by 24.57% and 26.62% in the one-week treatments. and up to 42.08% and 45.23% in two-week contracts. These results suggest that a longer exposure time and a higher concentration of *Spirulina platensis* improve bioremediation. Previous studies have reported comparable results. González-López et al. (2025) observed COD removal of up to 52.72 % when using *Spirulina sp.* in the treatment of landfill leachate under optimal conditions, thus supporting the potential of this microalgae to treat effluents with a high organic load.

However, the final BOD values obtained in the treatments when compared with the ECA-Water for category 4 – Conservation of the aquatic environment, whose allowed limit for BOD is 10 mg/L (MINAM, 2017), it is observed that none of the values obtained comply with this regulation, this suggests that the organic load is still high and that the bioremediation process needs optimization or combination with complementary technologies.

Heavy metal reduction analysis

The excessive accumulation of solid waste leads to the presence of various toxic pollutants, including heavy metals such as Pb, Zn, Fe, Cr, As

Table 7. Results of BOD and COD in experimental treatments

Code	BOD					COD			
	Initial	Final	Reduction value	Reduction level [%]	ECA-Water Category 4	Initial	Final	Reduction value	Reduction level [%]
N-01	431.1	362.2	68.9	15.98	10 mg/L	857	646.4	210.6	24.57
M-01	431.1	345.5	85.6	19.86		857	628.9	228.1	26.62
N-02	431.1	283.2	147.9	34.31		857	496.4	360.6	42.08
M-02	431.1	271.2	159.9	37.09		857	469.4	387.6	45.23

Note: N-01: Treatment with 550 mL of microalgae and M-01: Treatment with 750 mL of microalgae (duration of 1 week); N-02: Treatment with 550 mL of microalgae and M-02: Treatment with 750 mL of microalgae (duration of 2 weeks).

and Cd, these elements, even in very low concentrations, represent a high degree of toxicity and can cause adverse effects on living organisms. because its presence is common in leachate generated by waste decomposition, constituting a significant threat to the quality of groundwater and surface water (Hussein et al., 2021). The application of *Spirulina platensis* as a biological remediation agent demonstrated remarkable efficiency during the first week of treatment, a period in which the microalgae remained metabolically active. In this interval, a notable decrease in the concentration of contaminants was observed, however, after two weeks of treatment their efficiency decreased and there was even an increase in the concentration of some metals. This behavior is associated with the entry of the culture into the death phase, where cell lysis of microalgal biomass releases intracellular content, negatively affecting sustained removal of contaminants (Commonwealth Scientific and Industrial Research Organization [CSIRO], undated). In this context, a study with *Chlorella* sp. It mentions that although microalgae efficiently remove heavy metals such as hexavalent chromium (Cr(VI)) through biosorption and bioaccumulation, the contaminants remain associated with the biomass and if it is not properly removed, there is a risk that the contaminants will be released again when the microalgae die (Zhou et al., 2024).

In relation to the efficiency of *Spirulina platensis* in the removal of heavy metals, the results obtained reveal variable removal percentages depending on the type of metal and the time of exposure. According to Table 8 in Pb, removals were achieved between 69.33% and 80.67%, with samples treated for one week being more effective, especially in the M-01 treatment. In the case of Zn, the percentages ranged from 44.34% to 50.91%, with a slight improvement in the first week, suggesting greater efficiency

Table 8. Results of heavy metal concentration in experimental treatments

Heavy metals	Code	Initial	Final	Reduction value	Reduction level [%]	ECA-Water Category 4
Lead (Pb)	N-01	0.03	0.0092	0.0208	69.33	0.0025 mg/L
	M-01	0.03	0.0058	0.0242	80.67	
	N-02	0.03	0.0073	0.0227	75.67	
	M-02	0.03	0.0078	0.0222	74	
Zinc (Zn)	N-01	0.3196	0.1779	0.1417	44.34	0.12 mg/L
	M-01	0.3196	0.1569	0.1627	50.91	
	N-02	0.3196	0.168	0.1516	47.42	
	M-02	0.3196	0.1646	0.155	48.5	
Iron (Fe)	N-01	13.7065	4.0125	9.694	70.73	-
	M-01	13.7065	3.4692	10.2373	74.69	
	N-02	13.7065	3.328	10.3785	75.72	
	M-02	13.7065	3.6413	10.0652	73.43	
Chromium (Cr)	N-01	0.7894	0.2653	0.5241	66.39	-
	M-01	0.7894	0.1907	0.5987	75.84	
	N-02	0.7894	0.276	0.5134	65.04	
	M-02	0.7894	0.2784	0.511	64.73	
Arsenic (As)	N-01	0.2013	0.0752	0.1261	62.64	0.15 mg/L
	M-01	0.2013	0.0707	0.1306	64.87	
	N-02	0.2013	0.0775	0.1238	61.49	
	M-02	0.2013	0.0756	0.1257	62.44	
Cadmium (Cd)	N-01	0.00135	0.00038	0.00097	71.85	0.00025 mg/L
	M-01	0.00135	0.00036	0.00099	73.33	
	N-02	0.00135	0.00056	0.00079	58.52	
	M-02	0.00135	0.0006	0.00075	55.55	

Note: N-01: Treatment with 550 mL of microalgae and M-01: Treatment with 750 mL of microalgae (1 week duration); N-02: Treatment with 550 mL of microalgae and M-02: Treatment with 750 mL of microalgae (duration of 2 weeks).

in the early stages of treatment. Regarding iron (Fe), a significant removal was achieved in all samples from 70.73% to 75.72%, with no notable differences between weeks. For Cr, the results were consistent, with removals between 64.73% and 75.84%, with the highest efficiency observed in the M-01 sample. Similarly, As showed stable removals, between 61.49% and 64.87%, while for Cd, the percentages ranged between 55.55% and 73.33%, with one-week treatments being more effective. These findings demonstrate the potential of *Spirulina platensis* as a bioremediation agent, with a differential behavior depending on the type of metal, exposure time and treatment applied the one-week treatment with a higher number of microalgae (M-01) turned out to be the most effective in the removal of heavy metals. These values are comparable to those obtained by Randrianarison et al. (2021), who worked with mining wastewater reaching removals of up to 99 % for Fe, 95 % for Pb, 89 % for Zn and 94 % for Cu, especially under alkaline pH conditions (≥ 7.1) and with high biomass concentrations. However, they also observed that the simultaneous presence of several metals reduced the adsorption efficiency of some elements, such as Zn, whose removal decreased to only 52%, which is consistent with the variations observed in this study. On the other hand, Sánchez Aguirre (2022) evaluated the use of *Spirulina sp.* in contaminated surface waters of the Pias lagoon and managed to remove up to 95.6% of cadmium and 71.5% of arsenic in just 24 hours. Although they were not leachate, the results coincide with what was observed in the research, especially in relation to the effectiveness against the metals analyzed. Together, these studies reinforce what was found in this work and demonstrate that *Spirulina platensis* can adapt and be efficient in different types of contaminated waters, including leachate.

However, when comparing the results obtained with the ECA-Water in Category 4: Conservation of the aquatic environment, it is observed that, although a significant reduction of some metals was achieved, not all inorganic parameters reached the values established by the regulations. In the case of arsenic, its concentration remained below the permitted limit of 0.15 mg/L. However, other heavy metals such as cadmium, lead, and zinc had concentrations higher than the maximum values established by the ECA, which are 0.00025 mg/L, 0.0025 mg/L, and 0.12 mg/L, respectively (MINAM, 2017). These results show that the treatment with *Spirulina platensis* proved to be efficient in the partial removal of heavy metals, however it was not enough to comply with the ECA-Water, therefore, the implementation of complementary treatments is suggested, which allows achieving greater efficiency in the removal of metals and guarantee compliance with the permissible limits established by environmental regulations.

CONCLUSIONS

According to the results obtained, a reduction was recorded in physicochemical parameters, such as turbidity, BOD and COD, as well as in the concentrations of heavy metals present in the leachate treated with the microalgae. In the case of turbidity, significant decreases were observed, reaching final values of 35.9 NTU in treatment N-01 and 42.56 NTU in M-01, in the same way in treatments N-02 and M-02 final values of 45.3 NTU and 49.43 NTU respectively were obtained. Likewise, it was found that the treatment effectively reduced BOD and COD levels, with removal percentages ranging from 15.98% to 37.09% for BOD, and between 24.57% and 45.23% for COD, with M-02 and N-02 treatments standing out as the most efficient. Similarly, it was evidenced that the treatment with *Spirulina platensis* achieved significant reductions in heavy metal concentrations in the experimental samples, the removal efficiencies varied according to the type of metal and the treatment time, being higher for lead (80.67%), cadmium (73.33%), iron (75.72%) and chromium (75.84%). In the case of zinc and arsenic, intermediate percentages were recorded, with values of 50.91% and 64.87%, respectively. These results suggest that this biological treatment may be suitable as an initial stage within a comprehensive leachate treatment scheme, mainly the experimental sample M-01. It is worth mentioning that, after the application of the treatment with *Spirulina platensis*, only arsenic was reduced with values below the limit established by the ECA-Water in category 4: Conservation of the aquatic environment, which indicates that the treatment applied was partially effective and requires complementation with other types of treatment to ensure compliance with the established environmental standards.

The results obtained in the present study demonstrate the efficacy of the microalgae *Spirulina platensis* as a promising bioremediation alternative for the treatment of leachate generated in solid waste plants.

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