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PROPOSAL FOR BIORESTORATION OF DISTURBED SOIL OF THE INSTITUTE OF METALLURGY OF THE UASLP

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Abstract: In the work of Pérez-Castro and García-Meza (2019), the urgent need to carry out a restoration plan for the soil fragment located in front of the Institute of Metallurgy of the Autonomous University of San Luis Potosí is exposed, the importance The ecological nature of this fraction is due to the fact that it is a remnant of the last spring in the Valley of San Luis Potosí. Based on this need, a project was designed and applied in the period from February to May 2020 in order to evaluate the capacity of microalgae as the main colonizer in degraded soil, through a comparative analysis of organic matter and humidity. The results show a variation in the retention of liquid in the soil, resulting in an increase in the colonization of microalgae at higher humidity on the soil surface. Concluding that the best treatment for in situ colonization, considering both the development of microalgae on the surface and in depth, is the treatment that was irrigated with culture medium rich in nutrients, compared to soil irrigated with distilled water. In the future, the effect of microalgae on the possible increase of organic matter in soil samples must be analyzed.
Keywords: Microalgae, soil, colonization, organic matter.

INTRODUCTION

"Historically, humanity has taken advantage of and has established itself on the soil, using its attributes for collective benefit (obtaining food, fibers, construction material, fossil fuels, minerals, building sustenance). As a consequence, the soils have been degrading and, on occasions, disappearing" (Rodríguez and García-Meza, 2018, p.2). Such loss may be due to the reception of polluting compounds with more or less modifying effects on the physical, chemical and biological characteristics of soils.

The definitions coincide in pointing out that a soil,consists of horizons composed of weathered minerals, organic matter, air, water and that houses living organisms thanks to the geomicrobiological transformations that take place in it. As an indissoluble part of it, microorganisms are important formative agents, so its use for remediation and restoration is possible. In the particular case of microalgae,they are known to have originated 3.7 billion years ago; (García-Meza, 2008), likewise it is known that cyanobacteria can form organo-sedimentary structures (eg, biofilms, microbial crusts, stromatolites) and that among their main qualities is that of contributingin soil formationfrom its origin (García-Meza, 2008). These transformations happen thanks to relationships of mutualism or cooperation between microorganisms; for example, lichens (fungal-cyanobacterial association) that are key pieces in the formation and fertilization of soils and act as "primary colonizing" agents, whatever the environment, including areas as sterile as lava from volcanoes or ice from the tundra. Likewise, it is known that cyanobacteria and some microalgae of the Eukarya domain, the Viridiplantae (green algae), are tolerant to inorganic contaminants such as metals (Cu and Zn; Sánchez-Olvera et al. 2019), or organic compounds (Kesaano and Sym 2014). Therefore, it is possible to use microalgae in the remediation and restoration of soils affected by pollutants.

In response to the need presented in the project by Pérez-Castro and García-Meza (2019), in which it is concluded that "a restoration or rehabilitation plan is necessary for this fragment, which, although it is a very small area, has great importance for belonging to the last spring of the Valley of San Luis Potosí", since that soil lost structure, humidity, inorganic nutrients and organic matter. This and previous works in the study area indicate the presence of microalgae resistant to adverse conditions and high concentration of metals

(Zaragoza et al. 2017; Sánchez-Olvera et al. 2019), which can be used for soil colonization tests. altered. This project was designed and applied with the objective of evaluating the potential of microalgae to colonize a degraded altered soil, which allows to start a research work in soil biorestoration.

METHODOLOGY

1. CULTURE MEDIUM PREPARATION

A solid solution (agar) of Wholes-Hole was prepared where the microalgae samples were cultivated for 20 days, to later be inoculated in soil; Likewise, the Wholes-Hole culture medium, but in a liquid state, was used to irrigate the microalgae in the soil obtained.

Five macronutrient stock solutions, one micronutrient *stock solution and one* vitamin *stock solution were prepared:*

Macronutrient solution composition: Use 1 mL of each solution for 1 L of medium

$CaCl_2 2H_2O$	36.76g/L
$MgSO_4 7H_2O$	36.97g/L
$NaHCO_3$	12.60g/L
K_2HPO_4	8.71g/L
$NaNO_3$	85.01g/L

Composition of micronutrient solution: Use 1 mL of solution for 1 L of medium

$MnCl_2 4H_2O$	0.18g/L
$ZnSO_4 7H_2O$	0.022g/L
$(NH_4)_6Mo_7O_{24} 4H_2O$	0.0046g/L
$CuSO_4 5H_2O$	0.01g/L
H_3BO_3	0.06g/L

Once the macro and micronutrients are added, 500 mg/L of HEPES buffer is added. Adjust pH 7 with HCl or NaOH. Sterilize at 120 °C for 20 min. Subsequently, the vitamin solution is added:

Composition of vitamin solution: Add 0.5 mL to the culture medium, by filtration under sterile conditions.

biotin	0.1mg/mL
B12 vitamin	1.0mg/mL
Thiamin	100mg

As well as 1 mL of Fe-EDTA by filtration under sterile conditions, prepared with:

Na2EDTA	4.36g/L
$FeCl_3 6H_2O$	3.15g/L

2. MATERIAL STERILIZATION

Both the culture medium and three Petri dishes were sterilized by means of an autoclave. used to protect the solid culture medium with microalgae, likewise a container with three compartments used for the experiment was sterilized by means of UV light. It is important to specify that each compartment of the container measures 10 cm x 10 cm x 18 cm.

3. OBTAINING THE SOIL PROFILE

A 50 cm x 50 cm x 40 cm trench was excavated from the disturbed soil. Two samples were taken: one at the surface level and the other at a depth of 30 cm, approximately 1 kg of each sample was taken, then they were homogenized and sieved with a 250 μm mesh and both samples were stored in transparent plastic bags duly labeled.

4. COLLECTION OF MICROALGAE SAMPLES

The microalgae sample was collected from the runoff located at the Institute of Metallurgy, UASLP, in February 2020. It was scraped with a spatula at 3 different points previously selected and the microalgae obtained were

kept in a container with distilled water, homogenized and they were observed with an optical microscope (Leica Galen III 1420SP) for their identification.

5. PREPARATION OF ANTIBIOTIC FOR MICROALGAE

Before performing its inoculation in the solid culture medium, the sample was homogenized and separated into three test tubes. Two of these were exposed to 10 mgL^{-1} and 2 mgL^{-1} of ampicillin respectively, the third test tube was not added ampicillin, in these test tubes the cell culture was maintained for 48 hours.

6. PERCENTAGE OF HUMIDITY AND ORGANIC MATTER

These measurements were made in triplicate, three samples were taken, following the following methodology:

I. The crucibles were dried in an oven at approximately 60°C for 24 h.

II. 1 g of soil sample was added to the dry crucibles and allowed to dry for 24 h at 60 °C (Pi and A).

III. After 24 h, the crucibles with the sample were removed from the oven and weighed (MW).

IV. After weighing them, they were taken to the muffle (550 °C) for 2 h to remove the organic matter.

V. The crucibles were removed from the muffle and each was weighed again (B).

To determine the percentage of moisture, the following formula was used:

$$\%Humedad = \frac{(Pi - Pf)}{Pi} * 100$$

To determine the percentage of organic matter, the following formula was used:

$$\%M.O. = [(A - B)/A] * 100 * R^{OC}$$

$$R^{OC} = factor\ de\ corrección\ para\ carbono\ orgánico = 0.40$$

7. INOCULATION OF MICROALGAE IN SOLID CULTURE MEDIUM

Test tubes containing the culture and ampicillin were inoculated after 48 hours, under sterile conditions, in three Petri dishes with solid culture medium. The inoculated microalgae were incubated for 20 days at 25°C under light cycles; darkness of 12:12 h, until its growth is achieved to subsequently inoculate them to the soil samples of the container. Likewise, each compartment was labeled, having a first compartment irrigated with culture medium, a second compartment that was irrigated with distilled water and a third compartment which would be a control without microalgae that was irrigated with culture medium. In order to irrigate each compartment, two atomizers were used, one with the culture medium and the other with distilled water, each compartment was sprayed 4 times once a day, under sterile conditions.

8. MINERALOGICAL ANALYSIS BY MEANS OF ATOMIC ABSORPTION WITH GRAPHITE FURNACE

To identify and analyze the main soil nutrients, 5 g were taken. duplicate samples were pulverized in an agate mortar.

9. INOCULATION OF MICROALGAE IN SOIL SAMPLES

600 g of soil was poured into a container with three separate cells. Microalgae inoculation was carried out in a hood and under sterile conditions; two of the three Petri dishes with the highest amount of microalgae developed were scraped four times; For the scraping, two sterilized slides and 20 mL of culture medium were used to facilitate the handling of the microalgae on the soil samples.

10. DETERMINATION OF DENSITY OF MICROALGAE IN SOIL

In order to specifically identify the growth of microalgae, the colonized surface area was measured, as well as the depth covered visible through the transparent walls of the container; For this, three 9 X 9 cm acetate quadrants were used, subdivided into 1 X 1 cm squares.

11. DATA MANAGEMENT

For the registration of the development of the microalgae communities, a daily record was kept in an excel sheet, collecting data on depth and colonized area, in units of cm ^{2,} both measures in order to obtain a graph that shows the development of colonization of these communities.

RESULTS AND DISCUSSION

PERCENTAGE OF HUMIDITY AND ORGANIC MATTER

Moisture percentages indicate the amount or percentages of water in the soil per 1 g, likewise they show the maximum available amount of water that a soil can retain, which can increase with organic matter, since a healthy soil can retain almost 20 times its weight in water. The infiltration of water through the soil prevents the passage of contaminants towards the water table (FAO, 2015).

	Average	Standard deviation
% Organic material	0.06	0.01
% Humidity	0.17	0.01

Table 1 . Percentage of moisture and organic matter obtained and its standard deviation.

When comparing the results obtained with the data from the study by Pérez-Castro and García-Meza (2019), a difference in humidity was observed since 0.07% and 0.14% were obtained in the upper and lower horizons, respectively, which on average they represent 0.10% moisture; when comparing it with the percentage obtained in this work (0.17%) greater humidity is appreciated; the foregoing, probably due to the fact that there was greater humidity in the environment when the sampling of this work was carried out (February 2020) compared to the previous work (August 2019), according to the records of Conagua (2020). The humidity in the environment is directly proportional to the amount of moisture found in the soil.

According to Pérez-Castro and García-Meza (2019), this is still a soil with high porosity, which does not retain water in its pores and, as described in the USDA soil moisture estimation guide (1985), it is a dry soil with soil particles that are easily separated.

Likewise, it was observed that the organic matter of the soil increased when compared to the study by Pérez-Castro and García-Meza (2019), who reported 0.10%, and that obtained in this work was 0.17%. In both studies, since they are less than 1%, it is established that the soil is poor in organic matter and, therefore, has no presence of nutrients.

MINERALOGICAL ANALYSIS BY MEANS OF ATOMIC ABSORPTION WITH GRAPHITE FURNACE

The mineralogical analysis allows us to know about the cations ($Ca^{2+,}$ $K^{+,}$ Na^{+}) that the soil has and the way in which they can retain these nutrients, in the case of Ca^{2+} and Na^{+} in the form of salts. which are calcium carbonate ($CaCO_3$) and sodium chloride (NaCl). As for $K^{+,}$ it is found in the parent rock since it is one of the most abundant minerals in the earth's crust (Martínez, 2011).

Likewise, this study allowed us to determine the potential for the development and growth of living organisms in the soil; that is, it contains K^{+} and Ca^{2+} as macronutrients for microalgae.

	Average	Standard deviation
%Na $^{+}$	0.81	0.07
%K $^{+}$	0.86	0.03
%Ca2 $^{+}$	1.13	0.01

$^{+}$ values of less than 3% are reported (Table 2), a concentration that, according to studies by the FAO (2013), is the expected level for a sandy loam soil, which corresponds to the type of soil studied, according to Pérez. -Castro and Garcia-Meza (2019).

Table 2. Mean concentrations and standard deviations of nutrients and cations from soil samples.

Likewise, a low concentration of Ca $^{2+}$ was observed (Table 2) according to the FAO (2013). The FAO determines a range of Ca $^{2+}$ availability between < 2.51% (low) and 6.0% > (high); These percentages are related to the richness of the parent material and its degree of weathering. Ca $^{2+}$ is an important nutrient for all living organisms that can inhabit the soil, for example in microalgae it helps maintain cell structure.

Finally, a high concentration of K $^{+}$ was recorded (Table 2) compared to data from the FAO (2013), which reports low levels when it is <12% and high >0.3%. Although it is common for soils to have a high amount of K $^{+}$ normally, as it is one of the most abundant nutrients in soils on the planet ; In addition, as mentioned in the technical notes on soil fertility of the USDA (1985), most of the K $^{+}$ is found mainly in two different forms available for microalgae, in the interchangeable form and in solution in soil, at the time of carrying out the analysis. protein synthesis and osmotic regulation.

DATA MANAGEMENT AND DETERMINATION OF MICROALGAE IN SOIL

Figure 1 shows the record of microalgae colonization under different treatments in soil samples, both in surface area and depth; during the period February to May, 2020.

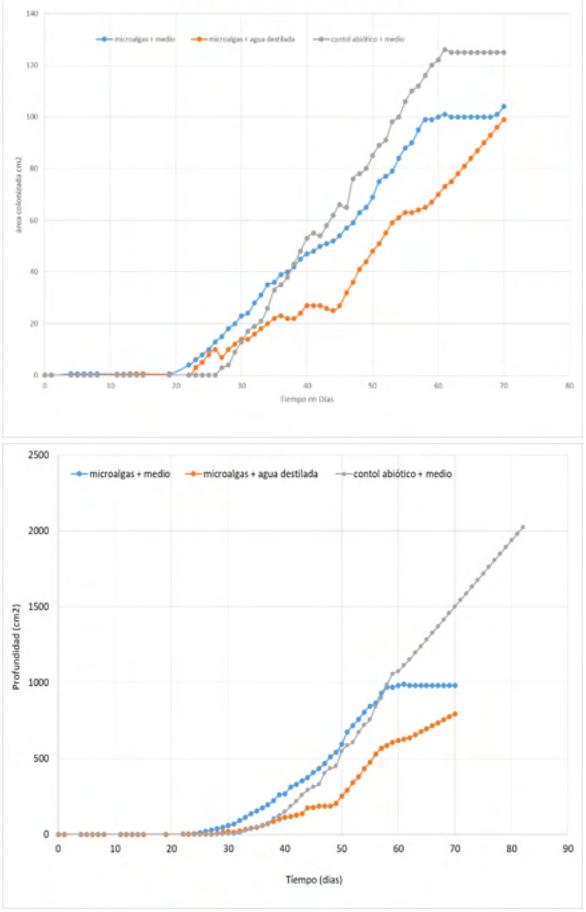

Figure 1. Results of (a) colonized area and (b) depth with respect to time.

The first observations made after 6 days of having inoculated the microalgae colonies, was an increase in infiltration, up to 3 cm deep in the compartment that was sprayed with distilled water; In the case of the compartment containing microalgae, culture medium was sprayed, as well as in the control, the infiltration was less than 1 cm deep. In addition, in the three compartments no growth of microalgae was observed (Fig. 1); after 16 days of not observing it, it was decided to saturate the soil in the irrigated compartments with culture medium or distilled water, as the case may be, pouring a total of 110 mL; this was justified by knowing that microalgae came from and grew in an

aqueous environment; that is, the availability of water in the soil was insufficient and could be limiting the growth of microalgae.

When saturating the soil with up to 20 mL of liquid in each compartment, maintaining the same incubation conditions, after 48 h it was observed that the compartments irrigated with culture medium were humid; Therefore, it can be suggested that the culture medium served as fertilizers for the soil samples and influenced their structure, allowing an increase in the field capacity in the compartments, since when compared to the sample that only contained distilled water, no neither water infiltration nor fluid retention was observed.

Six days after pouring 110 mL of distilled water and culture medium, it was observed that two seedlings grew, whose seeds were not sown. The seedlings, which were in the compartment with microalgae irrigated with culture medium, 3 days later one of the two seedlings drowned due to excess liquid but the second seedling remained after 42 days and then withered due to the possible wilt disease fungal, which happens when the soil is compacted; preventing the movement of the liquid and the growth of the roots (UCIPM, 2019). Furthermore, it was observed that the colonization of microalgae in this compartment increased, from 0 cm^2 to 100 cm^2 superficially (Fig. 1a) and from 0 cm^2 to 980 cm^2 in depth (Fig. 1b). It can be said that it was effective for the compartment with microalgae to irrigate keeping the surface saturated since it benefited the colonization of microalgae; although, after the microalgae developed, the colonized surface area no longer increased (Fig. 2c).

The colonization of microalgae in the compartments irrigated with the culture medium was mainly due to the macronutrients and micronutrients of the medium, which are used in the form of ions by the microalgae, each of these nutrients contributes in a different way; for example, Ca^{2+}, K^{+}, Na^{+}, which, although they are nutrients found in the soil, are necessary for the culture medium to contain them since they contribute to the production of biomass. Mg^{2+} is also necessary, which is part of the chlorophyll pigment and participates in the photosynthesis carried out by microalgae; Fe^{3+} acts as a redox catalyst in photosynthesis and participates in nitrogen assimilation; Zn^{2+} and Mn^{2+} are coenzyme activators. It also contains PO_4^{3-} and NO^{3-}, which the microalgae reduce to P and N, essential elements for their growth, amino acid and protein synthesis, the efficiency of the photosystem, generation and transformation of metabolic energy, all of which is essential for the development and reproduction of microalgae. When presenting a deficiency of micronutrients and macronutrients, as is the case of the compartment that was irrigated with distilled water, the general effect would be reflected in the deficiency of biomass increase (Martínez, 2011).

In the sample from the compartment irrigated with distilled water, colonization was slow (Fig. 2b); 2 seedlings grew after 1 week of having saturated the soil; after 2 weeks they drowned with the amount of stagnant liquid in the compartment, which remained for 42 days at a height of 1.5 cm^2 above the surface (Fig. 2b) and in depth; on 05/13/20 small spots of microalgae colonization were observed (Fig. 3b).

Figure 2. Comparison of soil sample surface with (a) microalgae irrigated in culture medium, (b) microalgae irrigated with distilled water and (c) control soil, from 05-13-20.

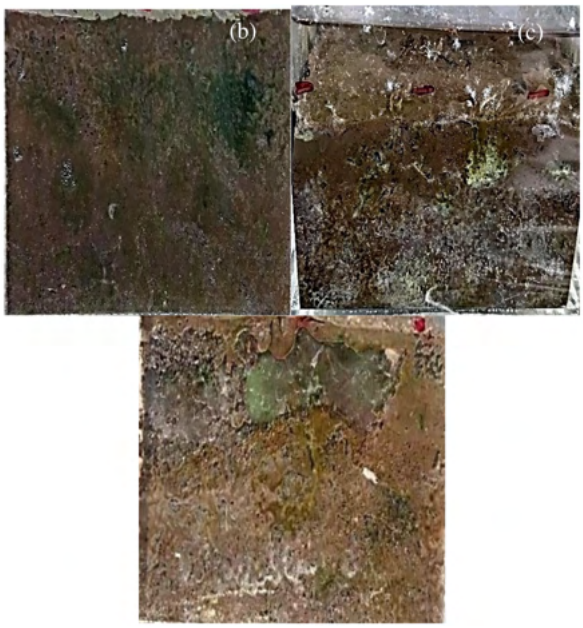

Figure 3. Comparison of soil sample depth with (a) microalgae irrigated in culture medium, (b) microalgae irrigated with distilled water and (c) control soil, from 05-13-20.

CONCLUSIONS

Variation in the retention of liquid in the soil was observed, as well as an increase in the colonization of microalgae in the three compartments. However, it is concluded that the best treatment to promote colonization in situ, considering both the development of microalgae on the surface and in depth, was the treatment that was irrigated with culture medium without inoculum (control), its colonization was accelerated.. The colonization of microalgae inoculated and irrigated with medium or distilled water was similar and remained stable for 70 days after saturating the liquid soil, achieving the same colonized surface area with both treatments.

Although the different populations of microalgae obtained could not be identified, it can be said that they belong to the Viridiplantae subkingdom, based on previous work (Zaragoza et al., 2017; Sánchez-Olvera et al., 2019), since obtained from the same place.

It is important to comment that the final analysis of soil samples from the different compartments was not carried out either, which allows knowing what nutrients were provided to the soil, if the organic matter and moisture it retains increased, as well as the structure of the soil.

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