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Impacts of Heavy Metals on Soil Microbial Activity

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Abstract

Concern about soil quality has been increasing due to environmental impacts from anthropogenic actions. The imbalance between its components alters activities in ecosystems. One of the main actions affecting soil quality is the presence of heavy metals, impairing the functioning of the ecosystem. This work evaluated the impacts of metal-contaminated soil on microbial activity after dam failure in Minas Gerais State, Brazil. Microbial respiration measurements and colony quantifications were used for evaluations. Thus, it is hoped that through these bioindicators, we can assess the quality of the environment and from these biostimulators restore the environmental balance, benefiting local communities affected by the disaster. After microbial biostimulation of the soil, there was an increase in the number of bacterial colonies as well as greater accumulation of CO_2 over the days. Thus, the addition of nutrients to the metal-impacted soil was essential for initiating the restoration of the affected ecosystem equilibrium.

Keywords: Microorganisms, Contamination, Environmental imbalance

1. Introduction

The sources of heavy metal pollution can be of natural or anthropogenic origin, coming from mining, industrialization or agriculture, being mining one of the most important sources of origin (Li et al., 2014).

Heavy metals interfere with the quality of the environment, water bodies, threats to the health and welfare of animals and humans through the food chain, and can lead to diseases such as cancer, renal dysfunction, hypertension, among others (Tchounwou et al., 2012).

Microorganisms have the ability to pick up heavy metals, either by bioaccumulation or by adsorption, from the microbial cell wall, which contains lipids, proteins, and some functional groups. However, the presence of heavy metals in the environment results in a decrease in the microbial population, due to the high toxicity of the elements, so it is necessary to use alternatives in order to keep the microbial population healthy (Chen et al., 2015).

Rathnayake et al. (2013) observed that the presence of cadmium and copper metals in soil reduced for the metabolic activity of *Bacillus megaterium, B. thuringiensis* and *B. simplex.*

Thus, in recent years, soil quality studies have been increasing due to the aggravation of environmental problems. Quality is related to the balance of all its elements, as well as its ability to maintain high productivity, causing minimal environmental disruption (Duval et al., 2013).

The indices for soil quality assessment should be sensitive to changes and easily determined (Armenise et al., 2013). Must be part of the physical, chemical and biological properties (Andrews et al., 2004). Bioindicators are species and / or biological activities that reveal the environmental condition of a given location that may come from anthropic actions (Baretta et al., 2006; Baretta et al., 2011; Kladivko et al., 2014).

Thus, looking for sustainable alternatives, biofertilizer has organic compounds and microorganisms capable of assisting in these factors, in a sustainable way and with low production cost (Aseri et al., 2008; D'andrea, 2002; Vessey, 2003; Wu et al., 2005).

Medina et al. (2012) studied the effects of the application of nutrient rich organic compounds in hydrocarbon contaminated soils, concluding that this practice favored the degradation of the contaminants.

In soil contaminated with the pesticide amethrin, the application of biofertilizer favored microbial activity, as measured by soil respiration, using the Bartha and Pramer respirometry technique, observed by Régo et al. (2014).

Thus, with its rich nutrient composition and wide use in various crops, it makes biofertilizer an alternative for use in soil xenobiotic degradation, improving soil quality (Jilani et al., 2007; Régo et al., 2014).

Also as a second sustainable alternative is the use of biochar for the removal of metal ions in water. It is a solid material obtained through the carbonization of biomass. It can be used to replace activated charcoal or coconut shell as a low cost and easily obtainable adsorbent to remove contaminants from water (Lehmann et al., 2011; Mohan et al., 2014).

The objective of this work was to evaluate the effects of heavy metals on soil microbial activities following the disaster that occurred in the city of Mariana, Minas Gerais State, Brazil, caused by the disruption of the Fundão dam, which resulted in the release of 34 million m^3 of mud from iron ore production by mining company Samarco, which is controlled by Vale and Britain's BHP Billiton. The fact occurred in november 2015.

2. Methodology

It collected metal contaminated mud in the city of Mariana, as well as collecting soil without the passage of mud, in a region near the city of Mariana, in Brazil.

In order to evaluate the microbial activity of contaminated soils, colony forming units per gram of soil were quantified by pour plate technique and the evaluation of respiration by CO_2 generation over time. The treatments consisted of:

- 0% - 50.0 g control soil + nutrients
- 25% - 12.5 g metal soil + nutrients
- 50% - 25.0 g metal soil + nutrients
- 75% - 37.5 g metal soil + nutrients
- 100% - 100.0 g metal soil + nutrients
- 50.0 g control soil
- 50.0 g control metal soil

For microbial biostimulation, bacteria selected from soil samples containing metals were used, together with 100 mL minimum medium enriched with coffee husk. After growth, 5 mL was inoculated into 50.0 g of dry soil.

2.1 Microbial Quantification

The quantification of the microbial population was performed by the plating technique in order to quantify the number of bacterial and fungal colonies per gram of soil. The culture media PCA (Plate Count Agar) for bacteria and PDA (Potato Dextrose Agar) for fungi were used and placed in sterile Petri dishes.

By means of 0.85% (w/v) sodium chloride NaCl solution, soil dilutions were made for counting colony forming units. 0.85 g of dissolved NaCl was weighed 100 mL of deionized water. After preparation, 6 mL of the solution was placed in test tubes for later sterilization.

50.0 g of soil was weighed into a 250 mL schott flask and 50.0 mL of deionized water was added for stirring at 200 rpm for 1 hour on a shaking table. After this period, the flasks were allowed to stand for 30 min and only the supernatant formed was used to start plating.

Plates were incubated for 72 hours at 28 °C for fungi, and for 48 hours at 35 °C for bacteria. After the incubation period, the readings of the forming colony units were performed per gram of soil.

2.2 Microbial Respiration

By Bartha and Pramer respirometers (1965), CO_2 generation over time was evaluated. The tests will be based on OECD (2002).

The CO_2 from microbial respiration will react with the sodium hydroxide solution over time. At times, aliquots of sodium hydroxide will be taken to be titrated with hydrochloric acid to quantify the generation of CO_2 over that period. The spent volume of hydrochloric acid will be entered into the following equation: (1):

$CO_2 = (A-B) \times 50 \times \theta_{HCl} \times 0.044$ (1)

Being that:

A= HCl volume spent for control

B = HCl volume spent for treatments

θ_{HCl} = Normality factor HCl

50 e 0.044 = Transformation factor from µmol to mg of CO_2

3. Results and Discussion

Microbial biostimulation was essential for increasing metabolic activities, both in increasing the number of colonies (Figure 1) and in increasing the accumulation of CO_2 over the days (Figure 2). For the soil control treatment, there was a growth of 42± 3 CFU g^{-1}, and for the metal soil there was 40±2 CFU g^{-1}.

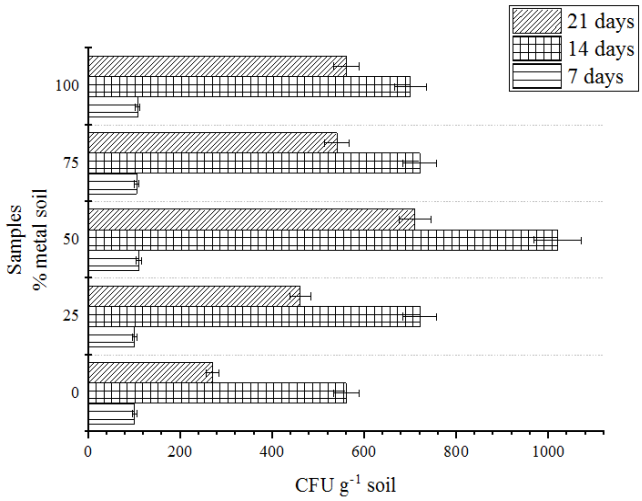

Figure 1. Quantification of bacterial colonies after microbial biostimulation process

In the first seven days of sample incubation, there was no significant difference between treatments. Differences began to occur after 21 days of incubation, with the largest number of treatment colonies 50%. This is due to the sources of nutrients in this treatment, which were essential for microbial growth, being an easy source for assimilation. Even with 100% of soil sample containing metals, there was still microbial growth, because the added nutrient sources were sufficient for the microbiota to overcome the stress of the environment.

After 21 days of incubation, there was a decrease in colonies growth, because there was a decrease in the amount of nutritional source for the native microorganisms.

The same can be observed in Figure 2, microbial respiration evaluation. From the moment that there was the addition of nutrient sources, microbial biostimulation, resulted in stimulation of microbial activity. Even in the face of stress arising from the presence of

metals in the soil, over the days, the microbiota managed to overcome the negative effects present in the environment.

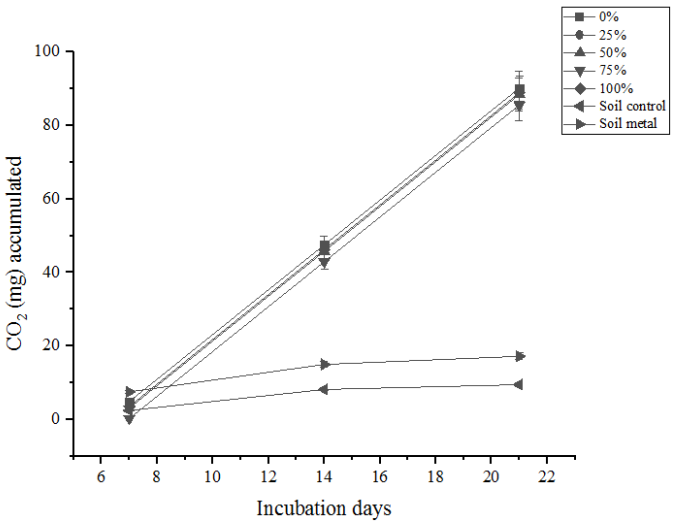

Figure 2. Quantification of CO_2 accumulation after microbial biostimulation process

Without the biostimulation performed, microbial activity was impaired over the days, due to the presence of metals in the soil, harming the local ecosystem, because the microbiota is responsible for nutrient cycling in the environment.

Microbial biostimulation was essential from control soil, without the presence of metals to samples containing metal tailings from the mining disruption (100%), favoring the beginning of the restoration of equilibrium of that affected site. The same was observed by Roy et al. (2018), when studying the effect of microbial biostimulation in soils affected by hydrocarbons, being fundamental for the aid of contaminant biodegradation.

Régo et al. (2014), studying the effects of ametryne herbicide on microbial activity, observed that the addition of biofertilizer was essential for microbiota biostimulation, favoring increased microbial respiration. The same observed effect was observed by causing biostimulation by the addition of Tween 80 surfactant, which is rich in carbon source (Régo et al., 2018).

4. Conclusion

Thus, it is important to add nutritional sources in contaminated sites, in order to favor the restoration of respiratory activities and increase the population of impacted microorganisms.

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