Use of lettuce (*Lactuca sativa* L.) As a parameter to prove the application of the landfarming bioremediation technique

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ABSTRACT
The environment has been suffering major problems that affect its quality. The pollution of water reserves, disorderly deforestation, too many fires and the misuse of soils that increasingly lose their essential characteristics for the survival of life on earth. The tailings of the adsorption process are plant biomass with a high content of the fuel adsorbed on its solid structure. This waste discarded in the soil can be removed or mitigated through the bioremediation technique. Bioremediation is a technique for decontaminating contaminated environments that uses living organisms, plants or microorganisms (bacteria and fungi), with the purpose of attenuating or recovering certain contaminants present in the environment. This study aimed to use lettuce (*Lactuca sativa L.*) as a parameter to prove the effectiveness of the Landfarming bioremediation technique in the recovery of soil contaminated with gasoline. An experiment was carried out with lettuce crops, in bioremediated and non-bioremediated soils, simulating a real field situation, with monitoring of the variables temperature, humidity and pH, in the plant growth period (35 days), followed by nutrient analyses on plants cultivated in these two types of soils. It was possible to observe that the plants were maintained throughout their cycle with healthy aspects, demonstrating that the soil had its bioremediation well executed, making it suitable for use in planting. There was no visual difference between the seedlings of the bioremediated soil and those of the soil collected at UEPB. The results of the analyses for macronutrients were within the normal range. However, for heavy metals, the lettuce samples analyzed, both in cultivation in natural soil (UEPB) and in bioremediated soil, presented levels above the standards determined by ANVISA. Therefore, these plants are not recommended for human consumption due to these high levels of existing metals.

Keywords: Contaminated soil, Landfarming bioremediation, Lettuce crop.

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INTRODUCTION

The environment has been suffering major problems that affect its quality. The pollution of water reserves, disorderly deforestation, excessive fires and the misuse of soils, which lose their fertility, compromising their productive capacity, even causing the desertification of large regions and waters, which increasingly lose their essential characteristics for the survival of life on earth. Numerous situations are the cause of these problems. The way natural resources are used, the deficiency in the preservation and monitoring of reserves and the lack of awareness in the applicability of environmental laws are among them. Industrial waste of the most diverse types is disposed of inappropriately, causing the contamination of soils and water sources. In the academic area, research waste also causes concern and the search for technologies that minimize these harmful effects is of great importance not only in academia, but also for the environment.

When mentioning the terminology "solid waste" it refers to an image of domestic waste, or at most, solid state waste, whether commercial or industrial. In fact, the definition of solid waste encompasses much more than these types of "waste". From the definition of the Brazilian Association of Technical Standards (ABNT), the term "solid waste" replaces the word "garbage"; and from the definitions of the National Solid Waste Policy (PNRS) the word "waste" appears to define solid waste that can no longer be reused, reused or reinserted in any way in the production cycle, or in consumption and production activities (BARROS, 2014).

In developed countries, such as the United States, Canada and several European countries, the biochemical technique of remediation has been widely used in works that are based, for example, on the treatment of soils contaminated by petroleum hydrocarbons. However, contrary to what has been observed in these countries, in Brazil, bioremediation projects are still in the field of theory, with few practical cases, although there is a real probability of expansion (ANDRADE et al. 2010).

Bioremediation is a technique for decontaminating contaminated environments that uses living organisms, plants or microorganisms (bacteria and fungi), with the purpose of attenuating or recovering certain contaminants present in the environment. In order to be efficient in this process, some factors become conditioning factors such as temperature, presence of oxygen, nutrients and pH (MORAIS FILHO; CORIOLANO, 2016).

In the field of bioremediation, one of the applications that has been most evident is related to the treatment of soils contaminated by oil and its derivatives. Petroleum hydrocarbons have a natural origin, so that, consequently, many microorganisms have a natural ability to degrade them (MENEZES et al., 2007). The microorganisms present in the soil are capable of degrading contaminants to harmless substances that are not harmful to the environment, such as carbon dioxide (CO2), water (H2O) and cellular biomass (MASHI, 2013).

Among the biotechnological options, the Landfarming bioremediation process has a considerable prominence due to the low operating cost and availability of treatment of large volumes of oily waste (SILVA, 2009).

Landfarming is one of the remediation technologies that consists of the application of residue on the soil surface for microbial biodegradation, this technique follows the conditions required for the treatment of soil with contaminated solid waste susceptible to degradation, described by ABNT - 1997 - NBR 13894 Soil Treatment (*Landfarming*). This process has low operating costs for large-scale treatment and does not require much maintenance (ABNT, 1997).

Lettuce (*Lactuca sativa L.*) is one of the most widespread and consumed vegetables in Brazil, having great importance in the country's economy and in the diet of the population (KIEHL, 1999; SMITH and HADLEY, 1989; SOUZA, 2005).

The objective of this work was to use the cultivation of lettuce (*Lactuca sativa L.*) as a parameter to prove the effectiveness of the Landfarming bioremediation technique in the recovery of soil contaminated with gasoline, as there is a need to provide a destination and use to this recovered soil, without causing environmental impacts to the disposal environment.

THEORETICAL FRAMEWORK

REMOVAL OF CONTAMINANTS FROM AQUATIC BODIES AND THE ADSORPTION PROCESS

In recent years, research has been carried out at the Universities, on a laboratory scale, with the objective of using techniques such as adsorption for the purification of water contaminated with petroleum derivatives using native biomass from their study regions. The tailings of the adsorption process are adsorbent biomass with a high contamination index, due to the adsorbed oil retained in its solid structure.

Adsorption is a mass transfer operation from a fluid phase to a solid phase, this phenomenon occurs on the surface of the solid. The larger the contact surface, the more favorable the adsorption process (RUTHVEN, 1984).

The main elements of adsorption are the fluid, the surface (usually a porous solid), and the components retained by the surface. The adsorbent is the solid in which adsorption will occur; the fluid in contact with the adsorbent is called the adsorptive, which can be a gas phase or a liquid solution, and the adsorbate is the phase made up of the components retained by the adsorbent (NASCIMENTO et al., 2019).

The first industrial applications of the adsorption process took place during the wars, in the 1920s, and it was used for the removal of alcohol and benzene from gaseous streams (developed by

Bayer AG, Germany) and for the recovery of ethane and heavy hydrocarbons from natural gas (developed by Union Carbide Corporation, USA) (PUPIM; SCHEER, 2005).

SOIL CONTAMINANTS AND TECHNIQUES FOR REMOVING THESE CONTAMINANTS

According to Rizzo et al. (2007), soil contamination by the introduction of oil into the environment is not new, on the contrary, there are records of this type of pollution since 1754. However, it was from the 1960s onwards that attention turned to this reality and several treatment techniques began to be adopted.

The various activities of the oil industry, such as drilling, production, transportation, processing and distribution, generate considerable amounts of solid waste, containing various classes of hydrocarbons, which can lead to serious environmental problems (FORMIGHIERI; JERÔNIMO, 2017).

According to CONAMA Resolution No. 001/86, environmental impact is defined as the alteration of the physical-chemical and biological properties of the environment. The treatment of this waste is essential to promote sustainable management of exploration and use of mineral resources.

According to Silva (2009), the treatment alternatives for waste contaminated with petroleum products are varied, including physical-chemical and biological processes, with the objective of removing organic pollutants at concentrations that are undetectable or, if detectable, at concentrations below the limits established as safe or acceptable by legislation.

The need to treat the waste generated from the gasoline adsorption process into natural biomass, through the bioremediation technique, arose from the need to reduce or neutralize the possible environmental implications that could be caused by the disposal of this waste in the soil in dumps that still exist in some cities in Brazil and also in landfills.

BIOREMEDIATION AND THE LANDFARMING TECHNIQUE

Bioremediation is a process in which living organisms, usually plants or microorganisms, are technologically used to remove or reduce (remediate) pollutants in the environment. This biotechnological process of remediation has been intensively researched and recommended by the current scientific community as a viable alternative for the treatment of contaminated environments, such as surface water, groundwater and soils, as well as industrial waste and effluents in containment landfills (Gaylard et al., 2005).

Biological processes such as bioremediation vary in terms of the type of treatment, which can be *in situ* when performed on-site and *ex situ* when performed outside the contaminated site (GAYLARDE et al., 2005). Both involve procedures that can be applied in the treatment of the surface or subsurfaces and depend on several aspects such as the heterogeneous distribution of the tailings, the concentration of the contaminant, the toxicity and persistence of the contaminant at the site, as well as the favorable conditions for the growth of microorganisms (MOREIRA and SIQUEIRA, 2006). After choosing the type of treatment, the most appropriate technique is chosen.

Landfarming is a technique in which oily residue is incorporated into the soil, under controlled conditions, to remove the degradation and immobilization of hazardous contaminants present by the soil microbiota. The residue is applied to the surface of an area and mixed with the soil through conventional equipment, such as tractors equipped with plows and/or harrows (*in situ*). The soil of the site must be contained in a properly impermeable cell, so that there is no contamination of the water table. This technique is appropriate for the treatment of non-recoverable oil containing impregnated absorbent materials (straw, sawdust and peat) and water-in-oil emulsions (CETESB, 2013).

The biological removal of petroleum products through *Landfarming* has been applied commercially on a large scale with relative success. The technology has been widely used due to its simplicity and cost-effectiveness. The dominant mechanisms of pollutant removal involved in *Landfarming* are the volatilization of volatile low molecular weight compounds during the first days of contamination or treatment, biodegradation and adsorption (MAILA; CLOETE, 2004).

According to the United States Environmental Protection Agency (USEPA), the *Landfarming technique* consists of disposing the residue in the reactive layer of the soil, so that the autochthonous microbiota acts as an agent of hydrocarbon biodegradation (TOMASONI et al., 2019).

CULTIVATION OF LETTUCE (LACTUCA SATIVA L.) AS A PARAMETER TO PROVE THE LANDFARMING TECHNIQUE

Lettuce is one of the most popular crops, planted and consumed throughout the Brazilian territory. The various cultivars of this vegetable adapt well to our diverse climate. Moisture management throughout the growing period is a critical factor for good quality production. Even in relatively short periods of inadequate moisture, it can affect the crop. During germination, temperatures of 7 to 24 ºC are required. Short days are ideal for growing lettuce. Days lasting more than 12 hours cause lettuce blooming (naandanjain.com.br, 2022).

ALMEIDA (2019) used the *Landfarming* technique as a means of reducing or completely removing the granite processing tailings (mud) added to decontaminated soil, and observed the appearance and growth of plants that served as a parameter to prove the effectiveness of bioremediation for possible application in agriculture. In this research, the effects on the growth and development of vegetables were evaluated through observation. The author concluded that it is feasible to use the bioremediation technique to reuse this waste as a complement to soils.

It is important to highlight that germination bioassays with plant seeds are carried out during the first days of their development, in which a series of important physiological processes occur and the presence of a toxic substance can cause adverse effects, resulting in a series of anomalies and even interfere with the survival of this plant (CASTRO, 2013).

METHODOLOGY

LOCATION OF THE EXPERIMENTAL AREA AND ANALYSES

The bioremediation and sowing experiments of the lettuce bed were carried out at the Laboratory of Extension, Teaching and Chemical Technology (LETEQ/CCT/UEPB). The physicochemical analyses of the soil were carried out at the Laboratory of Chemical and Physical Analysis of Soils of Embrapa Cotton, both located in the city of Campina Grande, PB. The lettuce analyses were carried out at the Plant Analysis Laboratory of the Federal University of Paraíba (CCA/UFPB), located in the municipality of Areia, PB.

MATERIAL

The soils used were collected at Embrapa Cotton and in the area next to the Laboratory of Extension, Teaching and Chemical Technology (LETEQ/CCT/UEPB). The lettuce seedlings were acquired from a vegetable grower in the region of Pocinhos, PB, with the ideal size for transplanting in the bioreactors.

BIOREACTORS

For the development of the research, a plastic tray measuring 8x55x35 cm, totaling 15.4 cm3, illustrated in Figure 1, and a glass bioreactor measuring 10x30x20 cm, totaling 6 cm3, were used as a horizontal bioreactor.

Figure 1 - Tray Horizontal Bioreator

Source: The authors(2022).

The procedure for planting lettuce was developed with bioremediated soil obtained in research developed according to Cavalcante, et al., 2021. Initially, the bioremediated soil was stored in the tray bioreactor, where the lettuce seedlings were later planted, where they remained for a period of 35 days to evaluate the growth. After this time, they were harvested, passed through each green plant and taken to a greenhouse at 65°C for 72 hours. After these 72 hours, the samples were taken from the oven and weighed again on a digital scale with two decimal places to verify the dry weight of each. Only after these steps were carried out were these samples sent for macronutrient and heavy metal analysis at the plant analysis laboratory of the Center for Agrarian Sciences - CCA/UFPB in Areia-PB.

Figure 2 illustrates the glass bioreactor measuring 10x30x20 cm, totaling 6 cm3.

Figure 2 - Landfarming bioreactor in glass

Source: The authors(2022).

In the glass bioreactor, natural soil was used without the application of the Landfarming bioremediation technique, collected from the area located next to the LETEQ/CCT/UEPB. In this bioreactor, four lettuce seedlings were planted to serve as a comparison with those planted in the bioremediated soil contained in the tray bioreactor. The planting and collection procedure was the same used in the tray bioreactor.

Figure 3 – Equipment for measuring temperature, humidity and pH

Source: The authors(2022).

In both bioreactors, the following parameters were monitored and quantified: temperature, humidity and hydrogen potential (pH), so that the soil was favorable to crop growth. The soil was moistened with tap water as the moisture was below 60%. Soil temperature and pH were checked twice a week, always in the same shift. A 3x1 pH meter was used (Figure 3) to measure the parameters mentioned above.

SOIL CHARACTERIZATION

The characterization of the bioremediated soil, used for lettuce planting, was carried out at Embrapa Cotton, Campina Grande, PB. The parameters analyzed were nutrients (N, P, K, Ca, Mg and S), pH and organic matter according to Embrapa's soil analysis methodology.

RESULTS AND DISCUSSION

VEGETATIVE ANALYSIS OF LETTUCE

Figure 4 illustrates the tray bioreactor with the lettuce transplanted for adaptation, observation and evaluation of the research.

Figure 4 - Lettuce transplanted in a tray bioreactor for adaptation, observation and evaluation of initial growth.

Source: The authors(2022).

It was possible to observe from the images of the seedlings (Figure 4) that on the first day after transplanting, they were in perfect physiological and vegetative conditions. They were adequately colored and well healthy, indicating that they had all the characteristics that they would have a normal development. Even though this type of container is not the most suitable for conducting this type of experiment, the amount of bioremediated soil used was satisfactory for the good progress of the experiment and an excellent development of the plants.

Figure 5 illustrates the tray bioreactor with the lettuce after 10 days of transplanting.

Figure 5 – Evolution of lettuce development in a tray bioreactor, 10 days after transplanting.

Source: The authors(2022).

Observing Figure 5, it is possible to state that the container used as a bioreactor, with bioremediated soil, proved to be appropriate for such practice, differently from what was initially thought, and it was possible to observe the appearance of the plants 10 days after their transplantation. And, in this way, it is true to say that from this number of days the plants began to take on new architectural configurations. Natural etiolation was already expected, due to the plants not being in direct contact with the sun, and it can be seen more clearly in Figure 6, with bioremediated and natural soil a few days before the harvest for weighing and analysis. It is worth noting that the choice of this crop was mainly due to the fact that it has a very short cycle, is easy to handle and has a lot of plant mass in which it would be essential to carry out the analyses proposed in the experiment.

Figure 6 – Development of lettuce in a tray bioreactor, with bioremediated soil and in a glass bioreactor, with natural soil.

Source: The authors(2022).

After 35 days after lettuce transplanting, during the follow-up of the experiment it was possible to observe the evolution of the seedlings of the crop, proving that the bioremediated soil is able to be reused for planting plant species for human consumption. However, only with the results of plant analyses will it be possible to say whether the plants are fit for human consumption or not.

Soil moisture and pH were also controlled twice a week, during the entire period of the 35 days of the experiment, verifying within this interval that soil moisture remained between 60 and 70% and pH was in the range of 6.5 to 7. This is a neutral pH, very conducive to the natural development of plants. With these values, it is possible to highlight that we can further improve the results of soil bioremediation by making few changes in the form of planting and in the total time of the experiment.

CHEMICAL ANALYSIS OF LETTUCE

Table 1 describes the results of the chemical analyses for macronutrients (N, P, K, Ca, Mg) and heavy metals (Cd, Cr, Ni and Pb) of lettuces, carried out 35 days after transplantation to the bioreactors. These analyzes were carried out at the Plant Analysis Laboratory of the Center for Agrarian Sciences of the Federal University of Paraíba in Areia.

Table 1 - Analysis of lettuce after 35 days of transplanting to the bioreactor.

Sample	In the LAB	N	P	K	Ca	Mg	Ni	Pb	Cd	Cr
		LETTUCE ANALYSIS								
Lettuce in natural soil	94521	29,58	1,94	61,28	19,81	4,47	2,23	5,78	0,22	1,29
Lettuce in bioremedied soil	94522	28,53	3,93	34,85	21,98	6,57	1,04	6,57	0,23	1,05

Legend: N, P, K, Ca and Mg: Digestion with H2O2 and H2SO4; Ni, Pb, Cd and CR: Digestion with HNO_3 and HClO4

After 35 days of transplanting, the lettuce plants were collected. Then, the green weight was carried out, taken to the greenhouse at 65 °C for 72 h. After this period, the plant material was taken for laboratory analysis to determine the chemical composition (N, P, K, Ca, Mg, Cd, Cr, Ni and Pb), as shown in Table 1.

In Brazil, the National Health Surveillance Agency (ANVISA) is the agency responsible for limiting the concentration of inorganic compounds in food. These limits are stipulated based on studies carried out by these organizations, with the help of foreign bodies, such as the World Health Organization (WHO) itself.

For the element cadmium (Cd), the National Health Surveillance Agency (ANVISA, RDC Nº 42 OF AUGUST 29, 2013) has a maximum limit of 0.20 mg.kg^{-1} for leafy vegetables, in such a way that the lettuce sample grown in natural soil (0.22 $^{mg.kg-1}$) and in bioremediated soil (0.23 $^{mg.kg-1}$) did not comply with the standards. It is worth mentioning that plants such as lettuce are cadmium accumulators, being quite tolerant to high concentrations, showing no symptoms of toxicity (MENDES; OLIVEIRA, 2004).

For the element lead (Pb), the National Health Surveillance Agency (ANVISA, RDC No. 42) has a maximum limit of 0.30 mg.kg^{-1} for leafy vegetables, where both lettuce samples showed a high level for this element.

With these results of heavy metals above those indicated by health agencies, these plants cannot be indicated for human consumption. We cannot say whether these heavy metals were from the soils used or from plants that have already been contaminated from their origin. However, these plants can possibly be indicated as removers of some of these metals from soils.

In the case of macronutrients (N, P, K, Ca, Mg) the results are presented in $^{g.kg-1}$. The nitrogen (N) values found in the analysis are considered adequate for lettuce, being close to the lower limit, which is 30 to 50 $^{g.kg-1}$ (TRANI and RAIJ, 1997).

Phosphorus (K) presented levels slightly below the desired for lettuce, as described by TRANI and RAIJ (1997). The concentration was below the lower limit of the range mentioned by these authors, which is 4.0 to 7.0 $^{g.kg-1}$. However, with lettuces from bioremediated soil, it is possible to observe a lower potassium content than lettuces from natural, non-bioremediated soil by approximately 50%.

CONCLUSION

Based on the results, it can be said that the application of the *Landfarming* technique can be one of the ways to minimize the effects of degradation of soils contaminated by organic waste.

The preparation of the bioreactor suitable for the favorable conditions for the treatment of the tailings is a fundamental step to facilitate the operations of homogenization, aeration and humidification of the soil.

The daily monitoring of pH, temperature and humidity parameters were essential for them to be corrected at the appropriate time, always maintaining conditions favorable to the efficiency of the system.

The experiment was installed with lettuce seedlings acquired in the region, with bioremediated soil and natural soil, and the results were very satisfactory, with the evolution of the plants within the normality for the environment in which they were installed.

The plants remained throughout their cycle with the appearance of healthy plants, demonstrating that the soil went through the bioremediation process properly, making it suitable for use in planting not only lettuce, but other vegetables as well.

Visually, no differences were detected between the lettuce plants from the bioremediated soil and the plants from the soil collected in the field of the LETEQ/UEPB laboratory.

The results of plant analyses for macronutrients (N, P, K, Ca and Mg) both in natural soil and for bioremediated soil were within the normal range. With few variations between one and the other. However, for heavy metals, in the lettuce samples analyzed, in both soils studied, the levels of Cd, Pb, Cr were above the standards determined by ANVISA. Therefore, these plants are not recommended for human consumption in this situation. They can be used to remove/extract part of these elements from the soils, improving them and, later, making them totally suitable for various crops.

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