

# Bioremediation of Agricultural Soils Applying Vegetable Activated Carbon and Mountain Microorganisms in the Peruvian Jungle

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Remediation is the set of operations carried out to reduce the level of contaminants in the soil and/or subsoil. The objective of the study was to bioremediate contaminated agricultural soils using activated carbon from *Cocos nucifera* and mountain microorganisms to improve physicochemical parameters and reduce cadmium in Peruvian Amazon soils. For the methodology, a total area of 2000 m<sup>2</sup> was used. Four monitoring points with 30 cm deep pits were used and 1 kg of soil was extracted from each sampling point. For the physicochemical analysis, activated carbon and mountain microorganisms were prepared. The treatments were arranged in a completely randomized design with a total of 7 treatments and 3 repetitions each: T1: soil without treatment or control group (GC); T2: activated carbon (AC) 100g; T3: activated carbon (AC) 200g; T4: activated carbon (AC) 300g; T5: mountain microorganisms (MM) 100 mL; T6: mountain microorganisms (MM) 200 mL; T7: mountain microorganisms (MM) 300 mL. The results show that T1 and T4 improve the physicochemical properties of the soil and absorb cadmium, while mountain microorganisms are only evident in T7.

## 1. Introduction

Soil contamination has increased considerably in recent years due to human activities such as deforestation, poor solid waste management, excessive use of fertilizers in the soil damaging its physical, chemical and biological properties. These factors influence nutritional requirements and food security (Shobhit et al., 2017). In addition, the environmental impact of industrial activities is progressively being subjected to stricter controls in order to limit pollution in the soil resource, using norms with quality parameters or standards (Elessawy et al., 2020).

Soil has a variety of nutrients that favor its metabolism. However, there are sources of heavy metal contamination caused by anthropogenic activities, such as poor agricultural practices, fertilization without dosage and many of these chemical inputs contain in their composition heavy metals such as cadmium (Cd), zinc (Zn), copper (Cu) and lead (Pb) that contribute to the accumulation in the soil. Cd is one of those elements with the highest toxicity to humans, causing severe toxicity even at low concentrations (Roberts, 2014).

To eliminate heavy metals and improve the nutrients in the soil, research was conducted using methods that helped to recover the degraded soil, including the use of nitrogen-fixing microorganisms, plants that absorb heavy metals, application of *Trichoderma sp.*, activated carbon from organic waste, among others, highlighting the values obtained before and after applying the experiments (Yaacoubi et al., 2014, Sandoval et al., 2021).

A promising alternative is to transform organic waste into activated carbon for environmental protection, especially for heavy metal adsorption, being affordable and eco-friendly methods for the environment (Yan et al., 2019, Abdul et al., 2019).

On the other hand, mountain microorganisms is another technique that favors the recovery of degraded soils, consisting of a consortium of liquid inoculants containing beneficial fermentation microorganisms such as lactic acid, yeast, photosynthetic bacteria, actinomycetes and fermenting fungi, favoring their proliferation in the soil, in addition to generating the mineralization of soil organic matter. In this sense, it is widely studied to improve soil quality and health (Olle and Williams, 2013).

For this reason, the present research proposes an eco-friendly and efficient methodology for bioremediation of contaminated agricultural soils using *Cocos nucifera* activated carbon and mountain microorganisms with the objective of improving their physicochemical parameters and reducing cadmium in the soil of the Peruvian Amazon.

## 2. Method

### 2.1. Measurements of the Study Area

The study was carried out in the Cacatachi district located in the San Martin region. The study area was 2000 m<sup>2</sup>, 50 m long x 40 m wide.

### 2.2. Sampling and collection of a degraded soil sample

For soil sampling, 4 monitoring points were used, 30 cm deep soil pits were dug and 1 kg of soil was extracted from each sampling point; the sample was placed in a labeled container to be transported to the "SGS" laboratory for physicochemical analysis.

Table 1: Coordinates of the sampling points of the experimentation site

Sample collection	Latitude	Length	Elevation	Precision	Date	Time
1	-6.729674	-2.637347	461.6±2 m	1.2 m	24.09.2022	11:52 am
2	-6.729746	-2.637244	451.5±2 m	1.3 m	24.09.2022	12:05 pm
3	-6.729747	-2.637152	458.5±2 m	1.3 m	24.09.2022	12:08 pm
4	-6.729752	-2.637359	457.6±2 m	1.3 m	24.09.2022	12:33 pm

### 2.3. Preparation and activation of carbon

250 g of *Cocos nucifera* endocarp were weighed on an electronic balance (FX - 300i, AND), washed and left to dry for 48 h at 35°C. After that, it was carbonized in the soil laboratory of the Universidad Nacional de San Martin located in Tarapoto, using a muffle (NABERTHERM, N3P), at 700°C for 45 min (Ahiduzzaman and Sadrul, 2016; Grima et al., 2016). Each sample was deposited in a mortar to refine the particles and sieved to separate the carbon from the ashes, obtaining 125g of *Cocos nucifera* as the final product.

Phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) was chosen as activating agent, in a 1:1 ratio until obtaining a 50% activating agent (Obregón and Sun, 2014). In a glass container, 125g of *Cocos nucifera* was placed, adding 5ml of H<sub>3</sub>PO<sub>4</sub> to the container for the solution to permeate the carbon (activation process) for 1 hour at 35 ° C. After activation, it was washed with distilled water to eliminate excess acid, leaving it to dry for 4 hours at 35 ° C, obtaining activated carbon with a diameter of 2-5mm of *Cocos nucifera*, measuring the particles in a digital Vernier (Stanley). (Sandoval et al., 2021).

### 2.4. Activation of Mountain Microorganisms

Activation was carried out with 20 L of distilled water; 1 L of mountain microorganisms and 0.5 L of molasses were added, and then the mixture was left to stand for 5 days. Finally, the mountain microorganisms were activated and were ready to be used (Ngilangil and Vilar, 2020).

### 2.5. Experimental treatments of activated carbon and mountain microorganisms

The 21 pots were adapted for the experimental units with a capacity of 5 kg of degraded agricultural soil contaminated with cadmium. The treatments were arranged in a completely randomized design with a total of 7 treatments and 3 repetitions each. T1: soil without treatment or control group (CG); T2: activated carbon (AC) 100g; T3: activated carbon (AC) 200g; T4: activated carbon (AC) 300g; T5: mountain microorganisms (MM) 100

mL; T6: mountain microorganisms (MM) 200 mL; T7: mountain microorganisms (MM) 300 mL. After application, the contents of the pots were mixed to homogenize and ensure adequate distribution. After 20 days, 1 kg of soil was collected for physicochemical analysis.

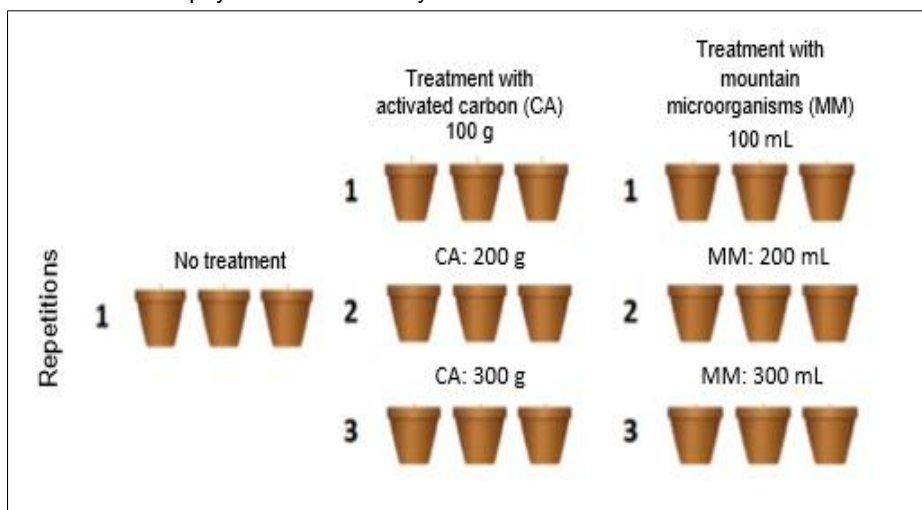


Figure 1. Experimental design with treatments

## 2.6. Statistical analysis

DUNCAN analysis was applied, which determined the significant differences between the experimental treatments.

## 3. Results

A physicochemical study was carried out on a soil sample without applying treatments and was compared with the environmental quality standards for the soil resource; the resulting values were: phosphorus 2.45 ppm (deficient), nitrogen 0.85 % (deficient), potassium 19.70 ppm (deficient), cation exchange capacity 5.28 Meq/100g (poor), pH 5.14 (acid), magnesium 0.82 Meq/100g (deficient), organic matter 1.02 % (poor) and cadmium 5.74 ppm (high). (MINAN, 2017)

Table 2: Analysis of physicochemical parameters of soil without treatment

Parameter	Unit of measurement	Value
Phosphorus	ppm	2.45
Nitrogen	Percentage	0.85
Potassium	ppm	19.70
Cation exchange capacity	Meq/100g	5.28
pH	-	5.14
Magnesium	Meq/100g	0.82
Organic matter	Percentage	1.02
Cadmium	ppm	5.74

The physicochemical study was carried out on a soil sample with activated carbon treatment of *Cocos nucifera* at different concentrations, it is observed that T4 (CA: 300 g) improved the parameters of phosphorus 9.95 ppm, nitrogen 2.78%, potassium 48.4 ppm, cation exchange capacity 21.8 Meq/100g, pH 7.33, magnesium 2.8 Meq/100g, organic matter 2.28% and the cadmium level decreased to 1.2 ppm. The use of activated carbon is an efficient and economical alternative to recover contaminated soils (López et al., 2019); the method contributes to the use of by-products to generate added value that minimizes negative impacts on the environment (Mallick and Mohanty, 2019)

Table 3: Analysis of soil physicochemical parameters with *Cocos nucifera* activated carbon.

Parameters	Treatments			
	T1 Control	T2 CA: 100g	T3 CA: 200g	T4 CA: 300g
Phosphorus (ppm)	2.45	4.52	5.45	9.95
Nitrogen %	0.85	1.15	1.84	2.78
Potassium (ppm)	19.70	28.8	39.7	48.4
Cation exchange capacity (Meq/100g)	5.28	9.47	15.25	21.8
pH	5.14	6.18	6.88	7.33
Magnesium (Meq/100g)	0.82	1.2	2.4	2.8
Organic matter %	1.02	1.21	1.74	2.28
Cadmium (ppm)	5.74	3.01	2.18	1.2

Table 4: Duncan's multiple comparison test for significant differences between treatments

Duncans honestly significant difference $\alpha = 0.05$			
$T_i - T_j$	$ \bar{y}_{i\cdot} - \bar{y}_{j\cdot} $	$w$	$ \bar{y}_{i\cdot} - \bar{y}_{j\cdot}  > HSD$
Difference de T1 y T2	10.451	0.5518	Significant
Difference de T2 y T3	11.954	0.5769	Significant
Difference de T3 y T4	14.971	0.5810	Significant
Difference de T1 y T3	14.452	0.5971	Significant
Difference de T2 y T4	12.208	0.5761	Significant
Difference de T1 y T4	16.755	0.7612	Significant

It is observed that there is a significant difference between the treatments with activated carbon; however, treatment 1, which was the control group compared to treatment 4, improved soil parameters as well as higher cadmium removal.

Table 5: Analysis of the physicochemical parameters of soil with mountain microorganisms

Parameters	Treatments			
	T1 Control	T5 MM: 100mL	T6 MM: 200mL	T7 MM: 300mL
Phosphorus (ppm)	2.45	2.14	3.15	6.45
Nitrogen %	0.85	0.24	0.81	1.52
Potassium (ppm)	19.70	21.2	29.8	39.1
Cation exchange capacity (Meq/100g)	5.28	7.17	9.85	14.74
pH	5.14	5.28	6.18	6.98
Magnesium (Meq/100g)	0.82	0.98	1.3	2.1
Organic matter %	1.02	1.01	1.42	1.94
Cadmium (ppm)	5.74	4.11	3.45	2.1

The physicochemical study of a soil sample with the treatment of mountain microorganisms at different concentrations: in T7 (MM: 300 mL) it improves the parameters of phosphorus 6.45 ppm, nitrogen 1.52%, potassium 39.1 ppm, cation exchange capacity 14.74 Meq/100g, pH 6.98, magnesium 2.1 Meq/100g, organic matter 1.94% and the cadmium level decreased to 2.1 ppm. Mountain microorganisms are future proposals that, through meticulous analysis and exhaustive treatments, can significantly contribute to the recovery of contaminated soil. (Xue et al., 2018)

Table 6: Duncan's multiple comparison test for significant differences between treatments with mountain microorganisms

Duncans honestly significant difference $\alpha = 0.05$			
$T_i - T_j$	$ \bar{y}_{i\cdot} - \bar{y}_{j\cdot} $	$w$	$ \bar{y}_{i\cdot} - \bar{y}_{j\cdot}  > HSD$
Difference de T1 y T5	11.154	0.6128	Significant
Difference de T5 y T6	12.579	0.6479	Significant
Difference de T6 y T7	15.812	0.6870	Significant
Difference de T1 y T6	16.579	0.7811	Significant
Difference de T5 y T7	16.586	0.7991	Significant
Difference de T1 y T7	19.421	0.9712	Significant

It is observed that there is a significant difference between the treatments; however, treatment 1, which was the control group compared to treatment 7, improved soil parameters as well as higher cadmium removal.

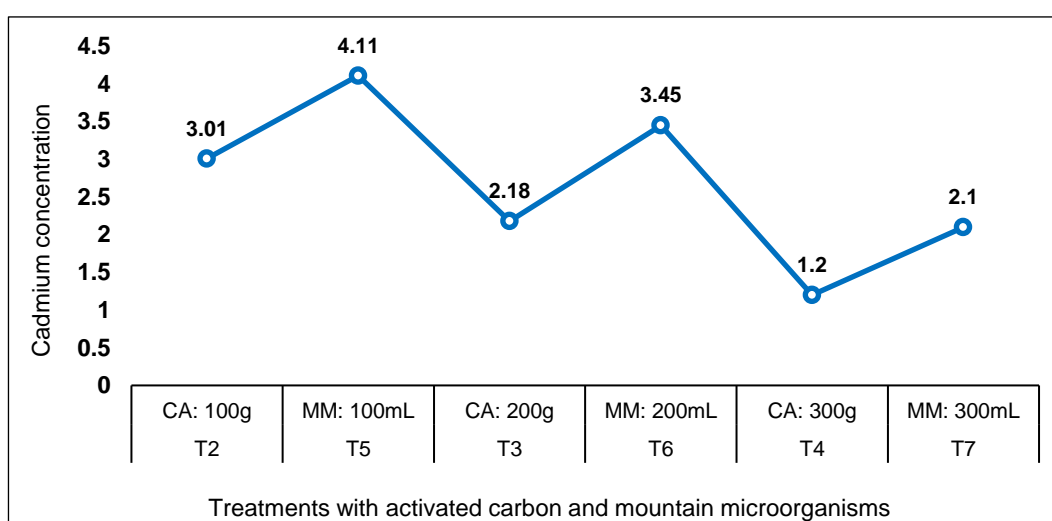


Figure 2. Removal of cadmium with activated carbon and mountain microorganisms

The treatment of activated charcoal from *Cocos nucifera* at different concentrations: at T2 (CA: 100 g) cadmium removal 3.01 ppm; in T3 (CA: 200 g) cadmium removal 2.18 ppm and in T4 (CA: 300 g) higher cadmium removal 1.2 ppm; the treatment of mountain microorganisms at different concentrations: at T5 (MM: 100 mL) cadmium removal 4.11 ppm; in T6 (MM: 200 mL) cadmium removal 3.45 ppm and in T7 (MM: 300 mL) higher cadmium removal 2.10 ppm. Cadmium is a heavy metal that causes health problems and its presence in agricultural soils is of concern due to its mobility and absorption by plants; however, activated carbon shows greater removal, probably due to its chemical composition and its conjugation capacity (Park et al., 2018); mountain microorganisms play a fundamental role in the removal of cadmium, but at a higher concentration a significant result would be expected. (Li et al., 2018)

#### 4. Conclusions

Activated carbon is a natural by-product used for various purposes such as industry, technology, medicine, ecology, etc. Its different functions can make an environment less polluted and thus contribute to new proposals for the recovery of contaminated agricultural soils. The use of mountain microorganisms would be an alternative to improve soil quality because among their metabolic functions are decomposition of organic matter, hormonal effects, promote foliage, flowering, degrade toxic substances and in some cases absorb heavy metals.

The highest cadmium bioremoval capacity in degraded agricultural soils and quality improvement with respect to its physicochemical properties was with *Cocos nucifera* activated carbon in treatment T3 (CA: 200 g) and treatment T4 (CA: 300 g). On the other hand, in the use of mountain microorganisms, improvements in their nutrients and cadmium removal were observed in the T7 treatment (MM: 300 mL).

The bioremediation method or technique could be a proposal to mitigate the negative environmental impacts caused by human activities, as these technologies are non-invasive, economical and environmentally friendly. The use of activated carbon is fundamental in different areas such as industry, technology, medicine and ecology, among others. Its multiple applications can contribute to the reduction of soil contamination and, therefore, promote new initiatives for a sustainable ecosystem. Other materials are more expensive and can generate some negative impact that contributes to pollution in the environment.

## References

- Abdul A.R., Rabat N.E., Johari K., Saman N., Mat H., 2019, Removal of lead (ii) ions from aqueous solution using desiccated coconut waste as low-cost adsorbent, *Chemical Engineering Transactions*, 72, 169-174
- Ahiduzzaman M y Sadrul A.K., 2016, Preparation of porous bio-char and activated carbon from rice husk by leaching ash and chemical activation. *Springerplus*, 5 (1):1248.
- Elessawy N.A, ElSayed E.M, Ali S., Elkady M.F, Elnouby M., Hamad H.A, 2020, Green synthesis in a magnetic nanocomposite vessel for adsorption characteristics, *Journal of Water Process Engineering*, 34, 101047.
- Grima O.C., Ramírez G.Á., Gómez L.D., Clemente J.C., 2016, Activated carbon from flash pyrolysis of eucalyptus residue, *Journal Heliyon*, 2 (9).
- Li J., Li L., Xiong X., Zhang R., Li W., 2017, Remediation of cadmium-contaminated soils by phytoremediation using *Sedum alfredii* Hance: Growth response, cadmium uptake and soil microbial community. *Ecotoxicology and Environmental Safety*, 145, 512-519.
- López M., Cervantes D., Juárez C., Alarcón A., Espinoza F., 2019, Bioremediation of soils contaminated with hydrocarbons using a bioproduct and activated carbon. *International Journal of Environmental Pollution*, 35(4), 711-720.
- Ministerio del Ambiente., 2017, Environmental Quality Standards for soil. Supreme decret N° 011-2017
- Mallick S., Mohanty M., 2019, Bioremediation of polycyclic aromatic hydrocarbon contaminated soil using activated carbon and bacteria. *Bioremediation Journal*, 23(2), 110-123.
- Ngilangil L.E, Vilar D.A, 2020, Effective microorganisms as a remedy for marginal soils in the Philippines, *Chemical Engineering Transactions*, 78, 253-258
- Obregón V.D y Sun K.M., 2014, Comparative cadmium adsorption study on activated carbon prepared from aguaje (*Mauritia flexuosa*) and olive fruit stones (*Olea europaea* L.), *Journal of Environmental Chemical Engineering*, 2, 2280 - 2288.
- Olle M., Williams I.H., 2013, Effective microorganisms and their influence on plant production – A review, *Journal of Horticultural Science and Biotechnology*, 88 (4), 380-386.
- Park J.H., Lee J.S., Shin W.S., 2018, Cadmium removal from aqueous solution using bamboo-based activated carbon. *Korean Journal of Chemical Engineering*, 35(2), 489-494.
- Roberts T.L., 2014, Cadmium Phosphorus Fertilizers: The Issues and the Science, *Procedia Engineering*, 83, 52-59.
- Shobhit R.V., Jay S.S., Naveen K.A, Surendra S., 2017, Soil-Plant-Microbe Interactions in Stressed Agriculture Management: A Review, *Pedosphere*, 27, 177 – 192
- Sandoval A.N., Sanchez L.J., Saavedra Y.M., Calla K.M., Alba R.A., Lloclla H., 2021, Reduction of Imidacloprid and Tebuconazole in *Oryza Sativa* Plantation Applying Strains of *Trichoderma* Spp., *Chemical Engineering Transactions*, 87, 631-636
- Sandoval A.N., Tavera J., Vela F., Calla K.M., Alba R.A., Lloclla H., Valverde Flores J.W., 2021, Adsorption of Gases by Internal Combustion of Trimobiles Using Activated Carbon Filter of *Mauritia Flexuosa* and *Cocos Nucifera*, *Chemical Engineering Transactions*, 85, 157-162.
- Xue Y., Gao H., Wang Y., Chen Y., 2018, Bioremediation of petroleum-contaminated soil by microorganisms isolated from high altitude cold mountainous areas. *Journal of Environmental Sciences*, 67, 111-118.
- Yaacoubi., Zidani O., Mouflih M., Gourai M., Sebti S., 2014, Removal of cadmium from water using natural phosphate as an adsorbent, *Procedia Engineering*, 83, 386-393.
- Yan K.Z., Ahmad M.A., Arsad A., Nasri N.S., 2019, Rubber seed shell based activated carbon by physical activation for phenol removal, *Chemical Engineering Transactions*, 72, 151 – 156.