

## BIOREMEDIATION OF SOIL SAMPLES CONTAMINATED WITH CRUDE OIL USING RICE HUCK-BASED BIOCARBON (*ORYZA SATIVA*)

Dayana Naily Díaz CAMACHO<sup>1, \*</sup>, Tatiana Lisbeth Sornoza MACÍAS<sup>1</sup>,  
María Antonieta RIERA<sup>1</sup>, Bladimir Jacinto Carrillo ANCHUNDIA<sup>1</sup>

<sup>1</sup>Department of Chemical Processes, Food, and Biotechnology, Faculty of Engineering and Applied Sciences,  
Universidad Técnica de Manabí, Portoviejo, Ecuador.

### Abstract

*This research consisted of the bioremediation of four soil samples contaminated with oil using biochar made from rice husks. For this, fourth samples of soil Balao (B), Tachina (T), Refinería (R), and Winchele (W), from the province of Esmeraldas, Ecuador, previously contaminated with oil from a refinery in the country. The rice husk (*Oryza sativa*) was characterized to determine its ash, organic matter, and moisture content. The soil samples were characterized to know their cation exchange capacity, pH, temperature, humidity, organic carbon, ash, organic matter, density, porosity, and texture. The crude oil used as a soil contaminant agent was determined by API, temperature, Conradson carbon, density, ash, organic matter, and viscosity of the crude oil. Each sample was contaminated, and the bioremediation process was evaluated for 90 days. In the zero analysis, the content of total hydrocarbons (TPH) in sample B was 2,691.78 mg/Kg, 2,505.99 mg/Kg for T, 2,950.99 mg/Kg in R and W with 2,708.24 mg /Kg. Once the biochar was obtained, its pH, initial yield, density, ash content, organic matter, phosphorus, calcium, and magnesium were known. After bioremediation, concentrations of 475.61mg/Kg, 209.65mg/Kg, 136.66mg/Kg, and 578.05mg/Kg of TPHs were reached for B, T, R, and B, achieving compliance with the permissible limit established in local legislation for contaminated soils. The results obtained indicate that the process used can be an alternative to soil bioremediation processes.*

**Keywords:** Hydrocarbons; Spills; Residual biomass; Restoration

### Introduction

The Ecuadorian state records oil exploitation as the first source of income for the country's economy. However, the exercise of this activity has historically caused environmental, health, and social disasters, either due to the development of the process or negligence of the people involved [1, 2]. As a consequence of these activities, it is easy to find environmental damage as destroyed soils, air with high levels of contamination, and bodies of water with alterations in their composition [3]. The soil is one of the natural resources affected by the effect of oil spills, which prevents the establishment of an ecosystem and the development of the necessary vegetation for humans and animals [4].

The constant oil spills in countries with oil activity led environmental institutions to find alternatives to mitigate contamination, taking legal measures and restoring affected areas [5, 6]. Hence, the importance of implementing alternative technologies to remedy contaminated soils and preserve the environment.

Biochar is an applicable product in soil remediation since it is a material rich in carbon content, water retention, increases pH, improves cation exchange capacity and base saturation,

\* Corresponding author: ddiaz9364@utm.edu.ec

removal of hydrocarbons and heavy metals, as well as the provision of nutrients for crops, preventing them from being objects of diseases or pests [7]. It is a biomaterial obtained in a circular economy framework, where organic waste, whether solid agricultural or urban, is subjected to incomplete combustion [8].

For this, biomasses with a high carbon composition are used, such as bamboo with 83.60% and rice husks with 57.20 to 72% [9]. The latter showed favorable results as biochar, contributing to the recovery of 97.85% of agricultural soil contaminated with petroleum derivatives [10]. The biochar obtained from rice husks also has the potential to immobilize metals since with dosages of 6%, 8%, and 10% in the sowing of a cocoa plant, it was possible to recover contaminated soil with cadmium in 88.22% [11]. On the other hand, due to the constant oil spills in Peru since 2020, researchers use biochar, achieving a 70% recovery in soils contaminated with diesel, a value that can increase if microorganisms adhere to that speed up the process. In this sense, biochar use contributes to the remediation of contaminated soils [12].

Rice is a grass-type plant originating in the Asian continent, which has become a basic cereal in the human diet up to now [13]. In its processing, by-products are generated which do not present any utility, such as the case of straw, chaff, and husk [14]. Of these, the rice husk is the one that has the most utility due to its characteristics and its high carbon and silica content [9]. According to what was stated by the Institute of Statistics and Censuses (INEC) and the Ministry of Agriculture and Livestock of Ecuador, about 1.6 million tons of rice are produced annually in the country, generating around 20% of husk [15, 16]. In this context, biochar was obtained from rice husks and applied to soil samples contaminated with crude oil extracted in Ecuador to evaluate its applicability as a restoration alternative [17].

## Experimental part

### *Materials*

The rice husks were collected in an agricultural field in the province of Manabí, on the coast of Ecuador. Oil samples were taken from the Trans-Ecuadorian Pipeline System and collected at the refining base of the Esmeraldas State Refinery, Ecuador. We worked with four soil samples from different sites in the province of Esmeraldas, Ecuador: Balao (B), Tachina (T), Refinery (R), and Winchele (W), to carry out the bioremediation process (Fig. 1).



Fig. 1. Contamination of the 4 soil samples

### *Methods*

#### *Characterization of rice husk*

The rice husk determined moisture, ash, and percentage of organic matter. The moisture content was determined using a BOECO - BMA 150 thermobalance. For the ash percentage, 1 gram of sample was placed in a Thermo Scientific - FB1410M muffle at a temperature of 500°C for 90 minutes and was calculated using Equation 1 [18]. The percentage of organic matter was determined using Equation 2.

$$\%ash = \frac{(Wc-Wb)*100}{(Wa-Wb)} \quad (1)$$

where: Wa is the weight of the crucible + sample (g), Wb is the weight of the crucible empty (g), and Wc is the weight of the crucible + sample after muffle (g).

$$\% \text{ organic matter} = 100 - \% \text{ ash} \quad (2)$$

***Obtaining soil samples***

Soil samples were collected in an area of 5 square meters from random sampling at each point, taking into account the guidelines established by ISO 10381-1 regarding the type of collection for subsequent analysis at the laboratory [19].

***Characterization of soil samples***

The characterization of the soil consisted of determining its cation exchange capacity (CEC). 5 grams of the uncontaminated sample and 25ml of 1 M ammonium acetate were stirred for 30 minutes and then filtered. 25ml of 10% (w/v) NaCl is added and filtered again. 10ml of formaldehyde and phenolphthalein were added and then titrated with NaOH until the change and determined the CEC with Equation 3 [20].

$$CEC \left( \frac{Meq}{100} g \right) = \frac{(V*0.071*100)}{pm} \quad (3)$$

where: V is mL of NaOH used in the titration, 100 is a reference mass unit to express the results, pm is the molecular mass of the sample (g/mol), and 0.071 is the molarity of the NaOH.

The pH and temperature were determined using a calibrated By acuaticos brand Soil4\_0987054324 electric sensor [21]. The moisture content was determined using a BOECO-BMA brand thermobalance. To calculate organic carbon was using spectrophotometry with a HACH UV/VIS DR 1900 spectrophotometer at a wavelength of 590nm [18]. The procedure already described for the determination of ash in the characterization of rice husk was used in the same way for soil samples [18].

Another aspect to determine is the apparent density, for which a moisture capsule with soil samples was placed in a Memmert SN55 oven at 105°C for 24 hours. Subsequently, the dry weight was related to the volume of the cylinder, using Equations 4 and 5. The real density was determined using a pycnometer and Equation 6 [20]. Porosity was determined using Equation 7. Based on the CEC, the texture was known [22].

$$Volume \text{ of the cylinder} = \Pi * r^2 * h \quad (4)$$

$$Da = \frac{Weight \text{ dry soil}(105^{\circ}C)}{Volume \text{ of the cylinder}} \quad (5)$$

$$\delta R = \frac{W_{pm}-W_p}{m+W_{pm}-W_{pmw}} * \delta w \quad (6)$$

$$PT = (1 - (Da/ \delta R)) * 100 \quad (7)$$

where: r is the radius (cm), h is the height (cm), m is the weight (kg), Wp is the weight of the pycnometer empty (kg), Wpm is the weight of the pycnometer with sample (kg), Wpw is the weight of the pycnometer with water (g), Wpmw is the weight of the pycnometer with sample and calibrated with distilled water, δR is the real density (Kg/m<sup>3</sup>), δw is the water density (Kg/m<sup>3</sup>), Da is the apparent density (Kg/m<sup>3</sup>), and PT is the total porosity (%).

### ***Biochar production***

For biochar production, the rice husk had to have a moisture content of less than 10%. 100 g of crushed husk was dried (1) in a Memmert SN-55 electric oven for 2 hours at 105°C. It was ground (2, 3) and subjected to pyrolysis at 500°C for 4 minutes (4) in a Genesis LT3140 model oven (Fig. 2) [23].



**Fig. 2.** Rice husk-based biochar production process

### ***Biochar characterization***

The ash content, moisture, pH, temperature, and density of the biochar were determined using the procedures already described in this document. Through volumetric and complexometric titration and Equation 8, the Ca and Mg content was obtained [24]. Phosphorus was determined using the HACH UV/VIS DR 1900 spectrophotometer at a wavelength of 590nm [25]. The biochar yield was calculated based on the amount of bioremediation material obtained from the husk mass used (Equation 9).

$$E = (Vt) * (N) * (Ve/Va) * (100/pm) \quad (8)$$

where E is the amount of meq/100g of the soil of the determined element, Vt is the total volume in mL of EDTA expended in the titration, N is the EDTA normality, Ve is the volume of extractant added (mL), Va is the aliquot taken (mL), pm is the molar mass of the sample (g/mol), 100 is the factor to refer to 100 grams of biochar.

$$\frac{\text{Weight of the biochar obtained}}{\text{Initial weight of the rice husk}} * 100\% \quad (9)$$

### ***Crude oil characterization***

API degrees and crude oil temperature were determined with a thermohydrometer as described in the ASTM D-1298 and ASTM D-1250 standards [26, 27]. The Conradson carbon residue methodology described in ASTM D-189 and Equation 10 allowed the carbon content to be found [28].

$$\%coal = \frac{A}{W} * 100 \quad (10)$$

where A is the weight of the coal (g), and W is the weight of the sample (g).

The density of crude oil was determined using Equation 11, and to find the specific gravity, the API grade of crude oil corrected to 60°F was used (Equation 12). The density of water at 60°F was then calculated and replaced [26].

$$\rho_{crudeoil} = SG * \rho_{H_2O} \quad (11)$$

$$\rho_{API} = \frac{141,5}{SG} - 131,5 \quad (12)$$

where: SG is the specific gravity.

The ash percentage was found by relating the ash mass and the mass of the sample, as described in the ASTM D-482 standard (Equation 13) [29], and then the organic matter content was obtained by weight difference.

$$\%ash = \frac{w}{W} * 100\% \quad (13)$$

where: w is the weight of the ash (g) and W is the weight of the sample (g).

The viscosity was determined using a No. Y776 viscometer, with the flow times in seconds as a function of the rise of the crude through the second and third bulbs of the material at a temperature of 40°C (Equation 14). The kinematic viscosity constants C and D of the viscometer model, whose values are 1.112 and 0.790cSt/s, respectively, were multiplied by the times obtained [30].

$$\begin{aligned} V_c &= t_1 * C \\ V_D &= t_2 * D \\ V &= \frac{V_c + V_D}{2} \end{aligned} \quad (14)$$

where:  $V_C$  is the second bulb flow time (s),  $V_D$  is the third bulb flow time (s),  $t_1$  is the time to rise to the second bulb (s),  $t_2$  is the rise time to the third bulb (s), C is the second bulb constant, D is the third bulb constant, and V is the kinematic viscosity (cSt).

### **Bioremediation process**

In the bioremediation process, four synthetic samples of soils were worked on (Balao, Tachina, Refinery, and Winchele), in which soil contamination with crude oil was simulated. The contaminant, biochar, and moisture concentrations were randomly assumed based on the total mass of each of the samples.

A 10% contaminant was added to the Balao sample (B) according to the efficacy of the biostimulation process with BIOIL-FC in an ex-situ process in contaminated plots at the same concentration of fuel oil and 30% biochar [31]. The Tachina (T) sample with 30% contaminant and 50% biochar simulates a bioremediation process in the event of a spill of 10 to 30% hydrocarbons, which can cause damage in an area of 5 hectares [32]. The refinery (R) sample had 40% oil and 70% biochar, representing a plot of soil contaminated with extra-heavy crude, and looked to evaluate the degradative activity of microorganisms present in the soil [33]. In the Winchele (W) sample, 50% of the contaminant and 100% of biochar, considering the concentration of  $\pm 2746\text{mg TPH/kg}$  of soil established by *A. Martínez et al.* [34] their study on a laboratory scale for the soil of sandy type.

The samples were aerated daily, guaranteeing a humidity of 20, 30, 40 and 50%, respectively, since according to *M. Viñas* [35] and *M. Ayotamuno et al.* [36], humidity between 20 and 90% favors the biodegradation of the crude, giving more effective restoration percentages. The dosage of biochar had a concentration greater than 10% of biochar, which improves the properties of the soil [37].

Over a total period of 90 days, four measurements were recorded for each treatment [38]. Total petroleum hydrocarbon content (TPH) was determined by reading on an HP model 6890 series G1530A Gas Chromatograph. The results obtained in Table 1 allowed us to know the most favorable conditions to achieve the highest percentage of remediation in the soil samples contaminated with crude oil.

**Table 1.** TPH control during 90 days of bioremediation

Sample	Contamination level	Biochar content	Moisture	TPH analysis (days)			
				0	25	55	90
B	10%	30%	20%	X	X	X	X
T	30%	50%	30%	X	X	X	X
R	40%	70%	40%	X	X	X	X
W	50%	100%	50%	X	X	X	X

## Results and discussion

### *Characterization of rice husk*

Table 2 presents the results of the characterization of the rice husk before its treatment. The ash and organic matter content were 19.05 and 80.95%, close to the values found for a rice husk from the Republic of Korea, with 18.80% ash on a dry basis and 67.30% in volatile matter [39]. To achieve higher performance in gasification and pyrolysis, organic matter content must be between 75% and 90%, and the silica ash must be up to 97% [40]. On the other hand, to avoid incomplete ignition or unexpected products, it is recommended that the moisture content in rice husk be less than 10% [23]. In this sense, the raw material used in this investigation was above those suggested, probably due to the environmental conditions of the storage place. Previous research indicates that under the effects of drying by sunlight, it is possible to reach a humidity of 4.72% in rice husks [41].

**Table 2.** Characterization of rice husk

Variable	Units	Value
Ash	% w/w	19.05
Organic matter	% w/w	80.95
Moisture	% w/w	10.75

### *Biochar characterization*

Table 3 shows the values obtained for each of the variables evaluated in the biochar made from rice husks.

**Table 3.** Physicochemical parameters of biochar

Variable	Units	Value
pH	--	7.00
Initial yield	% w/w	37.42
Real density	kg/m <sup>3</sup>	1041.67
Ash	% w/w	28.56
Organic matter	% w/w	71.44
P	mg/L	3.77
Ca	ppm	0.00
Mg	ppm	0.00

The initial biochar yield was 37.42%, a value similar to that obtained in the study by L. Oré *et al.* [42], who made this material based on *Theobroma cacao L.* (cocoa) shell, *O. sativa* (rice) and *C. arabica* husk (coffee) with yields of 9.70, 44.40 and 46.05%, respectively. Likewise, the pH of the present investigation was 7, and the ash content was 28.56%. *F. Ronsse et al.* [43] indicate that the pH of biochar subjected to a pyrolysis process increases proportionally with its ash content.

The amount of organic matter was 71.44%, and according to *J. Velázquez et al.* [44], the organic matter of biochar based on rice husks and conditioned with citric, maleic, and propionic acid at 5 and 10% (w/w) as catalysts, ranges between 65 and 71%. This parameter varies depending on the type of material and its composition. Sugarcane biochar has 18.80 to 26.20% organic matter [45], based on Australian pines 73.50% [46], and made with olive pruning remains 60.90% [47].

The phosphorus content of the biochar obtained was 3.77mg/g compared to that produced at 600°C in the study by *B. Bushra and N. Remya* [48], which varied from 0.22mg/g to 1.80mg/g. The real density was 1041.67kg/m<sup>3</sup>, and the Ca and Mg values were 0.0 ppm, showing the absence of minerals. In the research of *M. Garzón* [49], biochar based on pine pieces with 0.17 and 0.06kg/m<sup>3</sup> of Ca and Mg demonstrated that this bioremediation has zero or low concentrations of these elements. It is worth mentioning that the higher the value of macro and micronutrients in biochar, the higher fertility it will provide when in contact with the soil [23].

**Characterization of soil samples without contamination**

The parameters evaluated in the uncontaminated soil samples are shown (Table 4). The soil sample R reported a cation exchange capacity of 120.77Meq/100g; on the contrary, the W soil was the lowest with 49.203Meq/100g. A. Graterón [44] comments that sandy soil with low concentrations of CEC cannot retain and provide nutrients, as evidenced by the texture and level of CEC presented by soil W, unlike B, T, and R. The soils studied are slightly acidic and neutral, with the presence of nutrients, unlike very acidic or basic soils, according to the classification presented in the work of C. Sánchez [51]. Unlike T and W, R and B soils have agricultural use but with fertilization limitations, according to what was reported by the GADMCE [52].

**Table 4.** Characterization in the uncontaminated soil samples

Variable	Units	Soil sample			
		B	T	R	W
CE	Meq/100 g	102,75	86,06	120,77	49,203
pH	--	6,9	6,5	6,7	7
T	°C	26,3	26	27	27
Moisture	%(v/v)	18,004	16,378	16,256	10,926
Organic carbon	mg/g	386,63	188,21	8,101	36,350
Ash	%(w/w)	7,25	8,55	8,83	8,31
Organic matter	%(w/w)	2,75	1,46	1,17	1,77
Apparent density	kg/m <sup>3</sup>	957,37	907,37	957,37	915,7
Real density	kg/m <sup>3</sup>	1.977,13	2.663,27	2.153,46	3.295,25
Porosity	%(v/v)	55,61	65,93	55,54	72,91
Texture	--	Silty	Silty – sandy	Silty	Sandy

Four soil samples had an approximate bulk density of 1000kg/m<sup>3</sup>, similar to the study by *A. Bravo et al.* [53], who determined that an aliquot of clayey soil has 1100 kg/m<sup>3</sup> of this density and sandy soil of 1260kg/m<sup>3</sup>. Contaminated and untreated soils generally have a similar apparent density.

### Crude oil characterization

Table 5 presents the results of the characterization of crude oil.

Table 5. Characterization of crude oil

Variable	Units	Value
°API	--	23,2
T	°C	23
Conradson carbon	% w/w	11,24
Density	g/mL	0,90
Ash	% w/w	0,52
Organic matter	% w/w	99,48
Viscosity	cSt	59,49

The API<sub>60/60°F</sub> of the oil used was 23.2, corresponding to a medium-type crude according to the classification given by *A. Izurieta and A. Iza* [54], similar to 24.6°API of crude oil extracted from the Esmeraldas State Refinery [55]. The °API is slightly lower due to a mix between the pipelines that connect with this refining entity. In this sense, the Trans-Ecuadorian Pipeline System (SOTE) and the Heavy Crude Oil Pipeline (OCP) transport crude with °API between 24.0 and 24.6 and 17.2 to 18.2, respectively. The crude used in this investigation meets the specifications required by the buyer, as specified by EP Petroecuador [56] in its 2018 report.

The Conradson Carbon content of crude oil was 11.24% higher than the 8.34 and 8.44% reported by *A. Torres* [55] and *R. Villalba* [57], respectively. The ash content was 0.52%, which differs from that reported by *A. Torres* [55], which is 0.048%, and that reported by *P. Calle* [58] for Pungarayacu heavy crude 0.254%. In this sense, the Conradson coal and ash parameters occur in higher percentages in the analysis sample since each oil has different physicochemical properties depending on its origin or treatment [59].

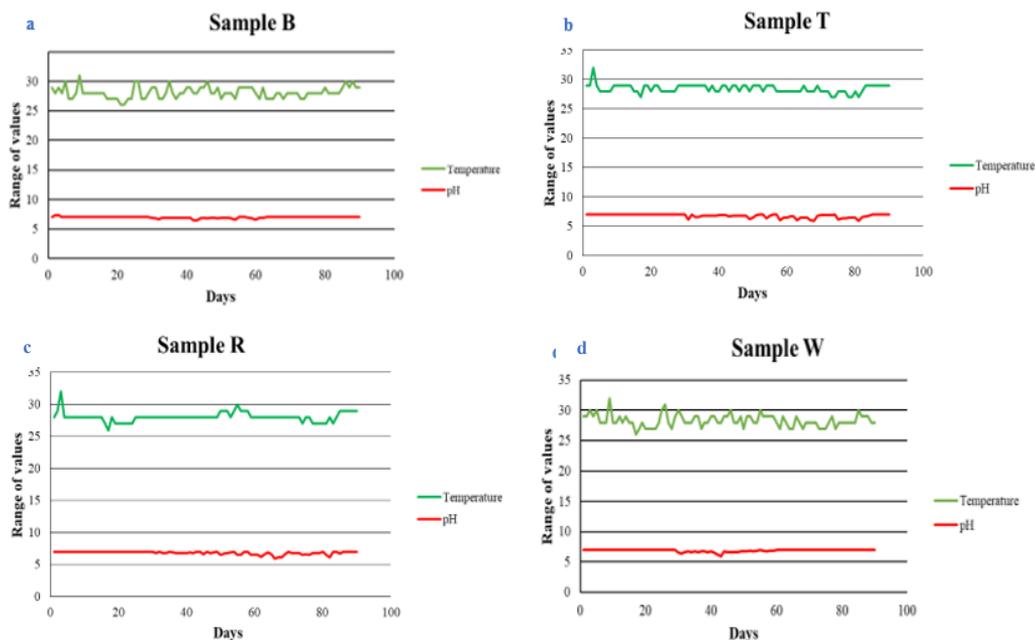
The density of the crude was 0.91g/mL. Crude oils with a density greater than 0.80g/mL and less than 0.93g/mL are paraffinic aromatic [60]. The viscosity of the oil crude at 23°C was 59.49cS; unlike a Venezuelan crude at 20°C, it presented a viscosity of 70cSt [43], concluding that the viscosity of the crude oil was close to the degree of temperature and the properties of the oil under study.

### Control of variables in the bioremediation process

#### Temperature and pH controls

Fig. 3 presents the results of the pH and temperature during the experimentation. The pH varied in all samples from 6.0 to 7.3, attributable to external factors such as humidity and minerals in the added water. A pH of 6.0 to 8.0, with an optimum of 7.0, is adequate for bioremediation soil because it extends bacterial life [61]. When the pH is not within this range, it is advisable to adjust it. In this research, the four samples stabilized their pH at neutrality.

These results are compared with the effect caused by the application of biochar in highly eroded soils, showing that a dose of 5% (w/w) benefits the physicochemical and biological properties with an incubation time of 105 days, achieving an improvement in soil pH from 3.9 to 5.1 [62]. Likewise, in the investigation by *J. Ordoñez* [63], doses of 5, 10 and 20% of acacia, urapan, and eucalyptus biochar were applied to soil samples contaminated with hydrocarbons for 30 and 60 days, achieving the reduction of TPHs and improving their structure and physicochemical properties. However, the bioremediation time was insufficient since it was only possible to reduce half of the initial concentration of TPH in each of the samples.



**Fig 3.** Temperature and pH control of samples during bioremediation process: (a) B, (b) T, (c) R, (d) W

For temperature, the result obtained most frequently for B and W was 28°C, for T 29°C, and for R 27°C. All samples oscillated between 26 and 31°C, characteristic temperatures of the Ecuadorian coastal region. The first 60 days of bioremediation coincided with the last months of the summer season in the country, for which the temperature was close to 31°C. The rest 30 days of the experimentation were carried out at the beginning of the winter season, registering temperatures close to 29°C. In both cases, the values obtained could be due to the climatic conditions of each season of the year.

An inadequate temperature impairs the bioremediation process. According to *J. Díaz* [64], temperature influences biodegradative processes by causing physicochemical changes in petroleum hydrocarbons, causing their metabolized as there is a directly proportional relationship between temperature and degradation. In addition, the biodegradation of hydrocarbons decreases at temperatures below 10°C and above 45°C. At lower temperatures, the viscosity of the oil increases, its volatilization decreases, and the solubility in the solution with the soil increases, causing a delay in the biodegradation potential [61, 65].

#### ***Control of Total Petroleum Hydrocarbons (TPH)***

Fig. 4 shows the results obtained during the control of the HPT from day 0 to day 90, resulting in a concentration for the first control (day 0) of 2691.75 mg/Kg for the B sample, T of 2505.99mg/Kg, R of 2950.99mg/Kg, and W of 2708.24mg/Kg, corresponding to 10, 30, 40 and 50% of contamination with crude oil, respectively. The TPH obtained does not have a direct relationship when compared to the concentration of the added contaminant, which may be due to the immediate degradative activity of the microorganisms present in the soil samples. *J. Díaz* [64] mentions that aerobic degradation is a fast and complete process in the metabolization of hydrocarbons, this being one of the mechanisms used by bacteria in the process of biodegradation

of oil contaminants, those that act by oxidizing oil and converting it into carbon dioxide (CO<sub>2</sub>), water (H<sub>2</sub>O) and energy.

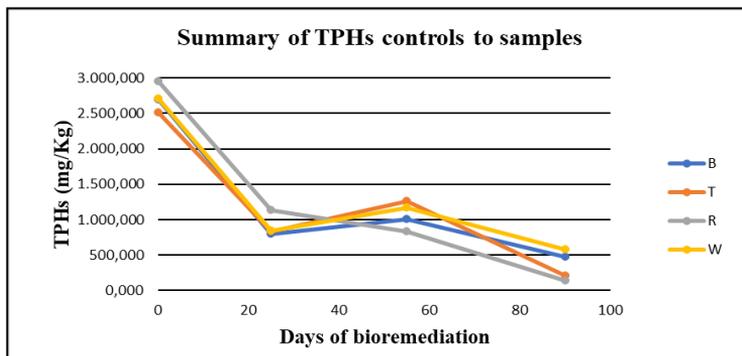


Fig. 4. TPH control in the bioremediation process

After 25 days, more than half of the TPH concentration decreased in each sample. At 55 days, an increase in TPH was observed for samples B, T, and W. This behavior can be related to the ease of volatilization of hydrocarbon-polluting compounds [66]. On the other hand, the % humidity in the samples can contribute to an increase or decrease in inorganic matter or minerals.

After 90 days of the bioremediation process, the levels of TPH for the R sample reached a degradation of 95.40%, in the T of 91.63%, in the B of 82.33%, and in the W of 78.66%. With the influence of the bioremediation element (biochar) and the control of factors such as humidity and removal, the contaminated soil samples could reduce their hydrocarbon content, complying with Annex 2 of Book VI of the Unified Text of Secondary Legislation of the Ministry of the Environment [67]: Environmental Quality Standard for Soil Resources and Remediation Criteria for Contaminated Soils, which establishes a permissible limit less than or equal to 620mg/Kg of contaminant to be considered a bioremediation soil.

Other variables that influenced the results obtained in the bioremediation process were the cation exchange capacity (CEC) of the soil, with R being 120.77Meq/100g, higher than the other samples and with a porosity of 55.54%. In the study by *S. Jien and C. Wang* [62], with a biochar dosage of 5% (w/w) in eroded soil, the CEC increased from 7.41 to 10.8Meq/100g, which is attributable to the total organic carbon of the biochar and its porous structure. Similarly, the porosity of biochar favors the soil by having free radicals that immobilize contaminants and, in turn, function as a binding agent by connecting soil microaggregates to form macroaggregates [62, 68].

Soil texture was a relevant variable in this process, presenting specific characteristics concerning pore size. As mentioned by *A. Acuña et al.* [69], the clay particles are larger than those of silt, and these are larger than those of sand. The more the thickness of the pores increases, the better the development of microorganisms. Therefore, the silty texture favors bioremediation. *E. Ramirez et al.* [70] state that silt particles are of greater importance for microbial taxa than sand particles. This fact is confirmed in the present investigation since the R, T, and B samples presented silty properties and were the soils with the highest percentage of bioremediation, unlike the W sample, which was sandy.

*B. Toledo* [71] affirms that some bacteria and fungi predominate in soils supporting external conditions. These represent approximately 104 or 106 cells per gram of soil. Bacteria

of the *Pseudomonas* class (*Aeruginosa*, *Cepacia*, *Putida*, and *Stutzeri*) are in contaminated soils and those undergoing remediation processes. In the case of *Pseudomonas Putida* and *Methanobacteria*, they have the beneficial property to degrade toluene, which shows that in the four samples, there was also a degrading action of the TPH by the microorganisms present.

Similarly, the daily removal of the samples favored the bioremediation process. According to *J. Olguín et al.* [72], soil aeration and the addition of nutrients in the soil favor the growth of microorganisms that degrade alkanes and, to a lesser extent, the population of microorganisms that degrade aromatic hydrocarbons.

In the contaminant degradation process, moisture control is of great importance. According to *G. Silva et al.* [66], with moisture between 15 and 25%, 78 to 100% of the n-alkanes and branched compounds are eliminated. Similarly, the degradation of branched and aromatic hydrocarbons is better with 30% moisture, reaching a removal rate of 64 and 74% of the contaminant. Therefore, when contaminating the samples with oil that had a Koup of 11.55, naphthenes or slightly substituted aromatics predominated in it, which possibly led to the aromatic hydrocarbons being degraded more in the Tachina sample because it was the one that remained in biotreatment at 30 % moisture. According to *G. Silva et al.* [73], the soil to be remedied must have a minimum of 40% and a maximum of 80% moisture. In this case, the R and W samples were at a humidity level of 40 and 50%, being within the recommended range.

The bioremediation process was possible by making biochar with easily acquired agro-industrial residues such as rice husks. In the same way, it was effective, obtaining degradation of TPH up to 95.40%, results similar to those achieved using other bioremediators. For example, in the research by *C. Simbaña* [66], it was possible to remove 92.11% of TPH using the fungus *Pleurotus ostreatus* as a bioremediator and leaving it to act for six weeks, or the research by *A. Martínez et al* [74], in which up to 93% of hydrocarbons were biodegraded using residual sludge for 50 days. The disadvantage of this process is the treatment time, which could decrease by complementing with other remedial factors, such as the use of earthworms or degradative bacteria characteristic of the soil, in such a way that they adapt to the environment in optimal living conditions.

## Conclusions

The bioremediation process using biochar based on rice husks allowed reaching a level of biodegradation of the TPH for the R sample of 95.40%, in T of 91.63%, in B of 82.33%, and in the W of 78.66%. The biochar dosages helped to decontaminate the samples up to levels of petroleum hydrocarbons below 620 mg/Kg, complying with the local environmental legislation established for this purpose. The biotreatment studied in this research could be applied in soils contaminated with crude oil or its derivatives to restore properties for agricultural, industrial, or residential use. However, in future research, it is suggested to carry out microbiological analysis of the soil to verify the presence of microorganisms that may contribute, together with biochar dosages, to the decrease in TPH concentrations.

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