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# Contamination by petroleum products on plant growth and soil microbiological indicators

# Impacto da contaminação por derivados de petróleo no crescimento vegetal e indicadores microbiológicos do solo

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ABSTRACT - Oil-derived compounds present in the soil can compromise plant growth and inhibit the development of the microbial population. These contaminants reduce soil porosity, hinder water absorption, and can disrupt plant metabolic processes. The aim of this research was to evaluate the effects of the presence of three hydrocarbons in the soil on the growth and development of forage species and on soil microbiological indicators. The experiment was carried out in a greenhouse using a randomized block design with four replications. Treatments were arranged in a 5×4 factorial, with the first factor consisting of the species evaluated (Pennisetum glaucum, Zea mays, Brachiaria ruziziensis, Panicum maximum and Sorghum bicolor) and the second factor corresponding to the presence of contaminants: benzene, toluene or xylene and soil without contaminants. The species were grown for 42 days. The tolerance of the species depended on the type of contaminant present in the soil. The total dry matter of P. glaucum was reduced by 26%, 10% and 32% for toluene, benzene and xylene, respectively. Z. mays had its growth reduced in the presence of toluene (13%) and benzene (21%). S. bicolor had its dry matter increased by 58% with xylene. B. ruziziensis and P. maximum were tolerant to all contaminants. The magnitude of the effects of contaminants on soil microbiological indicators depended on the species grown. The tolerance of plants and soil microbial community to contaminants depends on the forage species used and should be an important aspect when selecting plants to restore degraded areas.

**Keywords**: Petroleum hydrocarbons. Environmental pollution. Species for remediation.

RESUMO - Compostos derivados de petróleo presentes no solo podem comprometer o crescimento das plantas e inibir o desenvolvimento da população microbiana. Esses contaminantes diminuem a porosidade do solo, dificultam a absorção de água e podem interromper processos metabólicos das plantas. Assim, nesta pesquisa buscou-se avaliar os efeitos da presença no solo de três hidrocarbonetos no solo sobre o crescimento e desenvolvimento de espécies forrageiras e nos indicadores microbiológicos do solo. O experimento foi realizado em casa de vegetação no delineamento experimental de blocos casualizados, com quatro repetições. Os tratamentos foram dispostos em fatorial de 5×4, sendo o primeiro fator constituído pelas espécies avaliadas (Pennisetum glaucum, Zea mays, Brachiaria ruziziensis, Panicum maximum e Sorghum bicolor), e o segundo fator correspondendo a presença dos contaminantes: benzeno, tolueno ou xileno e solo sem contaminante. As espécies foram cultivadas por 42 dias. A tolerância das espécies dependeu do tipo de contaminante presente no solo. A massa seca total da P. glaucum foi reduzida em 26%, 10% e 32% para o tolueno, benzeno e xileno, respectivamente. Z. mays teve o crescimento reduzido na presença do tolueno (13%) e do benzeno (21%). S. bicolor teve sua matéria seca aumentada em 58% com o xileno. B. ruziziensis e P. maximum foram tolerantes para todos os contaminantes. A magnitude dos efeitos dos contaminantes nos indicadores microbiológicos do solo dependeu da espécie cultivada. A tolerância das plantas e da comunidade microbiana do solo aos contaminantes depende da espécie forrageira utilizada e deve ser aspecto importante para a seleção de plantas para recuperação de áreas degradadas.

**Palavras-chave**: Hidrocarbonetos de petróleo. Contaminação ambiental. Espécies para remediação.

**Conflict of interest:** The authors declare no conflict of interest related to the publication of this manuscript.



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## INTRODUCTION

Petroleum products are currently used for the production of energy, fuel, cosmetics, pigments, among other products, and their production is considered an important global economic activity. However, the increase in industrialization and production in the sector has caused a series of damage to the environment and to human beings (KOVALEVA et al., 2022). Among petroleum derivatives, benzene, toluene, and xylene (BTX) are the main representatives of the group of volatile organic compounds (VOCs) and are often found in water, air, and soil, exposing humans to a high risk of ingestion, inhalation, or absorption through the skin (YU et al., 2022).

The presence of these compounds in the soil can result in changes in physical, chemical, and biological characteristics, in addition to influencing the health status of plants (SHORES; HETHCOCK; LAITURI, 2018). In Brazil, according to CONAMA (2009) Resolution 420, levels of contamination in

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residential and industrial environments agricultural, are allowed. For benzene, toluene and xylene, the maximum level of contamination allowed in the soil is 0.03, 0.14 and 0.13 mg kg<sup>-1</sup>, respectively (CONAMA, 2009). In a study carried out in the state of Rio Grande do Norte, Brazil, contamination levels were reported in places close to gas stations, with concentrations of VOCs between 10.68 and 1368.44 mg kg<sup>-1</sup> (RAMALHO et al., 2014). In industrial environments and areas under livestock farming activities, the presence of these compounds in the soil was also found above the values allowed by the resolution, about 1735 mg kg<sup>-1</sup> (BELLO, 2020). These results reveal the contaminating potential of these compounds and the need for technologies to reduce their concentrations.

One of the alternatives for the restoration of contaminated areas is the use of plants capable of extracting and/or reducing petroleum products from the soil, in addition to stimulating degradation by microorganisms. Forage species, such as sorghum, rye, and wheat, have already shown potential for decontamination of areas with BTX, especially for having rapid growth, high biomass production, and great capacity to explore the soil and to absorb and accumulate contaminants in their structures, contributing to remediation (SHORES; HETHCOCK; LAITURI, 2018). This remedial capacity may also be related to interactions with soil microorganisms. Bacteria of the genera *Pseudomonas*, *Arthrobacter* and *Acinetobacter* showed the capacity to decompose almost 65% of aromatic compounds in oil-degraded soil (DINDAR; ŞAĞBAN; BAŞKAYA, 2013).

Areas with presence of BTX can be managed with species tolerant to contaminants and adapted to the cultivation site. Thus, studies to identify plant species with the capacity to tolerate different contaminants, as well as increasing the activity of soil microbiota, are essential to develop the technique to mitigate the effects of BTX in onshore production areas, land with deactivated oil wells, highways and surroundings of gas stations. Therefore, this study aimed to select and investigate potential forage plants capable of mitigating the environmental impacts caused by benzene, toluene and xylene, as well as evaluating the effects of these substances on soil microbiological indicators.

#### MATERIAL AND METHODS

#### **Experimental site and conditions**

The experiment was carried out in a greenhouse at the Federal Rural University of the Semi-Arid Region (UFERSA), located in the Northeast region in the municipality of Mossoró, RN, Brazil, between November 2022 and January 2023. The climate of the site is classified as very hot semi-arid, BSwh' in Köppen's classification, and the average temperature of the region during the experiment was 28 °C (ALVARES et al., 2013). The characteristics of the soil used were determined by the Soil Fertility and Plant Nutrition Laboratory of UFERSA (Table 1).

Table 1. Chemical and particle-size analysis of the soil used in the experiment.

Soil	N*	pН	EC	OM	P	$K^{+}$	Na <sup>+</sup>	Ca <sup>+2</sup>	$Mg^{+2}$	Al <sup>+3</sup>	H+Al	SB
	(kg)	$(H_2O)$	(S)	(kg)		(mol m <sup>-3</sup> )				mol m <sup>-3</sup>		
	0.32	7.8	0.02	2.9	14.2	1.96	0.54	16.5	4.50	0	0	23.51
A1	T	CEC	V	m	ESP	Fine S.	Coarse S.	Total S.	Silt	Clay		
		mol m³		%	Particle-size Fractions (kg)							
	23.51	23.51	100	0	2.33	0.25	0.63	0.88	0.08	0.03		

\*Nitrogen (N), Hydrogen potential (pH), Electrical conductivity (EC), Organic matter (OM), Phosphorus (P), Potassium ( $K^+$ ), Sodium (Na $^+$ ), Calcium (Ca $^{+2}$ ), Magnesium (Mg $^{+2}$ ), Aluminum (Al $^{+3}$ ), Potential acidity (H+Al), Sum of bases (SB), Effective cation exchange capacity (t), Potential cation exchange capacity (CEC), Base saturation (V), Aluminum saturation (m), Fine sand (Fine S.), Coarse sand (Coarse S.), Total sand (Total S.).

The experimental design was randomized blocks, with four replications. Treatments were arranged in a 5×4 factorial, and the first factor consisted of the species evaluated: pearl millet (*Pennisetum glaucum*), maize (*Zea mays*), Congo grass (*Brachiaria ruziziensis*), Massai grass (*Panicum maximum*) and sorghum (*Sorghum bicolor*) cv. Ponta Negra. These plants were chosen due to their high biomass production, adaptation to marginal locations and easy climatic adaptation. The second factor was related to the presence of contaminants: benzene, toluene or xylene and no contaminant.

The substrate containing soil and contaminant was prepared by placing 1 kilogram of soil, containing 30% cattle manure, in a plastic bag and enriching it with the contaminant solution (400 ppm), according to the treatment. Subsequently, the plastic bags were closed and left to rest for 72 hours for the reaction to reach equilibrium. The plastic bags were

placed in 0.0015 m<sup>3</sup> pots and arranged in a greenhouse for planting the species.

Eight seeds of each species were sown and, 5 days after emergence, thinning was carried out, leaving 4 plants per pot. The plants were irrigated daily, so as to maintain field capacity. Two fertilizations with NPK (10 - 50 - 00) and potassium chloride were carried out during the experimental period, as recommended for the species (EMBRAPA, 2008).

### Plant growth analysis

The experiment lasted 42 days after sowing. At the time of harvest, roots were separated from the shoots, and leaf area and root area were determined using Image J software (National Institute of Health, NY, USA) from images of the plants. Subsequently, the plant material was washed in

running water and dried in a forced air circulation oven at 70 °C until reaching constant weight for dry matter determination.

#### Analysis of sugar, chlorophyll and carotenoid contents

Total soluble sugars were quantified using 1 g of plant material, which was macerated and mixed in 10 mL of distilled water (DANTAS et al., 2005). The solution was then filtered and the content was determined by the anthrone method, proposed by Trevelyan and Harrison (1952).

For the determination of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, 1 g of plant material was weighed, macerated, transferred to tubes with 80% acetone solution and stored in an environment with no light until total discoloration. The liquid fraction was filtered and a 1-mL aliquot was collected for reading. Measurements were performed in a spectrophotometer, at wavelengths of 645, 652 and 663 nm for chlorophylls and 470 nm for carotenoids (SCOPEL; BARBOSA; VIEIRA, 2011). Contents of chlorophyll a, chlorophyll b, total chlorophyll and the estimated carotenoids were calculated according to Lichtenthaler and Wellburn (1983).

#### Microbiological analyses

The active soil microbiota of each experimental unit was determined using the technique of counting bacteria by the surface sowing method proposed by Pimentel (2016). At the end of the experiment, 1 g of soil free of roots and plant remains was put into test tubes with 9 mL of distilled and autoclaved water. This solution was homogenized on a vortex shaker, in a serial dilution of: 1:10, 1:100 and 1:1000. Then, 0.1 mL of this solution was pipetted into Petri dishes containing Potato Dextrose Agar (PDA) culture medium for fungal growth and bacteriological agar for the growth of total

and sporulating bacteria. The test tubes were placed in a water bath at 80 °C for 15 minutes, and then a 0.1-mL aliquot was collected and placed in a Petri dish with culture medium for the growth of sporulating bacteria. PDA was used for fungi and yeasts, and bacteriological agar was used for total bacteria growth. The Petri dishes were taken to a growth greenhouse at  $\pm\,28$  °C. Bacterial colony-forming units were counted after 24 hours, and fungal colony-forming units were counted after 96 hours.

Quantification was determined by colony-forming units per gram of soil (CFU g soil<sup>-1</sup> mean of the counts  $\times$  selected dilution  $\times$  10) (PIMENTEL, 2016).

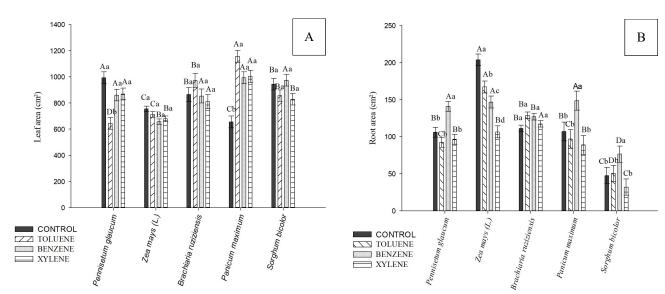
#### Statistical analyses

The results were analyzed using RStudio software (version 2023.06.1+524) and subjected to normality analysis using the Shapiro-Wilk test (SHAPIRO; WILK, 1965) and analysis of variance (ANOVA). In case of significance, Tukey's multiple comparison test was applied (p  $\leq$  0.05). The graphs were created with SigmaPlot 12.0 software.

#### RESULTS AND DISCUSSION

#### **Growth analysis**

The leaf area of *Pennisetum glaucum* was reduced by 35.18% in the presence of toluene in the soil (Figure 1A). On the other hand, the species *Zea mays*, *Sorghum bicolor* and *Brachiaria ruziziensis* were not affected by the contaminants. The leaf area of *Panicum maximum*, however, increased in soil contaminated with toluene, benzene and xylene by 43.34%, 33.94% and 34.73%, respectively, compared to the control.



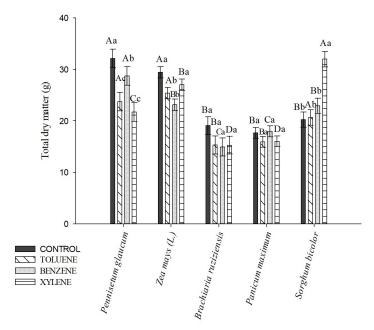
\*Different uppercase letters indicate significant differences between different species, while lowercase letters indicate significant differences between contaminants ( $p \le 0.05$ ). All data are presented as mean  $\pm$  standard deviation (n=4)

Figure 1. Leaf area (A) and root area (B) of forage species subjected to soil contamination with toluene, benzene and xylene.

Leaf area is an important variable related to plant growth, as it is directly associated with the photosynthetically active area. In this study, BTX showed little capacity to reduce the leaf area of plants, with a negative result only for *P. glaucum* in the presence of toluene. These results corroborate those found by Nguemté et al. (2018) in their study with grasses. The authors attribute this result to the capacity of these plants to tolerate and grow in contaminated soils, maintaining their leaf area even in the presence of toxic compounds.

The root areas of *Z. mays*, *P. maximum* and *S. bicolor* were reduced by 17.91%, 9.47% and 6%, respectively, in the presence of toluene (Figure 1B). On the other hand, benzene increased the root areas of *P. glaucum*, *P. maximum* and *S. bicolor* by 24.82%, 28.06% and 37.94%, respectively, while reducing the root area of *Z. mays* by 27.91%, compared to the control.

The results found for the root area of the studied species in the presence of BTX showed that the capacity to negatively affect plant roots depends on the pollutant and, in some cases, can cause an increase in root area. These results are in agreement with those found by Shores, Hethcock and Laituri (2018), who stated that the grasses tested (perennial ryegrass and foxtail barley) did not have their leaf area altered by the presence of BTX, probably due to the protection capacity that they have in their roots. The total dry matter of P. glaucum was reduced by 26.11%, 10.61% and 32.34% with toluene, benzene and xylene, respectively (Figure 2). Z. mays had its dry matter reduced in the presence of toluene (13.76%) and benzene (21.44%). S. bicolor had its dry matter increased by 58.05% with xylene, possibly due to the use of organic compounds as a carbon source (GLICK, 2010). B. ruziziensis and P. maximum did not differentiate from the presence to the absence of contaminants.



\*Different uppercase letters indicate significant differences between different species, while lowercase letters indicate significant differences between contaminants ( $p \le 0.05$ ). All data are presented as mean  $\pm$  standard deviation (n=4)

Figure 2. Total dry matter of forage species subjected to soil contamination with toluene, benzene and xylene.

Previous studies have reported that biomass production is directly influenced by the toxic effect caused by petroleum products, and crops such as wheat and peas showed reduction in dry biomass in an experiment carried out in pots (KOVALEVA et al., 2022). The species *B. ruziziensis* and *P. maximum* showed high biomass production in the presence of pollutants, a desirable characteristic for plants used in remediation. Dry matter production is an important parameter to evaluate the tolerance of species for decontamination of environments polluted by petroleum products (BARROSO et al., 2021).

# Analysis of total soluble sugars, chlorophylls and carotenoids

Analyzing chlorophyll contents is important to understand the effects of VOCs on photosynthesis and the

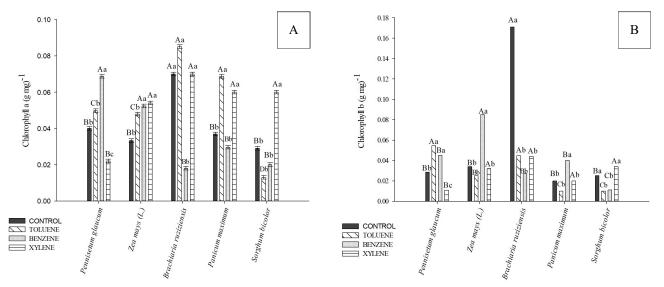
physiological state of plants, providing information about their response to adverse environmental conditions. According to the results, *B. ruziziensis* and *P. maximum* had chlorophyll *a* contents increased by 17.74% and 46.28%, respectively, in soil with toluene (Figure 3). Benzene negatively affected the chlorophyll *a* contents of *B. ruziziensis* (74.14%), *P. maximum* (25.25%) and *S. bicolor* (31.03%). Xylene reduced the chlorophyll *a* content of *P. glaucum* by 50%

Chlorophyll b contents were lower in the species Z. mays (26.47%), P. maximum (50%) and S. bicolor (60.15%) with toluene. For Z. mays and P. maximum, benzene increased chlorophyll b contents by 59.95% and 50%, respectively. Xylene affected the chlorophyll b content of P. glaucum (51%) and stimulated production in S. bicolor (26%); for the other species, there were no differences with this pollutant. The increase in chlorophyll b content in plants exposed to



BTX can be attributed to the adaptive response of plants to oxidative stress caused by the chemical compounds. When plants are exposed to BTX, especially at moderate concentrations, a defense response may occur that includes increased production of chlorophyll b. This chlorophyll aids

in the absorption of light under stress conditions, protecting plants from oxidative damage and improving their photosynthetic capacity under adverse conditions (HUANG et al., 2004).

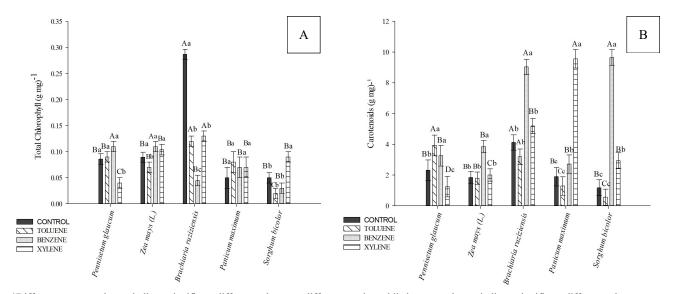


<sup>\*</sup>Different uppercase letters indicate significant differences between different species, while lowercase letters indicate significant differences between contaminants ( $p \le 0.05$ ). All data are presented as mean  $\pm$  standard deviation (n=4)

Figure 3. Chlorophyll a (A) and b (B) contents of forage species subjected to soil contamination with toluene, benzene and xylene.

Analyzing the total chlorophyll content is essential to evaluate the photosynthetic capacity and health status of plants under different environmental conditions. The results showed that the total chlorophyll content was reduced in the species *B. ruziziensis* under all contaminants (Figure 4A). In the species *P. glaucum*, total chlorophyll content decreased in soil with xylene, while the opposite occurred in *S. bicolor*. The reduction in total chlorophyll can negatively impact the

photosynthetic capacity of plants, resulting in lower biomass production and reduced growth. This is because chlorophyll is essential for light absorption and the conversion of light energy into chemical energy during photosynthesis. Plants with reduced chlorophyll contents are less efficient in capturing light and producing carbohydrates, which can compromise their development and yield (SHARMA et al., 2020).



\*Different uppercase letters indicate significant differences between different species, while lowercase letters indicate significant differences between contaminants ( $p \le 0.05$ ). All data are presented as mean  $\pm$  standard deviation (n=4)

Figure 4. Total chlorophyll (A) and carotenoid (B) contents of forage species subjected to soil contamination with toluene, benzene and xylene.

Carotenoid contents in the species varied with the soil contaminant (Figure 4B). It increased by 87.75% in *S. bicolor* with benzene in the soil, and xylene stimulated the carotenoid content of *P. maximum* by 80.12%. Among the species, *P. glaucum* showed more sensitivity to xylene and more tolerance to toluene, whereas *S. bicolor* and *P. maximum* were more sensitive to toluene. The other species did not show severe reductions, which may be an indication of their tolerance and sensitivity to these organic compounds (KOVALEVA et al., 2022).

The increase of more than 80% of carotenoids in *S. bicolor* and *B. ruziziensis* in the presence of benzene and *P. glaucum* with toluene is justified by the defense role of

carotenoids, which can be increased under conditions of oxidative stress as well as under conditions of abiotic stress, such as environmental stress by organic pollutants.

The effects on the amount of total soluble sugars (TSS) of the species in contaminated environments were also analyzed (Table 2). TSS contents in *P. glaucum* and *S. bicolor* were reduced in the presence of the three BTX compounds. The species *B. ruziziensis* had its TSS content reduced in the presence of toluene and benzene; however, in the presence of xylene, its TSS content increased by 40.72%. *Z. mays* had no statistically significant difference, while *B. ruziziensis* and *P. maximum* showed a higher amount of sugars in the presence of xylene, compared to the control.

**Table 2**. Amount of total soluble sugars (g) in forage species grown in soil with toluene, benzene and xylene.

Treatment	Control	Toluene	Benzene	Xylene
Pennisetum glaucum	7.8Ba	0.80Ab	0.72Ab	0.65Db
Zea mays	0.51Ca	0.88Aa	0.57Aa	1.00Da
Brachiaria ruziziensis	7.48Bb	1.28Ac	1.44Ac	12.62Aa
Panicum maximum	1.01Cb	0.88Ab	1.01Ab	7.14Ca
Sorghum bicolor	12.8Aa	0.83Ac	1.38Ac	9.69Bb

Uppercase letters differ in the column and lowercase letters differ in the row, Coefficient of variation (%): 16.94%.

Sugars play a crucial role as signalers in the face of environmental stresses and in plant osmotic regulation (HEIDARI et al., 2018). Organic pollutants, due to their hydrophobicity, reduce the capacity of water and minerals to enter through the roots. This can increase the soluble sugar content in plant leaves, a response to the lack of absorption by the roots. Such adaptation is essential to maintain the plant's homeostasis and metabolic functionality under adverse conditions, such as the presence of BTX compounds.

### **Microbiological Indicators**

In the quantification of total bacteria (Table 3), the sensitivity and tolerance to each contaminant used in the study

were evaluated. Toluene stimulated the growth of bacterial colonies in *S. bicolor* and *P. glaucum*, compared to the pot without a plant. Benzene significantly affected the growth of colonies in *P. glaucum* and *B. ruziziensis*. Xylene significantly increased colonies in *B. ruziziensis*; for the other species, except *Z. mays*, there was a reduction in colony reproduction.

Previous studies have identified bacteria present in the soil that degrade petroleum hydrocarbons and can also be stimulated by the presence of the pollutant (CHAUDHARY, 2016). Carbon can influence the growth and alter the C:N ratio, so these bacteria can be seen as biosensors to detect the level of toxicity in the environment (THACHARODI; JEGANATHAN; THACHARODI, 2019).

Table 3. Quantification of colony-forming units (CFU) of total bacteria (x10<sup>5</sup> CFU g<sup>-1</sup> soil) in soil contaminated by toluene, benzene and xylene.

Treatment	Control	Toluene	Benzene	Xylene
Not Planted	0.58Bc	0.72Cc	5.45Ab	7.35Ba
Pennisetum glaucum	0.56Bc	6.73Ba	0.59Bc	5.51Cb
Zea mays	3.69Ab	0.50Cc	4.71Ab	7.50Ba
Brachiaria ruziziensis	0.94Bb	0.45Cb	0.67Bb	8.98Aa
Panicum maximum	3.69Ab	0.66Cc	4.95Aa	5.60Ca
Sorghum bicolor	0.72Bd	10.3Aa	5.30Ab	3.95Dc

Uppercase letters differ in the column while lowercase letters differ in the row, Coefficient of variation (%): 17.09%.

The CFU count of sporulating bacteria in soil cultivated with *P. glaucum* and contaminated with xylene was higher than that observed in soil without contaminants (Table 4). The same pattern was found in the soil cultivated with *S.* 

bicolor in the presence of benzene. This increase in sporulating bacteria counts can be attributed to the ability of these bacteria to use volatile organic compounds, such as benzene and xylene, as carbon and energy sources, which

promotes their growth in contaminated environments (SILVA et al., 2022). In addition, recent studies show that the presence of certain plants can positively influence microbial activity in the soil, increasing the degradation of contaminants and improving the health of the ecosystem (FERREIRA; SOUZA, 2023).

On the other hand, the presence of contaminants

reduced bacterial populations in soil cultivated with *Z. mays*, *B. ruziziensis* and *P. maximum*. This reduction can be explained by the sensitivity of certain bacteria to the toxic compounds present in these contaminants, which can inhibit their growth or even cause the death of these bacterial cells (GOMES et al., 2022).

Table 4. Quantification of CFUs of sporulating bacteria (x10<sup>5</sup>, CFU g<sup>-1</sup> soil) in soil contaminated by toluene, benzene and xylene.

Treatment	Control	Toluene	Benzene	Xylene
Not Planted	0.85Cb	4.86Aa	6.36Aa	0.84Cb
Pennisetum glaucum	3.32Bb	3.77Bb	3.65Bb	6.72Aa
Zea mays	5.44Aa	0.34Cc	2.99Bb	0.63Cc
Brachiaria ruziziensis	5.20Aa	0.53Cc	3.42Bb	3.43Bb
Panicum maximum	6.00Aa	3.43Bc	2.98Bc	5.45Ab
Sorghum bicolor	0.35Cc	0.52Cc	6.36Aa	3.75Bb

Uppercase letters differ in the column, while lowercase letters differ in the row, Coefficient of variation (%): 23.51%.

Plants influence the remediation of environmental conditions, establishing favorable conditions for soil microbial activity in the degradation of pollutants. Association between microorganisms and plants to increase the degradation of petroleum hydrocarbons has been suggested and studied. Such association can increase the efficiency of bioremediation by combining the absorption and transformation mechanisms of plants with the degradative capacities of microorganisms, creating a synergistic

environment for removing contaminants from the soil (RODRIGUES; SILVA, 2021).

Toluene and xylene reduced the number of total fungi in uncultivated soils (Table 5). In soil with *Z. mays*, all contaminants reduced the number of fungi, while xylene increased the amount of fungi in soil with *P. maximum*. No change in the number of fungi was found for the other cultivated species.

**Table 5**. Number of total fungi (x10<sup>-3</sup>, CFU g<sup>-1</sup> soil) in soil contaminated by toluene, benzene, and xylene.

Treatment	Control	Toluene	Benzene	Xylene
Not Planted	78.62Aa	5.0Ac	80.00Aa	8.33Ab
Pennisetum glaucum	3.20Ba	5.40Aa	2.70Ca	3.21Ba
Zea mays	5.70Ba	0.62Cb	2.92Bb	0.77Cb
Brachiaria ruziziensis	3.06Ba	3.05Ba	2.79Ca	2.75Ca
Panicum maximum	3.06Bb	0.38Cb	3.15Bb	5.41Ba
Sorghum bicolor	5.96Ba	3.26Ba	4.70Ba	3.82Ba

Uppercase letters differ in the row while lowercase letters differ in the column, Coefficient of variation (%): 17.14%.

These results suggest that the presence of certain contaminants may have distinct effects depending on the type of soil and on the plant grown. In uncultivated soils, toxic compounds such as toluene and xylene can inhibit fungal growth, possibly due to the lack of plant exudates that could mitigate the effects of contaminants (SILVA et al., 2022). In contrast, the increase in the number of fungi in soil with *P. maximum* in the presence of xylene indicates that this plant can release specific exudates that stimulate fungal growth, even under adverse conditions (JOHNSON; LEE, 2023).

Forage species are cited in the literature as promising for remediation of hydrocarbon-contaminated soil along with the stimulation of microorganisms that are capable of degrading these compounds, using them as an energy source (MURATOVA et al., 2012). The degradation of VOCs in soil

is little known, and evaluating the dynamics of the microorganisms present is essential to understand and optimize the remediation of contaminated soils. Understanding the structure of the microbial community is necessary for further advances (LIAO et al., 2016).

The results reinforce the hypothesis that there is not just one type of interaction between pollutant, plant and soil. Even with plants under similar development conditions, it is necessary to evaluate the size of the contamination area, the soil retention capacity and the type of pollutant to be analyzed. The continuity of the study is essential for the identification, at the genus and species level, of the microorganisms present in the polluted soil, in addition to adjusting the environmental conditions for the degradation of monoaromatic compounds.



#### **CONCLUSION**

Different forage species showed varied responses to the Volatile Organic Compounds (VOCs) studied: *S. bicolor* was more sensitive to toluene, *P. maximum* and *B. ruziziensis* to benzene, and *P. glaucum* to xylene.

Z. mays proved to be the most tolerant species to toluene and benzene, while B. ruziziensis and P. maximum showed greater resistance to xylene.

These species stand out as potential candidates for the management of VOCs in soils aiming at environmental restoration.

#### REFERENCES

- ALVARES, C. A. et al. Koopen's climate classification map for Brazil. **Meteorologische Zeitschrift**, 22: 711-778, 2013.
- BARROSO, G. M. et al. *Mabea fistulifera* and *Zeyheria tuberculosa* can be indicated for phytoremediation programs of soils contaminated with hormonal herbicides. **International Journal of Phytoremediation**, 24: 1-8, 2021.
- BELLO, P. P. G. Environmental Investigation of the old Chemical Industry. **Holos Environment**, 20: 559-583, 2020.
- CHAUDHARY, D. K. Molecular tools in petroleum hydrocarbon degradation: an overview. **BAOJ Biotech**, 2: 1-18, 2016.
- CONAMA Conselho Nacional do Meio Ambiente. **Resolução nº 420, de 12 de fevereiro de 2009**. 2009. Available at: <a href="https://conama.mma.gov.br/images/conteudo/LivroConama.pdf">https://conama.mma.gov.br/images/conteudo/LivroConama.pdf</a>. Access on: Jan. 25, 2024.
- DANTAS, B. F. et al. Foliar Carbohydrates Content and invertase Activity in Vines at São Francisco River Valley-Brazil. **Revista Brasileira de Fruticultura**, 27: 198-202, 2005.
- DINDAR E.; ŞAĞBAN F. O. T.; BAŞKAYA H. S. Bioremediation of petroleum contaminated soil. **Journal of Biological and Environmental Sciences**, 7: 19-39, 2013.
- EMBRAPA Empresa Brasileira de Pesquisa Agropecuária. **Recomendações de adubação para o Estado de Pernambuco**. 2. ed. Recife, PE: Embrapa Solos, 2008. 212 p.
- FERREIRA, L. S.; SOUZA, R. M. Influência de plantas na atividade microbiana de solos contaminados. **Environmental Science Advances**, 18: 135-148, 2023.
- GLICK, B. R. Using soil bacteria to facilitate phytoremediation. **Biotechnology Advances**, 28: 367-374, 2010.
- GOMES, H. A. et al. Effects of toxic compounds on soil bacterial communities. **Microbial Ecology**, 44: 199-210, 2022.
- HEIDARI, S. et al. Physiological responses and phytoremediation ability of Eastern Coneflower (*Echinacea*

- purpurea) for crude oil contaminated soil, Caspian Journal of Environmental Sciences, 16: 149-164, 2018.
- HUANG, X. D. et al. Responses of three grass species to creosote during phytoremediation. **Environmental Pollution**, 130: 453-463, 2004.
- JOHNSON, C. M.; LEE, Y. H. Plant exudates and their role in enhancing soil microbial activity under contamination stress. **Environmental Microbiology Reports**, 15: 67-82, 2023.
- KOVALEVA, E. I. et al. The Response of Higher Plants to the Oil Contamination of Soils in a Pot Experiment. **Moscow University Soil Science Bulletin**, 77: 178-187, 2022.
- LIAO, C. et al. Biossurfactant-enhanced phytoremediation of soils contaminated by crude oil using maize (*Zea mays* L.), **Ecological Engineering**, 92: 10-17, 2016.
- LICHTENTHALER, H. K.; WELLBURN, A. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. **Biochemical Society Transactions**, 603: 591-592, 1983.
- MURATOVA, A. Y. et al. Remediating abilities of different plant species grown in diesel-fuel-contaminated leached chernozem. **Applied Soil Ecology**, 56: 51-57, 2012.
- NGUEMTÉ, P. M. et al. Potentialities of six plant species on phytoremediation attempts of fuel oil-contaminated soils. **Water, Air, & Soil Pollution**, 229: 1-18, 2018.
- PIMENTEL, I. C. Contagem de Bactérias pelo Método de Semeadura em Superficie: Protocolo I e II. In: DIONÍSIO, J. A. et al. **Guia Prático de Biologia do Solo**. Curitiba, PR: Sociedade Brasileira de Ciência do Solo, 2016. v. 1, cap. 3, p. 17-23.
- RAMALHO, A. M. et al. Study of contamination by benzene due diesel and gasoline leaks at a gas station in Natal /Brazil. **International Journal of Engineering & Technology Sciences**, 14: 49-54, 2014.
- RODRIGUES, A. L.; SILVA, M. P. Advances in plantmicrobe consortia for soil bioremediation. **Journal of Environmental Management**, 278: 1-24, 2021.
- SCOPEL, W.; BARBOSA, J. Z.; VIEIRA, M. L. Extração de pigmentos foliares em plantas de canola. **Unoesc & Ciência**, 2: 87-94, 2011.
- SHAPIRO, S. S.; WILK, M. B. An analysis of variance test for normality (complete samples). **Biometrika**, 52: 591-611, 1965.
- SHARMA, A. et al. Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. **Molecules**, 24: 1-22, 2020.
- SHORES, A. R; HETHCOCK, B.; LAITURI, M. Phytoremediation of BTEX and Naphtalene from produced-water spill sites using *Poaceae*. **International Journal of**



**Phytoremediation**, 20: 823-830, 2018.

SILVA, A. P. et al. Use of volatile organic compounds by sporulating bacteria in contaminated soils. **Journal of Environmental Microbiology**, 35: 215-230, 2022.

THACHARODI, A.; JEGANATHAN, C.; THACHARODI, D. Biomonitoring of Heavy Metal Polluiton by Bioluminescente Bacterial Biosensors, Indian Journal of Science and Technology, 12: 1-9, 2019.

TREVELYAN, W. E.; HARRISON, T. S. Dosagem de glicídios totais pelo método de antrona. **Journal Biochemical**, 50: 292-300, 1952.

YU, B. et al. BTEX in the environment: Na update on sources, fate, distribution, pretreatment, analysis, and removal techniques. **Chemical Engineering Journal**, 435: 1-54, 2022.