ORIGINAL ARTICLE

The Effect of Landfill Leachate and Pyrene on Sorghum Bicolor Growth Parameters and Soil Bacterial Communities

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Abstract

Background: Pyrene is one of the polycyclic aromatic hydrocarbons that has carcinogenic, mutagenic and teratogenic effects for living organisms. Landfill leachate is another environmental pollutant that covers a wide range of pollutants, especially heavy metals. The simultaneous presence of two types of pollutants with organic and inorganic structures can increase their toxicity.

Methods: In this experimental study, the single and simultaneous effect of Landfill leachate and pyrene on plant growth parameters and the number of heterotrophic soil bacteria was investigated. The study was conducted for 90 days at concentrations of 150, 300, 500, 750 and 1000 mg/kg^{-1} of pyrene and percentages 0, 30, 50, 70 and 100% of landfill leachate.

Results: The results showed that after 90 days, the highest dry stem and root biomass were obtained in irrigation treatment with pyrene and Landfill leachate (Blank)-non-contaminated municipal water with quantities of 8.2 and 3.5 g, respectively; moreover, the lowest stem and root biomass related to the treatment were observed in the simultaneous presence of 30% leachate and pyrene with a concentration of 300 mg/kg^{-1} with quantities of 5 and 1.8 g, respectively. Leachate did not produce any biological toxicity at any of the surfaces used, but the use of pyrene at concentrations of 1500 mg/kg^{-1} and above reduced the number of heterotrophic bacteria.

Conclusion: According to the results, the simultaneous presence of the two pollutants, pyrene and leachate, exacerbates the phytotoxicity due to possible interactions between them. Pyrene as a carbon source is decomposed by bacteria at low concentrations, but it inhibits metabolism and growth at high concentrations.

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Introduction

Due to population growth, industrialization and modern technologies, large amounts of organic, and inorganic pollutants have been introduced into the environment and have caused extensive contamination in soils. Soil contamination with organic and inorganic substances is a serious threat to human health and the food chain.[1] Polycyclic aromatic hydrocarbons are a group of chemical compounds with organic structure, which consist of two or more gasoline rings. Due to their toxicity and carcinogenic, mutagenic and teratogenic effects on aquatic and human organisms, the US Environmental Protection Agency (USEPA) has placed this group

of pollutants in the category of persistent *organic pollutants* (*POPs*).[2] Pyrene is one of these compounds with four gasoline rings that is highly resistant to natural degradation in the soil and can have toxic effects on soil organisms. The concentration of this compound in the soil varies according to geographical characteristics and extent of the environment and is often in the range of 100-1000 mg/kg.[3] Another environmental pollutant that covers a wide range of organic and inorganic pollutants is Landfill leachate. Landfills are always subject to biological, chemical and physical changes that produce various compounds, including leachate. Leachate is produced under anaerobic or aerobic decomposition of compounds in the landfill and the penetration of rainwater into it.[4] One of the most important contaminant groups in the Landfill leachate is heavy metals that are not biodegradable and have a high shelf life if they penetrate into the the soil. Heavy metals gradually accumulate through the food chain in the tissues of animals and plants and have deleterious effects on soil, plant and human microorganisms over time.[5] The simultaneous presence of two types of contaminants in soils with different organic and inorganic structures and considering the possible interactions between them and ultimately their impact on soil organisms are one of the most important issues we may face. Thavamani et al. investigated the microbial activity and its changes in mixed contaminated soil to polycyclic aromatic hydrocarbons and heavy metals. The results of their study showed that the presence of polycyclic aromatic hydrocarbons had a more negative effect on soil microbiological characteristics than the presence of polycyclic aromatic hydrocarbons in heavy metal contaminated soil.[6] Also, Bernard et al. investigated the toxicity of Landfill leachate in terms of alkalinity and ammonia on duckweeds.[7] But the toxicity of Landfill leachate and a polycyclic aromatic hydrocarbon on sorghum and the number of heterotrophic soil bacteria has not been investigated so far. Therefore, this study aimed to evaluate the simultaneous toxicity of Pyrene with organic structure and landfill leachate with a wide range of contaminants, including heavy metals with the mineral structure on two types of organisms such as sorghum bicolor and heterotrophic bacteria population of soil.

Methods

Design of Treatments

The study variables included the presence and absence of plant, different percentages of Landfill leachate (0, 30, 50, 70, and 100%), different concentrations of Pyrene (150, 300, 500, 750 and 1000 mg.kg-1) at times. It was 30, 60 and 90 days.[8] The variables defined are shown in Table 1 and the treatments designed are shown in Table 2.

Landfill Leachate Collection

Landfill leachate was collected from a landfill in Shiraz. The sampling interval was once every 7 days. The samples were stored in the refrigerator at 4°C until use. Landfill leachate properties were analyzed according to the Handbook of Standard Methods for Water and Wastewater Testing.[9] Table 3 shows the results of the landfill leachate characteristics measurement.

Collecting Soil Samples

The studied soil was collected from a field in Sepidan County, Fars province (30.0451 ° N, 52.2762 ° E) without any contamination with pyrene. Samples were taken from three different soil points at a height of 0–20 cm above the soil. Samples of all three points were poured into plastic containers after mixing and transferred to the laboratory. Soil samples were spread on a plastic sheet for drying for 72 hours in thin layers of 2-5 cm. Then, for uniform soil sampling, the samples were passed through sieves with a 3 mm mesh three times and kept in a dark room for one week.[10]

Contaminating the Soil with Pyrene

Five initial concentrations were considered to contaminate the soil with pyrene. These concentrations

Table 1: Variables defined in the study

Variable	Description
Concentration of Pyrene	150, 300, 500, 750, 1000 (mg. kg^{-1})
Percentage of leachate	a=0, b=30, c=50, d=70, e=100 (%)
Plant	Planting, Non planting
Time	30, 60, 90 Days

Table 2: Treatments designed in the study

Percentage of leachate	Pyrene	Plant	Treatments
a	-	+	blank
a	+	+	T_0
b	+	+	T_1
a ,b ,c ,d ,e	+	-	T_2
b	-	+	T_3

Table 3: Landfill leachate characteristics in the study

Parameters	Amount
Nitrate (NO_3_N)	942 mg l^{-1}
COD	38000 mg l^{-1}
Ammonia	280 mg l^{-1}
TDS	5200 mg l^{-1}
TSS	983 mg l^{-1}
Phosphate (po_4^{3-})	38 mg l^{-1}
pH	5.9

were: 150, 300, 500, 750 and 1000 mg/kg^{-1}. To prepare the contaminated soil at concentrations of 150 and 300 mg/kg, soil with concentrations of 1500 and 3000 mg/kg was prepared. For this purpose, 1.5 g and 3 g pyrene with 98% purity were dissolved individually in 1 liter of hexane. After complete dissolution of pyrene in hexane, one kg of non-contaminated pyrene was added to each solution and placed on the heater for 24 hours until complete soil drying. For soil preparation at concentrations of 150 and 300 mg/kg, each 1 kg of soil prepared at concentrations of 1500 and 3000 mg/kg was divided into 10 packs of 100 grams and each pack of 100 grams of soil contaminated mixed with 900 grams of non-contaminated soil. In order to properly mix the contaminated and non-contaminated soil and uniform it, the mixture was passed through sieves with a 4 mm mesh three times. Contaminated soils with concentrations of 500, 750 and 1000 mg/kg^{-1} were prepared by combining different weight ratios of contaminated soil with a concentration of 300 mg/kg^{-1} and non-contaminated soil.[11]

Planting and Irrigation

The seed of sorghum bicolor was purchased from the Pakan Bazr Company in Isfahan. Each plastic pot (15 cm in diameter, 20 cm in height) was filled with 1000 g soil and placed in the dark for one week. After one week, 8 seeds were planted per pot. After germination of the seeds, the pots were examined for germination or non-germination of the seed and 3 seeds with similar physical characteristics were left in the pot for further study and the other seeds were removed from the pot. The pots were kept in a greenhouse at a temperature of 25 °C and irrigated every 4 days for 90 days, according to the design of the study treatments with a 300 ml container.

Plant height was measured weekly and the average height of three plants per pot was reported as the final height. According to the study design, the plants were removed from the pot after 30, 60 and 90 days and the root and stem parts were separated. Then, they were carefully rinsed using distilled water and after measuring the root and stem wet weight, they were placed in an oven at 105°C for 24 hours to calculate the root and stem dry weight.

Counting of Heterotrophic Soil Bacteria

For counting the soil bacteria, the plate culture method was used. For this purpose, 2 g of soil sample was removed and dissolved in 100 ml distilled water. After dilution, 500 microliters of the sample was removed and spread on nutrient agar medium. The media were incubated at 35 °C for 24 h. The bacterial count results were reported as *CFU g^{-1} dry* soil.

Results and Discussion

Impact of Landfill Leachate on Plant Survival

After germination of the seeds, the plants irrigated with 50, 70 and 100% leachate had necrosis and chlorosis after two irrigation and finally dried. Only plants irrigated with municipal water and leachate 30% were not affected and remained until the end of the study. One of the biochemical changes that occur at environmental stresses, including heavy metals, is the production of a variety of reactive oxygen species that can cause major damage to the plant's membranes, fats, proteins and nucleic acids.[12] The presence of heavy metals in the leachate along with other toxic compounds can exacerbate this phenomenon in the plant. In the panda et al.'s study, high levels of lipid peroxidation were reported in plants under heavy metal stress.[13]

Effect of Landfill Leachate on Height and Biomass of Wet and Dry Plant

Figure 1 shows the results of the effect of leachate toxicity on dry and wet plant biomass. As the results

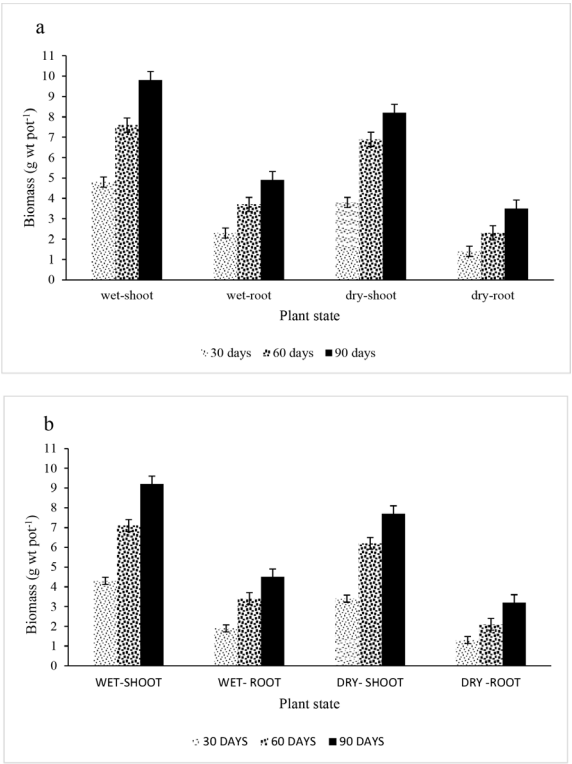

Figure 1: Impact of Landfill leachate on wet and dry biomass of plant in non-contaminated soil to pyrene. a) blank treatment (irrigation with municipal water); b) T_3 treatment (irrigation with 30% leachate)

Table 4: Average weekly height of plants in different treatments

Treatments	Average Weekly Height(cm)										
	2 week	3 week	4 week	5 week	6 week	7 week	8 week	9 week	10 week	11 week	12 week
Blank	9.8	11.4	15.7	19.2	24.8	32.9	40.4	49.7	57.5	64.4	68.9
T_0-300 mg kg^{-1}	5.9	8.7	11.8	15.3	18.1	23.3	30.1	38.4	47	55.8	59
T_0-150 mg kg^{-1}	6.2	8.8	12.1	15.5	18.3	23.7	30.2	38.6	47.1	56.1	59.2
T_1-300 mg kg^{-1}	5.6	8.4	11.3	14.8	17.5	22.6	28.8	36.9	45.7	53.8	57.6
T_1-150 mg kg^{-1}	5.8	8.5	11.6	15.1	17.8	22.8	29.1	37.1	45.9	54.1	57.9
T_3	6.4	9.1	12.4	15.8	18.7	24.1	30.6	38.9	47.4	56.7	59.6

show, leachate decreased wet and dry biomass of plants as compared to irrigation treatment with municipal water, so that the dry root and stem biomass of plants after 90 days in irrigation treatment with municipal water was 3.5 and 8.2 g, respectively, and was 3.2 and 7.7 g, respectively in irrigation treatment with leachate 30%. Wet biomass of root and stem of plants irrigated with municipal water after 90 days was 4.9 and 9.8 g and in 30% leachate irrigation was 4.5 and 9.2 g, respectively. Table 4 shows the plant height in different treatments. Landfill leachate had a negative effect on the plant height, so that plant height after 90 days was 9.3 cm lower compared with urban water irrigation treatment. The leachate contains a wide range of compounds, some of which are present at high concentrations. One of the compounds of leachate that can cause chronic biological toxicity is heavy metals. Concentrations of lead and cadmium in the soil irrigated with leachate 30% after 90 days were 12.05 and 4.33 mg/ kg^{-1}, respectively, which could be one of the factors causing negative impact on the plant growth parameters. The results of the Nagajyothi et al.'s study also showed that when plants are exposed to high concentrations of heavy metals, the wet and dry biomass of plants and stem and root lengths are reduced.[14] Ouzounidou et al. reported that the negative effect of heavy metals on the plant length could be due to unusual cell division and the inhibition of photosynthesis and respiration processes in the stem system and protein synthesis in the root.[15] Heavy metals decrease photosynthesis in plants by decreasing chlorophyll and increasing lipid peroxidation.[16] Heavy metals are also bound to the wall of the root cells at high concentrations, preventing the cell division and increase in its length.[17]

Pyrene Effect on Seed Germination

Seed germination is a very important step in plant growth that can be affected by the type of plant versus the concentration and type of soil pollutants. Seeds were only irrigated with municipal water until germination. All the seeds planted in contaminated soil at concentrations of 150 and 300 mg/Kg^{-1} germinated in two replicates. At a concentration of 500 mg/Kg^{-1}, one seed germinated in one replicate. None of the planted seeds in soils contaminated with concentrations of 750 and 1000 mg/Kg^{-1} germinated. It can be stated that the concentration of 500 mg/kg^{-1} is the threshold for the plant's inability to germinate, and toxicity in higher concentrations in the soil inhibits sorghum seed germination. Smreczak et al. investigated the germination of plants in the soil contaminated with polycyclic aromatic hydrocarbons and reported that PHHs had a phytotoxicity potential, but in soil contaminated with high concentrations of pyrene, the delay or absence of germination of seeds was observed.[18] During germination of plants under normal conditions, hydrolysis of carbohydrates stored in seeds reduces the potential of radical cells and results in increased water uptake and germination. The presence of petroleum hydrocarbons or their microbial metabolites in the soil, by entering the plant seed, prevents the hydrolysis and mobilization of hydrolysis products and prevents germination.[19]

Pyrene Impact on Height and Wet and Dry Biomass of Plant

Figure 2 shows the effect of pyrene toxicity on dry and wet plant biomass. According to the results, pyrene had a negative effect on plant biomass and was more than the negative effect of leachate on plant biomass. The dry biomass of the plant's stem and root in the soil treated with pyrene and irrigation with municipal water after 90 days at 150 mg/kg^{-1} concentration was 5.9 and 3 g, respectively, and 5.6 and 2.3 g, respectively in contaminated soil with a concentration of 300 mg/kg^{-1}. However, in the soils irrigated with Landfill leachate without contamination with dry biomass, stem and root biomass were 7.7 and 3.2 g, respectively. Comparison of wet and dry biomass of the stem and root at two concentrations of 150 and 300 mg/kg^{-1} of pyrene showed that these parameters at a concentration of 300 mg/kg^{-1} were lower and Phytotoxicity increased following an increase in the pyrene concentration from 150 to 300 mg/kg^{-1}. According to Table 4, pyrene toxicity on the plant height also decreased their length compared to blank treatment as well as irrigation treatment with pyrene-non-contaminated Landfill leachate, so that the plant height after 90 days in blank treatment, T_3 treatment, and T_0 treatment at a concentration of 150 mg/kg^{-1} was 68.9, 59.6, and 57.3 mg/kg^{-1}, respectively, and 56.1 cm at a concentration of 300 mg/kg^{-1}. The results of Chaineau et al.'s study were similar to those of our study. They investigated the toxicity of petroleum hydrocarbons on the germination and growth of some

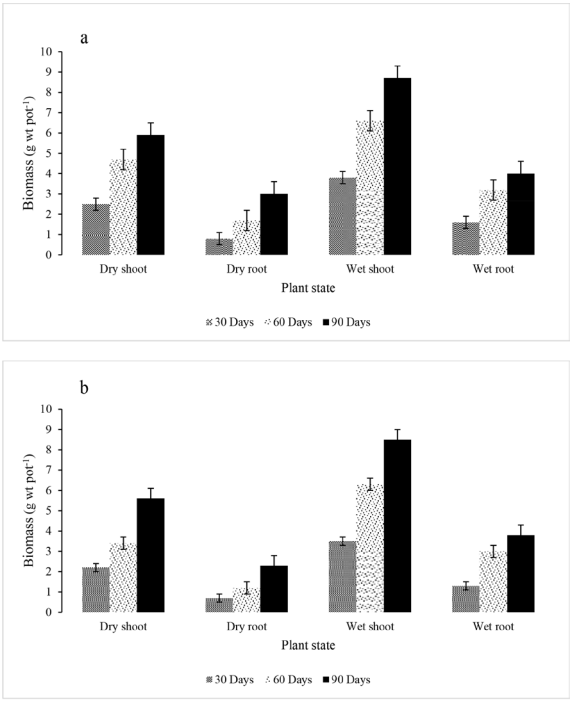

Figure 2: Pyrene impact on dry and wet plant biomass (irrigation with municipal water). a) 150 mg/kg $^{-1}$; b) 300 mg/kg $^{-1}$

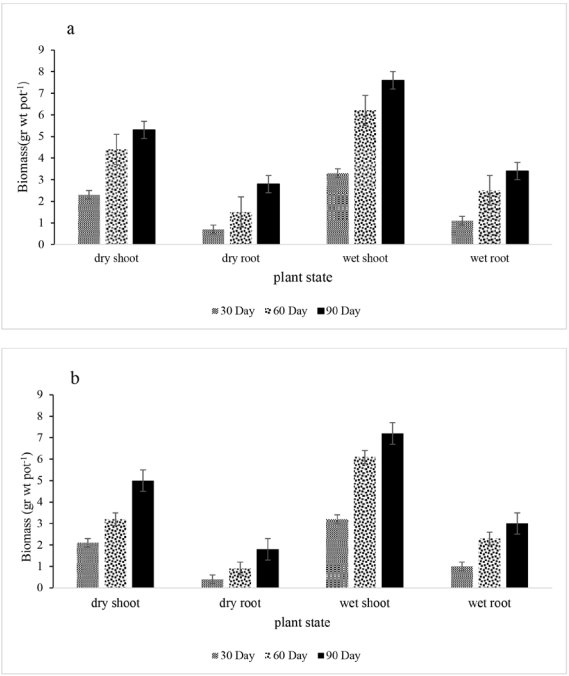

Figure 3: Wet and dry biomass of plant in T_1 treatment (pyrene- contaminated soil and irrigation with 30% leachate). a) Contaminated soil with a concentration of 150 mg/kg^{-1} ; b) contaminated soil at a concentration of 300 mg/kg^{-1}

plants and reported that the presence of petroleum hydrocarbons in soil inhibited the seed germination and reduced the plant growth.[20] In the study of Li et al., the presence of petroleum hydrocarbons in the soil had no effect on plant germination, but it had a decreasing effect on the plant growth.[21] The blockage of water absorption and nutrient following the attachment of pollutants to the root surface is one of the reasons for the reduction of plant biomass. In the case of petroleum hydrocarbons, in addition to binding to the root surface, toxicity to the apical meristem also reduces the plant biomass.[19]

Synergistic Effect of Landfill Leachate and Pyrene on the Height and Wet and Dry Biomass of Plants

Figure 3 shows the simultaneous impact of Landfill leachate and Pyrene on wet and dry biomass of the plants. Comparison of these results with other treatments showed that T_1 treatment had the most negative effect on the plant growth parameters when the plant was irrigated with leachate 30% in pyrene-contaminated soil. After 90 days, the lowest dry stem and root biomass at a concentration of 300 mg/kg^{-1} was 5 and 1.8 g, respectively, which were observed in this treatment. The same decreasing trend was observed for wet biomass and height. According to Table 4, the lowest weekly plant height after 90 days observed in T_1 treatment with a decrease in the concentration of 300 mg/kg^{-1} was stronger and reached 57.6 cm, which was 11.3 cm lower than the blanc treatment. Lin et al. investigated the growth of Zea mays L. in pyrene-and- Cu- contaminated soils and reported that the phytotoxicity caused by pyrene-and-Cu-contaminated soil was greater than single treatments. It is likely that the complex interactions of the two organic and inorganic contaminants together have created more toxicity than the presence of either of these compounds alone.[22] Lead and cadmium in the leachate are considered as two unnecessary elements for the plant; the measured concentration in the leachate used is shown in Table 3. The presence of Pyrene along with these elements can increase the phytotoxicity created.

Effect of Landfill Leachate and Pyrene on Soil Heterotrophic Bacteria in Non-Planting Treatments

According to Figure 4, the effect of different percentages of leachate on the number of heterotrophic bacteria in the pyrene-contaminated soil at concentrations of 150 and 300 mg/kg^{-1} indicates that the bacterial count increased as the percentage of leachate increased. Use of leachate alone at any of the surfaces used in our study did not cause toxicity to heterotrophic soil bacteria and even caused biological enhancement. After 90 days, the number of bacteria was significantly higher in irrigation treatments with leachate (P=0.001). Matejczyk et al. also investigated the toxicity of Landfill leachate in Poland and reported that chemical parameters of leachate did not cause toxicity to soil microbial conditions.[23] As Figure 5 shows, at concentrations of 150 and 300 mg/kg^{-1}, Pyrene had no negative effect on the number of soil heterotrophic bacteria and increased Pyrene concentration from 150 to 300 mg/kg^{-1} increased the number of bacteria. However, increasing the concentration of pyrene to 500 mg/kg^{-1} decreased the number of bacteria significantly

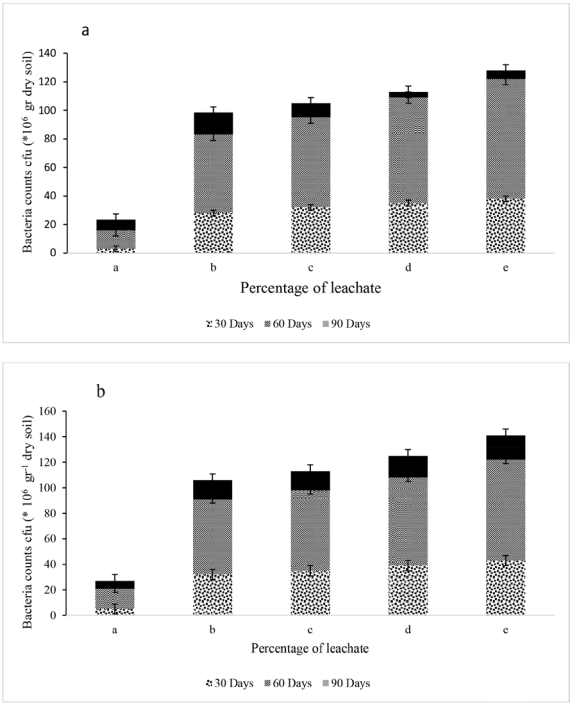

Figure 4: Effect of different percentages of Landfill leachate on the number of heterotrophic bacteria in the soil without planting. a) Pyrene-contaminated soil with a concentration of 1150 mg/kg^{-1}; b) contaminated soil at a concentration of 300 mg/kg^{-1}

(P=0.004). In other words, pyrene at concentrations of 500 mg/kg^{-1} and above, even with the same soil irrigation conditions with 30% leachate, caused biological toxicity in the soil and reduced the number of bacteria. Maximum pyrene toxicity arose at 500 mg/kg^{-1} concentration, in which toxicity was also higher than concentrations of 750 and 1000 mg/kg^{-1}. Pyrene at specific concentrations can be used as substrates for microflora because microorganisms can easily decompose it as a carbon source and this process results in increased metabolism. However, at high concentrations, pyrene reduces the microbial activity by causing toxicity.[24] The results of the study conducted by Hollender et al. showed that the bacterial count increased with increasing pyrene concentration and no toxicity was observed.[25]

Effect of Planting on Biological Toxicity of Landfill Leachate and Pyrene and Increase in the Number of Bacteria

Figure 6 shows the number of heterotrophic bacteria in planting treatments. The number of soil bacteria in planting treatment was higher than non-planting treatment. This increase in the number of bacteria is due to the growth of rhizosphere bacteria affected by root secretions. Plants alter the physical and chemical conditions of the soil and increase the soil ventilation. This increase in the bacterial population results from the growth of bacteria in the presence of plant roots because the roots of plants secrete substances such as amino acids, organic acids, enzymes, and other carbohydrate compounds that the

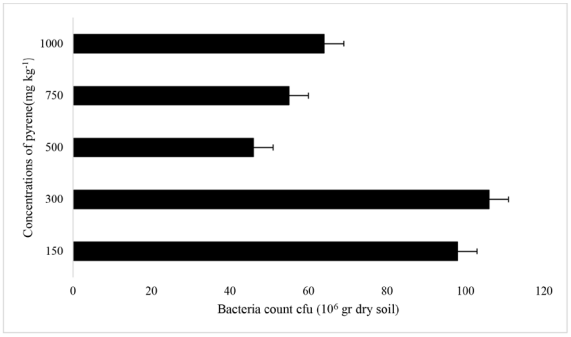

Figure 5: Effect of different pyrene concentrations on the soil bacterial count after 90 days (irrigation with 30% leachate with no planting)

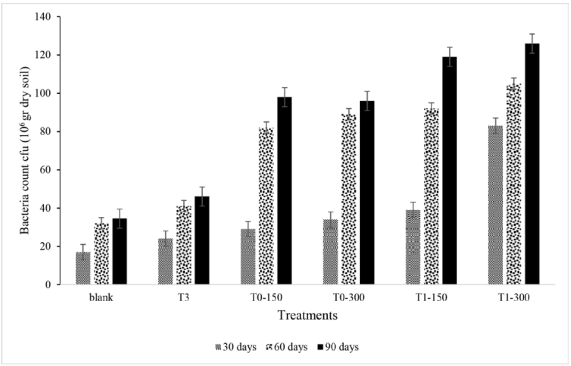

Figure 6: The number of soil bacteria in planting treatments. Blank: planting, irrigation with municipal water; without pyrene T_3: planting, irrigation with 30% leachate; without Pyrene T_0: planting, irrigation with municipal water; contaminated with pyrene T_1: planting, irrigation with 30% leachate, contaminated with pyrene.

bacteria can use as a source of carbon and energy. The same factor increases the bacterial population in the area.[26] For this reason, planting in contaminated soil can reduce the impact of Pyrene and leachate toxicity on the number of soil bacteria. Gabriele et al. investigated the impact of planting on the soil bacterial counts and reported that planting increased the number of rhizosphere bacteria.[27]

Conclusion

The present study investigated the synergistic toxicity of Landfill leachate and pyrene on sorghum growth parameters and the number of heterotrophic soil bacteria. According to the results of this study, pyrene at concentrations greater than 300 mg/kg and Landfill leachate inhibited germination and survival of plants by more than 30%, respectively. These two pollutants alone had negative effects on the plant growth parameters such as height and biomass of the stem and roots, and this effect was higher in the presence of concurrent conditions. Landfill leachate in the amounts used in this study had no toxic effect on the soil bacteria. Pyrene at concentrations less than 300 mg/kg^{-1} increased the number of bacteria, but at concentrations above 500 mg/kg^{-1} caused biological toxicity, which was less toxic

in plant cultivation treatments. In this study, the effect of low-concentration leachate was not investigated. Therefore, it is recommended that more studies should be done in future on the effect of low-concentration leachate(<30%) on the plant growth.

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Conflict of Interest: None declared.

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