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Interaction Effects of Sewage Sludge and Its Biochar on Anthracene Biological Degradation in a Pb Polluted Soil under Sunflower Cultivation

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Abstract

Background: This descriptive study was conducted to evaluate the interaction effects of sewage sludge and its biochar on anthracene biological degradation in a Pb polluted soil under sunflower cultivation

Methods: Treatments consisted of applying three rates of anthracene (0, 12.5 and 25 mg/kg soil), two levels of sewage sludge and its biochar (0 and 30 t/ha) in the presence of Azetobacter bacteria and Piriformospora indica (P. indica) fungus. After 60 days, plants were harvested and plant Pb concentration was measured using atomic absorption spectroscopy. In addition, the soil petroleum hydrocarbons concentration and soil microbial respiration were measured by the method described by Besalatpour et al. (2011). The catalyze enzyme activity was assayed.

Results: Plant inoculation with P.indica and Azotobacter significantly increased the degradation of anthracene in soil by 12.8% in the soil treated with 25 mg anthracene/kg soil. In addition, the soil Pb availability was decreased by 14.6% in the mentioned treatment. Applying 30 t/ha sewage sludge and its biochar significantly increased the plant biomass and anthracene degradation in the soil treated by 12.5 mg anthracene /kg soil by 13.5% and 12.6%, respectively.

Conclusion: Soil treated with sewage sludge and its biochar had a positive effect on plant resistance to abiotic stresses and degradation of anthracene in the soil. Among this, plant inoculation with P. indica and Azotobacter had also an additive effect on bio-remediation of anthracene in the soil that is a positive point in environmental studies. However, soil pollution with heavy metals had an adverse effect on it.

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Introduction

Petroleum hydrocarbons is a term used to describe a large group of chemical compounds that all originate from crude oil. Crude oil is used to produce numerous derived compounds that are added to the environment, causing water, soil and air pollution.[1] Crude oil is made up of many chemical compounds that are not easily measured in contaminated sites. Therefore, the term "total petroleum hydrocarbons" or TPHs is used to refer to these chemical compounds of crude oil or its by-products' origin.[2, 3] Petroleum hydrocarbons are a mixture of chemical compounds that are all composed of carbon and hydrogen and are, therefore, called hydrocarbons. These uniform compounds are chemically low soluble in water or insoluble in water and have less density than water. In addition to the carbon and hydrogen required for the formation of these compounds, nitrogen and sulfur may also be present in the structure of these compounds; in this case, after the addition of these types of petroleum hydrocarbons to the environment, by their reaction molecular oxygen, sulfur or nitrogen oxides and carbon monoxide are produced and enter the atmosphere and pollute it.[4, 5] Petroleum hydrocarbons may enter the environment in various ways: oil leakage from pipelines, overflow of crude oil tanks, crashes of crude oil carriers, release of refinery waste into the environment, and subsequent wars that burn the oil resources. Soil contamination with total petroleum hydrocarbons has emerged as a serious environmental and human health concern with a large percentage of used oil discharged into the ecosystem without any treatment.[6] Petroleum hydrocarbons are composed of carbon, oxygen, hydrogen, nitrogen, and sulfur. Saturated hydrocarbons, aromatic hydrocarbons, colloid and asphaltene are the four large TPH fractions that are toxic and cannot be quickly degraded by soil microorganisms. Furthermore, weathering could enhance the sorption of contaminants into the soil pores, leading to chronic soil aging problems and decreased bioavailability and biodegradability of microorganism to the pollutants. Crude oil and its by-products are one of the most important sources of environmental pollution of petroleum hydrocarbons, and its remediation from soils is necessary.[7-9]

Phytoremediation is one of the most important methods when compared with physical and chemical remediation methods due to its high efficiency, low cost and harmless products (mainly water and CO_2 and water). However, plant growth in central parts of the country is hampered by low soil organic matter content and soil pollution.[10-12] Therefore, using appropriate methods to increase the plant growth can speed up the bioremediation process.[13] Álvarez-López et al. reported that using organic amendments can affect the plant growth and consequently increase the soil quality.[14] In addition, using vitamins and substrates can stimulate the microorganisms' activities, thereby increasing the petroleum hydrocarbons degradation (biostimulation) indication that microorganisms stimulation by adding organic amendments can provide a main carbon source which tends to result in a rapid depletion of the available pools of major inorganic nutrients such as P and N.[15] In this regard, applying sewage sludge with the low amount of heavy metals can increase the soil organic carbon and consequently improve the soil microbial activity that help to increase the petroleum hydrocarbon degradation, specially in soils polluted with heavy metals. In this regard, Dindar et al. investigated the biodegradation of crude oil-contaminated soil treated with sewage sludge and concluded that applying sewage sludge as a carbon and macro- or micro-nutrient can stimulate the microorganism activities, thereby increasing the petroleum hydrocarbon degradation.[16]

It should be noted that in the central part of Iran, there is a co-contamination of heavy metals and petroleum hydrocarbons. Thus, it seems necessary to remediate the soils from these compounds. On the other hand, in arid and semi-arid regions of the country, there is a problem of soil organic matter deficiency which restricts the growth of the plants. Thus, adding organic amendments such as sewage sludge or its biochar can increase the soil organic carbon that helps to the growth of the plant. On the other hand, increasing soil organic carbon can alter the soil sorption properties that may affect the availability of heavy metals, thereby changing the soil microbial activities. On the other hand, the symbiotic effect of plant inoculation with fungi or bacteria can help to increase the plant's resistance to soil contaminants and thus promote the plant growth. On the other hand, plants can also affect the activity of soil microorganisms and consequently help to degrade the petroleum hydrocarbons compounds such as anthracene in the soil. Therefore, this research was done to evaluate the interaction effects of sewage sludge and its biochar on anthracene biological degradation in a Pb polluted soil under sunflower cultivation

Material and methods:

To investigate the interaction effects of sewage sludge and its biochar on anthracene biological degradation under sunflower cultivation, a naturally Pb-polluted soil with the low organic carbon was selected. This research was done as a factorial experiment in the layout of completely randomized block design in three replicates. Treatments consisted of applying three rates of anthracene (0, 12.5 and 25 mg/kg soil), two levels of sewage sludge and its biochar (0 and 30 t/ha) in the presence of Azetobacter bacteria and Piriformospora indica (P. indica fungus). The soil was collected around the Shazand Pb and Zn mine in Mrkazi province. The physic-chemical properties are shown in Table 1.

Table 1: The selected physico-chemical properties of soil in this study

Characteristic	Unit	Amount
Soil texture	-----	Loamy
pH	------	7.2
EC	dS/m	2.1
Organic carbon	%	0.1
Cation exchange capacity	Cmol/kg soil	10.2
Total Pb concentration	mg kg^{-1}	843.3
Total Cd concentration	mg kg^{-1}	2.8

The Pb polluted soil was treated with anthracene at the rates of 0, 12.5 and 25 mg/kg and incubated for two weeks to equilibrium. After that, sewage sludge and its biochar was added to the soil and incubated for two weeks to equilibrium.

The fungal strain of P. indica used in this study was obtained from the soil biology of water and soil research institute. The P. indica inoculum in this experiment was prepared according to the Zamani et al.'s method.[17]

To prepare Azotobacter chroococcum (A. chrooococcum) inoculant, we first cultivated the strain in Yeast Mannitol (YM) liquid medium for 24 h in a rotary shaker (150 rpm) at 28 °C. The culture was harvested when growth was complete and centrifuged at 3293 x g. Then, the cells of the bacterial were suspended in a 5% sucrose solution at the rate 1:5 (w:v). The bacterial was freeze-dried and amended to quartz sand to have a microbial concentration of approximately 1×107 CFU g^{-1}.[18]

Thereafter, the sunflower (Helianthus annuus L.) seeds were surface sterilized in 15% H_2O_2 thoroughly washed in distilled water, and pre-germinated on the moistened filter paper. After germination, the uniform sets of seedlings were selected (two seedling for each pot) for the experiment. The selected seedlings were inoculated with *P. indica* by immersion for 3 hours in inoculums (adjusted nearly to 2×10^6) under gentle shaking. The non-inoculated seedlings were dipped in sterilized distilled water containing 0.02% Tween. Then, the 5kg pots were filled with the treated soils, and the P.indica inoculated or non-inoculated seedlings were planted at a depth of 1cm in the top soil layer in the center of each pot and irrigated to reach near field capacity (FC). Thereafter, the inoculums of Azotobacter were added in the central part of each pot. For controlling the treatments, a 5% sucrose solution lacking bacterial cells was applied to substrate similar to the inoculated treatment.[18] The germinated seeds were counted after two weeks to calculate the germination percentage. After 60 day, the plants were harvested and the shoot and roots were dried at 60°C for 24 h and weighed. Then, the Pb concentrations in the plant biomass were measured in 100mg subsamples of plants, using atomic absorption spectroscopy (AAS) after incineration at 550ºC for 6 h and extracted with HCl 2 N. In addition, DTPA-extractable Pb (soil Pb availability) was measured according to the Lindsay method using AAS (Perkin-Elmer model 3030).[19]

Soil microbial respiration was determined by measuring the released CO_2 of the soil during 48 h of incubation. In addition, the anthracene concentration in the soil samples was extracted by soxhlet using n-hexane and a 1:1 (v/v) dichloromethane during 24 h and measured according to the Besalatpour et al.'s study.[2] The catalyzed enzyme activity was assayed according to the method illustrated by Kapur et al.[20]

Plant N concentration was measured after dry matter digestion in concentrated sulfuric acid, boric acid 2 %, distillation with NaOH (10 mol/L) and titration with HCl (0.1 mol/L), according to the Kjeldahl method.[21]

Statistical analyses were performed using ANOVA. The mean differences were calculated using the least significant difference (LSD) test. The 95 percentage (P=0.05) probability value was considered to determine the significant difference. Compliance of data dispersion with ANOVA model assumptions was verified using Shapiro-Wilcoxon's normality and Levene's equal variance tests. If necessary, data were $\log_{10}$ or square root transformed.

Results

The greatest seed germination percentage belonged to the plants cultivated in the Pb-polluted soil without receiving any anthracene pollution and treated with the greatest rate of sewage sludge and its biochar in the presence of P. indica fungus and Azetobacter (Figure 1). Increasing the application of sewage sludge and its biochar from 0 to 30 t/ha significantly increased the percentage of

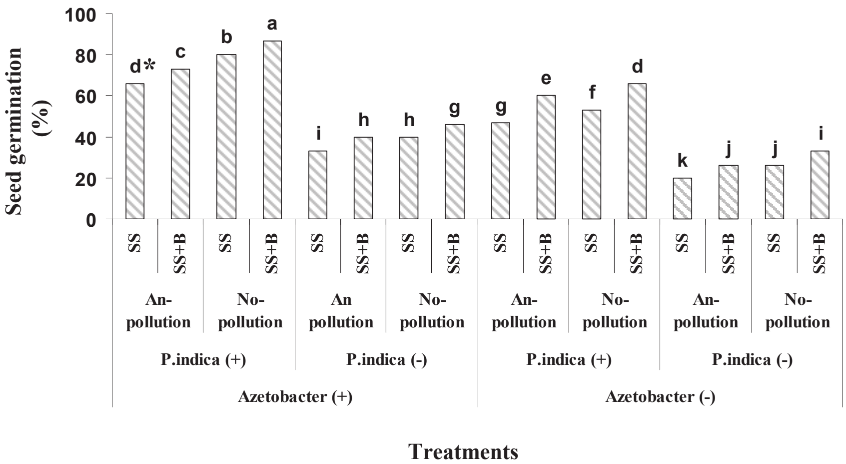

Figure 1: Effect of treatments on seed germination percentage, *means with similar letters are not significant (P=0.05), SS, SS+B, An-pollution, P. indica (+), P. indica (-), Azetobacter (+) and Azetobacter (-) are sewage sludge, sewage sludge and its biochar, anthracen pollution, the presence and absence of P. indica, and the presence and absence of Azetobacter, respectively.

seed germination by 12.1 and 18.2%, respectively. In addition, a significant increasie in the seed germination percentage by 17.2% was observed when the plants were inoculated with P. indica and Azetobacter. On the other hand, increasing the soil pollution to anthracene had negative effect on decreasing the seed germination percentage; as the results of this study showed, increasing soil pollution to anthracene significantly decreased the seed germination by 11.6%.

The greatest plant biomass was observed in the non-pyrene soil under cultivation of inoculated plants with P. indica and Azotobacter. Plant inoculation with P.indica or Azotobacter had a significant effect on the plant biomass (Table 2). However, they had additive effects as the results of this study showed that plant inoculation with P.indica, Azotobacter and co-inoculation significantly increased the plant biomass by 8.1, 10.6 and 14.2%, respectively. However, soil pollution to anthracene had adverse effects on the plant biomass. Increasing soil pollution to anthracene significantly decreased the plant biomass by 9.2% in the soil amended with 30 t/ha sewage sludge. Soil amended with sewage sludge or its biochar significantly increased the plant biomass. Accordingly, a significant increase by 16.2% in the plant biomass was observed when the sunflower was cultivated in the soil treated with 30 t/ha sewage sludge and its biochar.

The highest plant N concentration belonged to the plants cultivated in the soil receiving the greatest amount of sewage sludge and sewage sludge biochar, while the lowest concentration was observed in the non-treated soil (Table 3). Increasing the application of sewage sludge and its biochar from 0 to 30 t/ha significantly increased the Plant N concentration by 6.2 and 8.1%, respectively. Plant inoculation with Azotobacter had a significant effect on increasing the plant N concentration. However, co-inoculation had additive effects on increasing the plant N content. According to the results of this study, plant co-inoculation with Azotobacter and P.indica significantly increased the N concentration of the plants cultivated in the soil that received 30 t/ha sewage sludge by 11.3 %. Increasing soil pollution to anthracene significantly decreased the plant N concentrations.

The greatest soil Pb availability was observed in the anthracene-polluted soil under cultivation of inoculated plant with P.indica and Azetobacter. Increasing soil pollution to anthracene significantly increased the soil Pb availability by 8.2%. On the other hand, application of 30 t/ha sewage sludge and its biochar significantly decreased the soil Pb availability by 10.6% (Table 4). The presence of P. indica and Azetobacter had a significant effect on increasing thesoil Pb availability. Based on the results of this study, a significant increase in soil Pb availability by 5.6% was observed when the plants were inoculated with P.indica. In addition, Azotobacter showed an additive effect on increasing thesoil Pb availabity;

Table 2: The effect of sewage sludge and its biochar on the plant biomass in a Pb polluted soil under sunflower cultivation

Anthra-cene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	6.55g*	6.72e	6.22j	6.32i	6.32i	6.55g	6.12k	6.21j
	30	6.76e	7.25a	6.42h	6.61f	6.51g	6.71e	6.31i	6.43h
12.5	0	7.12b	7.29a	6.13k	6.21j	7.00d	7.12b	6.00i	6.12k
	30	6.52g	7.11c	6.31i	6.42h	6.41h	6.55g	6.22j	6.33i
25	0	6.52g	6.73e	6.00i	6.12k	6.21j	6.38i	5.73n	5.94m
	30	6.41h	7.02d	6.25j	6.32i	6.31i	6.45h	6.12k	6.21j

*Means with the similar letters are not significant (P= 0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

Table 3: The effect of sewage sludge and its biochar on plant N concentration (mg/kg DW) in soil in a Pb polluted soil under sunflower cultivation

Anthra-cene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	53.4f*	56.2c	50.4i	52.9g	52.1g	54.7e	49.3j	51.4h
	30	55.1d	60.3a	51.9h	55.2d	54.9e	58.8b	50.4i	53.7f
12.5	0	50.2i	55.8d	46.8l	49.6j	48.2k	53.6f	44.7n	46.9l
	30	53.5f	58.9b	50.3i	51.8h	52.2g	54.3e	48.2k	50.4i
25	0	51.6h	53.4f	45.4m	48.2k	44.1n	43.8o	42.6p	41.4q
	30	52.1g	56.1c	48.2k	49.4j	50.1i	52.3g	45.7m	46.9l

*Means with the similar letters are not significant (P=0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

Table 4: The effect of sewage sludge and its biochar on soil Pb availability in a Pb polluted soil under sunflower cultivation

Anthra-cene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	77.4c*	73.7h	67.7m	64.7o	73.8h	70.2j	64.1o	62.2p
	30	75.1f	70.2j	61.3q	58.8r	67.3m	62.5p	58.2r	50.2t
12.5	0	80.2b	77.3c	72.7i	70.3j	75.2f	72.8i	69.7k	66.3n
	30	77.6c	72.6i	64.5o	61.4q	70.2j	64.5o	61.3q	55.7s
25	0	83.4a	80.1b	76.1d	72.8i	80.2b	77.5c	74.2	70.1j
	30	80.2b	77.6c	73.9h	68.4l	76.2d	72.1i	70.2j	66.2n

*Means with similar letters are not significant (P=0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

Table 5: The effect of sewage sludge and its biochar on plant Pb concentration in a Pb polluted soil under sunflower cultivation

Anthra-cene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	69.4g*	67.6h	62.4m	60.3o	66.2i	60.3o	58.7q	55.1s
	30	65.2j	62.4m	59.4p	56.3r	62.7m	59.1p	55.1s	52.9t
12.5	0	72.1d	70.3f	66.3i	62.8m	69.2g	66.3i	63.8l	60.2o
	30	70.1f	67.8h	63.2l	60.4o	66.4i	64.1k	60.2o	58.6q
25	0	76.4a	74.9b	70.2f	67.1h	74.8b	71.3e	67.9h	64.8k
	30	73.1c	70.1f	67.2h	63.8l	70.1f	67.2h	64.7k	61.6n

*Means with the similar letters are not significant (P= 0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

the results of this study showed that plant inoculation with P. indica and Azotobacter caused anthracene significant increase in soil Pb availability by 13.4%.

Application of sewage sludge biochar showed a significant effect on plant Pb concentration (Table 5). The results of this study showed that application of 30 t/ha sewage sludge biochar and sewage sludge significantly decreased the plant Pb concentration by 10.9 and 11.6%, respectively. The presence of P.indica and Azotobacter had a significant effect on decreasing the plant Pb concentration; the results of this study showed that cultivation of plant co-inoculated with P.indica and Azotobacter in the non-anthracene polluted soil caused a significant decrease in the plant Pb concentration by 12.4%. Increasing soil pollution to anthracene significantly increased the plant Pb concentration.

The greatest degradation of anthracene in soil has belonged to the anthracene–polluted soil treated with the greatest level of sewage sludge and its biochar under cultivation of plant co-inoculated with P.indica and Azotobacter (Table 6). Plant inoculation with P.indica or Azotobacter significantly increased the anthracene degradation in soil. However, plant co-inoculation had additive effects. Based on the results of this study, cultivation of inoculated plant with P. indica in the anthracene-polluted soil significantly increased the anthracene degradation in soil by 14.6%. Increasing the soil pollution to anthracene significantly increased the anthracene degradation in the soil; a significant increase in degradation of anthracene in the soil by 18.2% was observed when the soil pollution with anthracene increased from 12.5 to 25 mg/kg soil.

The greatest soil microbial respiration in the soil belonged to the anthracene-polluted soil t treated with 30 t/ha sewage sludge and sewage sludge biochar, while the lowest respiration belonged to the non-anthracene polluted soil without receiving any sewage sludge or sewage sludge biochar (Table 7). Applying 30 t/ha sewage sludge and sewage sludge biochar significantly increased the soil microbial respiration by 15.3 and 17.1%, respectively. Plant co-inoculation with P.indica and Azotobacter had additive effects on increasing the soil microbial respiration. Simultaneous presence of P.indic and Azotobacter in anthracene-polluted soil (25 mg/kg soil) significantly increased the soil microbial respiration by 12.4%.

The greatest catalyze enzyme activity belonged to the plants cultivated in the Pb-polluted soil without receiving any organic amendments (Table 8). Application of 30 t/ha sewage sludge and sewage sludge biochar significantly decreased the catalyze enzyme activity by 13.6 and 15.2 %, respectively. In addition, plants inoculated with P.indica and Azotobacter significantly decreased the catalyze enzyme activity of the plants grown in the soil treated with 30 t/ha sewage sludge by 12.4%. However, the plant Pb concentration was also decreased by 8.9%. Increasing the soil pollution with anthracene significantly increased the catalyze enzyme activity

Table 6: The effect of sewage sludge and its biochar on degradation of anthracene in soil in a Pb polluted soil under sunflower cultivation

Anthracene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	NM*	NM	NM	NM	NM	NM	NM	NM
	30	NM	NM	NM	NM	NM	NM	NM	NM
12.5	0	55.4q**	59.8m	49.4u	54.1r	52.5s	56.1p	45.1v	49.1u
	30	61.9k	69.5 d	54.5r	57.9o	57.4o	60.9l	51.6t	54.3r
25	0	67.3f	70.3 c	62.1 j	65.4 h	65.1 h	68.4 e	58.5n	61.3 k
	30	70.9c	78.5 a	63.1 i	66.9 g	67.1 f	71.7b	60.2l	63.1 i

*NM: not measured, **Means with the similar letters are not significant (P= 0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

Table 7: The effect of sewage sludge and its biochar on soil microbial respiration in soil in a Pb polluted soil under sunflower cultivation

Anthracene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	10.1o	10.4n	9.65s	9.72r	9.82q	9.95p	9.42u	9.55t
	30	11.51h	11.62g	11.23k	11.37j	11.37j	11.49i	11.11l	11.22k
12.5	0	11.55h	11.62g	11.22k	11.32j	11.35j	11.49i	11.0m	11.13l
	30	11.75f	11.82e	11.55h	11.64g	11.62g	11.78f	11.32j	11.42i
25	0	12.00c	12.11b	11.72f	11.82e	11.83e	11.92d	11.55h	11.68g
	30	12.17b	12.22a	11.85e	11.94d	12.01c	12.11b	11.71f	11.82e

*Means with the similar letters are not significant (P= 0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

Table 8: The effect of sewage sludge and its biochar on catalyze enzyme activity in soil in a Pb polluted soil under sunflower cultivation

Anthracene (mg/kg)	Sewage sludge (t/ha)	Azetobacter (+)				Azetobacter (-)			
		P.indica (+)		P.indica (-)		P.indica (+)		P.indica (-)	
		Sewage sludge biochar (mg/kg soil)							
		0	30	0	30	0	30	0	30
0	0	17.00l*	17.21j	16.54q	16.71o	16.78o	16.86n	16.00t	16.22s
	30	17.22j	17.37i	16.69p	16.81n	16.89n	16.91m	16.31r	16.51q
12.5	0	17.15k	17.36i	16.85n	17.00l	17.03l	17.22j	16.54q	16.75o
	30	17.38i	17.76f	16.96m	17.21j	17.25j	17.32i	16.66p	16.71o
25	0	18.11d	18.32b	17.22j	17.52g	17.76f	18.12d	17.00l	17.21j
	30	18.22c	18.59a	17.76f	18.00e	18.11d	18.35b	17.21j	17.44h

*Means with the similar letters are not significant (P= 0.05), P.indica (+), P.indica (-), Azetobacter (+) and Azetobacter (-) are the presence and absence of the Piriformospora indica fungus and Azetobacter, respectively

by 12.1% in the plant grown in the soil receiving 30 t/ha sewage sludge. In this situation, the plant Pb concentration was also increased by 14.8%.

Discussion

Based on the results of this study, application of sewage sludge and its biochar significantly affected the plant biomass and seed germination. As the studied soil has a low percentage of organic matter and naturally contains high concentrations of Pb, using organic amendment increased the soil sorption properties, thereby decreasing the soil Pb availability. Decreasing the soil Pb availability had positive effects on increasing the plant biomass. Angelova et al. investigated the effect of organic amendments on the changes in the soil chemical properties and concluded that applying these amendments had significant effects on decreasing the soil heavy metal availability[22] that is similar to our results. However, they mentioned that the soil sorption and desorption properties are dependent on the soil chemical factors such as pH. In spite of all the points mentioned, the results of this study showed that adding sewage sludge and its biochar increased and decreased the soil organic matter and soil Pb availability, respectively.

It is noteworthy to mention that many studies have shown that adding sludge to heavy metal polluted soil can increase the heavy metals concentration in the soil,[23-25] but in cases where the soil is naturally contaminated with heavy metals, the use of non-polluted organic matter can help to decrease the heavy metal concentration of the soil.[26, 27] Generally, sorption and desorption is an important factor that regulates partitioning of heavy metals between soil liquid and solid phases and controls its availability.[28]

Using sewage sludge biochar also increased the plant biomass cultivated in the Pb polluted soil that is a positive point in environmental pollution. Application of sewage sludge with its biochar may have been able to increase the soil C/N ratio, thereby decreasing the soil Pb availability.[29] Accordingly, decreasing the soil Pb availability significantly increased the plant biomass. Alaboudi et al. investigated the effect of biochar manufactured from agriculture residues and concluded that applying these organic amendments had significant effects on decreasing the soil heavy metal availability[30] that is similar to our results. It is important to note that biochar application by increasing the uptake of nutrients such as phosphorus by plants can help to increase the plant biomass.[31] However, biochar has a liming effect when it is applied to the soil; therefore, possible increment in soil pH might occur.[30] Nevertheless, biochar has received great attention during the last few years, because of its positive role in enhancement of the soil quality and plant biomass.[32] de Figueiredo et al. investigated the short-term effects of a sewage sludge biochar on the changes in the soil heavy metal availability and reported that the sewage sludge biochar production is a suitable technology to transform the sewage sludge into a useful product for agro-environmental purposes. In addition, they mentioned that applying these products had a significant effect on decreasing the soil heavy metal availability.[33]

According to the results of this study, increasing the plant biomass due to applying organic amendments significantly increased the anthracene degradation in the soil that may be related to the role of plant root exudate on increasing the soil microbial activities (respiration); thereby increasing the degradation of anthracene in the soil. Generally, th plants' root exudate can stimulate the soil enzymatic and microbiological processes that have a positive effect on degradation of petroleum hydrocarbons in the soil.[34]

Plant inoculation with P. indica or Azetobacter had also a significant effect on increasing the plant biomass. However, their additive effects on increasing the soil's microbial respiration and anthracene degradation in it cannot be ignored. The important point of this research is that plant inoculation with Azetobacter directly and indirectly affected o the anthracene degradation in the soil. Plant inoculation with Azetobacter significantly increased the plant N concentration that is an important factor for increasing the plant biomass. Plant inoculation with P. indica has also shown a positive effect on increasing the plant biomass and anthracene degradation in the soil. In addition, the co-inoculation of P. indica and Azetobacter had additive effects on increasing the soil microbial respiration, consequently affecting the degradation of anthracene in the soil.

Abiotic stresses such as heavy metals produce oxidative stress in plants by producing reactive oxygen species such as H_2O_2. It is noteworthy to mention that ROS can act as a damaging or signaling factor, which depends on the equilibrium between radical production and scavenging mechanism.[35] Accordingly, finding a suitable way to decrease the radical production is necessary. According to the results of this study, synergistic effects of Azetobacter and P.indica significantly decreased the catalase enzyme activity. However, the plant Pb concentration and plant biomass were also decreased and increased, respectively. Accordingly, it can be concluded that measuring the plant Pb toxicity plays an important role in the amount of catalase enzyme activity. Aydina et al. investigated the effects of Cd and Pb toxicity on the amount of catalase enzyme activity in tomato plants and concluded that different rates of Pb and Cd concentration had direct effects on increasing the catalase enzyme activity[35] that is similar to the results of our studies.

Conclusion

Based on the results of our studies, plant Pb toxicity had significant effects on decreasing the plant biomass, thereby decreasing the anthracene degradation in the soil. Among this, plant inoculation with P.indica or Azetobacter, significantly increased the plant biomass through increasing the plant's available micro-nutrients or decreasing its Pb concentration. Increasing the soil pollution to anthracene significantly increased the soil microbial respiration, indicating that micro-organisms use anthracene as carbon sources rather than its toxicity. However, soil chemical properties have significant effects on degradation of anthracene in the soil that should be considered in future studies. On the other hand, interaction effect of other heavy metal pollution with petroleum hydrocarbons should be considered.

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References

1 Varjani SJ. Microbial degradation of petroleum hydrocarbons. Biores Technol. 2017; 223:277-86.

2 Besalatpour A, Hajabbasi M, Khoshgoftarmanesh A, Dorostkar V. Landfarming process effects on biochemical properties of petroleum-contaminated soils. Soil Sed Contam. 2011; 20(2):234-48.

3 Hussain F, Hussain I, Khan AHA, Muhammad YS,

Iqbal M, Soja G, et al. Combined application of biochar, compost, and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. Environ Exp Botany. 2018; 153:80-88.

4 Mosaed HP, Sobhanardakani S, Merrikhpour H, Farmany A, Cheraghi M, Ashorlo S. The effect of urban fuel stations on soil contamination with petroleum hydrocarbons. Iran J Toxic 12:. 2015; 9(30):1378-84.

5 Bieganowski A, Józefaciuk G, Bandura L, Guz Ł, Łagód G, Franus W. Evaluation of hydrocarbon soil pollution using E-nose. Sensor. 2018; 18(8):2463-69.

6 Ite AE, Ibok UJ. Role of plants and microbes in bioremediation of petroleum hydrocarbons contaminated soils. Int J. 2019; 7(1):1-19.

7 Hunt LJ, Duca D, Dan T, Knopper LD. Petroleum hydrocarbon (PHC) uptake in plants: A literature review. Environ Pollut. 2019; 245:472-84.

8 Kong L, Gao Y, Zhou Q, Zhao X, Sun Z. Biochar accelerates PAHs biodegradation in petroleum-polluted soil by biostimulation strategy. J Hazard Mater. 2018; 343:276-84.

9 Chen M, Xu P, Zeng G, Yang C, Huang D, Zhang J. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: applications, microbes and future research needs. Biotechnol Adv. 2015; 33(6):745-55.

10 Murray EW, Greenberg BM, Cryer K, Poltorak B, McKeown J, Spies J, et al. Kinetics of phytoremediation of petroleum hydrocarbon contaminated soil. Int J Phytoremediation. 2019; 21(1):27-33.

11 Yazdanpanah N, Mahmoodabadi M, Cerdà A. The impact of organic amendments on soil hydrology, structure and microbial respiration in semiarid lands. Geoderma. 2016; 266:58-65.

12 Foster EJ, Hansen N, Wallenstein M, Cotrufo MF. Biochar and manure amendments impact soil nutrients and microbial enzymatic activities in a semi-arid irrigated maize cropping system. Agr Ecosys Environ. 2016; 233:404-14.

13 Shahsavari E, Schwarz A, Aburto-Medina A, Ball AS. Biological Degradation of Polycyclic Aromatic Compounds (PAHs) in Soil: a Current Perspective. Current Pollut Report. 2019:1-9.

14 Álvarez-López V, Prieto-Fernández Á, Cabello-Conejo M, Kidd P. Organic amendments for improving biomass production and metal yield of Ni-hyperaccumulating plants. Sci Total Environ. 2016; 548:370-79.

15 Lee S-H, Lee S, Kim D-Y, Kim J-g. Degradation characteristics of waste lubricants under different nutrient conditions. J Hazard Mater. 2007; 143(1-2):65-72.

16 Dindar E, Şağban FOT, Başkaya HS. Biodegradation of crude oil-contaminated soil using canned-food-industry wastewater sludge for soil application. KSCE J Civil Eng. 2017; 21(5):1623-30.

17 Zamani J, Hajabbasi MA, Alaie E, Sepehri M, Leuchtmann A, Schulin R. The effect of Piriformospora indica on the root development of maize (Zea mays L.) and remediation of petroleum contaminated soil. Int J Phytoremediation. 2016; 18(3):278-87.

18 Van Oosten MJ, Di Stasio E, Cirillo V, Silletti S, Ventorino V, Pepe O, et al. Root inoculation with Azotobacter chroococcum 76A enhances tomato plants adaptation to salt stress under low N conditions. BMC Plant biol. 2018; 18(1):205.

19 Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese, and copper. SSSAJ. 1978; 42: 421-428.

20 Kapur D, Singh KJ. Zinc alleviates cadmium induced heavy metal stress by stimulating antioxidative defense in soybean [Glycine max (L.) Merr.] crop. J Appl Natural Sci. 2019; 11(2):338-45.

21 Muñoz-Huerta RF, Guevara-Gonzalez RG, Contreras-Medina LM, Torres-Pacheco I, Prado-Olivarez J, Ocampo-Velazquez RV. A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. Sensor. 2013; 13(8):10823-43.

22 Angelova V, Akova V, Artinova N, Ivanov K. The effect of organic amendments on soil chemical characteristics. Bulgarian J Agr Sci. 2013; 19(5):958-71.

23 Tytła M. Assessment of heavy metal pollution and potential ecological risk in sewage sludge from municipal wastewater treatment plant located in the most industrialized region in Poland- case Study. Int J Environ Res Public Health. 2019; 16(13):1-16.

24 Siddig NA, Ahmed AA, Saad SA, Koua FHM. Heavy metals accumulation from sewage sludge in the Nile tilapia Oreochromis niloticus (Trewavas, 1983) during a sludge-earthworm-fish short-term cycling. Ital J Food Safe. 2019; 8 (1): 7257-62,

25 Shakarami m, Maroufi S. Wastewater and sewage sludge effects on the absorption of some heavy metals in soil and mint. J Environ Stud. 2019; 45(1):1-15.

26 Penido ES, Martins GC, Mendes TBM, Melo LCA, do Rosário Guimarães I, Guilherme LRG. Combining biochar and sewage sludge for immobilization of heavy metals in mining soils. Ecotox Environ Safe. 2019; 172:326-33.

27 Bień J, Celary P, Morzyk B, Sobik-Szołtysek J, Wystalska K. Effect of additives on heavy metal immobilization during vitrification of tannery sewage sludge. Environ Protect Eng. 2013; 39(2):33--40.

28 Tang J, Cao C, Gao F, Wang W. Effects of biochar amendment on the availability of trace elements and the properties of dissolved organic matter in contaminated soils. Environ Technol Inno. 2019; 16: 100492.

29 Kabbashi N. Sewage sludge composting simulation as carbon/nitrogen concentration change. J Environ Sci. 2011; 23(11):1925-28.

30 Alaboudi KA, Ahmed B, Brodie G. Effect of biochar on Pb, Cd and Cr availability and maize growth in artificial

contaminated soil. Annal Agr Sci. 2019; 64(1):95-102.

31 Tripti, Kumar A, Usmani Z, Kumar V, Anshumali. Biochar and flyash inoculated with plant growth promoting rhizobacteria act as potential biofertilizer for luxuriant growth and yield of tomato plant. J Environ Manage. 2017; 190:20-27.

32 Gondek K, Mierzwa-Hersztek M, Kopeć M, Sikora J, Lošák T, Grzybowski P. Sewage sludge biochar effects on phosphorus mobility in soil and accumulation in plant. Ecol Chem Eng . 2019; 26(2):367-81.

33 de Figueiredo CC, Chagas JKM, da Silva J, Paz-Ferreiro J. Short-term effects of a sewage sludge biochar amendment on total and available heavy metal content of a tropical soil. Geoderma. 2019; 344:31-39.

34 Jin J, Wang M, Lu W, Zhang L, Jiang Q, Jin Y, et al. Effect of plants and their root exudate on bacterial activities during rhizobacterium–plant remediation of phenol from water. Environ Int. 2019; 127:114-24.

35 Aydin SS, Büyük İ, Gündüzer EG, Büyük BP, Kandemir I, Cansaran-Duman D, et al. Effects of lead (Pb) and cadmium (Cd) elements on lipid peroxidation, catalase enzyme activity and catalase gene expression profile in tomato plants. J Agr Sci. 2016; 22: 539-547.