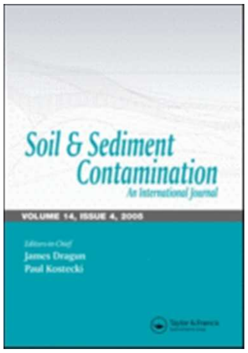

Using maize (Zea mays L.) and sewage sludge to remediate a petroleum contaminated calcareous soil

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Using maize (*Zea mays* L.) and sewage sludge to remediate a petroleum contaminated calcareous soil

Abstract:

Phyto-stimulation, the use of plants to stimulate activity of microorganisms in root zone, has been proposed as an approach to promote the degradation of petroleum hydrocarbons and thus the remediation of petroleum-polluted soils. In this study, we investigated the potential use of sewage sludge to enhance phyto-stimulating effects of maize (*Zea mays* L.) on the elimination of an aged petroleum contamination in a calcareous soil. In a pot experiment, maize was grown on the experimental soil for 2 months at three levels of sewage sludge application (0, 20, and 50 g dry matter of sludge per kg soil). The amendments increased root and shoot growth of the experimental plants approximately by a factor of two at the lower sludge treatment level and by a factor of five at the higher sludge treatment level. In a separate incubation experiment, sludge application also led to an immediate stimulation of soil respiration, which then further increased over time. The initial stimulation was three times larger at the higher than at the lower treatment level, but the rate of subsequent increase was similar in both treatments. The two sludge treatments also accelerated TPH elimination in the contaminated soil, and again the effect was approximately three times stronger at the higher than at the lower treatment level. The sludge effect on TPH elimination was much stronger than the effect of the plants. More than half of the initial contamination was reduced in combined treatment with maize and sludge application at highest rate. The results show that sewage sludge can substantially enhance the remediation of petroleum-contaminated soil, especially when applied in conjunction with a suitable plant such as maize.

Keywords: petroleum-contaminated soil, phyto-stimulation, biosolid, microbial respiration

Introduction

Contamination with petroleum hydrocarbons is a major threat to the environment in some areas and in particular to soils (Head et al., 2006; Besalatpour et al., 2011). These contaminants may damage soil fertility and pose a major threat to the health of humans and other organisms when they enter into waters and food chains (Besalatpour et al., 2010). Petroleum hydrocarbon contamination may occur in particular through ~~losses~~ wastes during oil extraction and refining, accidental spills, pipeline ruptures, leakage from storage tanks, and land disposal of petroleum wastes (Scott and Nelson, 2004; Diab, 2008; Soleimani et al., 2010; Al-Yemeni et al., 2010) and is a problem in both petroleum producing and consuming countries (Shirdam et al., 2009; Soleimani et al., 2010). There are various chemical and physical methods, such as soil washing, solidification, stabilization and thermal treatment, which can be applied to remediate petroleum-contaminated soils. However, these treatments are not only destructive~~distractive~~ to the soil, but also expensive and energy-intensive (Kaimi et al., 2006; Soleimani et al., 2010). Bioremediation on the other hand is an environmentally friendly method to eliminate organic soil contaminations (Alexander, 1999). To enhance its efficiency, ~~various authors~~many researchers proposed to combine this method with planting of suitable vegetation (Edema et al., 2011; Besalatpour et al., 2011; Soleimani et al., 2013).

Utilizing plants to stimulate microbial degradation of organic soil contaminants is called phyto-stimulation (Kuiper et al., 2004). The ability of plants to stimulate microbial degradation of petroleum compounds in their root zone has been demonstrated in many studies (e.g. Bertin et al., 2003; Bais et al., 2006; Diab, 2008; Tanee and Kinako, 2008; Al-Yemeni et al., 2010) and is thus considered a promising green and cost effective approach for the remediation of petroleum polluted soils and could be applied commercially on a large scale (Jørgensen et al., 2000). Often, however, microbial growth and activity in polluted soils is limited due to low organic matter content and lack of nutrients, in particular nitrogen and phosphorus (Zhang et al., 2011). Applying organic amendments like sewage sludge may in many cases improve these conditions and thus facilitate the degradation of organic contaminants, including petroleum hydrocarbons (Garcia-Gil et al., 2000; Dickinson and Rutherford, 2006; Al Zoubi et al., 2008; Agamuthu et al., 2013; Bourioug et al., 2014). For example, Rivera-Espinoza and Dendooven (2004) found that more ~~diesel~~diesels ~~was~~were mineralized, as indicated by CO_2 production, when sewage sludge was applied to a petroleum-polluted nutrient-poor soil. Rocchetti et al. (2011) showed that the rate of petroleum hydrocarbon bio-degradation was higher in the presence of fresh than mature compost 3 weeks after application, which they explained their finding with a larger amount of easily degradable organic substrates and stronger sorption of hydrophobic contaminants in the fresh compost than in the mature compost. In another study, the application of peat fertilizer enhanced petroleum degradation by 12.4% in the presence of burning bush (*Kochiascoparia* (L.) Schard) and by even 34.7% in combination with common flax (*Linumusitatissimum* L.) compared to soil with no amendment (Ravanbakhsh et al., 2009). Similar effects were also observed in hydrocarbon-contaminated soils planted with either maize (*Zea mays* L.) or cowpea (*Vignaunguiculata* (L.) Walp) after application of organic fertilizers such as poultry manure and cassava peels (Jidere et al., 2012).

While these are encouraging results, there is still little knowledge on how different biosolids affect the bio-degradation of petroleum hydrocarbons in soils and how they interact with the effects of plants grown for phyto-stimulation, particularly in the calcareous soils. Addressing this gap, the present study investigated the effect of sewage sludge application on the elimination of an aged petroleum contamination in a calcareous soil in combination with the cultivation of maize (*Zea mays* L.).

Materials and methods

Soil and sewage sludge ~~sampling and~~ characterization

The soil used for this study was collected from a petroleum-contaminated site around the Isfahan Oil Refinery Complex at Bakhtiardasht in central Iran (32°48' N, 51°32' E). The contamination was due to many years of oil leakage from the complex into the soil and the concentration of total petroleum hydrocarbons (TPH) was approximately 36.5 g kg^{-1}. The site, which has been used for Oil Refinery Complex since 1979, has an arid climate and long-term temperature mean and ~~mean~~ annual rainfall averages are 14.5°C and about 140 mm, respectively. Three composite soil samples were collected from 0 to 30 cm depth, thoroughly homogenized, and passed through a 2 mm sieve. Basic physical and chemical soil properties are given in Table 1.

The sewage sludge was obtained from the municipal Northern Waste Water Treatment Plant of the province of Isfahan (Shahinshahr). The sludge was air-dried, crushed using a wooden mallet, and manually mixed. Sub-samples were then taken to determine the properties given in Table 2.

Table 1

Physiochemical properties of the experimental soil

Table 2

Characteristics of the sewage sludge used in this study

Electrical conductivity (EC) and pH in soil were measured in a saturated extract by means of a glass electrode, while EC and pH in sewage sludge were measured in suspension with distilled water at a sludge:water ratio of 1:5 (w/v). Soil organic carbon (OC) was determined using the wet-combustion method of Nelson and Sommers (1982). Total nitrogen (N) was measured using the micro-Kjeldahl method (Bremner and Mulvaney, 1982). The particle size distribution of soil was determined using the pipette method (Gee and Bauder, 1986), and calcium carbonate by back-titration (Soil Conservation Service, 1972). ~~DTPA-extractable~~ Soil extractable metals were obtained by DTPA (Diethylene Triamine Pentaacetic Acid) ~~were obtained~~ using the extraction procedure of Leggett and Argyle (1983) and analyzed in the extracts by means of an atomic absorption spectrophotometer (PerkinElmer-AAnalyst 200). Available phosphorus (P) and potassium (K) were determined as described by Olsen and Sommers (1982) and- Knudsen et al. (1982), respectively. Bulk density of soil was determined from separately collected volumetric core samples (Blake and Hartge, 1986).

Total metal contents in sewage sludge were obtained using sample digestion with nitric and perchloric acid and atomic absorption spectrophotometry (PerkinElmer - AAnalyst 200) for the analysis of the digests. Wet digestion was also used to obtain total P and K of sewage sludge.

Sewage sludge application and greenhouse experiment

~~The sewage sludge was obtained from the municipal Northern Waste Water Treatment Plant of the province of Isfahan (Shahinshahr). The sludge was air-dried, crushed using a wooden mallet, and manually mixed. Sub-samples were then taken to determine the properties given in Table 2.~~

~~Table 2~~

~~Characteristics of the sewage sludge used in this study~~

~~EC and pH were measured in suspension with distilled water at a sludge:water ratio of 1:5 (w/v). Organic carbon and total N were determined using the same procedures as for the soil samples. Total metal contents were obtained using sample digestion with nitric and perchloric acid and atomic absorption spectrophotometry (PerkinElmer - AAnalyst 200) for the analysis of the digests. Wet digestion was also used to obtain total P and K.~~

The experiment was set up in a complete randomized factorial design with three different levels of sewage sludge application [0, 20 and 50 g sewage sludge per kg soil, denoted as S0, S20 and S50, respectively] and two levels of plant treatment [with and without maize (*Zea mays* L.)]. Each treatment combination was replicated 3 times. Plastic pots were filled with 3 kg soil each, into which 0, 20 or 50 g dry sewage sludge (by weight) had been mixed, depending on the treatment. All treatments received an initial fertilization with ammonium nitrate (4 g NH_4NO_3 per kg soil). After planting a single maize seed at 1-2 cm below the soil surface into each pot, the pots were kept under controlled climatic conditions (temperature $28\pm4^{\circ}$C, day/night cycle 15/9 h, and 48±7% relative humidity) in a greenhouse. Soil moisture was kept approximately at 70% field capacity during plant growth by periodical watering. After 2 months of growth, the maize plants were harvested and separated into shoots and roots. All plant samples were washed several times with distilled water, dried and weighed. Furthermore, a 10-gram soil sample was taken at 8 cm depth from each pot, including the unplanted controls, in order to determine (TPH) concentrations as described below.

In addition, an incubation experiment was performed to determine microbial respiration using the method described by Alef (1995). For this purpose, samples of about 500 g soil were taken from each sludge treatment at the beginning of the experiment and incubated for one day at 25°C in 500-mL glass containers closed with rubber stoppers in three replicates. Then, a test tube containing 10 mL of a 0.5 M NaOH solution was placed into the containers to trap the evolving CO_2. The trapped CO_2 was determined by titrating the excess in alkali with HCl. Three glass containers without soil were incubated in the same way as controls. The incubation was repeated using the same samples 3, 10, 17, 24, 31, 38, 45, 55, 65 and 75 days after sludge application.

TPH analysis

Total petroleum hydrocarbons were Soxhlet-extracted from10-gram air-dried soil samples using a 1:1 (v/v) mixture of 125 mL dichloromethane and n-hexane as described by Christopher et al. (1988). The extracts were sequentially purified and cleaned up using silica gel 60 (0.063–0.200 mm, Merck) to sorb polar compounds. The extractant was evaporated in a pre-weighed dish and the amount of TPH determined by taking the weight of the residue. All TPH concentrations are given relative to the final weight of the soil samples after drying at 60°C (USEPA, 1998).

Statistical analysis

The results of the experiment were analyzed using analysis of variance (ANOVA). Differences between treatment means were t-tested for Least Significant Differences. All statistical analyses were performed using SAS Version 9.1.3 (SAS Institute, 2005).

Results

The soil was calcareous and slightly saline (Table 1). The pH was in a range (7.5 to 7.8) considered optimal for mineralization of petroleum compounds (Dibble and Bartha, 1979).

The concentrations of available P and K indicated sufficient supply of these elements to the plants. In contrast, the unamended soil was rather poor in total N and OC. Application of sewage sludge not only provided OC, N, P, and K, but also micronutrient elements, including Fe, Zn, Cu and Mn (Table 2).

Root and shoot biomass of the experimental maize plants increased strongly with increasing level of sludge application (Figure 1). The S20 treatment approximately doubled root and shoot growth compared to the control (S0), while the S50 treatment even led to a roughly five-fold increase.

Table 3 shows that both factors, sludge application and incubation time, had highly significant effects on microbial soil respiration. Without sewage sludge application, respiration was below detection over the first 2-3 weeks of the incubation experiment and did never exceed 2 mg CO_2 d^{-1} at later stages (Figure 2). With the application of sewage sludge, microbial respiration showed a strong initial increase to around 10 mg CO_2 d^{-1} within 3 days in the S20 treatment and to around 30 mg CO_2 d^{-1} in the S50 treatment, after which it further increased much more slowly to reach around 45 mg CO_2 d^{-1} and 60 mg CO_2 d^{-1}, respectively, at the end of the experiment. While the higher dose of sludge application resulted in a higher respiration rate than the lower dose, the difference between the two sludge treatments remained approximately constant or was even slightly decreasing over time after the initial jump.

Table 3

ANOVA results for the effects of sludge application and incubation time on soil microbial respiration rate.

Also petroleum hydrocarbon elimination was strongly increased with increasing level of sludge application (Table 4, Figure 3). Without sewage sludge (S0) and plants there was less than 8% decrease in TPH over the 2-month duration of the experiment. After sewage sludge application the contamination was reduced by about 20% in the S20 treatment at the end of the experiment and by around 45% in the S50 treatment. The cultivation of maize also enhanced TPH elimination, but interestingly this effect decreased with the level of sewage sludge application and thus also relative to the sludge effect on root and shoot biomass shown in Figure 1. In the combined treatment with maize and sludge application at the highest rate (S50) the contamination was reduced by more than 50% of the initial TPH concentration of the soil.

Table 4

ANOVA results for the effects of sludge application level and presence of maize (*Zea mays* L.) on soil TPH concentration.

Discussion

The strong increase in TPH elimination after sewage sludge application is in agreement with the results of other studies showing similar enhancement effects of other organic soil amendments on the elimination of hydrocarbons in polluted soils (Rivera-Espinoza and Dendooven, 2004; Ravanbakhsh et al., 2009; Rocchetti et al., 2011; Jidere et al., 2012). Approximately half of the contamination was eliminated during the experiment at the upper rate of sludge application (S50), and there was no indication that this effect was close to approaching a maximum. On the contrary, the increase in TPH elimination from the lower (S20) to the higher (S50) application rate was roughly proportional to the effect of increasing the application rate from zero (S0) to the lower level. This suggests that substantial additional enhancement would still have been possible by further increasing the application rate. This conclusion is supported also by the findings of other authors. Namkoong et al. (2002), who investigated TPH degradation in diesel-contaminated soil at various mixing ratios between soil and either sewage sludge or compost, observed that the maximum rates of TPH elimination were reached for both amendments at a mixing ratio of 2:1. The same ratio was also found to be optimal for mixtures of petroleum-contaminated soils and field compost in the study of Wang et al. (2011), who achieved 38% TPH degradation with this mixing ratio after 150 days.

The sludge effect on TPH elimination did not depend on the presence of the maize plants. Although the effect was stronger in their presence than in their absence, the increase in the magnitude of the effect with increasing level of application rate was less in the presence of the plants than in their absence. In other words, the effects of sludge and plant were not only less than additive, the additional effect in response to the presence of the plants also decreased with increased level of sewage sludge application. This suggests that there was some negative interaction between the effects of the two types of treatment, such as competitive or even inhibitory interaction, that increased with the rate of sludge application. There are various ways how this could be explained. The presence of plant roots could enhance hydrocarbon degradation in soil directly, e.g. through the exudation of exo-enzymes, as well as indirectly, for example by exuding hydrocarbon-solubilizing compounds such as surfactants (Selberg et al., 2013) or by promoting microbial hydrocarbon degradation activity (Soleimani et. al., 2010). The latter could be achieved in particular by supplying nutrients, by stimulating co-metabolization of petroleum hydrocarbons through the supply of easily degradable organic carbon substrates, or by creating favorable physical soil conditions for the growth of hydrocarbon-degrading microbial communities (Van Epps, 2006). Similar effects on soil microbial activities can be expected from the sludge applications (Garcia-Gil et al., 2000; Dickinson and Rutherford, 2006). Negative interactions between the two treatments could thus have resulted for example from limitations in the availability of the petroleum hydrocarbons for degradation, from increased consumption of root-derived substrates by sludge-stimulated microbial populations at the expense of rhizosphere populations that were present also in the absence of sludge, or from enhanced decomposition of root exudates with degrading (such as enzymes) or mobilizing (such as surfactants) effects on hydrocarbons.

Negative interactions between the two treatments could have occurred also through competition of plants and microorganisms for growth-limiting soil resources. However, the maize biomass data do not show that the sludge treatments inhibited plant growth. On the contrary, they led to a strong increase in growth, below and aboveground. This could be due in particular to a fertilizer effect as well as to the removal of toxicity stress. Given the very low total N content of the unamended soil, it is likely that plant growth was primarily limited by the availability of N, for which the sludge on the other hand was a rich source (Table 2). Apart from a fertilizer effect, also inactivation of phytotoxic soil contaminants due to increased sorption, associated in particular with the input of organic matter, could have contributed to the enhanced plant growth in the sludge treatments. There are many reports of phyto-toxic effects of hydrocarbons in soil on crop plants (Chaineau et al., 1997; Palmroth et al., 2002; Merkl et al., 2005; Kechavarzi et al., 2007; Ravanbakhsh et al., 2009; Besalatpour et al., 2010). Chaineau et al. (1997) reported inhibited seed germination and reduced growth of maize, wheat and bean in soil contaminated with petroleum hydrocarbons. Although maize was much more tolerant to the contamination than wheat and bean, the aerial biomass of maize was decreased by up to 30% at a TPH concentration of 1.2%.

In contrast to plant growth and TPH elimination, the release of CO_2 from the soil responded very strongly at the lower level of application, while there was very little additional effect at the upper treatment level. Given that the release of CO_2 can be attributed primarily to microbial respiration in the plant-free treatments (Namkoong et al., 2002; Atagana, 2008), this decrease in responsiveness to the rate of sludge application suggests that microbial activity was close to an upper limit. A limitation in the availability of a degradable substrate can be excluded as the sludge was rich in organic carbon with a low C:N ratio. Likewise, a limitation in N, P or another major mineral nutrient can be excluded, as the sludge was rich also in these nutrients, and in fact plant growth did not show such a limitation. For similar reasons, toxicity effects are not a likely reason either. There were no indication of toxicity effects on plant growth, and the concentrations of toxic metals in the sludge were within the tolerance limits of the United States Environmental Protection Agency (USEPA, 1994), indicating that the sludge was of good quality for agricultural use. ~~Sewage sludge effect on microbial respiration indicator of microbial activity.~~

Whatsoever the reasons are for the discrepancy, a reduced responsiveness of soil respiration to sludge application at a treatment level at which TPH elimination and plant growth were apparently still fully responsive does not necessarily mean that there was no direct link between soil microbial activity and petroleum hydrocarbon degradation and does not contradict results indicating such links as reported e.g. by Wibbe and Blanke (1999) and Namkoong et al. (2002). It is plausible and even likely that only a small fraction of the microbial community co-metabolized petroleum hydrocarbons. This fraction may have become more dominant with the increase in sludge application rate relative to the majority of microorganisms that did not metabolize them, although one might argue that such dominance should rather be favored by a low and not a high availability of alternative easily degradable carbon substrates. Another, and perhaps more plausible explanation thus may be that the concentration of petroleum hydrocarbons that was available for degradation in the soil solution was the limiting factor and not the size of the microbial degrader populations and that this availability was increased with application rate through some hydrocarbon-mobilizing compound that was brought into the soil with the sludge or produced from a component of the sludge.

Conclusions

While still many questions remain open, the results of this study show that sewage sludge of adequate quality has the potential of being used as an efficient soil amendment to accelerate the bio-remediation of petroleum-contaminated land and to substantially enhance the phyto-stimulation effect of remediation plants such as maize. Although the additional remediation effect of the plants was substantial only in combination with sludge at the lower application rate and rather small at the higher rate, it should not be forgotten that combining such soil amendments with crop plant cultivation also has other potential benefits such as soil protection against erosion and generating revenue from the production of marketable biomass, such as biofuel, biochar or even livestock feed, depending on the quality of the produce.

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Figure Captions:

Figure 1. Root and shoot biomass of maize (*Zea mays* L.) after 2 months growth in petroleum-contaminated soil at three levels of sewage sludge application: 0 (S0), 20 (S20), and 50 (S50) g dry matter of sludge per kg soil. Error bars represent standard errors.

Figure 2. Evolution of soil microbial respiration rate during incubation of the petroleum-contaminated experimental soil after sewage sludge application at three different treatment levels: 0 (S0), 20 (S20), and 50 (S50) g dry matter of sludge per kg soil. Error bars represent standard errors.

Figure 3. Fraction (in % of initial concentration) of TPH eliminated from the experimental soil 2 months after sewage sludge application with and without cultivation of maize plants at three different sludge treatment levels: 0 (S0), 20 (S20), and 50 (S50)g dry matter of sludge per kg soil. Error bars represent standard errors, and bars with different letters are significantly different from each other ($p < 0.05$).

Table 1

Physiochemical properties of the experimental soil

Parameter	Unit	Mean value
Sand	%	72.5±3.5
Silt	%	13.6±1.8
Clay	%	13.9±3.2
Texture	---	Sandy Loam
Bulk density	g cm^{-3}	1.32±0.14
EC at 25 °C	dS m^{-1}	4.0±0.1
pH	---	7.8±0.08
$CaCO_3$	%	27.45±5
Organic carbon	%	0.23±0.03
Total N	mg kg^{-1}	602±37
Available P	mg kg^{-1}	502±29
Available K	mg kg^{-1}	250±14
$Fe_{\text{DTPA extractable}}$	mg kg^{-1}	22±6
$Zn_{\text{DTPA extractable}}$	mg kg^{-1}	13.0±3.5
$Cu_{\text{DTPA extractable}}$	mg kg^{-1}	8.0±2.1
$Mn_{\text{DTPA extractable}}$	mg kg^{-1}	15.2±1.6
C:N ratio	---	3.9±0.2

Table 2

Characteristics of the sewage sludge used in this study

Parameter	Units	value	U.S. EPA limit
Ash content	%	62.4±4.7	---
EC $_{25\ °C}$	dS m^{-1}	7.1±0.4	---
pH	---	7.2±0.2	---
OC	%	33.6±1.6	---
Total N	%	4.2±1.23	---
Available P	mg kg^{-1}	1710±36	---
Available K	mg kg^{-1}	1230±29	---
Total Fe	mg kg^{-1}	366±27	---
Total Zn	mg kg^{-1}	960±19	2800
Total Cu	mg kg^{-1}	62.5±2.3	1500
Total Mn	mg kg^{-1}	210±17	---
Total Pb	mg kg^{-1}	3.61±1.5	300
Total Cd	mg kg^{-1}	< 0.005	39
Total Ni	mg kg^{-1}	30.5±2.1	420
Total Co	mg kg^{-1}	4±0.6	---
C:N ratio	---	8±0.5	---
	g kg^{-1}	36.5±1.3	

Table 3

ANOVA results for the effects of sludge application and incubation time on soil microbial respiration rate.

Source of variation	Degrees of freedom	Mean squares
Sludge level	2	14492.48**
Time	9	640.25**
Time × sludge level	18	157.51**
Error	58	15.76

**significant at $P < 0.01$.

Table 4

ANOVA results for the effects of sludge application level and presence of maize (*Zea mays* L.) on soil TPH concentration.

Source of variation	Degrees of freedom	Mean squares
Sludge level	2	3.46^{**}
plant	1	0.35^{**}
Plant × sludge level	2	0.0006^{ns}
Error	10	0.0012^{ns}

“ns” no significant effect, **significant at P < 0.01.

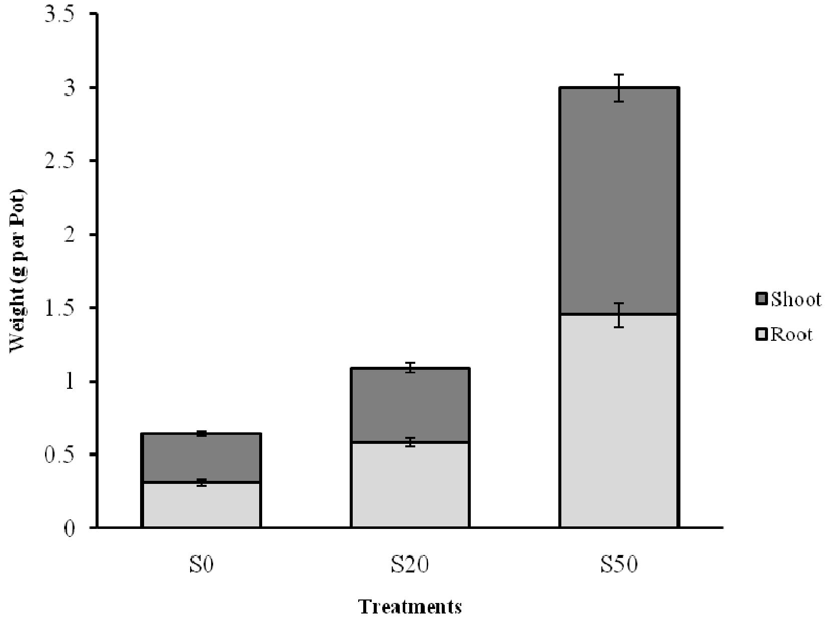

Figure 1. Root and shoot biomass of maize (*Zea mays* L.) after 2 months growth in petroleum-contaminated soil at three levels of sewage sludge application: 0 (S0), 20 (S20), and 50 (S50) g dry matter of sludge per kg soil. Error bars represent standard errors.

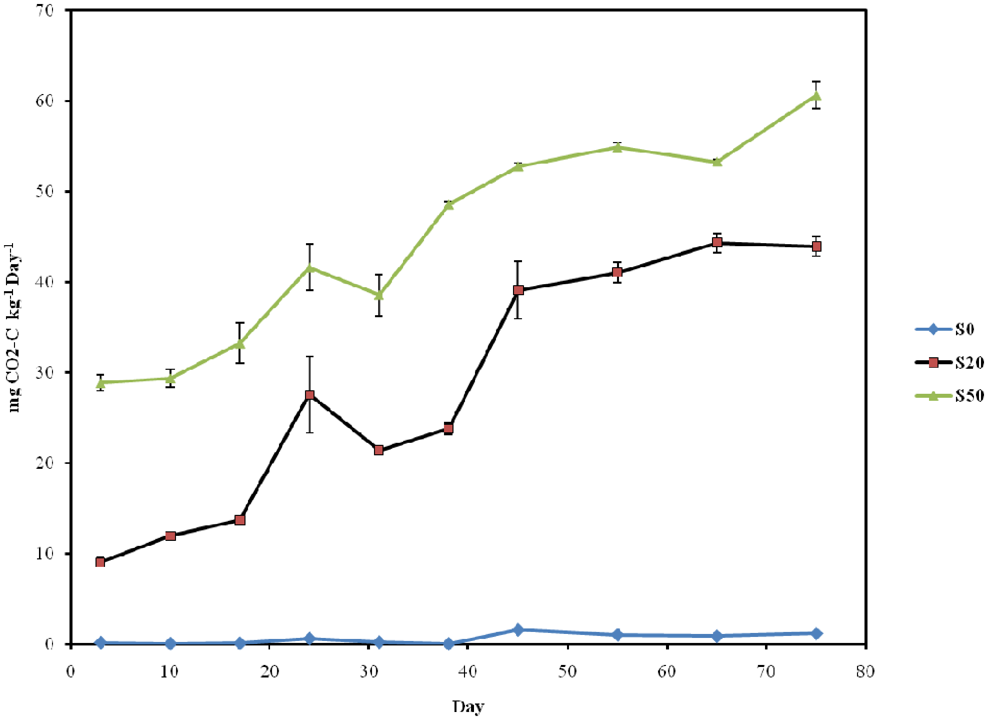

Figure 2. Evolution of soil microbial respiration rate during incubation of the petroleum-contaminated experimental soil after sewage sludge application at three different treatment levels: 0 (S0), 20 (S20), and 50 (S50) g dry matter of sludge per kg soil. Error bars represent standard errors.

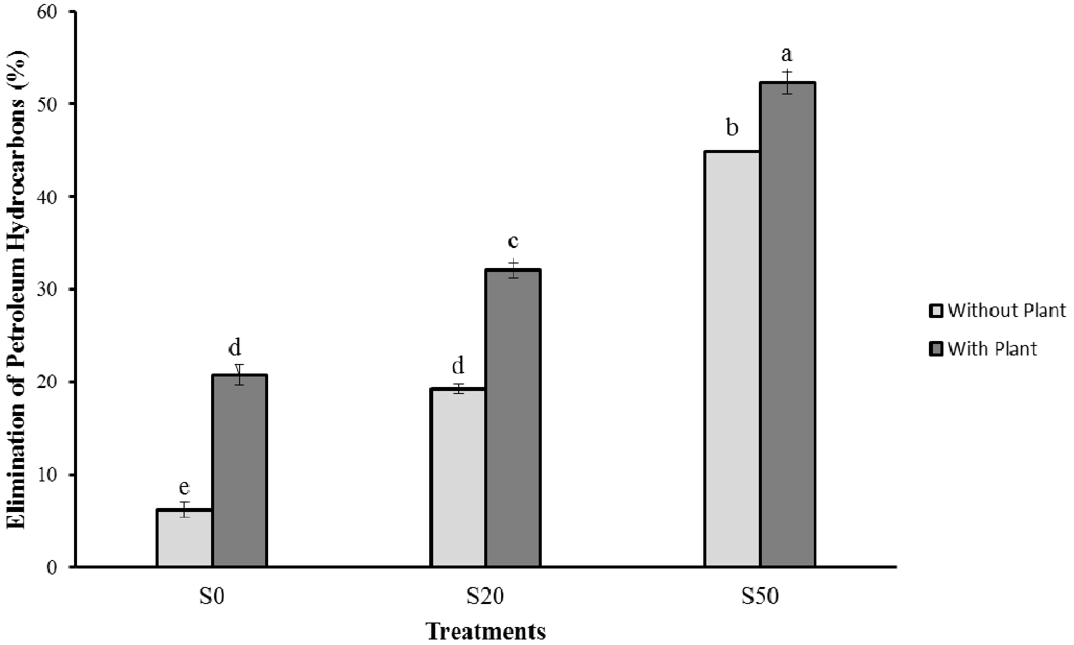

Figure3. Fraction (in % of initial concentration) of TPH eliminated from the experimental soil 2 months after sewage sludge application with and without cultivation of maize plants at three different sludge treatment levels: 0 (S0), 20 (S20), and 50 (S50)g dry matter of sludge per kg soil. Error bars represent standard errors, and bars with different letters are significantly different from each other ($p < 0.05$).