Effects of vegetable oil residue after soil extraction on physical-chemical properties of sandy soil and plant growth

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Received 3 January 2008; revised 31 January 2008, accepted 28 March 2008

Abstract

Vegetable oil has the ability to extract polycyclic aromatic hydrocarbons (PAHs) from contaminated sandy soil for a remediation purpose, with some of the oil remaining in the soil. Although most of the PAHs were removed, the risk of residue oil in the soil was not known. The objective of this study was to evaluate the effects of the vegetable oil residue on higher plant growth and sandy soil properties after soil extraction for a better understanding of the soil remediation. Addition of sunflower oil and column experiment were performed on a PAH contaminated soil and/or a control soil, respectively. Soils were incubated for 90 d, and soil pH was measured during the soil incubation. Higher plant growth bioassays with Avena sativa L. (oat) and Brassica rapa L. (turnip) were performed after the incubation, and then soil organic carbon contents were measured. The results show that both the nutrient amendment and the sunflower oil degradation resulted in the decrease of soil pH. When these two process worked together, their effects were counteracted due to the consumption of the nutrients and oil removal, resulting in different pH profiles. Growth of A. sativa was adversely affected by the sunflower oil, and the nutrient amendments stimulated the A. sativa growth significantly. B. rapa was more sensitive to the sunflower oil than A. sativa. Only 1% sunflower oil addition plus nutrient amendment stimulated B. rapa growth. All the other treatments on B. rapa inhibited its growth significantly. The degradation of the sunflower oil in the soils was proved by the soil organic carbon content.

Key words: soil; vegetable oil; plant growth; organic carbon; pH

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants, and they cause concern because of their toxic, carcinogenic, and mutagenic effects (Tao et al., 2004). Manufactured gas plant (MGP) sites are common in many countries. The primary organic pollutants of concern from MGP sites are PAHs (Hawthorne et al., 2002). Dissolution of contaminants from MGP contaminated soils using vegetable oil is a cost-effective method to remediate these soils (Isosaari et al., 2001). In our previous research, more than 90% of total PAH, including high molecular-weight congeners, was removed from the MGP soil using sunflower oil extraction (Gong et al., 2005a, 2005c, 2006). However, the sunflower oil had a high viscosity and 5%–10% of oil remained in the soil after extraction. The effects of the vegetable oil on soil characteristics and functions are still not well known. The objectives of this study were to evaluate the effects of sunflower oil residue on higher plant growth and biodegradation of oil residue shown by organic carbon contents.

Materials and methods

1.1 Soils and sunflower oil

A PAH-contaminated soil was collected near an abandoned MGP site in Berlin, Germany. It was shown in the previous study that some of oil remained in the soil after the extraction (Gong et al., 2006). To test different amounts of oil residue on soil properties and plant growth, a commercially available control soil was used, and 1%–15% of oil was artificially added to the control soil. The control soil, which was obtained from Agricultural Analytic and Research Centre, Speyer, Germany, was chosen because it has often been used as a control in soil ecotoxicological testing, and it is a natural soil of good quality (Loureiro et al., 2005, 2006). All the experiments described herein were carried out in the lab of Institute of Ecology, Berlin University of Technology. Typical characteristics of the MGP and control soils are presented in Table 1. Both the soils are sandy soils; thus, the comparability of the results is ensured.

The MGP soil was sieved through a 2-mm mesh, which was achieved by pressing the soil on the sieve, homogenized by mechanical mixing, and stored at 4 °C in refrigerator for the following experiments. The control soil...
had a particle size of < 5 mm. Sunflower oil, which was used for family cooking, was purchased from a supermarket in Berlin, Germany.

### 1.2 Soil treatments

Two kinds of soil treatments were first conducted: addition of sunflower oil (1%-15%, V/W) into the control soil, and column experiments on both the control and MGP soils. The control soil was artificially added with 10, 50, 100, and 150 ml of sunflower oil per kg soil dry mass (1%, 5%, 10%, and 15% of sunflower oil addition, V/W), and a blank was also set. Homogenization was then performed using a hand mixer. Column experiments were performed with the control and MGP soils for comparison of oil residue biodegradation and their effects on higher plant growth. The design of the soil column is shown in Fig.1. One liter of sunflower oil was poured on to the soil column. When this oil had percolated from the soil column, a second liter of sunflower oil was added. About 100 ml of the sunflower oil remained in the soil after the two percolations. Nearly 90% of individual PAHs were removed from the MGP soil by the sunflower oil. The details of the PAH removal are found in previous publications (Gong et al., 2005a, 2005c, 2006).

The individual oil-treated MGP and control soils were then divided into two parts, with one part being amended with nutrients by adding 0.35 g NH₄Cl, 0.1 g CaCl₂, 0.5 g NaNO₃, 1 g K₂HPO₄, 0.3 g MgSO₄·7H₂O, 0.1 g CaCl₂, and 0.05 g FeCl₂ per kg soil. To test oil residue degradation, all the soils, regardless of amendment, were incubated in 500-ml bottles at 20°C in darkness for 90 d. Every bottle was tightly sealed with a screw type lid. Water was occasionally added to maintain a sufficient moisture content. Duplicate soil samples were taken from the soils at 1, 28, and 84 d for soil pH measurements using an ISO method (ISO/DIS 10390). A summary of soil treatment nomenclature is presented in Table 2.

### 1.3 Plant growth bioassay

Plant growth bioassay was conducted on all the soils after the soil treatments according to an ISO (International Standard Organization) method (ISO 11269-2). A monocotyledonous and dicotyledonous plants were selected based on the species list presented in the ISO guideline: Avena sativa L. (oat) and Brassica rapa L. (turnip), respectively. During the bioassay, plastic pots with 150 g of the experimental soil were placed with 10 seeds at a depth of 1 cm from the soil surface, and water was added to the pots when needed to maintain a sufficient moisture content. All the pots were placed in a greenhouse at 20°C with 12 h light irradiation per day. After the emergence assessment within each pot, thin the seedlings to give a total of five evenly spaced representative specimens of the plants in the plots. After 15 d, plants were harvested and the growth measured as shoot fresh weight and recorded in all bioassays. All tests were carried out with four replicates.

### 1.4 Soil organic carbon analysis

All the soil samples after the plant growth bioassays were subjected to soil organic carbon analysis (C_{org}). One gram of the soil sample was added to a 50 ml test tube with 10 ml of dichromate-sulfuric acid and 5 ml of concentrated sulfuric acid. The test tubes were shaken for 90 min, and the supernatants were withdrawn and measured using a spectrophotometer at a wavelength of 578 nm to determine the organic carbon contents.

### 1.5 Statistical analysis

Average biomasses of A. sativa and B. rapa after the plant growth bioassay were compared statistically using
one-way analysis of variances (ANOVA). As the effects of the nutrient and column experiments on plant growth were very obvious and different, the treatments on the control soil with no nutrient added (C-0, C-1, C-5, C-10, and C-15), with nutrient added (C-0n, C-1n, C-5n, C-10n, and C-15n), and column treatments (C-C, C-Cn, MGP-C, and MGP-Cn) were compared separately. Whenever significant differences between treatments were found, the homogeneity of the variances was checked using Levene’s test, and posthoc multiple comparison using least square difference (LSDs) was conducted provided that equal variance could be asserted. All statistical analyses were conducted using the statistical software package SPSS 11.5, at 95% confidence.

2 Results

2.1 Soil pH variation in the incubation process

The nutrient mixture was acidic because after the nutrient amendments, all the soil pH decreased immediately (Tables 3 and 4). For the blank control without the oil addition, the soil pH was nearly uniform during the incubation process (C-0 and C-0n) (Table 3). However, the sunflower oil in the control and MGP soils without the nutrient addition (C-1, C-5, C-10, and C-15 in table 3, and C-C in Table 4) obviously reduced the soil pH values. The sunflower oil in the soil was gradually anaerobically degraded during the incubation due to the lack of oxygen, which resulted in acid production. Thus, the greater the oil was, the more significantly reduced the pH was. Soil treatments, C-1n, C-5n, C-10n, C-15n, and C-Cn, showed different pH profiles of the first 28 d incubation process, with the first two showing pH increasing and the last three showing nearly uniform pH. When the nutrient amendment and sunflower oil degradation worked together, the effects of these two processes were counteracted due to the consumption of the nutrients and oil removal, which explains the differences in the pH profiles. For C-1n and C-5n, the conditions were favorable for the oil degradation, resulting in the depletion of the added nutrients, which was also confirmed by the soil respiration curves in previous results (Gong et al., 2006), and soil pH increase. Both pH of the MGP soil (MGP-C and MGP-Cn (Table 4)) decreased after the incubation. For C-10n, C-15n (Table 3), and C-Cn (Table 4), as more oil was degraded with the aid of the nutrient, the pH decrease was counteracted by nutrient consumption. These results are consequently an important supplement to the soil respiration results previously produced (Gong et al., 2006).

2.2 Plant growth bioassay

Total masses of A. sativa and B. rapa exposed to the sunflower oil and/or PAHs in the control and sandy MGP soils were expressed as relative biomasses compared with the total masses of the two plant species in the control soil without sunflower oil and nutrient addition (C-0) (Fig.2). Growth of the A. sativa was adversely affected in the control soil with 1%–15% addition of sunflower oil (C-1 to C-15) (Fig.2). The intensity of the response is directly related to the increasing amount of sunflower oil. The control and MGP soils after the column experiments (C-C and MGP-C) showed similar growth inhibition to those control soil with 10%–15% of addition of sunflower oil (C-10 and C-15), revealing that the inhibitive effects on the plant growth were not only from PAHs but also from the sunflower oil and that sunflower oil played an important role. The nutrient amendment stimulated the A. sativa growth significantly.

### Table 3 Variation of pH of the control soil after sunflower oil and nutrient addition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-0</th>
<th>C-1</th>
<th>C-5</th>
<th>C-10</th>
<th>C-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>5.97</td>
<td>6.21</td>
<td>4.79</td>
<td>6.19</td>
<td>4.76</td>
</tr>
<tr>
<td>28 d</td>
<td>5.89</td>
<td>5.7</td>
<td>5.97</td>
<td>4.84</td>
<td>5.21</td>
</tr>
<tr>
<td>84 d</td>
<td>5.86</td>
<td>6.07</td>
<td>5.41</td>
<td>5.02</td>
<td>5.35</td>
</tr>
</tbody>
</table>

C-0, C-1, C-5, C-10, C-15: control soil without nutrient addition; C-0n, C-1n, C-5n, C-10n, C-15n: with nutrient addition. 

### Table 4 Variation of pH values of the control and MGP soils after column experiments and nutrient addition

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-C</th>
<th>C-Cn</th>
<th>MGP-C</th>
<th>MGP-Cn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>5.95</td>
<td>5.05</td>
<td>6.91</td>
<td>6.33</td>
</tr>
<tr>
<td>28 d</td>
<td>3.95</td>
<td>4.91</td>
<td>6.08</td>
<td>5.02</td>
</tr>
<tr>
<td>84 d</td>
<td>3.77</td>
<td>4.94</td>
<td>6.3</td>
<td>4.94</td>
</tr>
</tbody>
</table>

C-C, C-Cn, MGP-C, and MGP-Cn are column treatment.

Fig. 2 Relative biomass of A. sativa (a) and B. rapa (b) in the higher plant growth experiment. The dash lines separate the treatments into three groups and each group was compared separately. Error bar indicates one standard deviation (SD). In each group different letters mean significant difference among treatments.
Comparison of *A. sativa* and *B. rapa* growth experiments clearly indicated that *B. rapa* was more sensitive to the sunflower oil (Fig. 2a and 2b). When the control soil was added with 1% sunflower oil (C-1), *B. rapa* growth was significantly inhibited (Fig. 2b). The inhibiting effect on the *B. rapa* growth increased with the increasing addition of sunflower oil. When the control soil was amended with the nutrients, addition of 1% of sunflower oil (C-1n) enhanced the *B. rapa* growth, which might prescribe a good combination of oil degradation and nutrient consumption. All the other treatments on the *B. rapa* inhibited its growth significantly.

### 2.3 Soil organic carbon

Organic carbon contents of the soils after the plant growth bioassay are shown in Fig. 3. The degradation of the sunflower oil in the soils can be indirectly reflected by the soil organic carbon contents, which also showed that the nutrients enhanced sunflower oil degradation. However, after 90 d incubation and plant bioassay, there was still some sunflower oil remaining in the soil, and this remaining oil should be further degraded.

The growth of the oil-treated seedlings including height, leaf number, and biomass was significantly reduced. It is suggested that active reactive oxygen species including superoxide, hydrogen peroxide, and hydroxyl free radicals were produced in excess under environmental stresses such as heavy metals and oil pollution, caused lipid peroxidation and damaged cell, seriously disrupted normal metabolism (Parida et al., 2004; Zhang et al., 2007). Raymond et al. (1976) suggested that both petroleum and its metabolites affected the plant growth. The pH variations of the oil treated soils after incubation show that oil metabolites had a significant effect on soil properties; these metabolites might directly or indirectly affect plant growth. The germination of some plants might also be inhibited by oil because their propagules are relatively small in size and are completely covered by oil. Profiti et al. (1995) also showed that oiling depressed stem growth, leaf production, and maximum leaf size of *Rhizophora mangle*. The oil degradation in the soils also increased soil salinity, which was proved in our study by salt content analysis, and it may also affect plant germination and growth. Some...
other scientists also demonstrated that oil pollution could further aggravate the high salinity problem and reduce the salt-stress tolerance of the seedlings (Page et al., 1985). Yousef (2002) reported that the interaction between hydrocarbon and salinity significantly reduced the number of germinated plant seeds. The relative proportion of sand, silt, and clay may affect the movement and retention of oil in soil and pattern of mixing with water (Suprayogi and Murray, 1999). In sandy soils because of its large pore spaces, the oil discharged onto the soil surface would penetrate more rapidly and deeply, even reached the root zone, damaging the root system and posing more negative effects. On the other hand, the rate of microbial breakdown of oil was relatively fast due to the large pore spaces for the growth of microorganism. In addition to particle size, Pezeshki et al. (2000) reported that sediments rich in organic matter had higher affinity to adsorb toxic organic pollutants and reduced their bioavailability and toxicity. Li et al. (2007) demonstrated that the vegetable oil in sediments that inhibited the activity of V. fischeri, the test organism of the Microtox SPT assay was present during the early stage of anaerobic biodegradation and that the inhibition decreased over time and was eventually eliminated after 8 weeks of incubation. Their results demonstrated that the toxicity of oil-contaminated sediments decreased over time as the oil degraded. The degradation of vegetable oil in soils was also confirmed by the soil organic carbon analysis (Fig.3). However, the degradation in our experiment was not as fast as that by Li et al. (2007) because of different matrices, environmental factors, and ecosystems. It can be assumed that more vegetable oil will be degraded with longer incubation. The interactions between vegetable oil degradation and environmental factors and the toxic effects of vegetable oil on plant growth and microorganisms require more attention. Comprehensive studies that evaluate the effects of temperature, nutrients, and other potentially limiting factors on the rates of production and removal of toxic intermediates are needed.

4 Conclusions

Both the nutrient amendment and the sunflower oil degradation resulted in soil pH decrease. Effects of these two processes were counteracted when they worked together due to the nutrient consumption and oil removal. Sunflower oil and nutrient amendments showed different effects on A. sativa and B. rapa growth. Growth of A. sativa was adversely affected by the sunflower oil, and the nutrient amendment stimulated A. sativa growth significantly. All the treatments on B. rapa, with 1% sunflower oil addition plus nutrient amendment as an exception, inhibited its growth significantly. Soil organic carbon content showed that some soil oil remained in the soil after 90 d incubation and sunflower oil degradation was partly achieved.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20707030) and the National Basic Research Program (973) of China (No. 2004CB418506). Scholarships from TU Berlin and BMBF to Z. Gong are gratefully acknowledged. The authors thank Maike Mali and Ruth See for technical assistance. We also thank the anonymous reviewers for their constructive reviews of the manuscript.

References