



Growth response of *Zea mays* L. in pyrene–copper co-contaminated soil and the fate of pollutants

Qi Lin, Kai-Li Shen, Hong-Mei Zhao, Weng-Hong Li*

Department of Environmental Engineering, Zhejiang University, Huajiachi, 268 Kaixuan Road, Hangzhou, Zhejiang 310029, People's Republic of China

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Abstract

Phytoremediation, use of plants for remediation, is an emerging technology for treating heavy metals or a final polishing step for the high-level organic contamination, and may be suitable for remediation of heavy metal and organic co-contaminated soil. The aim of this study was to investigate the influence of co-contamination on the growth of *Zea mays* L. and the fate of both heavy metal and organic pollutants, using Cu and pyrene as the model pollutants. Results showed that shoot and root biomass were affected by the copper–pyrene co-contamination, although maize grown in spiked soils showed no outward signs of phytotoxicity. With the initial concentration of 50, 100 and 500 mg/kg, pyrene tended to alleviate the inhibition of Cu to *Z. mays* L. Pyrene in both planted and non-planted soil was greatly decreased at the end of the 4-week culture, accounting for 16–18% of initial extractable concentrations in non-planted soil and 9–14% in planted soil, which indicated that the dissipation of soil pyrene was enhanced in the presence of vegetation probably due to the biodegradation and association with the soil matrix. With the increment of Cu level, residual pyrene in the planted soil tended to increase. The pyrene residual in the presence of high concentration of Cu was even higher in the planted soil than that in the non-planted soil, which suggested that the change of the microbial composition and microbial activity or the modified root physiology under Cu stress was probably unbeneficial to the dissipation of pyrene. A more thorough understanding of the mechanisms by which metals affect the dissipation of organic pollutants in the rhizosphere could provide a much better framework on which to base manipulation. Unlike pyrene, heavy metal copper cannot be degraded. Decontamination of Cu from contaminated soils in this system required the removal of Cu by plants. It was observed that the ability of Cu phytoextraction would be inhibited under co-contamination of high level of pyrene in highly Cu-polluted soil. In the treatment of 400 mg Cu/kg and 500 mg pyrene/kg, the accumulation of Cu was less than half of that in 400 mg Cu/kg treatment.

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1. Introduction

Since accumulated pollutants are of concern relative to both human and ecosystem exposure and potential impact [1], efforts are underway in many countries to control the release of contaminants [2] and to accelerate the removal or breakdown of existing contaminants by appropriate remediation techniques. Phytoremediation, use of plants for remediation, is one such highly appealing technology [2–6]. There are some promising results suggesting that these techniques might become viable alternatives to mechanical and chemical approaches in remedia-

tion of metal contaminated soils or a final polishing step for the high-level organic contamination [7–9].

Despite the wide study of phytoremediation in heavy metal or organic contaminated soil, little information was still available regarding the effectiveness and processes of phytoremediation of sites co-contaminated with organic and metal pollutants. It was reported that 40% of hazardous waste sites in the United States are co-contaminated with organic and metal pollutants [10,11]. Metals most frequently found include cadmium, chromium, copper, zinc and lead. Common organic co-contaminants include petroleum hydrocarbons (TPHs), and polycyclic aromatic hydrocarbons (PAHs) coming from the exploration and consumption of fossil fuel, polychlorinated biphenyls (PCBs) widely used in the industrial process, and other chlorinated aromatics used as PCB replacement such as polychlorinated terphenyls

* Corresponding author. Tel.: +86 571 86971157.
E-mail address: linqi@zju.edu.cn (Q. Lin).

(PCTs), halogenated compounds like perchloroethylene (PCE) and trichloroethylene (TCE) and pesticides like atrazine and bentazon [12]. As the presence of metals can inhibit a broad range of microbial processes including nitrogen and sulfur conversions, dehalogenation, and reductive processes in general [13–19], and the changed bioavailability of heavy metals in the presence of organic co-contamination might occur [20], the efficiency and mechanism of phytoremediation probably was quite different.

It is well known that phytoremediation of organics is based on the beneficial effects of roots on degradation. A multitude of changes occur in soil in the presence of roots that may be apprehended as changes in its chemical characteristics, modified microbial composition, and enhanced microbial activity. In heavy metal–organic pollutant combined system, heavy metal not only cause the positive or negative effects on the root growth and thereafter affect the root enhanced dissipation, but also exert direct effects on microorganisms and cause direct and indirect effect for degradation of organic pollutants. Lin et al. [21] reported that in copper co-contaminated soil with the initial pentachlorophenol (PCP) concentration of 50 mg/kg, plants grew better with the increment of soil Cu level (0, 150 and 300 mg/kg), implying that combinations of inorganic and organic pollutants sometimes exerted antagonistic toxic effects on plant growth. The observed higher PCP dissipation in soil spiked with 50 mg/kg PCP in the presence of Cu and the less difference of PCP residual between strongly and loosely adhering soils further suggests the occurrence of Cu–PCP interaction and the enhanced degradation and mass flow are two possible explanations. In copper co-contaminated soil with the initial PCP concentration of 100 mg/kg, however, both plant growth and the microbial activity were inhibited with the increment of soil Cu level. The lowered degrading activity of microorganisms and the reduced mass flow were probably responsible for the significantly lower levels of PCP dissipation in copper co-contaminated soil. Besides, Zhu et al. [22,23] reported that the exposure of bacteria to a combination of PCP and copper at non- or sub-toxic concentrations resulted in enhanced cytotoxic effects in a synergistic mode as measured both by growth inhibition and colony-forming ability. Pollutant biodegradation was thus inhibited due to the decrease of microbial biomass and activity [24]. In some cases, however, addition of metals has also been observed to stimulate microbial activity. It is suggested the stimulatory effect may also be due to metals reducing competition for equivalents or nutrients between metal-resistant degraders and metal-sensitive non-degraders [11]. Kuo and Gentner [24] reported that the addition of some metals at low levels stimulated biodegradation. Hexavalent chromium (0.01 mg total chromium/L) increased the biodegradation rate of phenol by 177% and that of benzoate by 169% over controls containing no metals. Copper and cadmium (both at 0.01 mg total metal/L) increased the benzoate biodegradation rate by 185% and the 2-chlorophenol biodegradation rate by 168%.

Unlike organic pollutants, the most effective but also technically the most difficult phytoremediation strategy of heavy metal is phytoextraction [6]. Several bottleneck processes limiting heavy metal accumulation in plants include the mobilization

of poorly available contaminant trace elements in the soil, root uptake, symplastic mobility and xylem loading, as well as detoxification and storage inside the shoot [25]. The effect of organic pollutant on the phytoextraction of heavy metal is not fully understood yet. However, organic chelators increase metal ion uptake and translocation in plant is widely researched. For example, when ethylene diamine tetra-acetic acid (EDTA) is added to lead contaminated soils, there is a >100-fold increase in the uptake and transport of the lead–EDTA–chelate into stems and leaves [26,27]. Therefore, in stress condition, plants altered to increase their secretion of particular organic matter such as organic acids will probably increase the uptake and translocation of metal pollutants.

In sum, phytoremediation of sites co-contaminated with organic and metal pollutants is very complex. The objective of this paper was to investigate the influence of co-contamination on the growth of *Zea mays* L. and the fate of pollutants in soil and plants. Cu and pyrene were selected as the model pollutants. Maize (*Z. mays* L.) was chosen as the tested plant species because of its high biomass yields and heavy metal tolerance. Ali et al. [28] studied maize tolerance and proposed this plant as a possible solution for the stabilization and restoration of Cu-polluted soils. Additionally, maize may create particularly good environmental conditions for soil microorganisms and microfauna [29–31]. Dillewijn et al. [32] reported that the extractable TNT content in rhizosphere soil associated to maize roots decreased by more than 96% in 60 days regardless of inoculation and considered that under field conditions, maize is potentially useful alternative to remediation surface soils contaminated with medium levels of TNT.

2. Material and methods

2.1. Soil and subsamples

Soil samples used in the present study were collected from Jiaxin county, Zhejiang province, China. The soil is classified as Paddy soil (course-loamy, nonacid). Soil testing results showed pH (1:2.5 water) 6.10; OM content 5.2% and CEC 4.28 cmol/kg. The levels of Cu and pyrene added into soil were 0, 200, 400 mg Cu/kg soil and 0, 50, 100, 500 mg pyrene/kg soil. Briefly, the bulk soil was first mixed thoroughly with Cu (as CuSO₄), urea (0.11 g/kg), KH₂PO₄ (0.10 g/kg) and KCl (0.11 g/kg) in an aqueous solution, incubated at a moisture condition for 4 weeks. Then the subsamples were air-dried naturally, fully homogenized again and stored for use. The subsamples containing pyrene was prepared with the above subsamples. High purity pyrene in acetone was sprayed onto the soil. After acetone had evaporated off, the spiked soils were sieved again through 2 mm sieve to ensure the homogeneity and stored for use.

2.2. Incubation experiment

Two hundred grams of each subsample (including control subsamples without an addition of Cu and pyrene) were put in open plastic pots and pre-incubated in the greenhouse for 1 week with 60% of the water holding capacity. Then, two pre-

germinated seeds of maize (*Z. mays* L.) were sown in each pot. The seedlings were thinned 5 days after emergence to leave one plant per pot and maintained in the greenhouse for 4 weeks. The water content of the soil in the pots was regularly adjusted. The unplanted treatments were also cultured in same condition. Three replicates were performed.

2.3. Sampling and analysis

Prior to the harvests (4 weeks after seedling) pots were left without watering for 1 day. At harvest, shoots and roots were washed with distilled water, dried, and then weighted. Soils were carefully collected and homogenized. Part of soil was air dried and passed through 60 mesh standard sieve for Cu analysis. Others were stored at -20°C for pyrene analysis.

Heavy metal contents were determined by flame atomic absorption spectrophotometry (Perkin-Elmer AA100). For water-soluble heavy metals in the soil, 4 g of fresh soil were shaken at 25°C for 2 h with 20 ml of double-distilled water. Suspensions were then centrifuged (10 min at 4000 rpm) and filtered through a $0.45\ \mu\text{m}$ filter.

Residual of pyrene in soils were extracted by ultrasonication extraction. Two grams of soil sample were ultrasonicated in 10 mL of dichloromethane for 1 h followed by centrifugation. Then 3 mL of supernatant was filtered through 2 g of silica gel column with 10 mL 1:1 (V/V) elution of hexane and dichloromethane. The solvent fractions were then evaporated, exchanged by methanol with a final volume of 2 mL and quantified with the Agilent 1100 serials HPLC system. The HPLC analyses were performed with an Agilent 1100 Serials liquid chromatograph (equipped with a vacuum degasser, quaternary pump, autosampler, column compartment, diode array and multiple wavelength detectors, and a hypersil reversed-phase ODS-C-18 column made by the Agilent Company, USA). The conditions for measuring were: a mobile phase made of water and methanol in the proportion of 10/90 (V/V) at a flow rate of $1.0\ \text{mL}\ \text{min}^{-1}$, and signal wavelength of 235 nm. The solvents used for chromatographic analyses were HPLC grade.

Plant samples were digested using a mixture of HNO_3 – HCl – HClO_4 acids [33]. Briefly, the shoot and root samples were digested by a mixture of concentrated HNO_3 and HCl in the ratio of 3:1 at room temperature for 1 day, then heated to dryness, after that 5 mL of HClO_4 was added and heated until white fog appeared, finally a few drops of HNO_3 were added to solubilize the residue. Copper in plants was determined by AAS.

The selected properties of the soil measured with the routine analytical methods [34]. Soil pH was determined from suspension of 10 g of soil and 25 mL of water. Soil organic matter was determined using Walkley–Black wet oxidation method. Cationic exchange capacity was determined with 1.0 M NH_4Ac (pH 7.0).

2.4. Data processing

The results were presented as the average of three replicates. Statistical analyses were carried out using analyses of variance

(ANOVA) or paired *T*-tests. The level of statistical significance is represented by * for <0.05 , ** for <0.01 and ns for not significant.

3. Results and discussion

3.1. Growth response

Shoot and root biomass were affected by the copper–pyrene co-contamination, although maize grown in spiked soils showed no outward signs of phytotoxicity (Table 1). The decrease of root dry weight and a slight but non-significant ($P < 0.05$) decrease of shoot dry weight were observed in the Cu spiked soil compared with that in the control un-spiked soil. Pyrene co-contamination, however, did not decrease the plant dry weight further. On the contrary, with the initial concentration of 50, 100 and 500 mg/kg pyrene, the yields of shoot tended to increase, suggesting certain concentration of pyrene can alleviate the inhibition of Cu to maize. These results were different to that of Gao and Zhu [35], who reported that the yields of both root and shoot of flowering Chinese cabbage tended to decrease with the increment of soil contaminant levels of pyrene and phenanthrene. Besides, Chekol et al. [36] reported that PCB spiking (100 mg/kg) of the soil did not significantly affect the biomass of all tested grass species and shoot weights of flatpea and sericea lespedeza. Therefore, the growth respond of plants to organic contamination might be relation to the plant species and the characteristics of organic pollutants. Since the interaction of Cu–pyrene was complex, further research concerning the effect of pyrene on the Cu toxicity need to be done.

3.2. Dissipation of pyrene in soil

At the end of the 4-week culture, extractable pyrene in both planted and non-planted soil was greatly decreased, accounting for 16–18% of initial extractable concentrations in non-planted soil and 9–14% in planted soil. The mechanisms of the dissipation of pyrene in soil were biodegradation, photodegradation, volatilization, plant uptake and metabolism, and incorporation of the contaminant into soil organic material. The extractable

Table 1
Plant dry matter yields of maize as affected by the co-contamination of pyrene

Cu added (mg/kg)	Pyrene added (mg/kg)	Shoot (g)	Root (g)
0	0	0.689 ± 0.048	0.355 ± 0.006
200	0	0.599 ± 0.008	0.211 ± 0.012
400	0	0.593 ± 0.102	0.260 ± 0.035
0	50	0.746 ± 0.140	0.249 ± 0.072
200	50	0.769 ± 0.066	0.259 ± 0.059
400	50	0.805 ± 0.119	0.334 ± 0.087
0	100	0.860 ± 0.127	0.299 ± 0.010
200	100	0.740 ± 0.106	0.243 ± 0.023
400	100	0.777 ± 0.082	0.284 ± 0.014
0	500	0.799 ± 0.082	0.201 ± 0.057
200	500	0.769 ± 0.121	0.205 ± 0.023
400	500	0.644 ± 0.055	0.150 ± 0.020

Two-way ANOVA. Values are means \pm S.E. Shoot: $F = 3.439$, $P < 0.05$; Cu: ns; pyrene: *. Root: $F = 4.094$, $P < 0.01$; Cu: ns; Pyrene: **.

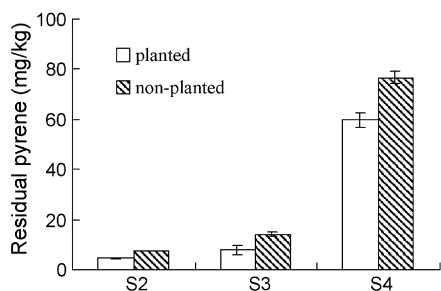


Fig. 1. Residual of pyrene in planted and non-planted soil after 4 weeks' culture in pyrene spiked soil. S2, S3, S4 represent for soil with initial pyrene of 50, 100, 500 mg/kg, respectively.

pyrene in the planted soil was significantly lower than in the non-planted soil (Fig. 1). This result was in complete agreement with the findings of previous experiments with a mixture of aliphatic hydrocarbons or with PAHs such as anthracene and pyrene [37–41]. Since phytovolatilization of pyrene was negligible, plant metabolism was not significant [42] and contributions of plant off-take of such organic compound to the total remediation enhancement in the presence of vegetation was less than 0.24% [35], plant promoted dissipation of pyrene was probably contributed to the biodegradation and association with the soil matrix.

Fig. 2 shows the effect of Cu on the pyrene dissipation in the planted soil. With the increment of Cu level, residual pyrene in soil tended to increase. It was observed that the extractable pyrene in the absence of Cu was 4.65, 7.82, 59.75 mg/kg after 4-weeks culture in the treatment of 50, 100, 500 mg/kg pyrene, respectively. While in the presence of Cu, it was 7.36, 13.03, 60.45 mg/kg in 200 mg/kg Cu treatment and 8.31, 14.39, 73.25 mg/kg in 400 mg/kg Cu treatment, respectively. Since phytoremediation of organics is based on the beneficial effects of roots on biodegradation and the formation of bound residues, the increased residual of pyrene in the presence of Cu was probably attributed to the two aspects. One was the change of the microbial composition and microbial activity. Kuo and Genthner [24] reported that metals adversely affecting degraders in a consortium. Addition of low levels of metals (0.1–2.0 mg total metal/L) lengthened acclimation periods and decreased dechlorination and biodegradation rates. Besides, metals also affected

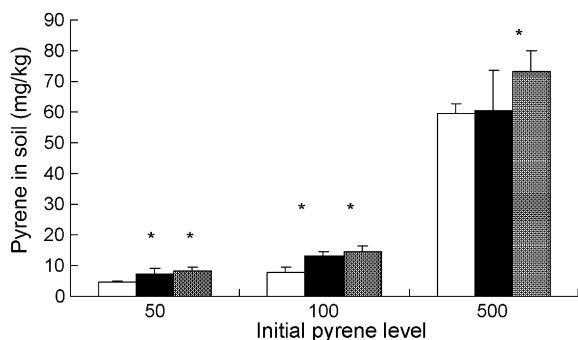


Fig. 2. Extractable pyrene in planted soil as affected by co-contamination of Cu. □, control; ■, 200 mg/kg Cu; and ▨, 400 mg/kg Cu. * indicates significant difference between control and copper treatment.

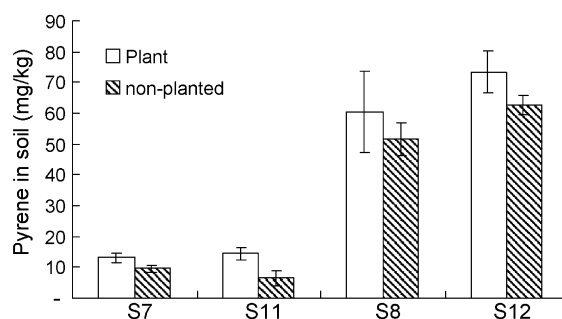


Fig. 3. Extractable pyrene in planted soil and non-planted soil in Cu–pyrene co-contaminated soil. S7, spiked with initial pyrene concentration of 100 mg kg⁻¹ and Cu of 200 mg kg⁻¹; S11, spiked with initial pyrene concentration of 100 mg kg⁻¹ and Cu of 400 mg kg⁻¹; S8, spiked with initial pyrene concentration of 500 mg kg⁻¹ and Cu of 200 mg kg⁻¹; S12, spiked with initial pyrene concentration of 500 mg kg⁻¹ and Cu of 400 mg kg⁻¹. Paired *T*-test between planted and non-planted soil. *t* = 4.345, *n* = 4 and *P* < 0.05.

non-degrading consortium members that play a vital but indirect role in the degradation process [24,43]. Similarly, our research group also found the significant decrease of microbial biomass in the heavy metal-polluted soil and suggested that the adverse effect of heavy metals on the soil microbial number might be the reason of the slow degradation of pentachlorophenol [21]. Therefore, in this experiment, the decreased microbial biomass and degraders of the parent compound were suggested to be the possible explanation to the increased pyrene residual. The other reason for increased residual of pyrene in soil was the modified root physiology. It was well known that the most important factor that caused a multitude of changes occur in the rhizosphere was the input of large quantities of readily available organic substrates in the form of root exudates. The increased leakage or the modified composition of exudates under Cu stress might cause the decreased bioavailability of pyrene with formation of pyrene–organic matter bound which could also be extracted by solvent. Walton et al. [44] suggested that rhizosphere could stabilize pollutants by polymerization reactions such as humification. They cited an experiment with ¹⁴C PAHs which showed that ¹⁴C in fulvic/humic acids was higher in rhizosphere than

Table 2
Water extractable Cu in planted and non-planted soil as affected by the co-contamination of pyrene (mg/kg soil)

Cu added (mg/kg)	Pyrene added (mg/kg)	Planted soil	Non-planted soil
0	0	0.34 ± 0.07	0.26 ± 0.02
200	0	0.43 ± 0.04	0.33 ± 0.07
400	0	0.58 ± 0.03	0.54 ± 0.10
0	50	0.48 ± 0.03	0.33 ± 0.06
200	50	0.67 ± 0.12	0.49 ± 0.03
400	50	0.69 ± 0.03	0.68 ± 0.05
0	100	0.51 ± 0.20	0.46 ± 0.04
200	100	0.71 ± 0.02	0.58 ± 0.06
400	100	0.73 ± 0.11	0.69 ± 0.12
0	500	0.43 ± 0.07	0.56 ± 0.03
200	500	0.73 ± 0.17	0.51 ± 0.07
400	500	0.62 ± 0.03	0.60 ± 0.06

Paired *T*-test between planted and non-planted soil. *t* = 2.784, *n* = 12 and *P* < 0.05.

Table 3
Copper concentration and accumulation in maize after 4 weeks' culture

Treatment		Concentration of Cu in maize			Accumulation of Cu in maize		
Cu added	Pyrene added	Shoot (μg)	Root (μg)	S/R ^a	Shoot (μg)	Root (μg)	Total
0	0	7.29 a	15.63 a	0.47	5.28 a	5.54 a	10.82 a
200	0	16.24 abc	56.58 b	0.29	9.74 b	11.89 b	21.63 bcd
200	50	15.39 ab	75.76 bd	0.20	11.33 bc	17.05 c	28.38 d
200	100	14.41 ab	34.11 a	0.42	10.63 b	8.16 a	18.79 bc
200	500	18.31 bc	59.70 b	0.31	12.93 bcd	11.54 b	24.46 cd
400	0	23.03 c	113.53 c	0.20	14.04 cd	31.79 e	45.83 f
400	50	16.06 abc	69.99 b	0.22	11.89 bcd	26.39 d	38.28 e
400	100	19.26 bc	96.18 cd	0.20	14.69 d	27.20 de	41.89 ef
400	500	10.70 a	53.61 b	0.20	6.88 a	8.05 a	14.93 ab

Mean within column followed by the same letter are not significantly different as determined by LSD multiple comparison procedure using one way ANOVA ($P < 0.05$).

^a Shoot/root ratio of Cu concentration.

non-rhizosphere soil. In this experiment, we also observed an important result that in the presence of high concentration of Cu, the pyrene residual was higher in the planted soil than that in the non-planted soil (Fig. 3). These results suggested that phytoremediation of organic pollutants in heavy metal co-existed soil was very complex and quite different from that in the single pollution system. In highly Cu co-contaminated soil, the negative effect of Cu on the dissipation of organic pollutants should be fully considered. As Olson et al. [45] proposed that differences in the quantity and quality of nutrients released by root exudation and root mortality likely lead to variable and potentially opposing effects on microbial PAH degradation. Mueller and Shann [46] found that PAH dissipation and mineralization were not affected by planting and amendment with decaying fine roots inhibited PAH degradation by the soil microbial community. In light of the complex nature of root dynamics, the absence of a positive planting effect is not surprising in highly Cu co-contaminated soil. But a more thorough understanding of the mechanisms by which metals affect the dissipation of organic pollutants in the rhizosphere could provide a much better framework on which to base manipulation.

3.3. Copper extraction in soil and accumulation in maize

Unlike pyrene, heavy metal copper cannot be degraded. Decontamination of Cu from contaminated soils in this system requires the removal of Cu by plants. For phytoextraction to occur, Cu must be bioavailable (ready to be absorbed by roots). Bioavailability depends on metal solubility in soil solution. Table 2 was the water extractable Cu in planted and non-planted soil as affected by the co-contamination of pyrene. It was clear that copper concentration in water extracts was significantly increased in planted soil after 1 month's culture. Due to the slightly increase of soil pH value in planted soil as compared with the non-planted soil (data not shown), the increase of water extractable Cu in planted soil was apparently not contributed to the change of pH in the rhizosphere. A possible explanation could be the complexing properties of soluble exudates and the solubilization of microorganisms in the rhizosphere. Chen et al. [20] reported that water extractable Cu in soil was increased

after planted with ryegrass and copper complexes were the predominant species for Cu in the soil solution. Similarly, Cattani et al. [47] suggested that maize cultivation in a polluted vineyard soil could increase the potentially available fraction of copper. The soluble and the potentially bioavailable (determined using the technique of diffusive gradients in thin film) copper concentrations were sixfold increase in the rhizosphere of the polluted soil compared with that in the bulk soils. Besides, pyrene co-contamination tended to increase the Cu concentration in water extracts in both planted and non-planted soil, though it was not statistically significant. Therefore, the results related to the Cu concentration in water extracts indicated that both vegetation and organic co-contamination would change the content of heavy metal in soil solution and more attention should be paid to the potential risk of heavy metal accumulation and phytotoxicity in plants and soil ecosystem.

Table 3 was the Cu concentration and accumulation in maize after 4 weeks' culture. In the absence of pyrene, shoot and root Cu concentrations in maize increased with increasing soil Cu level. The ratio of shoot to root, however, decreased from 0.47 in control soil to 0.29, 0.20 in 200, 400 mg Cu/kg soil, respectively, which also suggested the root acquisition of Cu intensified with the increment of soil contaminant levels. This conclusion is in agreement with the experimental results of Cattani et al. [47], who found no significant difference between the polluted vineyard soil (183 mg Cu/kg soil) and unpolluted forest soil (18.4 mg Cu/kg soil) as comparing the copper content of maize leaves; but the root concentration is approximately fourfold greater in the vineyard soil compared to the forest soil. Pyrene co-contamination had an effect on the Cu concentration and accumulation in maize. But the interaction between pyrene and Cu might also be related to the various levels of Cu treatment. In 200 mg Cu/kg soil, for example, Cu accumulation in maize in the presence of pyrene was not significantly different from that in the absence of pyrene. While in 400 mg Cu/kg soil, both concentrations and accumulation of Cu in maize tended to decrease with the pyrene co-contamination. Especially, in the treatment of 400 mg Cu/kg and 500 mg pyrene/kg, the accumulation of Cu was less than half of that in 400 mg Cu/kg treatment. That is to say, the co-contamination of pyrene would cause the decrease

of phytoextracting efficiency of Cu in highly Cu-polluted soil. This is another important result in this experiment.

4. Conclusions

Phytoremediation is an appealing technology for sites contaminated with organic and metal pollutants. But it was noticing that the growth response of *Z. mays* L., dissipation of pyrene in soil and accumulation of Cu by *Z. mays* L. might be affected by the co-contamination due to the interaction between heavy metal and organic pollutants. With the initial concentration of 50,100, 500 mg/kg pyrene, the yields of shoot under Cu-contaminated soil tended to increase as compared with the pyrene absent treatment, suggesting certain concentration of pyrene can alleviate the inhibition of Cu to *Z. mays* L. The dissipation of soil pyrene was enhanced in the presence of vegetation. But with the increment of Cu level, residual pyrene in the planted soil tended to increase, suggesting the change of the microbial composition and microbial activity or the modified root physiology under Cu stress was unbeneficial to the dissipation of pyrene. Besides, results related to the Cu concentration in soil solution and *Z. mays* L. indicated that both vegetation and organic co-contamination would change the bioavailability of heavy metal in soil. The ability of Cu phytoextraction would be inhibited under co-contamination of high level of pyrene in highly Cu-polluted soil.

Acknowledgments

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References

- [1] J.E.J. Weckx, H.M.M. Clijsters, Oxidative damage and defence mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper, *Physiol. Plantarum* 96 (1996) 506–512.
- [2] J.L. Schnoor, L.A. Licht, S.C. McCutcheon, N.L. Wolfe, L.H. Carreira, Phytoremediation of organic and nutrient contaminants, *Environ. Sci. Technol.* 29 (1995) 318A–323A.
- [3] S.D. Cunningham, T.A. Anderson, A.P. Schwab, F.C. Hsu, Phytoremediation of soils contaminated with organic pollutants, *Adv. Agron.* 56 (1996) 55–114.
- [4] L. Raskin, R.D. Smith, D.E. Salt, Phytoremediation of metals: using plants to remove pollutants from the environment, *Curr. Opin. Biotechnol.* 8 (1997) 221–226.
- [5] D.E. Salt, R.D. Smith, L. Raskin, Phytoremediation, *Annu. Rev. Plant Physiol. Plant Mol. Biology* 49 (1998) 643–668.
- [6] U. Krämer, Phytoremediation: novel approaches to cleaning up polluted soils, *Curr. Opin. Biotechnol.* 16 (2005) 133–141.
- [7] K.C. Jones, *Organic Contaminants in the Environment*, Elsevier Applied Science, New York, NY, 1991.
- [8] M.B. Leigh, J.S. Fletcher, X. Fu, F.J. Schmitz, Root turnover: an important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants, *Environ. Sci. Technol.* 36 (2002) 1579–1583.
- [9] N. Singh, M. Megharaj, R.S. Kookana, R. Naidu, N. Sethunathan, Atrazine and simazine degradation in Pennisetum rhizosphere, *Chemosphere* 56 (2004) 257–263.
- [10] T.R. Sandrin, A.M. Chech, R.M. Maier, A rhamnolipid biosurfactant reduces cadmium toxicity during biodegradation of naphthalene, *Appl. Environ. Microbiol.* 66 (2000) 4585–4588.
- [11] T.R. Sandrin, R.M. Maier, Impact of metals on the biodegradation of organic pollutants, *Environ. Health Perspect.* 111 (2003) 1093–1101.
- [12] S. Saleh, X.D. Huang, B.M. Greenberg, B.R. Glick, Phytoremediation of persistent organic contaminants in the environment, in: A. Singh, O. Ward (Eds.), *Soil Biology: vol. 1. Applied Bioremediation and Phytoremediation*, Springer-Verlag, Berlin, 2004, pp. 115–134.
- [13] H. Babich, M.A. Devanas, G. Stotzky, The mediation of mutagenicity and clastogenicity of heavy metals by physicochemical factors, *Environ. Res.* 37 (1985) 253–286.
- [14] R.D. Bardgett, S. Saggar, Effects of heavy metal contamination on the short term decomposition of [¹⁴C] glucose in a pasture soil, *Soil Biol. Biochem.* 26 (1994) 727–733.
- [15] C. Burkhardt, H. Insam, T.C. Hutchinson, H.H. Reber, Impact of heavy metals on the degradative capabilities of soil bacterial communities, *Biol. Fertil. Soils* 16 (1993) 154–156.
- [16] B.P. Knight, S.P. Mcgrath, A.M. Chaudri, Biomass carbon measurements and substrate utilization patterns of microbial populations from soils amended with cadmium, copper, or zinc, *Appl. Environ. Microbiol.* 63 (1997) 39–43.
- [17] A. Masakazu, S. Itaya, Effects of copper on the metabolism of ¹⁴C-labeled glucose in soil in relation to amendment with organic materials, *Soil Sci. Plant Nutr.* 41 (1995) 245–252.
- [18] I.P. Pankhania, J.P. Robinson, Heavy metal inhibition of methanogenesis by *Methanospirillum hungatei* Gp1, *FEMS Microbiol. Lett.* 22 (1984) 277–281.
- [19] J.E. Rogers, S.W. Li, Effect of metals and other inorganic ions on soil microbial activity—oil dehydrogenase assay as a simple toxicity test, *Bull. Environ. Contam. Toxicol.* 34 (1985) 858–865.
- [20] Y.X. Chen, Q. Lin, Y.F. He, G.M. Tian, Behavior of Cu and Zn under combined pollution of 2,4-dichlorophenol in the planted soil, *Plant Soil* 261 (2004) 127–134.
- [21] Q. Lin, Z.W. Wang, S. Ma, Y.X. Chen, Evaluation of dissipation mechanisms by *Lolium perenne* L, and *Raphanus sativus* for pentachlorophenol (PCP) in copper co-contaminated soil, *Sci. Total Environ.* 368 (2006) 814–822.
- [22] B.Z. Zhu, M. Cheyion, Mechanism of the synergistic cytotoxicity between pentachlorophenol and copper–1,10-phenanthroline complex: the formation of a lipophilic ternary complex, *Chem.–Biol. Interact.* 129 (2000) 249–261.
- [23] B.Z. Zhu, S. Shechtman, M. Cheyion, Synergistic cytotoxicity between pentachlorophenol and copper in a bacterial model, *Chemosphere* 45 (2001) 463–470.
- [24] C.W. Kuo, B.R.S. Genthner, Effect of added heavy metal ions on biotransformation and biodegradation of 2-chlorophenol and 3-chlorobenzoate in anaerobic bacterial consortia, *Appl. Environ. Microbiol.* 62 (1996) 2317–2323.
- [25] R.B. Meagher, Phytoremediation of toxic elemental and organic pollutants, *Curr. Opin. Plant Biol.* 3 (2000) 153–162.
- [26] J.W. Huang, J. Chen, W.R. Berti, S.D. Cunningham, Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction, *Environ. Sci. Technol.* 31 (1997) 800–805.
- [27] A.D. Vassil, Y. Kapulnik, I. Raskin, D.E. Salt, The role of EDTA in lead transport and accumulation by Indian mustard, *Plant Physiol.* 117 (1998) 447–453.
- [28] N.A. Ali, M.P. Bernal, M. Ater, Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*, *Plant Soil* 239 (2002) 103–111.
- [29] J.M. Lynch, J.M. Whipps, Substrate flow in the rhizosphere, *Plant Soil* 129 (1990) 1–10.
- [30] R. Schulin, G. Geiger, G. Furrer, Heavy metal retention by soil organic matter under changing environmental conditions, in: W. Salomons, W.M. Stigliani (Eds.), *Biogeochemistry of Pollutants in Soils and Sediments: Risk Assessment of Delayed and Nonlinear Responses*, Springer, Berlin, 1995, pp. 53–85.

- [31] P.F.A.M. Romkens, L.A. Bouwman, G.T. Boon, Effect of plant growth on copper solubility and speciation in soil solution samples, *Environ. Pollut.* 106 (1999) 315–321.
- [32] P.V. Dillewijn, A. Caballero, J.A. Paz, M.M. Gonzalez-Perez, J.M. Oliva, J.L. Bamos, Bioremediation of 2,4,6-trinitrotoluene under field conditions, *Environ. Sci. Technol.* 41 (2007) 1378–1383.
- [33] Environmental Protection Agency of China, *Monitoring and Analytical Methods of Environment*, Chinese Environ. Sci. Press, Beijing, 1999, pp. 352–354.
- [34] Agrochemistry Commission (AC), *Routine Methods for Soil and Agrochemical Analyses*, Soil Science Society of China, Science Press, Beijing, 1983, p. 457 (in Chinese).
- [35] Y.Z. Gao, L.Z. Zhu, Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils, *Chemosphere* 55 (2004) 1169–1178.
- [36] T. Chekol, L.R. Vough, R.L. Chaney, Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect, *Environ. Int.* 30 (2004) 799–804.
- [37] A.P. Schwab, M.K. Banks, Biologically mediated dissipation of polycyclic aromatic hydrocarbons in the root zone, in: T.A. Anderson, J.R. Coats (Eds.), *Bioremediation Through Rhizosphere Technology*, American Chemical Society, Washington, DC, 1994, pp. 11–26.
- [38] T. Günther, U. Dornberger, W. Fritsche, Effect of ryegrass on biodegradation of hydrocarbons in soil, *Chemosphere* 33 (1996) 203–215.
- [39] K.A. Reilley, M.K. Banks, A.P. Schwab, Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere, *J. Environ. Qual.* 25 (1996) 212–219.
- [40] P. Binet, J.M. Portal, C. Leyval, Dissipation of 3-6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass, *Soil Biol. Biochem.* 32 (2000) 2011–2017.
- [41] E.J. Joner, C. Leyval, Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular Mycorrhiza, *Environ. Sci. Technol.* 37 (2003) 2371–2375.
- [42] S. Trapp, M. Matthies, I. Scheunert, E.M. Topp, Modeling the bioconcentration of organic chemicals in plants, *Environ. Sci. Technol.* 24 (1990) 1246–1252.
- [43] D.J. Roberts, N. Venkataraman, S. Pendharkar, The effect of metals on biological remediation of munitions contaminated soil, *Environ. Eng. Sci.* 15 (1998) 265–277.
- [44] B.A. Walton, A.M. Hoylman, M.M. Perez, T.A. Anderson, T.R. Johnson, E.A. Guthrie, R.F. Christman, Rhizosphere microbial communities as a plant defense against toxic substances in soils, in: T.A. Anderson, J.R. Coats (Eds.), *Bioremediation Through Rhizosphere Technology*, American Chemical Society, Washington, DC, 1994, pp. 82–92.
- [45] P.E. Olson, T. Wong, M.B. Leigh, J.S. Fletcher, Allometric modeling of plant root growth and its application in rhizosphere remediation of soil contaminants, *Environ. Sci. Technol.* 37 (2003) 638–643.
- [46] K.E. Mueller, J.R. Shann, PAH dissipation in spiked soil: impacts of bioavailability, microbial activity, and trees, *Chemosphere* 64 (2006) 1006–1014.
- [47] I. Cattani, G. Fragoulis, R. Boccelli, E. Capri, Copper bioavailability in the rhizosphere of maize (*Zea mays* L.) grown in two Italian soils, *Chemosphere* 64 (2006) 1972–1979.