

# Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation

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## Abstract

Accumulation of polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) by crop plants from contaminated soils may pose health risks. A greenhouse pot experiment using lettuce (*Lactuca sativa* L.) as a representative vegetable was conducted to assess the concentrations of PAHs and HMs in vegetables grown in wastewater-contaminated soils. The concentrations of total PAHs were ranged from 1.5 to 3.4 mg kg<sup>-1</sup> in the contaminated soils, while 1.2 mg kg<sup>-1</sup> in the reference soil. Linear regression analyses showed that the relationships between soil and shoot PAH concentrations were stronger for LMW-PAHs ( $R^2$  between 0.51 and 0.92) than for HMW-PAHs ( $R^2$  0.02 and 0.60), suggesting that translocation for LMW-PAHs is faster than HMW-PAHs. Furthermore, the data imply that root uptake was the main pathway for HMW-PAHs accumulation. The plant shoots were also highly contaminated with HMs, particularly Cd (0.4–0.9 mg kg<sup>-1</sup>), Cr (3.4–4.1 mg kg<sup>-1</sup>), Ni (11.7–15.1 mg kg<sup>-1</sup>) and Pb (2.3–5.3 mg kg<sup>-1</sup>), and exceed the guidance limits set by State Environmental Protection Administration (SEPA), China and the World Health Organization (WHO). This study highlights the potential health risks associated with cultivation and consumption of leafy vegetables on wastewater-contaminated soils.

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**Keywords:** Heavy metals; Plant uptake; Polycyclic aromatic hydrocarbons; Soil contamination; Wastewater irrigation

## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) are becoming increasingly prevalent in soil environments as a result of wastewater irrigation, sludge applications, solid waste disposal, automobiles exhaust and industrial activities [1,2]. In countries facing freshwater shortages, sewage water and industrial effluents are useful water sources that are commonly used for agricultural production. In metropolitan regions of northern China, the domestic wastewater and industrial effluents are biologically treated and subsequently used for irrigation. However, the wastewater contains substantial amount of nutrients, and organic and inorganic pollutants, which are creating both opportunities and problems for the farming sector [3,4].

It is well known that PAHs are recalcitrant and mutagenic/carcinogenic pollutants, and there is serious concern about their presence in the environment, especially their tendency for bioaccumulation in food chains [5]. The accumulation of PAHs and HMs in the soil environment is of increasing concern because of their impacts on soil health, food safety and potential health risks. Food chain contamination is one of the important pathways for the entry of these toxic pollutants into the human body. Vegetables cultivated on the wastewater-contaminated soils may take up these pollutants in sufficient quantities to cause health problems for the consumers. Plant uptake of PAHs varies significantly, and is affected by several factors including initial soil concentrations, plant species and soil microbial population [6]. Previously, numerous studies have demonstrated that vegetables accumulate high concentrations of PAHs, grown in PAH-contaminated soils [7,8]. According to Fryer and Collins [9], and Wild et al. [10], several mechanisms, including uptake through transpiration stream, volatilization and subsequent re-

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deposition on leaves and sorption from direct contact with soil particles, are responsible for the transfer of organic pollutants from soil to plant tissues.

In recent years, a number of articles have addressed the sources, accumulation and transfer of HMs of wastewater-contaminated soils [11–13]. However, information regarding the combined uptake, translocation and accumulation of PAHs and HMs present in wastewater-contaminated soil is still under study. This study highlights that the agricultural soils irrigated with wastewater containing a mixture of PAHs and HMs result in elevated uptake of these pollutants into vegetables. The potential health risks caused by the consumption of these contaminated-vegetables could be very severe. This study was conducted in order to understand the appropriateness of using wastewater-contaminated farmlands for vegetable cultivation, to investigate the uptake of both PAHs and HMs by vegetable from long-term wastewater-irrigated soils, and to assess the potential harm to public health. To achieve these objectives, pot trails were conducted in a greenhouse with lettuce as a representative leafy vegetable.

## 2. Materials and methods

### 2.1. Area description and soil sampling

Located near Beijing and adjacent to the Bohai Sea, Tianjin is one of the largest industrial cities in China. Soil contamination with PAHs and HMs in Tianjin is caused by long-term wastewater irrigation, sludge applications, solid waste disposal and automobiles exhaust [2]. According to Cao et al. [14], the total PAH concentrations varied from 1800 to 35,000 ng L<sup>-1</sup> in surface water and 227–600 ng L<sup>-1</sup> in reclaimed water in Tianjin. Majority of the rivers in this area have also been heavily contaminated with wastewater from Tianjin and Beijing cities. Due to high demand and shortage of freshwater, farmlands (approximately 3500 km<sup>2</sup>) have been irrigated with wastewater for more than 40 years [15].

In Tianjin, wastewater-contaminated soils were collected in 2006 from four sites such as XiaoDian (T1), DaGu (T2), HanGu (T3) and NingHe (T4), and one reference soil from XinAn (T5), as shown in Fig. 1. The reference soil (T5) was irrigated with river water that is not totally clean, as mentioned above. At each site, a composite soil sample was collected in the upper horizon (0–20 cm). After transportation to the laboratory, soil was sieved through a 2 mm mesh, then sealed in solvent-cleaned glass containers and stored at –20 °C until analysis. Sub-samples were air-dried and used to measure the physico-chemical properties according to standard procedures with results shown in Table 1. Soil pH was measured with both H<sub>2</sub>O (1:2.5 ratio) and CaCl<sub>2</sub> (1:10 ratio). Water soluble organic carbon (WSOC) and its fractions (including humic acid and fulvic acid) were measured as described by Chen et al. [16].

### 2.2. Design of pot experiment

Lettuce (*Lactuca sativa* L.) was chosen as one of the representatives of leafy vegetable because it is regularly cultivated in



Fig. 1. Location map of the study area indicating the sampling sites.

farms and routinely used as uncooked green leaves. Plastic pots were filled with 1 kg of sieved soil (<2 mm) and four replicates were prepared for each sample. Seeds of lettuce were disinfected in 30% (w/w) H<sub>2</sub>O<sub>2</sub> solution for 10 min, followed by thorough washing with deionized water. These seeds were then germinated in petri dishes with moist papers at 28 ± 1 °C. After 3 d, five uniform seedlings at the two-leaf stage were transplanted to each pot and seedlings were thinned to 3 after 1 week.

The experiment was conducted in a greenhouse under sunlight 12 h, with day temperature about 25 ± 4 °C and night temperature about 19 ± 3 °C and relative humidity of 65 ± 2%. Soils were irrigated with deionized water to maintain the water content at 70% of the field moisture capacity. The pots were re-randomized at regular interval to compensate for light and temperature differences inside the greenhouse. Plastic plates were placed at the height of plant shoots to collect the soil and dust particles for measuring the aerial supply of PAHs to shoot tissues. The leaf surface area and dust mass were calculated as proposed by Fismes et al. [17]. To distinguish the PAHs uptake through root from aerial deposition, the soils were covered with uncontaminated sand to prevent the possible contact of leaf with soil, and also to minimize the evaporation of LMW-PAHs. Plants were harvested 8 weeks after transplanting, and separated into shoots and roots. Shoots were rinsed briefly with deionized water while roots were first washed with tap water and then with deionized water to remove all adhering soil particles. After drying with tissue paper, shoots and roots were freeze-dried (at 530 ± 17 Pa and –50 ± 2 °C) in the freeze drier, and dry weights were recorded.

### 2.3. PAHs extraction and analysis

All solvents purchased from Beijing Chemical Factory were of analytical grade and redistilled in an all-glass system before

Table 1  
Initial physico-chemical characteristics of the soils used

Properties	T1	T2	T3	T4	T5
pH (water)	7.83	8.28	8.25	7.95	8.15
Soil particle size (%)					
<2 $\mu\text{m}$	12.1	38.4	2.61	4.19	4.47
2–50 $\mu\text{m}$	30.4	50.8	21.4	61.1	79.8
50–200 $\mu\text{m}$	57.4	10.8	76.1	34.7	15.7
Carbons fractions ( $\text{mg kg}^{-1}$ )					
WSOC <sup>a</sup>	99.7	146	107	72.0	160
FA <sup>b</sup>	93.5	123	54.3	45.0	110
HA <sup>c</sup>	6.15	23.0	52.3	27.0	50.0
HMs ( $\text{mg kg}^{-1}$ )					
Cd	2.58	1.24	1.35	1.28	0.80
Cr	77.5	75.5	76.6	67.6	64.2
Cu	41.5	36.0	61.8	25.3	22.8
Ni	38.5	35.8	33.8	33.0	28.1
Pb	35.0	38.4	97.9	52.1	29.7
LMW-PAHs ( $\mu\text{g kg}^{-1}$ )					
Naphthalene (Na)	546	502	300	414	246
Acenaphthene (Ace)	28.3	26.3	15.1	19.0	14.6
Acenaphthylene (Acy)	207	352	515	677	131
Fluorene (Fl)	19.1	23.5	19.1	25.1	18.7
Phenanthrene (Ph)	165	41.7	103	111	91.6
Anthracene (An)	484	471	229	287	196
HMW-PAHs ( $\mu\text{g kg}^{-1}$ )					
Fluoranthene (Flu)	30.1	35.3	64.5	15.9	30.8
Pyrene (Pyr)	401	259	61.0	109	88.3
Benzo(a)anthracene (BaA)	288	204	46.6	97.3	77.9
Chrysene (Chr)	128	140	47.0	35.5	32.8
Benzo(b)fluoranthene (BbF)	232	243	51.8	70.8	51.5
Benzo(k)fluoranthene (BkF)	304	301	53.5	74.0	79.5
Benzo(a)pyrene (BaP)	94.6	49.2	ND	69.3	70.5
Dibenzo(ah)anthracene (DBA)	263	117	ND	ND	ND
Indeno(1,2,3-cd)pyrene (InP)	101	ND	ND	ND	ND
Benzo(ghi)perylene (BghiP)	79.2	114	ND	ND	74.6

T1–T4: wastewater-irrigated soils; T5: reference soil. ND: not detected.

<sup>a</sup> WSOC: water soluble organic carbon.

<sup>b</sup> FA: fulvic acid.

<sup>c</sup> HA: humic acid.

use. Silica gel, alumina and sodium sulfate were purchased from Qingdao Haiyang Chemical Co. (Shandong, China) and activated before using at 180, 250, and 450 °C for at least 12 h, respectively, and then kept in a desiccator. Ten grams of freeze-dried soil and plant samples were weighed and mixed with 2 g anhydrous sodium sulfate. The samples were Soxhlet extracted for 24 h with 200 mL dichloromethane (DCM). The solvents were removed using a rotary evaporator and the volume was reduced to 1 mL under nitrogen. The extract was then cleaned using silica adsorption chromatography prepared with silica gel, alumina and capped with anhydrous sodium sulfate and eluted with 60 mL mixture of hexane and DCM (7:3, v/v). Afterward, the eluted fraction was again reduced to 1 mL under nitrogen for analysis of PAHs.

The final concentrated extracts from the environmental samples were analyzed using gas chromatograph mass spectroscopy (GC–MS, Agilent 6890, USA), equipped with a 5973 mass selective detector under the selected ion monitoring mode. An HP-5 silica fused capillary column (60 m  $\times$  0.25 mm inner

diameter  $\times$  0.25  $\mu\text{m}$  film thickness) was used with helium as the carrier gas at a constant flow rate of 1 mL min<sup>-1</sup>. The GC oven temperature was programmed from 50 °C (2 min) to 200 °C (2 min) at 10 °C min<sup>-1</sup>, then to 290 °C at 20 °C min<sup>-1</sup> and then held for 8 min. The injector and detector temperatures were 280 and 290 °C, respectively. Mass spectra were acquired at the electron ionization mode with an electron multiplier voltage of 1906 eV. For identification, selected ion monitoring (SIM) mode was carried out using the molecular ions selective for individual PAHs. Chromatographic data were collected and processed using GC–MS Chemstation software. At a concentration of 2000 mg L<sup>-1</sup> for each PAH, the stock reference standard mixture of 16 PAHs (Cat. No. 47930-U, Supelco Co.), includes naphthalene (Na), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Ph), anthracene (An), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(123-cd)pyrene (InP), dibenz(ah)anthracene

(DBA), and benzo(ghi)perylene (BghiP). The GC–MS detection limit was 0.001–0.01 mg L<sup>-1</sup>.

#### 2.4. HM analysis

Dried plant material was ground and about 0.25 g weighed into clean, dry digestion tubes. Concentrated HNO<sub>3</sub> (5 mL) was added and the samples were left overnight. On the following day, the tubes were placed on a heating block and the temperature was raised to 80 °C for 1 h and then to 120–130 °C for 20 h. After digestion, the cooled solutions were diluted to 50 mL with ultrapure water (Easy-pure, Dubuque, Iowa, USA) and filtered into plastic bottles pre-washed with acid. The concentrations of HMs in the acid digests were measured using graphite furnace atomic absorption spectrophotometer (GFAAS, Shimadzu-6300, Japan). Similarly, total soil HM concentrations were extracted by aqua regia at 160 °C and metal concentrations in the digests were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer OPTIMA-2000, USA).

#### 2.5. Quality control

Experiments on PAH recovery were carried out by spiking a known concentration of PAH standards (2 µg g<sup>-1</sup>) in uncontaminated soil and plant. The results showed satisfactory recovery, ranged from 92.5 ± 5.3% to 106.9 ± 4.1% for soil and plant, respectively. Similarly for HMs, a reagent blank and standard reference soil and plant materials (from the National Research Center for Standards in China) were included in the sample batch to verify the accuracy and precision of the digestion procedure and subsequent analysis.

#### 2.6. Data analysis

##### 2.6.1. Shoot and root transfer factors

PAHs and HM concentrations in the extracts of soils, roots and shoots were calculated on the basis of dry weight. Root concentration factors (RCFs) and shoot concentration factors (SCFs) are often used for contaminant concentrations in plants because soil-to-plant transfer is one of the major pathways for pollutants to enter the food chain. RCFs and SCFs were calculated as follows:

$$(i) \quad RCF = \frac{C_{\text{root}}}{C_{\text{soil}}}$$

$$(ii) \quad SCF = \frac{C_{\text{shoot}}}{C_{\text{soil}}}$$

where  $C_{\text{root}}$ ,  $C_{\text{shoot}}$  and  $C_{\text{soil}}$  represent the contaminant concentration in root, shoot and soil on dry weight basis, respectively.

##### 2.6.2. PAHs and HMs in dusts

$$Dd = SA \times AdDd$$

where Dd, SA and AdDd represent the daily dust deposition on lettuce (mg), surface area of the lettuce (m<sup>2</sup>), and average daily

dust deposition (mg m<sup>-2</sup>) as calculated from the plates.

$$TAd = Dd4 + \frac{Dd8}{2} \times 56 \text{ d}$$

where TAd, Dd4 and Dd8 represent the total average dry dust deposition on lettuce (mg), and daily dust deposition on lettuce (mg) at 4 and 8 weeks, respectively.

$$ATd\text{-tPAHs} = C\text{-tPAHs} \times TAd \times 10^{-6}$$

where ATd-tPAHs and C-tPAHs represent the average total deposition of the total PAHs (µg) on lettuce, and the average total PAHs concentration in the dust. However, HM concentrations in the dust samples were below the detection limits of ICP-OES, Perkin-Elmer OPTIMA-2000, USA.

The data were statistically analyzed using the statistical package SPSS 11.5. The measures were expressed in terms of mean, while the figures presented the mean values and standard deviation of four replicates. Statistical significance was computed using Duncan's multiple range test and Paired-samples *t*-test, with a significance level of  $P < 0.05$ .

### 3. Results

#### 3.1. Soil pollution

Table 1 summarizes the results of PAHs and HMs in soil samples collected from the study area. A wide range of soil PAH concentrations were observed and ranged from 12.1 to 546 µg kg<sup>-1</sup> for LMW-PAHs, while from 30.1 to 402 µg kg<sup>-1</sup> for HMW-PAHs. In the analyzed PAHs included those 16 PAHs considered as carcinogens by the US Environmental Protection Agency (US-EPA). The mean concentration of total LMW-PAHs in soil samples ranged from 798 to 1534 µg kg<sup>-1</sup>, and total HMW-PAHs varied from 324 to 1922 µg kg<sup>-1</sup>. The highest pollution levels were observed for soil samples collected from sites T1 and T2. The soil Cd, Cr, Cu, Ni and Pb concentrations ranged from 0.8 to 2.58, 64.2 to 77.5, 22.8 to 61.8, 22.8 to 38.5, and 29.7 to 97.9 mg kg<sup>-1</sup>, respectively. The results indicated that all metal concentrations were markedly higher than the reference values (T5). Furthermore, all the metal concentrations except for Cd, were below the Environmental Quality Standards set by the State Environmental Protection Administration (SEPA) for soils in China [18].

#### 3.2. Plant biomass

Plants grown in contaminated and reference soils were compared for dry root and shoot biomass (Fig. 2). In the contaminated-soil, the biomass of shoots and roots were significantly lower ( $P \leq 0.01$  to  $P \leq 0.001$ ) than the reference soil (T5). In soil T5, shoot and root biomass reached 4.52 and 1.29 g pot<sup>-1</sup>, respectively; while the lowest shoot dry weight (0.30 g pot<sup>-1</sup>) and root dry weight (0.17 g pot<sup>-1</sup>) was observed in soil T1.



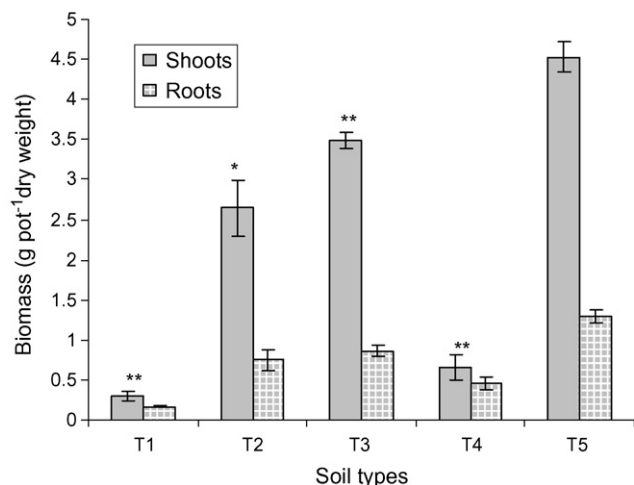


Fig. 2. Root and shoot biomass on dry weight basis. The error bars indicate the standard deviation while asterisks (\* and \*\*) indicate significantly low biomass for whole plant at the  $P \leq 0.01$  and  $P \leq 0.001$ , respectively, for contaminated-soil grown plants than reference soil grown plants.

### 3.3. PAHs in plant tissues

The selected PAHs were divided into two categories as low molecular weight PAHs (LMW-PAHs) having two or three benzene rings and high molecular weight PAHs (HMW-PAHs) having four or more benzene rings. Figs. 3 and 4 show the variation in percentages of PAH compounds with different molecular weights in shoots and roots. Total LMW-PAH concentrations in the root samples ranged from 457 to 718  $\mu\text{g kg}^{-1}$ , and were significantly higher ( $P \leq 0.01$ ) in the contaminated soils than in the reference soil (294  $\mu\text{g kg}^{-1}$ ). Except T3, the measured concentrations of LMW-PAHs in the root samples increased with increasing concentrations in soils. Root concentrations of LMW-PAHs were two-to-three times lower than the soil concentrations (Table 1). LMW-PAH concentrations in the shoot ranged from 209 to 338  $\mu\text{g kg}^{-1}$ , and were significantly higher ( $P \leq 0.001$ ) in the contaminated soils than in the reference soil (143  $\mu\text{g kg}^{-1}$ ). Shoot concentrations of LMW-PAHs were four-to-five times lower than the corresponding soil concentrations.

Except for T3 and T4, the total HMW-PAH concentrations in root samples varied from 179 to 925  $\mu\text{g kg}^{-1}$ , and were significantly higher ( $P \leq 0.01$ ) in the contaminated soils than in the reference soil (223  $\mu\text{g kg}^{-1}$ ). Fig. 4 shows the variation patterns of total HMW-PAHs in roots and shoots. The measured concentrations of HMW-PAHs in the root samples increased with increasing soil concentrations. Root HMW-PAH concentrations were two-to-three times lower than the soil concentrations. Except T3 and T4, HMW-PAH concentrations (42.3–139  $\mu\text{g kg}^{-1}$ ) in the shoots were also significantly higher ( $P \leq 0.05$ ) in contaminated soils than in the reference soil (45.2  $\mu\text{g kg}^{-1}$ ). Shoot concentrations were 10–16 times lower than the respective soil concentrations.

The values of RCFs and SCFs for PAHs are given in Table 2. RCFs generally decreased with increasing soil PAH concentrations. Similarly, linear regression analysis was performed to examine the relationship ( $R^2$ ) between the concentrations

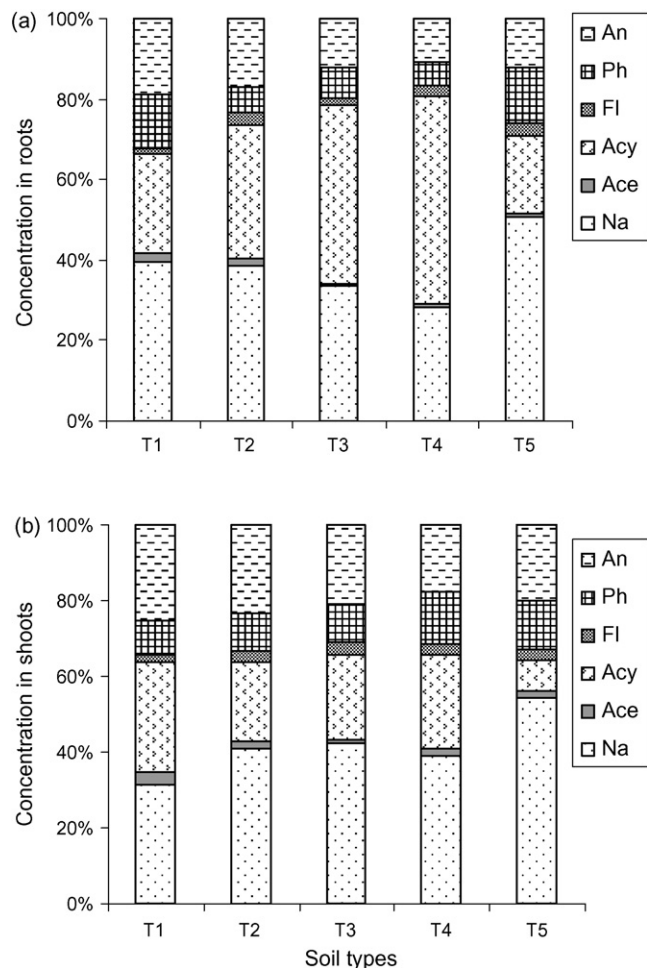


Fig. 3. The profile of LMW-PAHs concentrations in root and shoot tissues on dry weight basis: (a) LMW-PAH concentrations in roots; (b) LMW-PAH concentrations in shoots.

Table 2  
PAH soil-to-plant transfer factors (on dry weight basis,  $n = 20$ )

PAHs	RCFs		SCFs	
	Mean	S.D.	Mean	S.D.
<b>LMW-PAHs</b>				
Na	0.64	0.14	0.33	0.07
Ace	0.43	0.11	0.29	0.14
Acy	0.71	0.10	0.24	0.21
Fl	0.78	0.14	0.42	0.06
Ph	0.67	0.20	0.41	0.21
An	0.31	0.05	0.22	0.04
<b>HMW-PAHs</b>				
Flu	0.84	0.07	0.32	0.10
Pyr	0.55	0.11	0.09	0.04
BaA	0.55	0.15	0.11	0.03
Chr	0.43	0.13	0.09	0.01
BbF	0.47	0.29	0.06	0.03
BkF	0.52	0.18	0.06	0.03
InP	0.28	0.03	0.07	0.02
BghiP	0.53	0.30	0.12	0.03

S.D.: standard deviation.

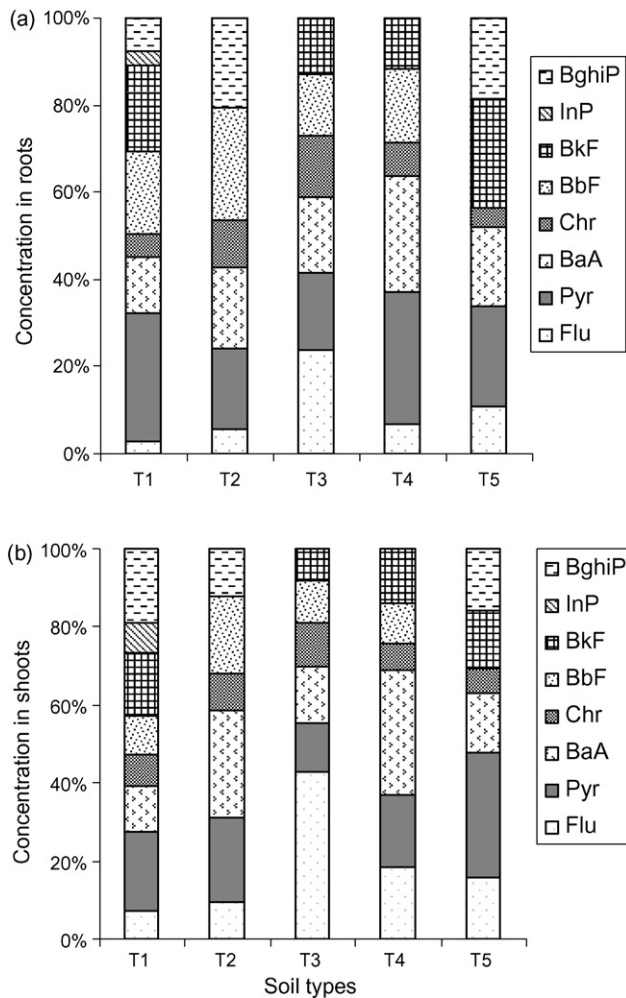


Fig. 4. The profile of HMW-PAH concentrations in root and shoot tissues on dry weight basis: (a) HMW-PAH concentration in roots; (b) HMW-PAH concentration in shoots.

of soils, roots and shoots for both LMW and HMW PAHs (Figs. 5 and 6). The  $R^2$  values ranged from 0.34 to 0.90 for correlation between the concentrations of root and shoot of LMW-PAHs, while values varied between 0.48 and 0.73 for HMW-PAHs. Similarly, the  $R^2$  values changed from 0.72 to 0.93 for root and soil concentrations of LMW-PAHs, and from 0.62 to 0.97 for HMW-PAHs. The  $R^2$  values for shoot and soil ranged from 0.51 to 0.92 for LMW-PAHs and varied from 0.02 to 0.60 for HMW-PAHs. Similarly, the  $R^2$  value was 0.89 for total PAHs in soils and roots, and 0.99 for total PAHs in shoots and roots.

#### 3.4. PAHs and HMs in dust samples

The surface area of lettuce leaves was calculated to estimate the dust deposition using the plate's data. The daily mass of the dust particles deposited on plates was  $92 \text{ mg m}^{-2}$ . The average SA of lettuce grown in T1, T2, T3, T4, and T5 soils, was 100, 196, 289, 121, and  $400 \text{ cm}^2$  at 4 weeks, respectively, and 169, 900, 1024, 289, and  $1225 \text{ cm}^2$  at 8 weeks (harvest), respectively. TAd values on lettuce grown in T1,

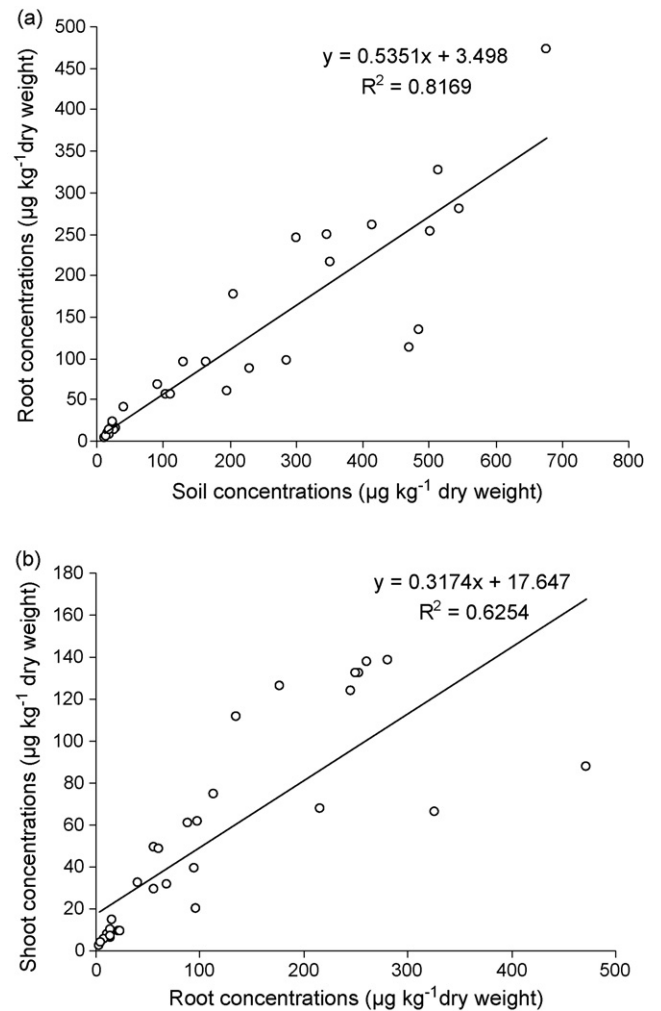


Fig. 5. Linear regression model for LMW-PAH concentrations in soil, root and shoot tissues on dry weight basis: (a) relationship between soil and root concentrations; (b) relationship between root and shoot concentrations.

T2, T3, T4, and T5 soils, were 44.5, 233.6, 266.4, 75.6, and  $319.2 \text{ mg}$ , respectively. In the dust samples, the average total PAH concentration was  $7.3 \text{ mg kg}^{-1}$ . Furthermore, the ATd-tPAHs values on lettuce grown in T1, T2, T3, T4, and T5 soils were 0.3, 1.7, 1.9, 0.6, and  $2.3 \mu\text{g}$ , respectively. Hence the aerial supply of PAHs through dust deposition was 0.1, 0.5, 0.8, 0.2, and 1.2% of the total shoot PAH concentrations in the plants grown in T1, T2, T3, T4, and T5 soil samples, respectively.

#### 3.5. HMs in plant tissues

A summary of the selected HM concentrations in plant tissues are shown in Fig. 7 and total soil metal concentrations are given in Table 1. Heavy metal concentrations in the root and shoot tissues of plants were compared with the standards set for vegetables and fruits in China. According to the SEPA, China [19], the maximum permissible limits of Cd, Cr, Cu, Ni, and Pb for vegetables and fruits are 0.2, 0.5, 20, 10, and  $9 \text{ mg kg}^{-1}$ , respectively, on a dry weight basis. The Cd concentrations in the root and shoot samples were exceeded the SEPA limit for

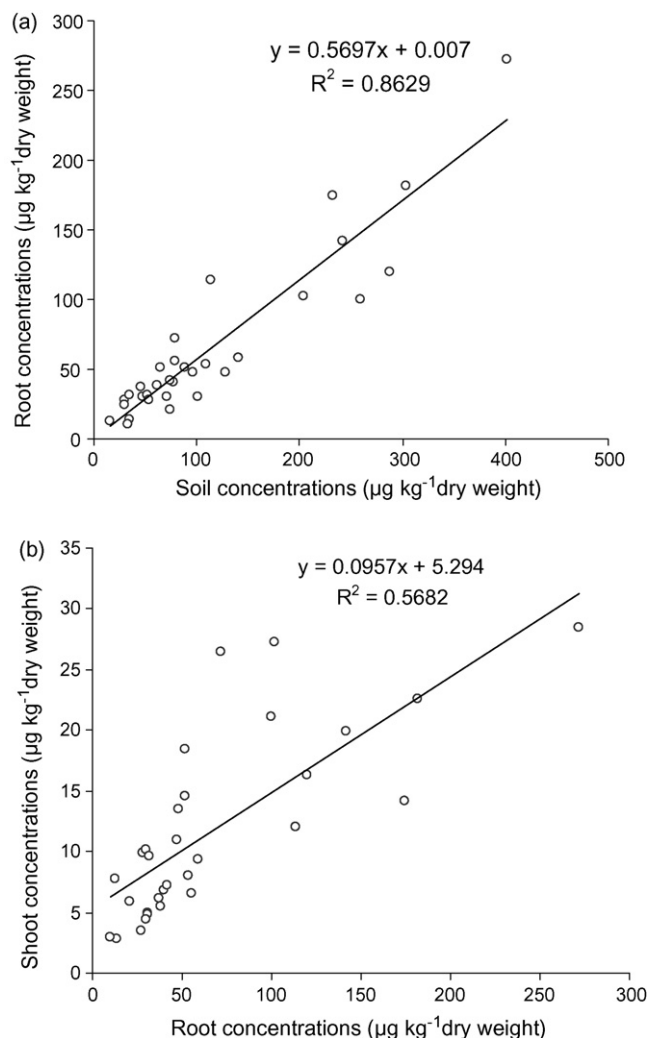


Fig. 6. Linear regression model for HMW-PAH concentrations in soil, root and shoot tissues on dry weight basis: (a) relationship between soil and root concentrations; (b) relationship between root and shoot concentrations.

vegetables and fruits in China (Fig. 7a). Similarly, the Cr and Ni concentrations in the root and shoot tissues were also exceeded the SEPA limit (Fig. 7b and c). Among the analyzed metals in plant tissues, only Cu concentration was below the permissible limit set by the WHO for plants.

The values of RCFs and SCFs for HMs are given in Table 3. For all selected metals, RCFs ranged from 0.06 to 0.93, while SCFs values varied between 0.02 and 0.61. Regression anal-

Table 3

HM transfer factors (on dry weight basis) for plants grown in wastewater-contaminated soils ( $n = 20$ )

Metals	RCFs		SCFs	
	Mean	S.D.	Mean	S.D.
Cd	0.51	0.14	0.42	0.13
Cr	0.09	0.01	0.05	0.01
Cu	0.23	0.07	0.20	0.07
Pb	0.15	0.06	0.07	0.03
Ni	0.69	0.15	0.39	0.07

S.D.: standard deviation.

Table 4

Linear regression ( $R^2$ ) of soil concentrations with shoots and roots; and soil concentrations with transfer factors

Metals	Soil with shoots and roots		Soil with transfer factors	
	Shoots	Roots	SCFs	RCFs
Cd	0.62 ns	0.82*	0.96*	0.13 ns
Cr	0.94**	0.33 ns	0.95*	0.91**
Cu	0.13 ns	0.62*	0.99**	0.60 ns
Pb	0.17 ns	0.77*	0.95*	0.88**
Ni	0.82*	0.09 ns	0.95*	0.15 ns

ns: not significant; \*significant at  $P \leq 0.05$ ; \*\*significant at  $P \leq 0.01$ .

ysis was performed to identify the relationships between the metal concentrations in soil and shoot and also for soil and root concentrations (Table 4).

#### 4. Discussion

Results from the present and previous studies [8,13,20–22] demonstrate that the plants grown on contaminated-soils may contain PAHs and HMs in their tissues which pose a major health concern. PAHs may be taken up through several pathways including the aerial absorption of volatile compounds by leaves, deposition and penetration of contaminated soil particles and dusts on leaves, and soil-to-root transfer of contaminants followed by subsequent translocation by the transpiration stream. However, root uptake is implicated as the main pathway for PAHs accumulation in plant tissues, particularly for HMW-PAHs [17]. The highest biomass was noticed in the reference soil (T5), possibly because of its high value of WSOC and low pollution level for both PAHs and HMs as compared to wastewater-irrigated soils. In this study, the plants grown in T1 and T4 soils posed high plant toxicity most probably because of the high concentration of total LMW-PAHs, and particularly naphthalene which has 20 times more toxicity than HMW-PAHs because of its volatilization [23]. The HMW-PAHs with low solubility are generally less mobile and have low vapor pressure and high octanol–water ( $K_{OW}$ ) and octanol–air ( $K_{OA}$ ) partitioning coefficients. According to Sverdrup et al. [24], PAHs (such as Ph, Pyr, Flu, and Fl) with log  $K_{OW}$ -values ranging between 3.5 and 5.2 reduced the early stage growth of terrestrial plants. In addition, LMW-PAHs are more mobile in the soil and more likely to adsorb onto the root surface and easily transfer further into the root interior [25]. Our data indicated that PAH concentrations in lettuce grown on contaminated-soils were markedly high and generally similar to those values reported in the literatures [7,22]. However, PAH concentrations of this study were higher than the LMW and HMW PAH concentrations in lettuce grown on industrial contaminated soils [25]. Furthermore, PAHs from aerial deposition through contaminated soils and dust particles was nearly negligible, as indicated from the PAH measurements of dust samples recovered from plates used in this experiment.

A wide range of variation in RCFs for both LMW-PAHs and HMW-PAHs could be contributed to their difference in soil concentrations and soil physico-chemical properties. Similarly,

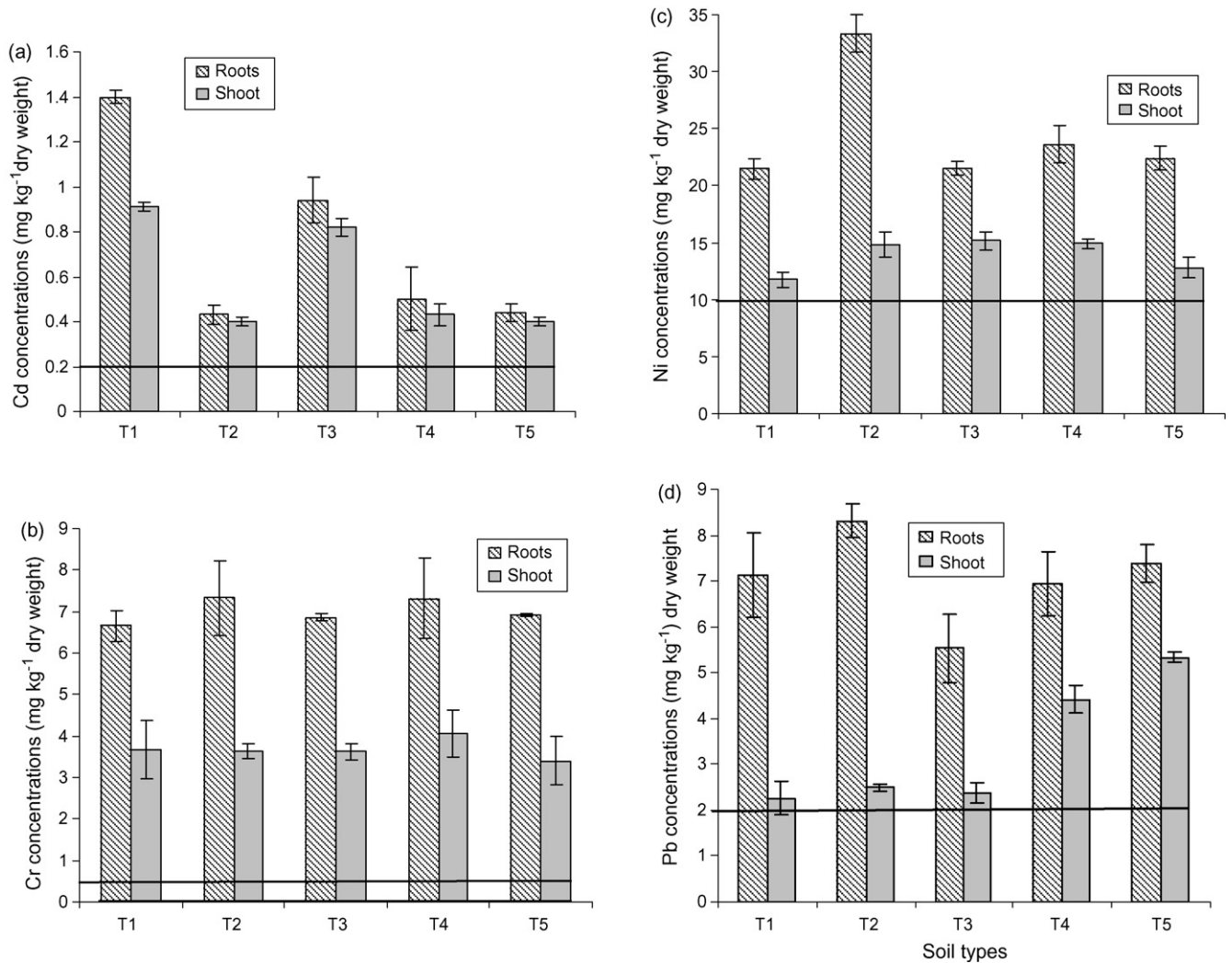


Fig. 7. HM concentrations in the root and shoot tissues on dry weight basis: (a) Cd; (b) Cr; (c) Ni; (d) Pb. The error bars indicate the standard deviation, while horizontal lines indicate the SEPA limits for Cd, Cr and Ni set for vegetables and fruits in China and WHO limit for Pb.

SCFs also varied widely for LMW-PAHs, but less for HMW-PAHs because most of the HMW-PAHs might not easily be translocated from root to shoot, particularly for Chr, BbF, DBA and BghiP, due to their low solubility and low concentration in soils. However, a fraction of the PAHs in the roots may be those strongly adsorbed on the root epidermis [25]. The linear regression also shows a strong relationship between the concentrations in soil and root, suggesting that root epidermis contributes a portion of PAHs. However, soil and shoot PAH concentrations were also plotted using linear regression model and this correlation for LMW-PAHs ( $R^2$  from 0.51 to 0.92) was stronger than HMW-PAHs ( $R^2$  from 0.02 to 0.60), suggesting that translocation for LMW-PAHs is easier than HMW-PAHs. However, the health risk associated with PAHs contamination in soils and vegetables cannot be predicted based on exposure level because the identification of most hazardous PAHs and their mode of action in producing specific health effects remain uncertain, making it difficult to quantify the risk precisely. In addition to food chain, other pathways, including inhalation and dermal contact, contribute to the human exposure to environmental carcinogenic

PAHs [26]. Environmental quality standards and guidelines for PAHs in soil and vegetables have not been established in many countries, including China. In order to limit human exposure to PAHs, setting environmental standards should be of primary concern for public health policy.

Accumulation of HMs by agricultural crops is also a public health concern [20,27]. This study shows that elevated concentrations of HMs were accumulated in plants grown in contaminated soils. In the study area, soil contamination with HMs is mainly due to the wastewater irrigation and application of sludge to the farmlands. HM concentrations in plants were inconsistent with the results in literature for both leafy and non-leafy locally grown vegetables [28]. Concentrations of Cd, Cr and Pb were higher than those in lettuce plants grown on wastewater-irrigated-farmlands in Beijing [13]. However, the concentrations of Cu and Ni found in this study were lower than those in spinach and Egyptian clover plants irrigated through sewage water in India [11]. Similarly, the Pb concentration was lower than the concentration in lettuce plants grown in contaminated-soil collected from Barber Orchard, Haywood



County, NC, United States [29]. The Cd, Cu and Pb concentrations were also lower than those detected by Muchuweti et al. [30] in vegetables irrigated by wastewater in Zimbabwe. As mentioned above, plants were contaminated with Cd, Cr, Ni and Pb and exceeded the guideline limits set by SEPA and WHO for vegetables and fruits. The consumption of such highly contaminated foodstuffs is likely to cause human health risks.

Soil-to-plant transfer factors are often used to estimate metal uptake ability of plants and it is one of the key components of human exposure to metals through food chain. In order to investigate the health risk associated with wastewater-contaminated soils, it is essential to assess the transfer of metals from soil to plants [27]. The RCFs and SCFs were highest for Cd and Ni, and varied widely for plants cultivated in different soil samples, followed by Cu, Pb and Cr (Table 3). The high transfer values for Cd and Ni from soil to plant indicate a strong accumulation of the respective metals. The metal transfer from soil to plants measured in this study was lower than those reported in literature for lettuce [27], likely because of the differences in soil properties, particularly pH. The total transfer factor (RCFs + SCFs) of Cr, Cu, Pb and Ni decreased with increasing respective total metal concentrations in soils indicating an inverse relationship between transfer factors and total metal concentrations. This relationship was also reported by Wang et al. [31] for several leafy and non-leafy vegetables. However, for Cd, the soil to plants transfer factor increased with increasing total Cd concentrations in soils; this depends on metal availability as reported by our previous study [32].

Regression analyses showed that the relationship was strong for soil Cr and Ni with plant shoots ( $R^2$  value 0.94 and 0.82, respectively) while its values were high for soil Cd, Cu and Pb with plant roots ( $R^2$  value 0.82, 0.62, and 0.77, respectively). The regression model was also used for establishing the relationship between the metal concentrations in soil and the SCFs or RCFs values. Results indicated a strong relationship between the soil concentrations and shoot or root concentrations (Table 4). According to Wang et al. [31], the soil-to-plant transfer factor of a given crop–metal system should be estimated from the regression model between the transfer factor values and the corresponding soil metal concentrations.

In this study we focused our attention on PAHs and HMs uptake and translocation in plant grown on wastewater-contaminated soils. Results indicate that the lettuce was severely contaminated with HMs particularly with Cd, Cr, Ni, and Pb. This finding also suggests that the consumption of such polluted vegetables grown in wastewater-irrigated soils could pose a threat to the health of the consumers. According to Wang et al. [28], the consumption of vegetables and fish contaminated with the HMs such as Cd, Cr, Cu, Hg, Pb, and Zn is the most likely route for human exposure in Tianjin, China. Furthermore, they investigated that the Cd and Hg are the major contributors counting for 45% and 51% to the target hazard quotients. Our data further demonstrate that metals and PAHs in vegetables grown in wastewater-contaminated soils may represent a significant pathway for human exposure to these two groups of contaminants.

## 5. Conclusion

Leafy vegetables, particularly *L. sativa* L. grown on wastewater-contaminated soils contain PAHs and HMs in shoots and roots with elevated concentrations. The LMW-PAHs dominated in shoots and roots due to their high solubility thus greater uptake and translocation of PAHs into plants. The concentrations of LMW-PAHs in the roots were two-to-three times lower than the soil concentrations, while LMW-PAH concentrations in shoots were four-to-five times lower than the respective soil concentrations. Shoot and root concentrations are positively related to soil concentrations. Similarly, the HMW-PAH concentrations in the root samples were two-to-three times lower, while shoot concentrations were 10–16 times lower than the respective soil concentrations. RCFs and SCFs also suggest that plant concentrations were higher for PAHs with a high solubility. Furthermore, this study suggests that soil-to-plant transfer is one of the major pathways of PAHs transport into shoot and root of plants grown in wastewater-contaminated soils. It was also concluded that vegetables grown in wastewater-irrigated soils were contaminated with Cd, Cr, Pb and Ni exceeding the permissible limits for vegetables. The transfer factor for HMs was found to be in the order of Ni > Cd > Cu > Pb > Cr. As far as human health risks are concerned, such contaminated-farmlands should not be used for cultivation of leafy vegetables.

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