Assessing radish (*Raphanus sativus* L.) potential for phytoremediation of lead-polluted soils resulting from air pollution

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ABSTRACT

The objective of this study was to investigate the capability of radish to extract lead from soils contaminated with lead resulting from air pollution. A randomized block experiment design was performed. The soil was contaminated with $PbNO_3$ and the treatments consisted of 180 (standard), 250, 350, 450, 800 and 1000 mg/kg lead. After development, plants were harvested and divided into shoots and roots. The lead content of each plant part as well as the soil-lead were measured. The results indicated a non-linear positive relation between the lead concentrations in soil and that accumulated in plant roots and shoots. By increasing the lead concentration in soil, its accumulation in plant tissues was also increased. Most of the extracted lead was accumulated in the roots (208.1 mg/kg) compared to shoots (27.25 mg/kg). Since radish can be seeded up to five times a year, and its yield may reach up to 20 t/ha, it can be used to remediate lead-polluted topsoils (0–10 cm).

Keywords: lead; phytoextraction; radish; soil pollution; *Raphanus sativus* L.

Environmental pollution is one of the most important current concerns for human's daily life. The most common heavy metals in polluted areas are reported to be Cd, Pb, Cr, Cu, Hg and Ni (United States Environmental Protection Agency 1997). Among these, Cd and Pb are the most important toxic contaminants that enter from different sources into the environment, plants and food chain. Consequently, conducting any research on decontamination of these environmental hazardous elements and minimizing their influence on food chain is vital. Soil is the final recipient of many waste components of human activities, especially those related to mining, industrial emissions, disposal or leakage of industrial wastes, and car-related activities. Usually lead concentration varies between 1 to 200 mg/kg in soil; its average concentration is about 13 mg/kg. Combustion of leaded fuel releases large amount of lead into the air; released lead is then deposited on soil surface and enters into the soil with rain or irrigation water. Coarse lead particles releasing from vehicle pipes usually disperse and precipitate on soil surface in the distance of about 50 to 100 m from the roads. However, the dispersed lead particles with less than 2 μm in diameter can move much further from this area. Ecological consequences of soil pollution with heavy metals are directly related to the movement and solubility of heavy metals. These two factors govern the pollutant transfer from soil surface into the water table, soil microbial availability, soil microorganisms, agricultural production, and human populations (Leeper 1972, Emmerich et al. 1982). Lead accumulation in human body may cause different symptoms such as ceasing heme production in blood or serious brain damages, particularly in children.

One technology to remediate contaminated soils is the so-called phytoextraction. Phytoextraction technology can be considered as a sustainable, environment-friendly and inexpensive technique. It has minimum destructive impact on the ecosystem (USEPA 1997). In this method, the so-called hyperaccumulator plants are used to clean up the contaminated soils. Numerous studies by several investigators were conducted on different aspects of phytoremediation (e.g. Kos et al. 2003, Vysloužilová et al. 2003, Finžgar et al. 2007, Kalaji and Loboda 2007, Komárek et al. 2007, Turan and Esringü 2007, Grejtovský et al. 2008, John et al. 2008) and all confirm that this technology to clean up the contaminated sites is efficient and environment-friendly. Phytoextraction is a time-consuming method, but does not require high technical knowledge. The idea to use plants for extracting heavy metals from soil was introduced by Baker and Brooks (1989) and Raskin et al. (1994). The first recognized plants with high capabilities to absorb heavy metals belonging to Brassicaceae and Fabaceae families (Chaney et al. 2000). However, development of phytoremediation methods still need more effort and extensive research on widely different scientific disciplines including biochemistry, breeding, microbiology and environment (Bennett et al. 2003). Selecting suitable plants for phytoextraction is one of the most important issues to remediate polluted sites. To achieve optimal results, the plants that are able to concentrate high amounts of heavy metals in their roots, shoots and leaves with preferably high biomass should be selected, as the amount of metal uptake strongly depends on plant biomass. Different types of plants including trees, vegetables, grasses and weeds for accumulating wide range of heavy metals have been recognized; several hundreds of plants are recognized worldwide as metal hyperaccumulators. Among these, however, only few plants are reported to accumulate lead. The highest amount of accumulated lead is reported for *Brassica juncea* (Baker and Brooks 1989, Shen et al. 2002). This plant not only absorbs large amount of lead in its root, but also can transport the absorbed lead from roots to its shoots, which is an important characteristic for phytoextraction purpose. When the plants were grown in nutrient solution, more than 1.5% Pb^{2+} was found in their buds tissues (Kumar et al. 1995). The phytoextraction coefficient for *Brassica juncea* is reported to be 1.7 (USEPA 1997). The plant tolerance to specific pollutant in soil to use for phytoextraction purpose is very important. Since phytoremediation technology is based on the use of plants to clean up polluted soils, the difference between total concentration of metal in soil and the plant concentration threshold value is an important factor to check phytoextraction feasibility. Lead absorption by roots is a passive process. The lead absorbed by roots is the result of a cation exchange process at cell wall; traps between cell spaces and vacuoles may accumulate it as lead carbonate in root cell walls (Epstein et al. 2000). The amount of lead absorbed by roots from soil depends on physiological characteristics of plants such as plant type, age and root system and physicochemical characteristics of soil such as CEC, calcium carbonate content, organic matter, pH and phosphorus concentration (Jarvis and Leuang 2002). It is obvious that the metals within such plants can be recycled by means of gasification, extraction with acid, non-aerobic digestion or extraction of plant oil (Schnoor 1997). By taking up an element or by decomposition of pollutant components in the rhizosphere or selective absorption of a metal, plants can reduce contamination from the polluted sites (Pulford et al. 2003). Reviewing the available literature shows that only few studies have been conducted to study phytoextraction of soils polluted with lead from cars exhaustion. For instance, soils in the vicinity of highways and green spaces in Tehran contain considerable lead concentrations particularly at the depth of 5–10 cm. The objective of this study was to investigate the capability of radish (*Raphanus sativus* L.) to extract lead from contaminated soils with high content of Pb. Since the rooting depth of radish is about 10–15 cm, its capacity to take up high lead content from soil as a hyperaccumulator plant was the main concern of this study. Other objectives were then to assess the capability of radish to phytoextract lead from polluted soils and to obtain the efficiency of radish for phytoextraction purpose. Consequently, an extensive experiment was conducted to find out if radish can be introduced as a Pb-hyperaccumulative plant.

MATERIAL AND METHODS

A randomized block experiment design with six treatments and five replicates was established in the greenhouse under uncontrolled environmental conditions. The designed treatments consisted of 180 (standard), 250, 350, 450, 800 and 1000 mg/kg lead. The lead concentration in the standard treatment (Pb1) was 180 mg/kg. The reason for such high Pb concentration was that these soils were taken from the sides of highways in Tehran that are continuously exposed to exhaustion of vehicles.

Table 1. Some physical and chemical properties of the experimental soil

Sand (%)	Silt (%)	Clay (%)	Soil texture	EC_e (dS/m)	CEC (meq/ 100 g soil)	Total lead concentration (mg/kg)	OM (%)	pH_e
56	25	19	Sandy clay loam	1.2	13	180	1.08	7.3

EC_e – electrical conductivity of a saturated soil paste extract; CEC – cation exchange capacity; OM – organic matter; pH_e – pH of saturated soil paste

To get some information on lead concentration of the soils, six samples were taken randomly from the soils near the highways where the possibility of lead pollution was high. To contaminate the experimental soils, the soils were first thoroughly mixed with Lead Nitrate. The soil contamination process was performed under three continuous steps until the target concentrations were achieved. After that, 5 other treatments including 250, 350, 450, 800 and 1000 mg/kg lead denoted as Pb2, Pb3, Pb4, Pb5, Pb6 were applied. In order to obtain a reliable set of data, five replicates for each treatment were established. The treatments with their replicates were left for 80 days to obtain chemical equilibrium, receiving water every 24 hours. When the chemical equilibrium between lead and soil was obtained, different lead forms in the soil treatments were measured. The soil pH was measured with a pH meter, the electrical conductivity of saturation extract with a conductivity meter, calcium carbonate content with titration method, particle size distribution with hydrometer method, cation exchange capacity, soil organic matter concentration with Walkley and Black method, and total soil lead with extraction method proposed by USEPA (1986). Table 1 gives some physical and chemical properties of the experimental soils.

The soils were carefully packed in the pots to obtain a uniform bulk density of 1.35 g/cm^3. The soil containers with 20 cm in diameter and 10 cm in height with were established in the greenhouse. The seeds were then seeded in the pots in the amount of 250 seeds per square meter. The target number of seeds per each pot was 8, but 12 seeds were first seeded and then thinned to 8. Having a standard treatment, the soil water content was always held at field capacity to prevent any water stress during the whole growth period. Since the growth period of radish is relatively short (about 45 days), no pesticide was used. When plants were fully developed, radishes were harvested and divided into shoot and root parts. Different chemical forms of lead concentrations in the soil were then measured with continuous extraction method (USEPA 1986). The lead concentration in shoots and roots was measured by digestion with complex of nitric acid-perchloric acid and sulfuric acid. The soil lead concentration was measured, using the Atomic Absorption apparatus. Finally, the effect of soil lead concentration on lead absorbed by different parts of radish including shoot and roots was performed with statistical comparison of averages, using the Duncan's multiple range test method with MSTATC software.

RESULTS AND DISCUSSION

To distinguish the influence of different soil lead forms on phytoextraction of lead by radish, different amounts of lead forms were measured. The results of these chemical analyses are given in Table 2. As can be seen in this table, the lowest lead concentrations belong to the soluble lead form. It is obvious that among different soil lead types, only the soluble and available forms can be absorbed by plants. Only soluble and exchangeable forms of lead thus determine capability of plant to absorb this metal. However, according to the obtained results lead has little availability. Similar results are also reported by Blaylock et al. (1997) and Salt and Kramer (2000). As can be seen in Table 2, the soluble lead comprised only

Table 2. Soluble, exchangeable and organic lead concentrations in the experimental soil treatments after equilibrium (mg/kg)

Treatment	Soluble	Exchangeable	Organic
Pb1	0.18	1.06	11.72
Pb2	0.25	1.53	15.12
Pb3	0.31	2.22	21.99
Pb4	0.47	2.94	28.60
Pb5	0.83	5.03	51.18
Pb6	1.06	6.92	69.21

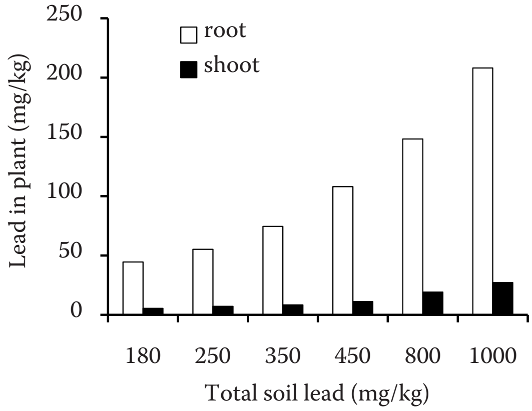

Figure 1. The relationship between total soil Pb and the lead absorbed by roots and shoots

0.05% of the total lead, while the organic lead and exchangeable lead comprised 3.2 and 0.32% of the total soil lead, respectively. Therefore, total available lead for plant is only 0.37% of the total soil lead. Still, even such little available concentration is considerable to remediate soil.

Among different factors influencing phytoremediation, two important limiting factors are pollutant fixation by soil particles and low absorption and/or transportation by plants. The starting point to recognize suitable plants for phytoremediation of a pollutant is to measure its total concentration and soluble and non-soluble forms. These concentrations for the experimental soil treatments are given in Table 2; the relationship between available lead and its different forms in soil is nonlinear. The relationship between soil Pb concentrations and lead accumulated in radish roots and shoots are presented in Figure 1. The results obtained from statistical comparison of average accumulative lead in roots and shoots with the Duncan's multiple range test indicate that the highest lead absorption occurred in the treatment with 1000 mg/kg Pb ($P = 0.01$). The results of statistical analysis of accumulated lead in radish roots and shoots are given in Tables 3 and 4, respectively. It was further observed that by increasing the lead concentration in the soil, its accumulation in plant tissues was also increased. Furthermore, there was no toxicity for radish up to 1000 mg/kg soil lead. Consequently, it may be concluded that radish can be used as a hyperaccumulator plant to remediate lead-polluted soils. Figure 1 shows the influence of total soil lead on its absorption by radish.

The overall results obtained in this study indicate that there exists a non-linear positive relationship between the lead concentrations in the soil and that accumulated in plant roots and shoots. It was also observed that by increasing the lead concentration in soil, its accumulation in plant tissues increased. The major lead accumulation occurred in the roots rather than shoots. The measured lead accumulated in roots was 208 mg/kg while it was 27.25 mg/kg in harvested shoots. Since radish can be seeded up to five times a year in the same soil, and because its yield may reach up to 20 t/ha, it can be used to remediate topsoils polluted with lead from air. Since radish is a vegetable, if used for lead phytoextraction, it should be treated as hazardous waste immediately after harvesting.

Table 3. Statistical analysis of accumulated lead in radish roots

	df	*SS*	*MS*	*F*	*P*
Between	5	97788.9	19557.784	4244014	0.0001
Within	24	0.111	0.005		
Total	29	97789			

CV = 0.06%

Table 4. Statistical analysis of accumulated lead in radish shoots

	df	*SS*	*MS*	*F*	*P*
Between	5	1780.831	356.166	30035.097	0.000
Within	24	0.285	0.012		
Total	29	1781.116			

CV = 0.083%

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