Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2022.28430

262 (2022) 111–120
June

Lead phytoremediation potential of *Ligustrum texanum* (Wax-leaf Privet) in the presence of humic acid

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Received 11 November 2021; Accepted 23 March 2022

ABSTRACT

Assisted phytoremediation technique is greatly progressed, but is still in development and needs further study. Thereby, in this study, *Ligustrum texanum* (Wax-leaf Privet) was exposed to soil treated with the various concentrations of lead nitrate ($Pb(NO3)_2$) (0, 100, 300, 500, and 700 mg L^{-1}) and humic acid (HA) (0 and 300 mg L^{-1}) irrigation solution for the three-month period, in order to evaluate the species' ability to phytoremediation of soil Pb^{2+}; and the effect of HA on this process. Un-planted pots were also used and treated with Pb in order to investigate the direct role of plants in soil remediation. The effect of Pb and HA on various plant morphological traits (plant tissue's fresh and dry weight, plant and root length, number of leaves, and collar diameter) were also detected. Up to 200 μg g^{-1} Pb in soil (as a result of 500 mg L^{-1} Pb irrigation solution), Pb led to a positive (stimulative) effect on various plant morphological traits, in contrast, Pb in higher concentrations had a negative (repressive) effect on plant growth. Humic acid had increased plant morphological traits whilst is not promising for contaminated soil phytoremediation process due to reducing uptake and accumulation of Pb through plant roots. According to the results, this evergreen species is prominent for Pb phytoremediation in contaminated soils due to its high tolerance to Pb contamination, high Pb uptake, and plant affordability.

Keywords: Phytoremediation; Phytostabilization; Soil contamination; Lead; Humic acid

1. Introduction

With the development of industry, mining activity, fertilizer application, use of wastewater for irrigation and the application of sewage sludge, soil heavy metals contamination has become a worldwide concern. Heavy metals have been and continue to be used for industrial, agricultural, and domestic purposes such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and military operations that all used heavy metals such as Cd, Cu, Pb and Zn [1–3]. Some heavy metals such as Mn, Fe, Cu, Zn, Mo, and Ni are essential or beneficial micronutrients for plants whereas, others such as Cd, Pb, and Hg are not essential for them and also, in high concentrations have strong toxic effects on plants and threaten the environment; many types of research have devoted to inhibiting the movement of heavy metals from soil to the food chain [4–6]. Due to the high toxicity of Pb and its compounds, actions to prevent and repair environmental contamination are common in our days. Materials and devices containing Pb must not be disposed of in the environment, especially in domestic sewage

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in order to avoid the presence of this toxic element in the food chain. The effects of its toxicity are varied and can modify bones, the central nervous and cardiovascular systems, the kidneys and the liver [7]. The maximum limit of Pb in agricultural crops and soil is 0.5–10 and 72 mg kg^{-1} [8]. The emerging field remediation technique called phytoremediation is one of the cleanest, most convenient, environmentally acceptable and cost-effective means by which plants can accelerate the decontamination of soil contaminants, directly or indirectly [9–11]. In this technique, metals are collected by plant roots and then accumulate in plant tissues [12]. Phytoremediation has two main subsets, phytoextraction which used plant and soil amendments it into uptake contamination and transformed to aerial and harvestable parts in order to remove contamination; and phytostabilization that used for reducing the mobility of contaminants through accumulation in roots or immobilization within the rhizosphere so reduces metal dispersion [13,14]. The plants that have a deep root system, high tolerance, and high biomass production and also, have the ability to accumulate target contamination, grow fast and easily propagate are prominent species for phytoremediation of contaminated soils [13,15]. There are many methods for reducing the accumulation of heavy metals in plants. Among these methods, soil amendments are applied to remediate heavy metals, which is considered a realistic and cost-effective method [16]. Humic substances, which are part of soil organic matter, comprise substances that have quite high molecular weights formed by a secondary synthetic reaction; that enhances soil fertility and physicochemical properties were used as a soil amendment [17]. These humic substances are naturally derived from organic materials of high molecular weight found in soils and sediments as well as decomposition of animal and plant residues [18]. Humic acid contains acidic groups such as carboxyl and phenolic OH functional groups [19] and therefore provide organic macromolecules with an important role in the transport, bioavailability and solubility of heavy metals [20]. More than 85% of the total Pb absorbed by the plants was retained in the roots of leguminous tree species [21]. *Ligustrum texanum* (Wax-leaf Privet) is the high tolerance, evergreen and fast-growing Japanese shrub so was used in this experiment as a Pb-contaminated soil accumulator (never used and studied before) and humic acid (HA) was used as a soil amendment as well. In this experiment, the phytoremediation potential of *L. texanum* (with and without HA) and the effects of Pb and HA on morphological traits of this species were investigated.

2. Materials and methods

2.1. Sample preparation

Thirty seedlings of biennial *L. texanum* were purchased from a botanic garden (Babolsar, Mazandaran, Iran, 2019-6-8). Seedlings were kept for two weeks in a greenhouse (University of Mazandaran, Babolsar, Mazandaran, Iran), with the normal ambient condition to adapt to the environmental conditions before starting the experiment. Seedlings pots in 10 groups (for each treatment) were irrigated for a three-month period (90 d) in the growing season (summer, 2019-6-22 to 2019-9-20) with $Pb(NO3)_2$ (99%, Merck, Germany) and HA (80%, Daesin Crop., Korea) irrigation solution in different concentrations including, 0, 100, 300, 500 and 700 mg L^{-1} every 3 d (30 times, each time 100 mL solution) and 0 and 300 mg L^{-1} each 6 d (15 times, each time 50 mL solution) for Pb and HA respectively. Each treatment was performed in triplicates. The weather condition of these three months was average high and low temperature 29.4°C and 21.23°C respectively, average daylight and sunshine 13.4 h and 6.8 h respectively, and 80% humidity. For each concentration of Pb (five treatments), three replicates of pots of the same size and containing an equal amount of soil but without planting any seedlings were used and treated as non-planted (NP) treatments.

2.2. Soil characteristic

Plants were planted in polyethylene pots (20 cm height, 10 cm^2 surface area and 200 cm^3 volume) filled with two kg (dry weight) sandy loam soil. Soil composition included clay, silt, sand, cow manure, compost fertilizer and rice bran in a volume ration of 1:2:3:1:2:1. The sand, clay and silt fractions of the initial soil were determined using the hydrometer method according to the method described by Bouyoucos 1952 [22]. Soil texture classification was defined by McDonald et al. [23] method. Organic carbon (OC) was evaluated using oxidation by the dichromate digestion method [24]. Organic matter content (OM) was determined by the Walkey-Black method [25]. The pH was measured by the $CaCl_2$ method [26]. Electrical conductivity (EC) was measured in saturation extracts according to Rhoades [27]. Total nitrogen (N) was measured by Walkley and Black method [28]. To measure the available potassium (K) and sodium (Na) Junsomboon and Jakmunee method was used [29], with an extract and use a photometer (Photolab S6, WTW Co, Germany). To prepare the extract, 5 g soil was mixed with a total of 25 g marine sand and placed into a glass column with 2 cm diameter wide and 33 cm height. Then 1 cm of sand, 25 mL of ammonium acetate (98%, Merck, Germany) were added, and then 10 mL of extract sample was collected and subsequently, the extract was diluted whit deionized water to 100 mL in a balloon.

2.3. Plant morphological traits measurement

After the irrigation period (90 d), seedlings were harvested and separated to root, shoot and leaves in order to fresh weight measurement that is determined by scales (accurate to 0.001 g; AND Co, DJ-V 320A, China). Then separated tissues were rinsed with deionized water to remove any surface contamination, dried between laboratory papers, and finally, oven-dried (SH-DO-54NG, SH-Scientific Co., South Korea) at 70°C to a constant weight [30] in order to measure dry weight by scales (accurate to 0.001 g; AND Co., DJ-V 320A, China). Plant and root length were also measured by a meter (accurate to 1 mm) and collar diameter was determined by a digital caliper (accurate to 0.01 mm).

2.4. Pb analysis

1 g of the soil of treatments was transferred to 10 mL hydrofluoric acid (48%, Merck, Germany; Ultrapure) and

heated in an oven for 60 min at 200°C. Then, 16 mL nitric acid (65%, Merck, Germany; Suprapur), 4 mL perchloric acid (70%, Merck, Germany; Ultrapure) and 10 mL hydrochloric acid (37%, Merck, Germany; Ultrapure) were added. The tubes were shaken for 1 h with a shaker (145 rpm; Vibromix50, Domel, Slovenia). Finally, the extract was diluted to 50 mL with deionized water. Then each sample was filtered using the filter paper (595, Schleicher and Schuell Filters, pore size 4 lm) and the Pb concentration in the solution was performed by AAS at 217.0 nm wavelength (Perkin-Elmer 1100B) [31].

In order to Pb concentrations determination in plant tissues, roots, shoots and leaves were dried at 70°C for 48 h (when their weight remained constant) in the oven (SH-DO-54NG, SH-Scientific Co, South Korea). Thereby dry samples were powdered with a homogenizer (MillMix20, Domel Co, Slovenia) and mixed (subsample ca. 0.01 g) with a nitric acid (65%, Merck, Germany; Suprapur), sulfuric acid (95%, Merck, Germany; Ultrapure) and perchloric acid (70%, Merck, Germany; Ultrapure) in a volume ratio of 8:2:1 respectively. After 24 h, samples were heated at 100°C for 30 min to remove acidic vapors and after cooling down, samples were diluted up to 50 mL with deionized water. Subsequently, the concentrations of Pb were analyzed by using flame AAS at 217.0 nm (Perkin-Elmer 1100B; Gupta et al. 2008). Final concentrations were expressed as µg in g dry weight.

The standard for the AAS calibration was prepared in the extraction solution by the addition of appropriate quantities of Pb [32].

2.5. Calculation methods

2.5.1. Bio-concentration factor

Bio-concentration factor (BCF) demonstrates the plant's ability for metal accumulation in plant tissues (root, shoot and leaves). It is calculated as a ratio of Pb concentration in plant tissues to Pb concentration in soil of each treatment [34].

$$\mathrm{BCF} = \frac{\text{metal concentration in plant}(\mu g / g)}{\text{metal concentration in medium}(\mu g / g)} \quad (1)$$

2.5.2. Translocation factor (TF %)

Translocation factor (TF) demonstrates the plant's ability to translocate metals from roots to harvestable parts (shoots and leaves). It is calculated as a percentage ratio of Pb concentration in shoots (for S/R %) and leaves (for L/R %) to Pb concentration in roots [33,34].

$$\mathrm{TF} = \frac{\text{Pb concentration in leaves and shoots}(\mu g / g)}{\text{Pb concentration in roots}(\mu g / g)} \times 100 \quad (2)$$

2.5.3. Plant resistance index (PRI %)

Plant resistance index (PRI) demonstrates the plant resistance to heavy metals and calculates the percentage ratio of treatment dry weight to control dry weight.

$$\mathrm{PRI} = \frac{\text{Treatment dry weight}(g)}{\text{Control dry weight}(g)} \times 100 \quad (3)$$

2.5.4. Plant phytoremediation efficiency (PPE %)

Plant phytoremediation efficiency (PPE) demonstrates the plant's ability to remediate contamination from soil and is calculated as the ratio of the Pb concentration of nonplanted to planted pot soil.

$$\mathrm{PPE} = \frac{\begin{array}{c}\text{Pb concentration of nonplanted}\\ \text{soil treatment}(\mu g / g)\end{array}}{\begin{array}{c}\text{Pb concentration of planted}\\ \text{soil treatment}(\mu g / g)\end{array}} \times 100 \quad (4)$$

2.6. Quality assurance and quality control

Due to not planting seeds, soil and plant contamination conditions were assessed before purchase, after confirmation of no contamination, plants with almost the same height and weight were purchased. Thirty pots with seedlings were situated at a one-meter distance between the pots in order to exploit all plants equally from environmental conditions and prevent the overshadowing of plants. Humic acid and Pb solutions were shaken to dissolve the Pb or HA particles completely, then added to the soil surface to penetrate deep into the potting soil. The irrigations with Pb and HA were done in separated days in order to prevent any interference between them and impede soil saturation. A pot plate was placed under each pot in order to check that no amount of irrigation solution in each irrigation exited from the bottom of the pots. Glass roof of the greenhouse was prevent rainfall or any other external water from entering the pots. No other fertilizer or material except HA and Pb was added to the soil during the experiment. All acids used in the study were ultra-pure grade (Merck, Germany). To eliminate any adsorbed metals and contamination, all containers used in this experiment were kept in 10% HNO_3 for 24 h and finally rinsed with ultrapure water. Analysis of Pb performed in triplicates. Blanks and standard materials were used for the accuracy and precision of the analysis. The precision was found to be less than 5%. Recoveries ranged between 94% and 101.5%.

2.7. Data analysis

Based on the results gathered, we calculated for further analysis bio-concentration factor (BCF), translocation factor (TF), plant resistance index (PRI) and plant phytoremediation efficiency (PPE) according to formulas presented in the Supplementary material. All data were tested for goodness of fit to a normal distribution with Shapiro–Wilk's test. The data were not normally distributed, so we used non-parametric procedures. The Kruskal–Wallis test was used to evaluate plant morphological traits between various concentrations of Pb and HA, and the Mann–Whitney test was used to compare Pb concentrations in various plant tissues and phytoremediation efficiency factors. A value of 0.05

was set as a significance level. Microsoft Excel ver. 2016 (Microsoft Corp., Redmond, WA, USA) and SPSS ver. 22 (SPSS Inc., Chicago, IL, USA) were used for data manipulation and all statistical analyses.

3. Results

3.1. Plant morphological traits

Lead and HA affected all measured plant morphological traits (plant and root length, number of leaves, collar diameter, plant tissues fresh and dry weight and saturated leaves weight) and there was a significant difference between each of these traits in various treatments ($p < 0.05$). Plant morphological traits increased with enhancing the Pb concentration of irrigation solution to 500 mg L^{-1} whereas in 700 mg L^{-1} these traits decreased as well as wilting symptoms appeared. Humic acid led to increasing plant morphological traits in comparison to the non-HA treatments with the same Pb concentration. The highest and the lowest plant length was observed in Pb 500 HA (72.67 ± 6.03 cm) and Pb 700 non-HA (43.67 ± 5.03 cm, Table 2). Root length also increased to 15.83 ± 2.02 cm in (Pb 500 HA) and decreased to 8.17 ± 0.76 cm (Pb 700 non-HA, Table 2). The number of leaves increased in treatments under 500 Pb, and the highest and lowest amount was measured in Pb 500 HA (244.33 ± 16.65) and in Pb 700 (73.67 ± 2.52) respectively (Table 2). There was a significant decrease in collar diameter (27.27% on average) in Pb 700 and Pb 700 HA in comparison to other treatments (Table 2). Plant fresh weight increased to 192.67 ± 12.01 g (Pb 500 HA) and decreased to 90.67 ± 5.13 g (Pb 700 non-HA, Table 3). The highest fresh weight of root, shoot and leaf was measured 73.67 ± 8.50 g, 47.67 ± 8.32 g, 0.34 ± 0.01 g respectively, all in Pb 500 HA. While, the lowest fresh weight of the root, shoot and leaf was measured all in Pb 700 non-HA in order: 24.33 ± 3.05 g, 12.67 ± 6.43 g and 0.10 ± 0.02 g respectively (Table 3). The highest root, shoot and leaf dry weight also was measured in order: 36.67 ± 2.52 g, 34.67 ± 2.08 g, 190.33 ± 8.02 mg respectively, all in Pb 500 HA and the lowest was measured in order: 11.33 ± 1.53 g, 14.67 ± 2.52 g, 66.33 ± 19.42 mg all in Pb 700 non-HA (Table 3). Saturated leaf weight had the same trend also; the highest and the lowest were measured in Pb 500 HA (0.61 ± 0.10 g) and Pb 700 non-HA respectively (0.24 ± 0.21 g, Table 3).

Table 1
Physico-chemical characterizations of control soil

Parameter	Value
Sand (%)	70
Silt (%)	23
Clay (%)	7
Soil texture	Sa·L[a]
OC (%)	2.55
OM (%)	4.44
pH in water	6.74
EC (mS)	2.3
Total N (%)	0.13
Available Na (μg g^{-1})	227
Available K (μg g^{-1})	383
Soluble Pb (μg g^{-1})	0.05

[a]Sandy loam soil texture;
OC – Organic carbon; OM – Organic matter; EC – Electrical conductivity.

3.2. Pb concentration in plant tissues

Generally, the trend of the Pb concentration in plant tissues was in the range of root > shoot > leaves. There was a significant difference between various Pb concentrations of plant tissues (root, shoot and leaves; $p < 0.05$; Fig. 2). The lowest amount of Pb concentration was almost 0 μg g^{-1} in control and the highest was 1,675 ± 5.86 μg g^{-1}, 196.33 ± 5.03 μg g^{-1}, 14.6 ± 0.65 μg g^{-1} in the root, shoot and leaves respectively, all in Pb 700 non-HA (Fig. 1). In all these three tissues, when the Pb concentration of irrigation solution was increased subsequently, the Pb concentration of plant tissues was raised as well (Fig. 2).

Table 2
Morphological traits of *Ligustrum texanum* in expose to various concentrations of Pb and HA after the three-month period

Treatments	Plant length (cm)	Root length (cm)	Number of leaves	Collar diameter (mm)
Control	47.33 ± 12.09^{ad}	11.33 ± 2.08^{cd}	124.67 ± 33.08^{ab}	9.63 ± 2.11^{cdef}
Control HA	53.67 ± 5.51^{ad}	13.33 ± 0.58^{abcd}	142.67 ± 30.28^{abc}	10.33 ± 1.53^{abcdef}
Pb 100	58.33 ± 2.52^{ad}	13.33 ± 1.15^{abcd}	165 ± 40.36^{adc}	10.36 ± 2.51^{abcdef}
Pb 100 HA	66.67 ± 19.55^{ab}	13.83 ± 2.02^{abcd}	166.00 ± 53.67^{abc}	11.00 ± 2.27^{abdef}
Pb 300	67.33 ± 8.39^{ab}	15.33 ± 4.72^{abc}	199.33 ± 53.97^{bc}	12.36 ± 3.30^{abcde}
Pb 300 HA	69.33 ± 7.77^{ab}	15.33.08^{abc}	203.00 ± 56.95^{bc}	12.33 ± 0.51^{abcde}
Pb 500	72.33 ± 6.43^{b}	15.52 ± 4.44^{abc}	239.67 ± 21.13^{c}	12.03 ± 0.70^{abcde}
Pb 500 HA	72.67 ± 6.03^{b}	15.83 ± 2.02^{abc}	244.33 ± 16.65^{c}	12.37 ± 1.09^{abcde}
Pb 700	43.67 ± 5.03^{a}	7.17 ± 2.36^{e}	73.67 ± 2.52^{a}	7.73 ± 1.27^{f}
Pb 700 HA	45.33 ± 11.93^{ad}	8.17 ± 0.76^{de}	76.67 ± 18.58^{a}	8.40 ± 0.53^{def}

Values with the same letters did not differ statistically.

Table 3
Morphological traits (weights) of *Ligustrum texanum* in expose to various concentrations of Pb and HA after the three-month period

Treatments	Plant fresh weight (g)	Root fresh weight (g)	Shoot fresh weight (g)	Average leaf fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)	Average leaf dry weight (mg)	Average saturated leaf weight (g)
Control	140 ± 37.75^{acd}	43.00 ± 9.16^{f}	22.15 ± 3.51^{ab}	0.25 ± 0.03^{abc}	14.33 ± 2.52^{abc}	17.67 ± 3.05^{ab}	148.44 ± 14.01^{abc}	0.43 ± 0.08^{ab}
Control HA	158 ± 20.30^{acc}	48.33 ± 4.16^{e}	28.67 ± 4.51^{abc}	0.27 ± 0.02^{abcd}	16.00 ± 4.36^{abc}	19.67 ± 1.53^{abc}	119 ± 12.49^{fbcd}	0.44 ± 0.08^{ab}
Pb 100	168 ± 10.58^{abc}	52.66 ± 10.11^{bd}	34.67 ± 3.51^{bcd}	0.28 ± 0.03^{abcd}	17.00 ± 3^{abc}	22.33 ± 1.53^{abc}	134.33 ± 19.50^{cde}	0.44 ± 0.05^{ab}
Pb 100 HA	176 ± 47.57^{bc}	55.66 ± 2.89^{bd}	36.00 ± 4.36^{bcd}	0.29 ± 0.03^{bcd}	19.33 ± 2.52^{abc}	24.33 ± 6.51^{abcd}	140.33 ± 13.01^{cde}	0.45 ± 0.14^{ab}
Pb 300	186 ± 22.54^{c}	58.00 ± 7.21^{b}	38.67 ± 3.51^{cd}	0.28 ± 0.03^{bcd}	23.33 ± 4.16^{bcd}	27.33 ± 2.52^{bcde}	154.67 ± 12.22^{def}	0.49 ± 0.16^{ab}
Pb 300 HA	189.33 ± 51.54^{c}	62.33 ± 6.51bd	39.67 ± 4.93^{cd}	0.30 ± 0.02^{bcd}	24.67 ± 6.43^{cd}	28.67 ± 4.51^{cde}	168.67 ± 3.51^{ef}	0.51 ± 0.15^{ab}
Pb 500	190.67 ± 24.70^{c}	69.33 ± 10.26ac	46.33 ± 2.52^{b}	0.31 ± 0.03^{cd}	29.00 ± 6.08^{de}	32.33 ± 1.53^{de}	175.67 ± 6.51^{ef}	0.52 ± 0.17^{ab}
Pb 500 HA	192.67 ± 12.01^{c}	73.67 ± 8.50ac	47.67 ± 8.32^{b}	0.34 ± 0.01^{d}	36.67 ± 2.52^{e}	34.67 ± 2.08^{e}	190.33 ± 8.02^{f}	0.61 ± 0.10^{b}
Pb 700	90.67 ± 5.13^{a}	24.33 ± 3.05ef	12.67 ± 6.43^{a}	0.10 ± 0.02^{e}	11.33 ± 1.53^{a}	14.67 ± 2.52^{a}	66.33 ± 19.42^{a}	0.24 ± 0.21^{a}
Pb 700 HA	92.67 ± 13.65^{ad}	24.67 ± 2.89ef	18.00 ± 3.60^{a}	0.11 ± 0.03^{e}	12.33 ± 2.52^{ad}	15.33 ± 4.51^{a}	78.67 ± 30.17^{ab}	0.27 ± 0.04^{a}

Values with the same letters did not differ statistically.

3.3. Pb concentration in soil

There was a significant difference between soil Pb concentrations in various treatments (Fig. 2; $p < 0.05$). The lowest Pb concentration was measured in the control (0.05 μg g^{-1}, Table 1). The highest amount of Pb concentration was measured in Pb 700 HA (254 μg g^{-1}) and Pb 700 NP (986 μg g^{-1}), in planted and non-planted treatments, respectively. With increasing the Pb concentration of irrigation solution, the Pb concentration of soils increased in both planted and non-planted treatments. Soil Pb concentration of non-planted pots was significantly higher in comparison to planted treatments with the same irrigation solution (Fig. 2).

3.4. Phytoremediation and tolerance factors

There was a significant difference in each tolerance (PRI) and phytoremediation factor (BCF, TF and PPE) of various treatments [$p < 0.05$; Table 4, Eqs. (1)–(4)]. The highest and lowest BCF was observed in Pb 700 non-HA (7.37) and Pb 300 HA (3.42), respectively (without considering control, Table 4, Eq. (1). The highest S/R % and L/R % (TF %) were observed in Pb 700 (13.7% ± 0.35%) and Pb 100 (1.6% ± 0.1%) respectively [Table 4, Eq. (2)]. With increasing the Pb concentration of irrigation solution, PRI was increased up to 137.61% ± 8.58% in Pb 500 however, it decreased significantly to 64.76% ± 3.66% in Pb 700 [Table 4, Eq. (3)]. PPI was constantly increased with increasing the Pb concentration of irrigation solution. The lowest and the highest PPI (without considering control) were in Pb 100 (150.33% ± 48.95%) and Pb 700 HA (418% ± 21.63%) respectively (Table 4).

4. Discussion

We found that *L. texanum* uptakes Pb and remediates contaminated soil. Irrigating plants with high Pb concentration solutions reduced plant growth whilst, lower Pb concentration stimulated it. Humic acid increased plant growth however, it reduced plant phytoremediation efficiency due to the reduction of root Pb uptake and soil Pb disruption, so it caused Pb retention in soil. This species is appropriate for the phytostabilization process due to its high root accumulation and high tolerance to soil contamination.

4.1. Bioremediation potential

Plant growth in contaminated soil depends on different factors such as plant stress tolerance, kind of contamination, contamination concentration, soil pH and soil organic matter content [35,36]. Pb concentration of plant tissues were in order root > shoot > leaves and with increasing the Pb concentration of irrigation solution, the Pb concentration of plant tissues (root, shoot and leaves) increased as well, so they had a direct relation (same result as *Crambe abyssinica* and *Brassica juncea*; [21,37]. Lead primarily accumulates in root cells, because of the blockage by Casparian strips within the endodermis; besides, lead is trapped by the negative charges that exist on roots' cell walls [37]. The enhanced removal rates of the contaminant in planted vs. unplanted soil were mainly

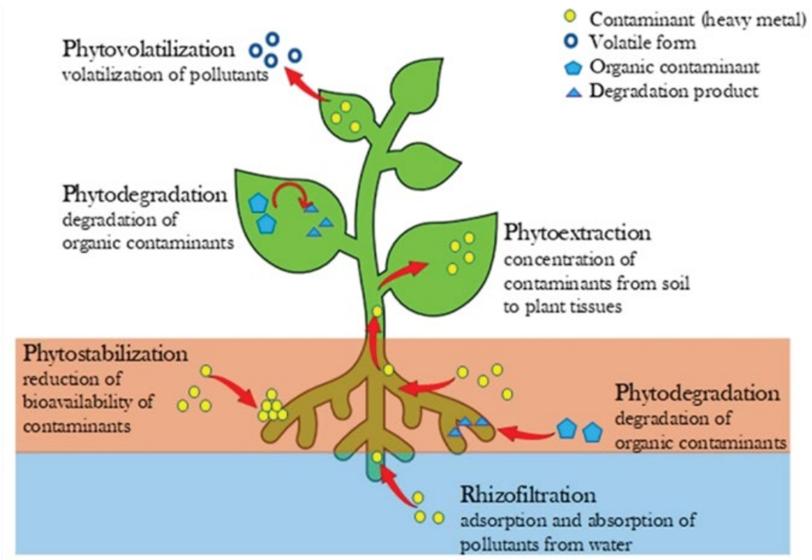

Fig. 1. Schematic representation of different phytoremediation approaches.

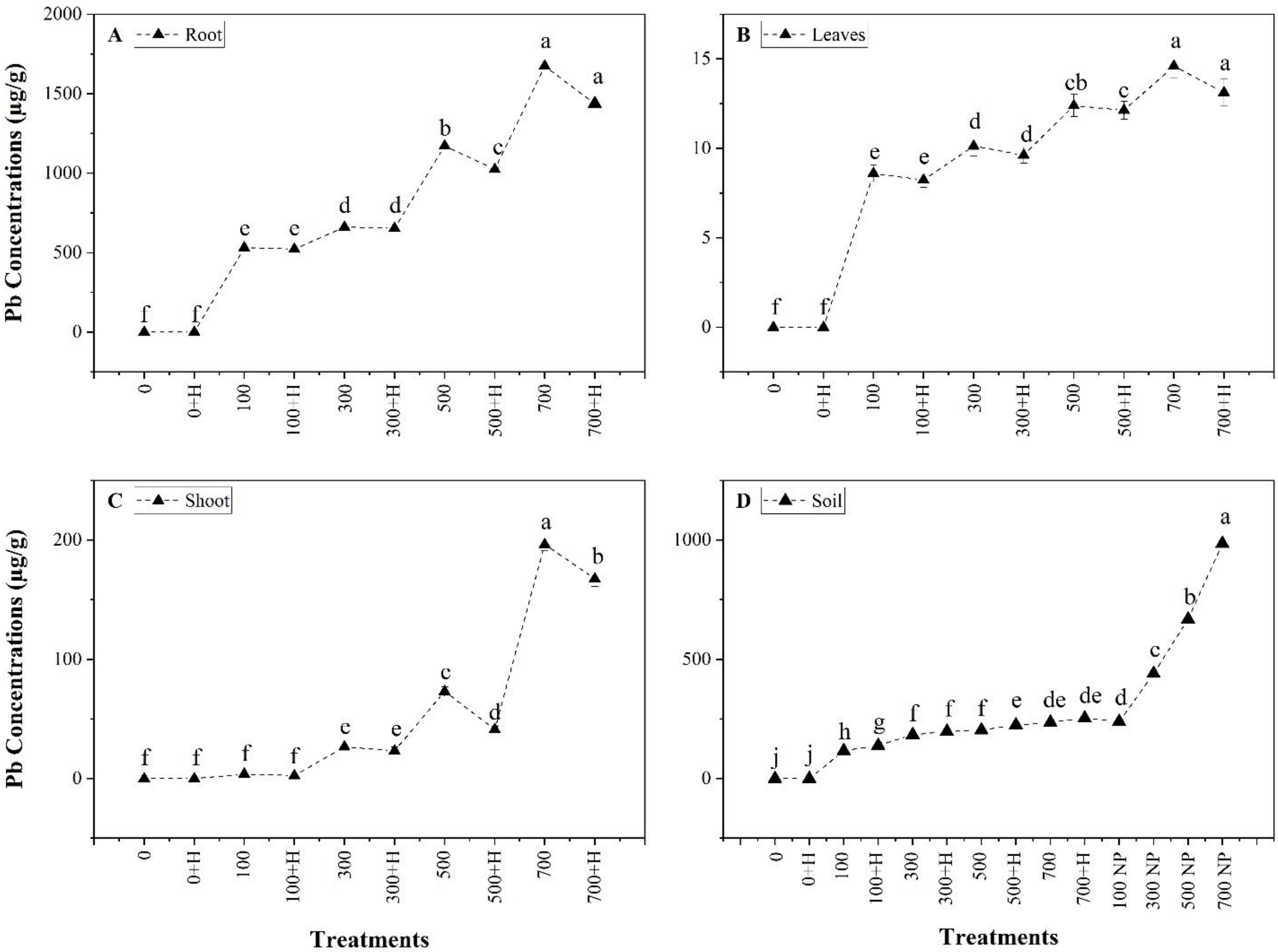

Fig. 2. Pb concentration of *Ligustrum texanum* tissues (root, shoot and leaves) and pot soils after three-month irrigation with various concentrations of Pb and HA; Values with the same letters did not differ statistically.

ascribed to plant-promoted biodegradation. BCF reflects both Pb concentration in soil and plant, so it can be used for demonstration of the Pb accumulation from medium to plant or maintenance of Pb in soil (Formula 1). BCF was relatively high (BCF > 1) in *L. texanum*, which indicates that this species has a great ability to uptake and accumulate Pb from soil to plant tissues. In order to detect plant species' ability for phytoextraction or phytostabilization strategies Mendez and Maier [38] suggested values of TF > 1 (or 100%) for an efficient plant application of phytoextraction and TF < 1 (or 100%) for the phytostabilization process. According to the values of TF obtained in this study (TF < 100%), *L. texanum* resulted to be more adequate for phytostabilization in contaminated sites (same as *Brassica juncea*, Table 4, Formula 2) [21]. Heavy metal excluders accumulate Pb more in roots than aerial parts [39], so they can be used to prevent the entrance of heavy metals into the food chain. PPE showed the plant phytoremediation efficiency and the direct role of plants in soil remediation due to comprising soil Pb concentration in planted and un-planted treated soils. Therefore *L. texanum* has prominent efficiency to use in the soil phytoremediation process due to its high PPE (Table 4, Formula 4).

4.2. Influence of Pb irrigation

We have found that Pb in high concentrations (more than 500 mg L^{-1} and 200 μg g^{-1}, in irrigation solution and soil respectively) had negative effects on plant morphological traits (plant tissues fresh and dry weight, saturated leaf weight, plant and root length, number of leaves and collar diameter) whereas, low concentrations of Pb (under 500 mg L^{-1} and 200 μg g^{-1}, in irrigation solution and soil respectively) can stimulate plant growth (same result as *Crambe abyssinica* and *Brassica juncea*) [37,21]. A number of studies also reported that high concentrations of heavy metals can inhibit plant growth [40] and biomass production [41]. In contrast, some other studies reported the growth-promoting impact of different contaminants like Pb [36,42]. The contamination with heavy metals reduced photosynthesis, increased breathing as well as leading to a negative effect on mineral nutrition and productivity in general [37]; It seems, trace amounts of heavy metals stimulate plant growth, while high concentrations of that can lead to inhibition of growth and cause injury in plants [36]. Pb once in its roots can cause difficulties or even block the water and nutrient entries, thus reducing nutrients absorption and translocation [37]. Average Saturated leaf weight indicates the effect of Pb on leaves' water content and there was a reverse relation between Pb concentrations and saturated leaves weight (Table 3). PRI showed the plant resistance to contamination due to comprising treated plants to control dry weight, so results indicate that up to Pb 500, seedlings had a great tolerance to Pb but in Pb 700 PRI decreased significantly and the plant loosed its resistance [Eq. (3)]. So, the Pb contamination tolerance threshold for *L. texanum* is a number between 500 to 700 mg L^{-1} and 200–235 μg g^{-1} for irrigation solution and soil respectively.

4.3. Influence of humic acid supplementation

Humic acid had positive effects on plant morphological traits (plant growth) whereas, it had negative effects on plant phytoremediation efficiency. Generally, plant morphological traits in treatments with HA were higher than non-HA treatments (Tables 2 and 3). Similarly, Atiyeh et al. [43] found that plant growth traits were increased with the addition of HA in the range of 100–1,000 mg kg^{-1}. Many studies have shown that HA improves plant growth and increases plant biomass in both contaminated and uncontaminated soil [42,15] and there is a positive relationship between plant biomass production and effective phytoremediation [44]. There were lower plant tissue Pb concentrations in treatments with HA in comparison to non-HA treatments (with the same Pb concentration of irrigation solution; Fig. 2). Thereby, HA reduced Pb accumulation and uptake in plants growing in contaminated soil [36]. Hence there were higher soil Pb concentrations in HA treatments than non-HA treatments (with the same Pb concentration of irrigation solution, Fig. 2). Humic acid

Table 4
Phytoremediation and tolerance factors of *Ligustrum texanum* in exposure to various concentrations of Pb and HA after the three-month period

Treatments	BCF	TF (S/R) %	TF (L/R) %	PRI%	PPE%
Control	0.89 ± 0.08^{d}	0.00 ± 0.00^{e}	0.0 ± 0.00^{d}	100.00 ± 0.00^{ab}	0.00 ± 0.00^{f}
Control HA	0.78 ± 0.05^{d}	0.00 ± 0.00^{e}	0.0 ± 0.00^{d}	100.00 ± 0.00^{ab}	0.00 ± 0.00^{f}
Pb 100	4.86 ± 0.52^{b}	0.67 ± 0.06^{e}	1.6 ± 0.10^{a}	119.10 ± 7.56^{a}	205.00 ± 20.88^{de}
Pb 100 HA	3.91 ± 0.41^{c}	0.43 ± 0.60^{e}	1.6 ± 0.10^{a}	128.09 ± 23.54^{a}	150.33 ± 48.95^{e}
Pb 300	5.70 ± 0.24^{b}	4.00 ± 0.40^{d}	1.5 ± 0.10^{a}	132.85 ± 16.09^{a}	239.00 ± 8.54^{cd}
Pb 300 HA	3.42 ± 0.14^{c}	3.50 ± 0.40^{d}	1.5 ± 0.05^{a}	135.23 ± 23.50^{a}	222.67 ± 10.50^{d}
Pb 500	7.26 ± 0.23^{b}	6.10 ± 0.40^{c}	1.2 ± 0.05^{b}	136.19 ± 17.65^{a}	328.67 ± 6.11^{b}
Pb 500 HA	5.37 ± 0.24^{a}	4.00 ± 0.20^{d}	1.1 ± 0.05^{bc}	137.61 ± 8.58^{a}	297.00 ± 10.58^{bc}
Pb 700	7.37 ± 0.40^{a}	13.70 ± 0.35^{a}	1.0 ± 0.01^{b}	64.76 ± 3.66^{b}	418.00 ± 21.63^{a}
Pb 700 HA	6.80 ± 0.26^{a}	11.60 ± 0.41^{b}	0.8 ± 0.05^{c}	66.19 ± 9.75^{b}	405.33 ± 31.13^{a}

Values with the same letters did not differ statistically;
BCF – Bio-concentration factor, TF S/R – Translocation factor between shoots and root, TF L/R – Translocation factor between leaves and root, PRI – plant resistance index, PPE – plant phytoremediation efficiency; Formulas 1, 2, 3 and 4

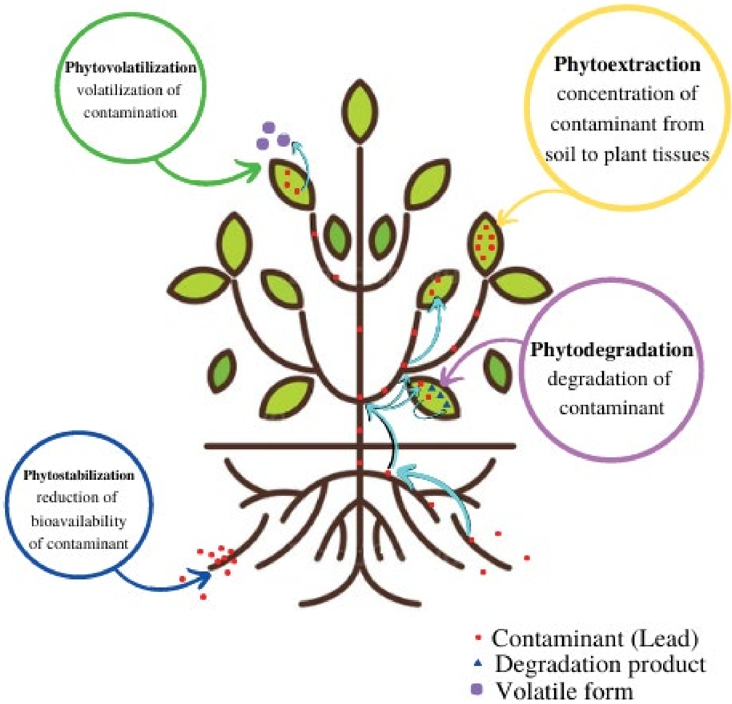

Fig. 3. After one month, symptoms of necrosis began to appear on leaves of *Ligustrum texanum* irrigated with high concentrations of lead (Pb 500 and Pb 700 treatments).

is not promising for the phytoremediation process but it can be used in polluted areas such as industrial and mine vicinity sites in order to reduce heavy metals accumulation in edible plants [45]. The addition of HA to heavy metal contaminated soil decreases the fraction of heavy metals uptake by plants roots, so they remained more in the soil [46] and thereby, there is a lower BCF in HA containing than non-HA treatments with the same Pb concentration of irrigation solution [Table 4, Eq. (1)]. Humic acid not only reduces plants' metal uptake but also decreases the plant's ability to mobilize heavy metals such as Pb to their aerial parts [45], so there is a lower TF (S/R % and L/R) in HA containing in comparison to non-HA treatments with the same Pb concentration of irrigation solution [Eq. (2)].

4.4. Limit of the study

We have found that *L. texanum* is useful in Pb phytoremediation with a tolerance limit of ca. 200 $\mu g\ g^{-1}$ of Pb in soil (a result of Pb 500 irrigation). We based this threshold, on the disruptions of Pb 700 irrigation resulting in ca. 235 $\mu g\ g^{-1}$ soil Pb concentration. The exact tolerance limit thus matches a range of soil Pb concentrations between 200 and 235 $\mu g\ g^{-1}$. Further studies should therefore include denser concentrations of irrigation in order to discover the precise limit of tolerance.

5. Conclusion

According to the results, *L. texanum* is a prominent species for Pb phytoremediation of contaminated soil purposes due to its high tolerance to Pb contamination, high Pb uptake and accumulation, fast-growing and evergreen traits. Humic acid is not promising for the phytoremediation process whilst could be prominent for use in contaminated sites to prevent Pb disruption to edible plants due to its ability to reduce plant Pb uptake from soil. Most of the Pb was accumulated in the roots of *L. texanum* than the aerial parts of the plant which makes this species suitable for stabilizing contamination (phytostabilization) purposes to minimize the danger of transferring heavy metals to the food chain.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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