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Phytoremediation of Lead-Contaminated soil

Phytoremediation of Lead-Contaminated Soil by *Sinapis arvensis* and

*Rapistrum rugosum*

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## Abstract

Nowadays, public concern relating to ecological deleterious effects of heavy metals is on the rise. To evaluate the potential of *Rapistrum rugosum* and *Sinapis arvensis* in lead-contaminated phytoremediate, a pot culture experiment was conducted. The pots were filled by soil treated with different rates of leadoxide (PbO) including 0 (control), 100, 200, 300, 400, and 500 mg Pb per 1 kg soil. Germinated seeds were sown. Surprisingly, with increasing concentration of Pb, dry weight of *R. rugosum* and *S. arvensis* did not decrease significantly. In both of species, the concentration of Pb was higher in roots than shoots. In general, *S. arvensis* was absorbed more Pb compared to *R. rugosum*. The results revealed high potential of *R. rugosum* and *S. arvensis* in withdrawing Pb from contaminated soil. For both species, a positive linear relation was observed

between Pb concentration in soil and roots. However, linear relationship was not observed between Pb concentration in the soil and shoots. Although both species test had low ability in translocation Pb from roots to shoots but they showed high ability in uptake soil Pb by roots. Apparently, these plants are proper species for using in phytoremediation technology.

## **Keywords**

Heavy metals, Lead, Phytoremediation technology, *Rapistrum rugosum*, *Sinapis arvensis*.

## **Abbreviations**

BCF-Bioconcentration factor, BAC- Biological Accumulation Coefficient, Pb-Lead, HMs- Heavy metals, TF-Translocation factor, TE-Translocation efficiency.

**Introduction**

Anthropogenic activities and industrialization have increased the concentration levels of heavy metals (HMs) in the environment (Ali et al. 2013; Saifullah et al. 2009). Metallic elements with atomic weights more than  $63.5 \text{ g mol}^{-1}$  and specific gravity greater than  $5 \text{ g cm}^{-3}$  are defined HMs (Gumpu et al. 2015). They are poisonous at very low concentration (Akpor et al. 2014) and known as a major group of contaminants that belong to non-degradable of inorganic pollutants (Salt et al. 1998). Some HMs such as copper (Cu), selenium (Se) and zinc (Zn) are essential in trace amount to maintain the body metabolism but they are toxic at higher amount (Salt et al. 1998). Others have no biological activity, the body does not need them for healthy growth, and trace amounts of them are poisonous (Salt et al. 1998). Most HMs are poisonous while some of them are not very toxic (Salt et al., 1998).

When infected HMs plants consumed by herbivores, they enter to food chain. Biomagnification of HMs will be more toxic, when consumed by humans. Therefore, control the human activities that release HMs into the environment and cleaning up the contaminated soil and water is recommended (Nica et al. 2012).

The most common toxic HMs include arsenic, lead, mercury, cadmium, chromium, copper, nickel, silver, and zinc (Akpor et al. 2014). HMs have several negative impact on plants. Some impacts of HMs on plants include, decrease of seed germination and lipid content by cadmium, decrease enzyme activity and plant growth by chromium, the inhibition of photosynthesis by copper and mercury, the reduction of seed germination by nickel and the reduction of chlorophyll production and plant growth by lead (Gardea-Torresdey et al. 2005).

Phytoremediation is employing plants for cleaning up and improving the environmental quality. It is described as an emerging ‘green bioengineering technology’ for environmental restoration and using plants for the removal of pollutants (Pilon-Smits, 2005). During phytoremediation, plants accumulate a toxic metal or immobilize the contaminant. Therefore, Phytoremediation may lead to decrease the HMs contents of polluted soils and waters to environmentally acceptable levels (Ghosh and Singh, 2005). It has been suggested as an inexpensive and sustainable in situ biotechnology approach to help rehabilitate contaminated soils by HMs without destructive effects on soil properties (Salt et al. 1998; McGrath et al. 2002; Pilon-Smits 2005).

Lead (Pb) is among the most widespread, persistent, and toxic soil contaminants (Jarup, 2003). Anthropogenic activities have increased concentration levels of Pb at many locations worldwide (Saifullah et al. 2009). Industrial activities such as Pb based paints, shotgun pellets made of Pb, lead arsenate pesticide application, coal burning, gasoline, explosives, and the disposal of municipal sewage sludge enriched in Pb such as lead batteries (Saifullah et al. 2009; Tian et al. 2010; Zheng et al. 2011) are the primary sources of Pb in Soil.

Short-term exposure to high levels of Pb can cause brain and kidney damage as well as gastrointestinal disorder in humans, while long-term exposure can affect the central nervous system, blood, liver, and reproductive system (Han et al. 2008; Zaier et al. 2010). Therefore, the clean-up process for these Pb-contaminated soils represents a significant expense to various industries and governmental agencies (Saifullah et al. 2009).

Herbal plants have many advantages such as having medicinal and ornamental values, increasing biodiversity, decreasing erosion and phyto-remediating some pollutant material (Zimdahl, 2007).

High levels of short-term biomass production of herbal plants may able there to absorb greater amount of HMs (Wei et al. 2005; Kumar et al. 2013).

The Brassicaceae consists mostly herbaceous plants with annual, biennial or perennial lives pans. *Rapistrum rugosum* and *Sinapis arvensis* are two members of this family. They cause a significant yield reduction in crops, even when present at a low density (Whish et al. 2002).

Members of Brassicaceae family have a key role in phytoremediation technology. Many wild species of this family are known hyperaccumulator of heavy metals and tolerate the toxic effects of a wide range of metals (Anjum et al. 2012). The Brassicaceae contains a large number of hyperaccumulator species include 87 species from 11 genera: *Alyssum* (46), *Arabis* (1), *Arabidopsis* (2), *Bornmuellera* (4), *Cardamine* (1), *Cochlearia* (1), *Peltaria* (2), *Pseudosempervivum* (2), *Stanleya* (1), *Streptanthus* (1), and *Thlaspi* (28) (Anjum et al., 2012).

Accordingly, the main goal of this study is (i) to evaluate the potential of lead phytoremediation by *R. rugosum* and *S. arvensis* and (ii) to assess the values of lead transported from root to shoot.

## **Materials and methods**

### ***Plant Materials, Growth Conditions and soil properties***

The *R. rugosum* seeds were collected from plants of Experimental Fields of the Ferdowsi University of Mashhad (latitude 36°, 17' , 44" N, longitude 59°, 36' , 42" E and altitude 985 m), Mashhad, Iran. Seeds were kept in the dark at the refrigerator (4±1 °C) for further use. Seeds were surface sterilized by immersing into 5% (v/v) sodium hypochlorite for 10 min to prevent fungal growth and then carefully rinsed with distilled water properly.

Germination percent of naked compare with encapsulated seeds of *R. rugosum* was markedly greater. Therefore, prior to begin experiment for increasing seed germination, the fruits were

dehulled and the seeds were placed in 11 cm-diameter Petri dishes on top of a single layer of filter paper (Whatman International, Maidstone, UK). The petri dishes were transferred to an incubator with 25 °C temperature under dark conditions (Ohadi et al. 2011; Chauhan et al. 2006). The seeds in petri dishes were allowed imbibe and emerge the radicle. After the emergence of the radicle, ten emerged seeds were transplanted in 2 L plastic pots filled with a silty loam soil containing different concentrations of Pb.

The *S. arvensis* seeds were obtained from the Plant Protection Research Institute in Tehran, Iran. The seeds were surface sterilized by immersing into 5% (v/v) sodium hypochlorite for 10 min. Then rinsed with distilled water properly. Sterilized seeds were placed in 11 cm-diameter Petri dishes on top of a single layer of filter paper (Whatman International, Maidstone, UK) and they were placed for 7 days at 4–5 °C under dark conditions (Paolini et al. 2001). Then, transferred to incubator (16 h at 20 °C and 8 h at 10 °C, with 45% and 65% relative humidity, respectively). After seed radicle emerged, ten seeds were transplanted in 2 L plastic pots. The pots were placed in a controlled environment under greenhouse conditions with a light/dark period of 16/8 h at 30/15 °C and 45/65% relative humidity. The illumination amount of 2250 lux was obtained from 400 W high-pressure sodium vapor lamps (Osram Sylvania, Lynn, MA, USA). The pots were irrigated with tap water every other day. For both plants, at the one-leaf stage, the seedlings were thinned to four per pot.

### ***Treatments***

Bioassay was conducted during October 2014 to February 2015 in Research Greenhouse at College of Agriculture Ferdowsi University of Mashhad, Iran. The soil for the experiment was

secured from the nearby greenhouse. The soil characteristics summarized in Table 1. The soil samples were air-dried in the shade and passed through 4 mm mesh to remove stones.

*Table 1 near here*

The concentration of 100, 200, 300, 400 and 500 mg/kg PbO (MERCK) was prepared by mixing 0.108, 0.216, 0.323, 0.431, and 0.539 g kg<sup>-1</sup> soil, respectively. These soil samples were mixed every two days. After four weeks, the samples were uniformly mixed and placed into pots (2 kg soil per pot).

### ***Measurements***

Ten weeks after planting, plants of the experimental units were harvested and separated into roots and shoots. The roots were rinsed with tap water followed by distilled water. The plant samples were dried at 75 °C for 48 h and weighed. Each plant sample was grinded with a stainless mill (Restch, 5657 HAAN, N: 21468, 1100Watt, volt, 220/380). 0.3 g of the dried shoot and root samples were acid digested using 10 mL HNO<sub>3</sub>:HClO<sub>4</sub> (9:4 v/v) (Ramana et al. 2015). After 24 h, they were heated (220 °C) until clear solution was obtained. The solutions were filtered by Whatman no. 1 filter paper (International, Maidstone, UK) and diluted with deionized water. The concentration of Pb was determined using flame atomic absorption spectrometry (Shimadzu AA 670) and expressed as mg kg<sup>-1</sup>. A series of known standard solutions used for calibration and standard curve was used to analyze the samples.

The bioconcentration factor (BCF), which is defined as the ratio of the total concentration of HM in the harvested plant tissue to its concentration in the soil where the plant was growing, was calculated by using the following formula proposed by Zhuang et al (2007):



$$BCf = \frac{HM \text{ harvested}}{HM \text{ soil}} \quad (1)$$

where *HM* harvested, is the concentration of the *HM* harvested from plant tissues (roots plus shoots) and *HM* soil, is the concentration of the *HM* in the soil.

Biological Accumulation Coefficient (BAC), which is defined as the ratio of the *HM* in shoot to the concentration in the soil (Surat et al. 2008).

$$BAC = \frac{HM \text{ Shoot}}{HM \text{ Soil}} \quad (2)$$

where *HM* shoots is the concentration of the *HM* in shoots and *HM* soil is the concentration of *HM* in soil.

Translocation factor (TF) is defined as the ratio of the total concentration of HM in the aerial parts of the plant to the concentration in the root, using following formula proposed by Padmavathiamma and Li (2007):

$$TF = \frac{HM \text{ Shoots}}{HM \text{ Roots}} \quad (3)$$

where *HM* roots is the concentration of *HM* in roots.

Translocation efficiency (TE %) was calculated using the following formula proposed by Meers et al (2004):

$$TE\% = \left( \frac{HM \text{ Content in the shoots}}{HM \text{ Content in the whole plant}} \right) \times 100 \quad (4)$$

### ***Statistical Analysis***

The experiment was conducted based on a completely randomized design in factorial arrangement with four replications per treatment. The means of measurement were determined using Microsoft EXCEL and were reported for each of the studied parameters.

The curves were depicted with SIGMA PLOT (version 12.5), and analysis of variance was performed using Two-way analysis. Separation of means was done by using least significant difference (LSD) test at %5 probability ( $p < 0.05$ ). Statistical analysis was performed using SAS software version 9.1 (SAS Institute, Cary, NC).

Relation between Pb concentration in soil and content of Pb in root was linear (5) and relation between Pb concentration in soil and content of Pb in shoot by using a three parametric Gaussian model (6) was determined as the best-fitted curve.

$$Y = a \times bx \quad (5)$$

$$Y = a \times \exp \left[ -0.5 \left( \frac{x-x_0}{b} \right)^2 \right] \quad (6)$$

where  $a$  is the maximum Pb concentration in shoot,  $x_0$  is the concentration of Pb in the soil that showed maximum Pb concentration in shoot, and  $b$  indicating the response rate of the curve or steepness of the curve.

## Results and discussion

### *Plant biomass*

Pb-contaminated soil did not effect on biomass production by *R. rugosum* and *S. arvensis*. Actually, significant decrease did not observed in the presence of Pb concentration compared with control (Fig. 1). Maintaining growth was observed even under high concentration of Pb.

These results are in agreement with Wu et al. (2004) and Epstein et al. (1999) who have been stated no effect of Pb on Indian mustard (*B. juncea*) biomass.

Lead causes detrimentation of chloroplast, inhibition of leaf and root growth, limitation of photosynthesis and damaging cell membrane (Gupta et al. 2011), and therefore, it decreases production biomass. The Pb accumulator plants compare with non-Pb accumulators, higher activity of super oxide dismutase, guaiacol peroxidase, and lipoxygenase may observed. Higher activity of these enzymes induce at lower Pb level in accumulator species than non-accumulators (Haung et al. 2012). These mechanisms may explain no reduction in biomass of *R. rugosum* and *S. arvensis* in the presence of Pb in soil.

#### ***Lead Uptake and Translocation in R. rugosum and S. arvensis***

The accumulation of Pb in roots and shoots of *R. rugosum* and *S. arvensis* are shown in Table 2. Pb-concentration in root and shoot depends to Pb-concentration in soil as increasing Pb concentration in the soil lead to increase uptake of Pb by both species. The concentrations of Pb in roots and shoots of *R. rugosum* and *S. arvensis* were significantly higher than control plants. The results of this study revealed high potential of Pb in *S. arvensis* and *R. rugosum* (Table 2). Due to high uptake Pb potential by these plant species, they play an important role in phytoremediation technology. *R. rugosum* and *S. arvensis* have high growth rate and therefore, they create high biomass in a short period. They uptake high amounts of HMs elements. Both species grown in Pb-contaminated soil were found to accumulate a large amount of Pb in roots (967 and 1640 mg kg<sup>-1</sup> DW) and shoots (221 and 218 mg kg<sup>-1</sup> DW) respectively (Table 2). High rate of absorbing Pb by these species is a distinct advantage due to ability presence and survival a high range of ecological conditions.

The data in Table 2 indicated that *R. rugosum* and *S. arvensis* roots accumulated higher amount of Pb than their shoots. In all concentrations of soil Pb, roots of these plants have higher values of Pb than shoots. Bioconcentration factor (BCF) of both plant species was decreased by increasing levels of Pb (Table 3). Similar result was reported by Mertens et al (2005). Whereas Ramana et al (2015) reported increased BCF in the presence of higher levels of HMs. BCF higher than 1 shows plant potential for phytoremediation. In all concentrations of Pb, BCF was higher than 1. At 100, 200, 300, 400, and 500 mg kg<sup>-1</sup> Pb treated soil, BCF for *R. rugosum* were 3.35, 3.04, 2.79, 2.77, and 2.38 and for *S. arvensis* were 4.36, 4.19, 3.90, 3.82 and 3.72, respectively. Therefore, *S. arvensis* had higher Pb removal potential than *R. rugosum*.

Biological Accumulation Coefficient (BAC) of both plant species was decreased by increasing levels of Pb. Likewise, Surat et al. (2008) decrease of BAC in contaminated-Pb soil at 10 and 20 mg kg<sup>-1</sup> on *S. arvensis* was reported. Decreasing in BCF and BAC was more intense in 500 mg kg<sup>-1</sup> than other concentrations (Table 2). Translocation factors of Pb by *S. arvensis* from roots to shoots with increasing Pb levels was decreased whereas translocation of Pb by *R. rugosum* did not showed a constant procedure, increased followed by a decreased was observed in translocation to shoots (Table 3). In hyperaccumulator species, BCF and TF are very important factors. *R. rugosum* and *S. arvensis* have low potential in transferring Pb from roots to shoots but they have high power in uptake of Pb from the soil. Therefore, high uptake of Pb from soils by *R. rugosum* and *S. arvensis* is appropriate for phytostabilization. Brooks (2000) stated that to known a plant as a hyperaccumulator of Pb, it must removed more than 1000 mg Pb per kg<sup>-1</sup> plant and Tf >1, So, *R. rugosum* and *S. arvensis* may consider Pb accumulator because both of them has Tf <1 however BCF >1. There are several reasons for avoiding transport of lead from

roots to aerial plant parts. These reasons include immobilization by negatively charged pectins within the cell wall (Kopittke et al. 2007; Arias et al. 2010), precipitation of insoluble lead salts in intercellular spaces (Islam et al. 2007; Meyers et al. 2008), accumulation in plasma membranes (Islam et al. 2007; Jiang and Liu, 2010), or sequestration in the vacuoles of rhizodermal and cortical cells and sequestration in casparian strip (Seregin et al. 2004; Kopittke et al. 2007).

Translocation efficiency (TE %), showed the same results as TF and TE % for *S. arvensis* was decreased with increasing Pb levels but *R. rugosum* did not showed a constant procedure. High concentration of Pb in roots not only decreases healthy risk of environmental pollution but also limit entering Pb into the food chain by herbivores (Pourrut et al. 2011; Gupta et al. 2013). Our result is an evident for hypothesis dependence of uptake and translocation from roots to shoots of HMs to plant species (Pourrut et al. 2011).

Figure 2 shows the relationship between Pb concentration in soil and Pb concentration in roots of *S. arvensis* and *R. rugosum*. Increasing concentration of Pb in soil lead to increase Pb in roots of *R. rugosum* and *S. arvensis*. Similar results, was observed for *Festuca arundinacea* and *Lolium preenne* at different of Zn concentrations (Zamani et al. 2015), *Solanum nigrum* at different of Cd concentrations (Wei et al. 2013), *Fagopyrum esculentum* at different of Pb concentrations (Tamura et al. 2005). Figure 2 shows the relationship between Pb concentration in soil and Pb concentration in roots. For both plant species the relationship is positively linear and with increasing concentration of Pb in soil, Pb concentration in roots was also increased. Parameters of linear function are shown in Table 4. These parameters show very good estimation for determining relation between concentration of Pb in soil and concentration of Pb in roots.

Figure 3a and 3b shows the relationship between Pb concentration in soil and Pb concentration in shoots of *S. arvensis* and *R. rugosum* respectively. Figure 3 and Table 4 shows a peak function between concentrations in soil and shoots in both of plant species. Parameters and adjusted coefficients were estimated by the Gaussian function (Table 4). This information showed a good relationship between concentration of Pb in soil and shoots. A strategy to avoid Pb toxicity is to restrict Pb in root cells. Whereof, Pb is a heavy metal with high atomic mass, mobility in plant cells is very low. Most of the Pb absorbed by roots is retained at root and do not transfer to vascular tissues (Pourrut et al. 2011; Gupta et al. 2013). Decrease Pb concentration in shoots at 500 mg kg<sup>-1</sup> concentration in soil may be related to detoxification of Pb. In this condition Pb uptake is predominantly interacellular or sequester in cell wall (Pourrut et al. 2011; Gupta et al. 2013).

### **CONCLUSIONS**

The results of this study show that *R. rugosum* and *S. arvensis* did not only survive in soil highly contaminated with Pb, but they also took up Pb even at high concentration in soil. Based on the results, *S. arvensis* and *R. rugosum* have high potential for absorbing Pb. *S. arvensis* takes up higher amounts of Pb than *R. rugosum*. Although, they were able to absorb high amount of Pb in root cells, the amount of Pb translocated from roots to shoots was reduced in both species. Both species had fast vegetative growth and accumulated high amounts of Pb in a short period. Therefore, they may used in phytoremediation technology.

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Table 1. Chemical and physical characteristics of soil

characteristics	OM%	EC(ms)	PH	Sand	Clay	Silt	Soil texture	Na	P	K
quantity	0.70	1.07	7.3	23.20%	19.10%	57%	silty loam	0.23	12	210

<sup>a</sup>The concentration of nutrients unit was  $\text{mg kg}^{-1}$

Table 2. Concentrations of Pb (mg kg<sup>-1</sup>) in roots and shoots

part	Control	C100	C200	C300	C400	C500
<i>R. rugosum</i>						
Root	2.8 a	254±11.3 b	456±16.7 c	618±20.9 d	841±24.7 e	967±29.2 f
Shoot	0.5 a	81±3.4 b	152±5.6 c	220±9.1 d	269±10.4 e	221±8.1 d
<i>S. arvensis</i>						
Root	4 a	350±17.3 b	682±22.1 c	956±27.1 d	1257±32.1 e	1640±37.4 f
Shoot	0.4 a	86±5.3 b	157±7.2 c	216±14.2 d	270±11.2 f	218±8.9 e

The means followed by the same letter, in the same row were not significantly different at  $p < 0.05$ .

Table 3. Effect of different levels of Pb on Bioconcentration factor (BCF), Translocation factor (TF) and Translocation efficiency (TE)

	Control	C100	C200	C300	C400	C500
<i>R. rugosum</i>						
BCF	NM	3.35	3.04	2.79	2.77	2.38
BAC	NM	0.81	0.76	0.73	0.67	0.44
TF	NM	0.32	0.33	0.36	0.32	0.23
TE%	NM	24.2	25.0	26.2	24.2	18.6
<i>S. arvensis</i>						
BCF	NM <sup>a</sup>	4.36	4.19	3.90	3.82	3.72
BAC	NM	0.86	0.79	0.72	0.68	0.44
TF	NM	0.25	0.23	0.22	0.22	0.13
TE%	NM	19.7	18.7	18.4	17.6	11.7

a: NM; Not Measure

Table 4. Parameters and adjusted coefficients were estimated by the linear and Gaussian function

Linear function				
Plant species	<i>a</i>		<i>b</i>	$R_{adj}^2$
<i>R. rugosum</i>	83.53 (11.99)		1.79 (0.036)	0.99
<i>S. arvensis</i>	21.83 (10.76)		3.18 (0.053)	0.99
Gaussian function				
	<i>a</i>	<i>b</i>	$X_0$	$R_{adj}^2$
<i>R. rugosum</i>	260.12 (3.12)	186.16 (4.78)	392.14 (4.06)	0.98
<i>S. arvensis</i>	257.53 (3.98)	193.53 (6.91)	399.15 (5.99)	0.97



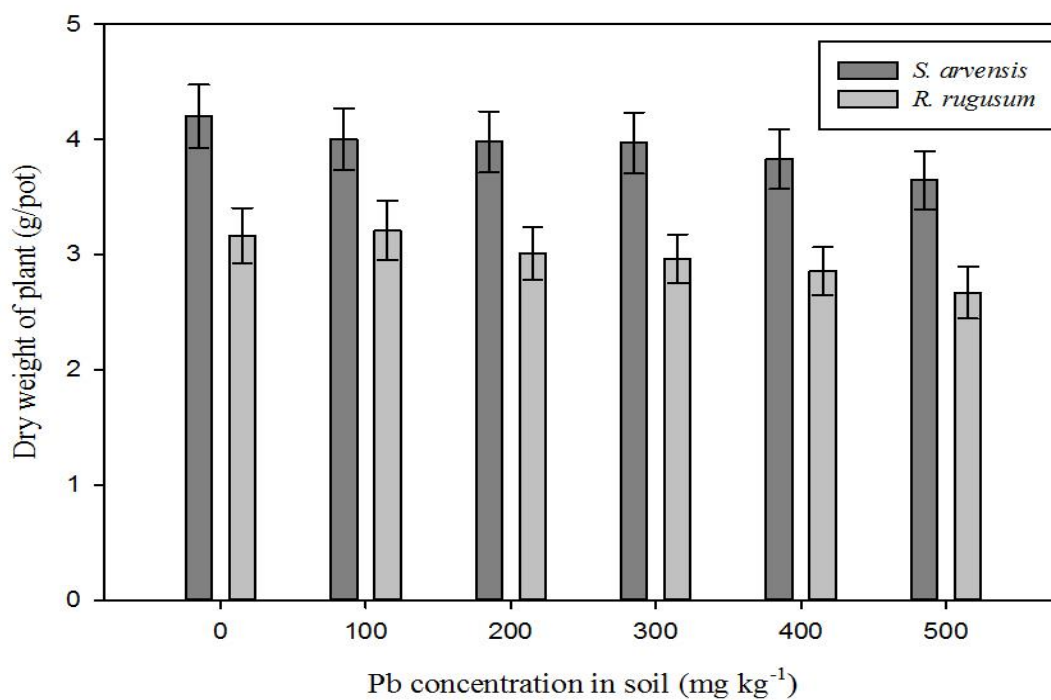


Figure 1. Biomass of *R. rugosum* and *S. arvensis* in the presence of Pb

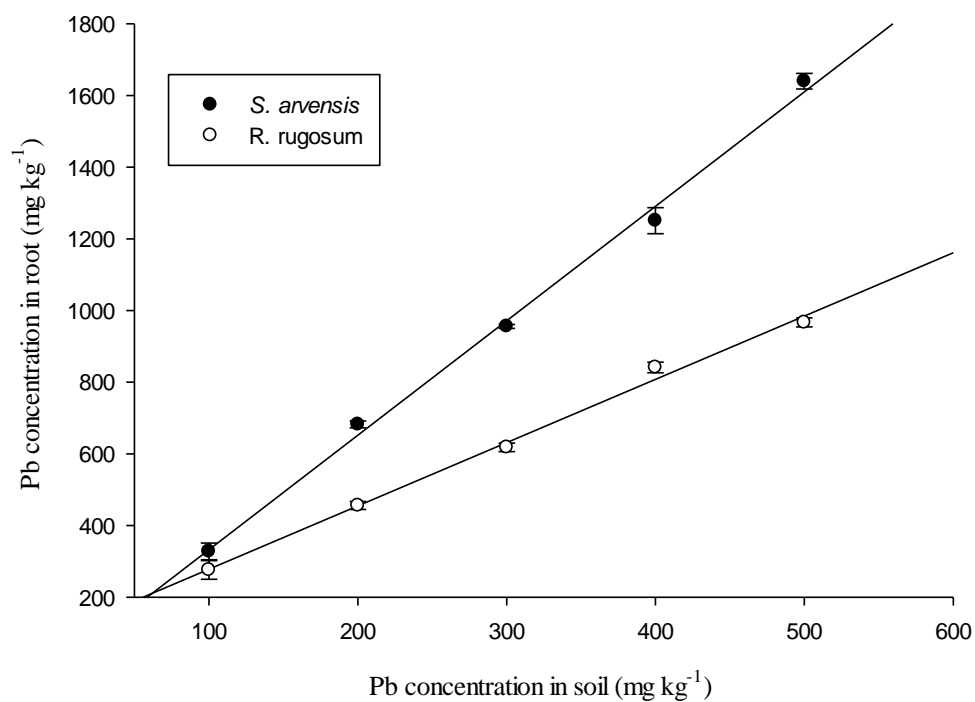
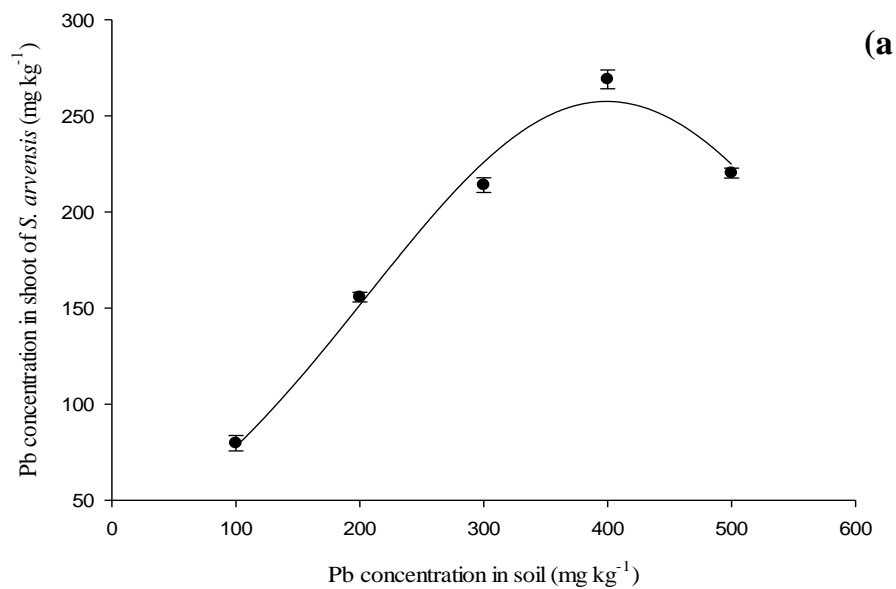
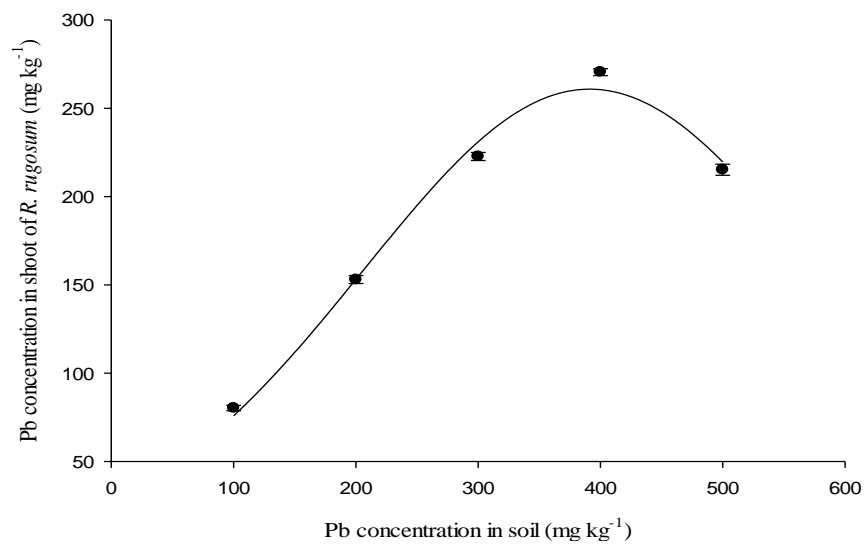


Figure 2. Relationship between Pb concentration in soil and Pb concentration in roots of *R. rugosum* and *S. arvensis*



(a)



(b)

Figure 3. Relationship between Pb concentration in soil and Pb concentration in shoots of a) *S. arvensis* and b) *R. rugosum*