Accumulation and distribution of lead (Pb) in plant tissues of guar (Cyanopsis tetragonoloba L.) and sesame (Sesamum indicum L.): profitable phytoremediation with biofuel crops

Hira Amin, Basir Ahmed Arain, Taj Muhammad Jahangir, Muhammad Sadiq Abbasi and Farah Amin

Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan; National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan; National Centre of Excellence in Analytical Chemistry, Quaid-e-Awam University of Engineering, Science & Technology, Nawabshah, Pakistan; National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, Pakistan

ABSTRACT
Contamination of lead indicates one of the major threats to soil system. Phytoremediation technique utilized plants which are able to tolerate and accumulate metals within their tissues. It has recently been suggested that biofuel plants are more suitable for both utilization and remediation of metal contaminated soil. This study reported Pb phytoremediation potential of Cyanopsis tetragonoloba L. in comparison with Sesamum indicum L. in the framework of a pot-experiment. Plants were subjected to seven Pb concentrations (0, 100, 200, 400, 600, 800 and 1000 mg kg⁻¹ soil) for 12 weeks. Our results demonstrated that both C. tetragonoloba and S. indicum were able to tolerate Pb concentrations up to 1000 mg kg⁻¹ which confirms the plant ability to grow well in higher Pb levels. Significant metal accumulation was observed in root along with reduced biomass for both plant species. Furthermore, both plant species could possibly be used for phytostabilization, with success in marginally polluted soils where their growth would not be impaired and decontamination of Pb could be maintained at satisfying levels. However, bioconcentration factor (BCF), bioaccumulation coefficient (BAC) and translocation factor (TF) values proposed that C. tetragonoloba was more efficient for phytoremediation than S. indicum at higher Pb levels.

1. Introduction
Soil pollution with toxic trace metals and their accumulation in soil is of great concern in agricultural production owing to the adverse effects on crop growth i.e., metal phytotoxicity, and soil micro-organisms (Nagajyoti, Lee, & Sreekanth, 2010). Toxic trace metals have entered into agricultural soils primarily because of rapid industrialization, inappropriate utilization and disposal of toxic trace metal containing wastes, excessive use of fertilizers and pesticides (Amel et al., 2016; Bashmakov, Lukatkin, Anjum, Ahmad, & Pereira, 2015), which become hazardous to human and environmental health. Trace metals, such as lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), iron (Fe) (Nagajyoti et al., 2010), are major environmental pollutants, particularly in areas with high anthropogenic activities.

Among soil pollutant, lead (Pb) is one of the toxic metal pollutants (Shahid, Pinelli, Pourrut, Silvestre, & Dumat, 2011); widely used in many industrial processes and occurs as a contaminant in all environmental compartments including soil, water, and living organisms (Puniamiya et al., 2010); known to induce a broad range of toxic effects to morphological, physiological, and biochemical activities of living organisms. Lead impairs plant growth such as root elongation, seed germination, seedling development, transpiration, chlorophyll production, lamellar organization in the chloroplast, and cell division (Gupta, Huang, Yang, Razafindrabe, & Inouhe, 2010; Maestri, Marmiroli, Visioli, & Marmiroli, 2010). However, the extent of these effects varies and depends on the lead concentrations, the duration of exposure, the intensity of plant stress, and the particular organs studied.

Lead occurs naturally in the earth’s crust (Arias et al., 2010) and its natural levels remain below 50 mg kg⁻¹ (Pais & Jones, 2000), but anthropogenic activities often modify the amount and nature of lead species present in soil. Anthropogenic sourced lead (Pb) include lead-based paints, lead arsenate pesticide application, gasoline, coal burning, explosives, lead batteries and from the disposal of municipal sewage sludge (Tian, Lu, Yang, et al., 2010; Zheng, Liu, Lütz-Meindl, & Peer, 2011).

In soils, lead may occur as a free metal ion, complexed with inorganic constituents (e.g., HCO₃⁻, CO₃²⁻, SO₄²⁻...
Sesamum indicum and Cyamopsis tetragonoloba, in situ advantages, cost effective, and less energy-intensive to remediate metal-contaminated soils because it offers resources. Phytoremediation is a promising technique (Adriano, 2001), required large investments and technological agricultural lands (Oh, Li, Cheng, Xie, & Yonemochi, 2013), required large investments and technological physical and chemical methods are not suitable for countries has tried to reduce lead emissions using lead-free fuels (Adriano, 2001). Remediation of lead-polluted soils by traditional physical and chemical methods are not suitable for agricultural lands (Oh, Li, Cheng, Xie, & Yonemochi, 2013), required large investments and technological resources. Phytoremediation is a promising technique to remediate metal-contaminated soils because it offers in situ advantages, cost effective, and less energy-intensive than high-cost traditional clean-up methods. Phytoremediation involves the use of plants to reclaim high amount of metals from soil into the harvestable parts (i.e., underground roots and aboveground shoots) (Mahmood, 2010). The harvested biomass then can be safely processed by drying, ashing, composting, and storage at a landfill, anaerobic digestion, pure plant oil production, microbial, physical, or other chemical means (Ginneken et al., 2007).

The capacities of heavy metal uptake and accumulation, mechanisms of metal concentration, exclusion and compartmentation vary among different plant species and also between various parts of plants (Lone, He, Stoffella, & Xe, 2008; Sharma, Singh, & Manchanda, 2014). Various plant species belongs to botanical families, in particular the Brassicaceae, Asteraceae, Fabaceae, Poaceae, and Chenopodiaceae showing phytoremediation potential, are well documented in the literature (Stanislaw & Gawronski, 2007; Anjum, Umar, & Iqbal, 2014) but few or less work has been reported on Cymopisis tetragonoloba L. and Sesamum indicum L.

Guar (Cymopisis tetragonoloba L.) is one of the important annual legume crops belongs to family Fabaceae. The endosperm of guar seeds contain gum, which is gaining importance as non-food item (Ashraf, Aliouane, Pradere, & Dumat, 2009; Vega, Andrade, & Covelo, 2010). Generally, lead may accumulates on the surface layer of soil (Cecchi et al., 2008) and caused disturbance of soil system. Thus, in order to reduce the level of lead from the natural environment many developed countries has tried to reduce lead emissions using lead-free fuels (Adriano, 2001).

Table 1. Plant species selected for pot experiment.

| No. | Botanical name                  | Vernacular name | Family       |
|-----|---------------------------------|-----------------|--------------|
| 1   | Cymopisis tetragonoloba L.      | Guar            | Fabaceae     |
| 2   | Sesamum indicum L.              | Sesame          | Pedaliaceae  |

2. Material and methods

2.1. Seeds procurement and sterilization

Seeds of the guar variety BR-99, from the Institute of Fodder Research Program and sesame variety TH-6, were collected from the Institute of Oilseeds Research Program, National Agricultural Research Centre (NARC), Islamabad (Table 1). In order to avoid any microbial contamination, seeds were surface sterilized with 0.1% HgCl₂ for 10 min and washed 7 times with sterilized water (Pourakbar, Khayami, Khara, & Farbidina, 2007).

2.2. Experimental soil collection

Soil samples were collected from Pb-free agricultural fields located in Jamshoro, Sindh, Pakistan, at depth of 0–15 cm using hand shovel. Equidistant (2 m) collected samples were homogenized to prepare one bulk sample. For the greenhouse experiment, soil was air dried at room temperature for 15 days and ground to a final particle size of 2 mm. To enhance soil porosity, sand was mixed in 3:1 proportion with soil samples soil.

2.3. Soil samples measurements

Soil pH was measured with a pH-meter (InoLab-WTB GmbH; Weilheim, Germany) using glass electrode at the 1:2 (w/v) ratio of soil to water suspension (Rachit, Verma, Meena, Yashveer, & Shrey, 2016). The electrical conductivity (EC) was measured with an electrical conductivity meter (WTW – 330i) at the 1:2 (w/v) ratio of soil to water suspension (Rachit et al., 2016). Organic matter (OM) and organic carbon (OC) (%) were
measured according to Walkley and Black (chromic acid titration) method (Fanrong et al., 2011). The properties of the soil are shown in Table 2.

### 2.4. Preliminary screening for Pb

In order to select the Pb concentrations for treatment, various doses of Pb(NO₃)₂ (0, 50, 100, 200, 500, 700, 1000, 1500, and 2000 mg kg⁻¹) were tried in the preliminary screening of the *C. tetragonoloba* and *S. indicum* for 20 days. Based on the Pb toxicity symptoms and morphological growth of the seedlings, the following doses (0, 100, 200, 400, 600, 800, 1000 mg kg⁻¹) were finally selected (Table 3).

### 2.5. Pot experiment

Plastic pots were filled with 5 kg sieved soil, after which soil was artificially spiked with Pb (aqueous solution) using Pb(NO₃)₂ salt to each pot in increasing concentrations (100, 200, 400, 600, 800, 1000 mg kg⁻¹), each with three replicates and kept for 2 weeks to attain equilibrium. The clean soil without Pb spiking was used as control. Pots were arranged in a completely randomized design. After 15 days of equilibration, 20 surface-sterilized seeds were sown per pot. One week after seed germination, plants were thinned to 5 per pot. A plastic tray was kept below the treatment pot to collect any leachate, which was returned to the pots at next watering. The experiment was conducted in a greenhouse for 3 months. Any symptoms of metal toxicity exhibited by plants were visually noted during the experimental period. Plants were harvested 12 weeks after germination. Soil samples (in triplicate) were also collected for analysis of Pb content by an Atomic Absorption Spectrophotometer (Perkin-Elmer, AAnalyst 800). Plant growth and biochemical parameters were also measured.

### 2.6. Germination percentage (%)

The germination percentage, expressed as percentage of germinated seeds to the total number of viable seeds, calculated by the following equation: (Talebi, Nabavi, & Sohani, 2014)

\[
\text{Germination percentage} (%) = \frac{\text{Total No. of germinated seeds}}{\text{Total No. of seeds sown}} \times 100
\]  

### Table 2. The properties of experimental soil.

| Parameter          | Value       |
|--------------------|-------------|
| pH                 | 6.89±0.04   |
| E.C. (μs cm⁻¹)     | 1662±11     |
| Organic carbon, %  | 2.29±0.04   |
| Organic matter, %  | 3.79±0.02   |
| Pb (total), mg kg⁻¹| ND          |

Notes: Similar letters in same column are statistically non-significant according to Duncan’s Multiple Range Test (p < 0.05). Data are means (n = 3 ±SD), *n* superscript represent significantly highest followed by later alphabets for lower means; ND = Not detected.

### 2.7. Morphological parameters

Plants were taken from each replicate pot to measure morphological parameters. Root length, shoot length were measured with the help of scale. Root and shoot fresh weights were also measured with the help of analytical balance. Plant samples were air dried for one week. Then, oven-dried at 80 °C to a constant weight and dry weights were recorded.

### 2.8. Estimation of photosynthetic pigments

Photosynthetic pigments, in fully expanded leaves from each treatment, were extracted using 0.5 g of fresh material, ground with 10 mL of 80% aqueous acetone. After filtering, 1 mL of the suspension was diluted with a further 2 mL of acetone, and optical density were determined with a UV−visible spectrophotometer (Biochrom Libra S22), using two wavelengths (663 and 645 nm) against blank. Chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll contents (mg g⁻¹ f.w) were obtained by calculation, following the method of Arnon (1949).

\[
\text{Chlorophyll a} = (12.7 \times OD663) - (2.69 \times OD645) \times \frac{V}{1000} \times \frac{1}{\text{wt}} \text{mg/g FW}
\]  

\[
\text{Chlorophyll b} = (29.9 \times OD645) - (4.68 \times OD663) \times \frac{V}{1000} \times \frac{1}{\text{wt}} \text{mg/g FW}
\]  

\[
\text{Total Chlorophyll} = (20.7 \times OD645) - (8.02 \times OD663) \times \frac{V}{1000} \times \frac{1}{\text{wt}} \text{mg/g FW}
\]  

### 2.9. Determination of tolerance index

Tolerance Index (TI) is expressed as the ratio between the growth parameters (root/shoot length, root/shoot fresh, and dry weight) of the plants in contaminated soil in relation to the growth parameters of plants from non-polluted soil calculated according to Chen et al., 2011.

\[
\text{Tolerance index} (%) = \frac{\text{[Growth parameter]}_{\text{Pb contaminated soil}}}{\text{[Growth parameter]}_{\text{Control soil}}} \times 100
\]  

### 2.10. Quality control and quality assurance

All the glassware used during the present experimentation was of high quality, acid resistant Pyrex glass. The analytical grade reagents with a certified purity of 99% and stock metal standard solution (1000 ppm) for...
AAS analysis were procured from E. Merck (Germany). Working standards were prepared by appropriate dilutions of stock standard solutions with double distilled water.

2.11. Plant samples preparation and Pb determination

To determine Pb accumulation in different plant tissues (i.e., root, stem, leaf, and pod), harvested plant parts were rinsed thoroughly by means of tap water, and after that with deionized (DI) water to clean adhered components of soil, then oven-dried at 80°C till steady weight. The oven-dried plant tissues were ground carefully using an electric grinder and passed through a 1.0 mm mesh strainer. The ground plant tissue samples (0.5 g) were digested with 12 mL of 3:1 (v/v) HNO₃/HClO₄ mixtures on a hot plate for 2 h. After cooling, the digested solution was filtered through Whatman’s filter paper and finally makes up the volume up to mark 50 mL by adding deionized (DI) water.

The quantification of lead (Pb) in respective tissues was carried out using atomic absorption spectrometer (Perkin-Elmer, AAnalyst 800) equipped with a lead cathode lamp, under optimum analytical conditions for the estimation of lead. The optimum conditions for AAS used throughout these studies given in Table 4. The standard calibration method was adopted for the quantification of results and triplicate samples were run to ensure the precision of quantitative results. The Pb concentration and accumulation in plant root and shoot were calculated by the following formula: (Monni, Salemaa, & Millar, 2000)

\[
Pb \text{ Conc. (mg/kg)} = \frac{\text{AAS interpretation (reading)} \times \text{dilution factor}}{\text{dry wt. of plant tissues}}
\]

\[
Pb \text{ Acc. (μg/plant)} = Pb \text{ conc.} \times \text{dry wt. of plant tissues}
\]

2.12. Soil sample preparation and Pb determination

Soil samples were air dried at room temperature, ground, mixed well, and kept in plastic (polyethylene) sealed lock bags used for subsequent metal analysis. Digestions of soil samples were done using aqua regia method. To quantify the Pb content in soil, sample of 1 g soil was digested by means of wet acid digestion method through HNO₃ along with HCl in proportion of 3:1 (v/v) and heated on a hot plate for 2 h until the solution becomes clear. After cooling, the volume was completed to 50 mL by adding distilled water. The solution was filtered through Whatman’s filter paper and consequently, examine for Pb contents with Atomic Absorption Spectrophotometer.

2.13. Evaluation of phytoremediation efficiency

To evaluate the phytoextraction/phytostabilization potential of C. tetragonoloba and S. indicum, the following factors were calculated:

2.13.1. Bioconcentration factor (BCF)

The bioconcentration factor (BCF) was represented as the Pb concentration ratio in plant roots to soil, calculated as follow:

\[
\text{Bioconcentration Factor [BCF]} = \frac{[\text{Pb}] \text{ root}}{[\text{Pb}] \text{ soil}}
\]

2.13.2. Bioaccumulation coefficient (BAC)

The bioaccumulation coefficient (BAC) was expressed as a ratio of Pb in shoot to that in soil, calculated as follow:

\[
\text{Bioaccumulation Coefficient [BAC]} = \frac{[\text{Pb}] \text{ shoot}}{[\text{Pb}] \text{ soil}}
\]

2.13.3. Translocation factor (TF)

The translocation factor (TF) was determined as a ratio of heavy metals in plant shoot to that in plant root, calculated as follow:

\[
\text{Translocation Factor [TF]} = \frac{[\text{Pb}] \text{ shoot}}{[\text{Pb}] \text{ root}}
\]

2.14. Statistical analysis

All experiments were conducted with three replicates and the data collected were analyzed statistically using PASW’ Statistics 18 (SPSS Inc., Chicago, IL, U.S.A.). To compare the means of the treatments, analysis of variance (ANOVA) was performed followed by Duncan’s multiple range Post Hoc tests at significance level of \( p < 0.05 \), to observe significance difference among means.

3. Results and discussion

3.1. Soil characterization

The soil was sandy loam with slightly acidic to neutral pH (6.89), organic carbon (2.20%), organic matter contents (3.79%), and electrical conductivity (1662 μS cm⁻¹). Among soil properties, soil pH has strong effects on solubility and speciation of metals both in the soil and
particularly in the soil solution (Fanrong et al., 2011), whereas, organic matter content has a strong influence on cation exchange capacity, buffer capacity as well as on the retention of heavy metals. Thus, metals present in organic rich soils contaminated with heavy metals are less mobile and less bioavailable than metals present in mineral soils (Olaniran, Balgobind, & Pillay, 2013). The mobility and availability of heavy metals in the soil are generally low, especially when the soil is high in pH, clay, and organic matter (Rosselli, Keller, & Boschi, 2003).

Dede et al. (2012) reported that Pb is an immobile metal in soil, since it readily forms a precipitate with a low aqueous solubility within the soil matrix, and in many cases it is not readily bioavailable. So, in accordance with soil properties such factors may act individually or in combination with each other and may alter the soil behaviour of the lead present, as well as the rate of uptake by plants.

### 3.2. Pb-induced phytotoxic effects

Gradual increase in Pb concentration significantly ($p < 0.05$) reduced all tested growth and biochemical parameter in two plant species. In the current investigation, the germination percentage of *C. tetragonoloba* and *S. indicum* seeds were significantly ($p < 0.05$) affected at 1000 mg Pb kg$^{-1}$ as compared to control (Table 5). The reduced germination percentages (64.76 and 80.00%) were recorded at 1000 mg Pb kg$^{-1}$ in *C. tetragonoloba* and *S. indicum*, respectively. It has been well documented in the literature that germination is an essential process to determine the effects of Pb toxicity on different plant species. Germination is strongly inhibited at very low concentrations of Pb, even at micromolar levels (Kopittke, Asher, Kopittke, & Menzies, 2007).

In this study, *C. tetragonoloba* and *S. indicum* seeds were able to germinate in the presence of low to moderate level of Pb concentrations in soil. The inhibition of seed germination may also result from the interference of lead with protease and amylase enzymes (Sengar et al., 2009).

Seedling’s height (root and shoot length) is also among primary determinants of plant growth. Addition of Pb was significantly ($p < 0.05$) inhibited growth in terms of root and shoots length in *C. tetragonoloba* and *S. indicum* (Table 5). In *C. tetragonoloba* and *S. indicum*, the longest roots (17.85 and 13.76 cm) and shoots (134.30 cm and 119.04 cm) were observed in control treatments with 0 mg kg$^{-1}$ Pb, respectively. Pb is not an essential nutrient and at high concentrations inhibits plant growth. At 1000 mg Pb kg$^{-1}$ concentration, root length decreased by 13.60 cm and 10.67 cm while shoot length reduced by 100.47 cm and 85.33 cm in both *C. tetragonoloba* and *S. indicum*, respectively. Higher concentration of Pb-induced morphological changes in plants i.e., root and shoot elongation in different plants showed a great sensitivity to excessive Pb. Lead exposure in plants strongly limits the growth and development of seedlings (Gopal & Rizvi, 2008). At high concentrations, lead inhibits the growth of roots which directly influences root growth and decreasing the capacity of water and nutrients absorption, ultimately leading to reduction in growth of the plant species.

Plant growth is an important parameter used to assess the survival and adaptation of a given species to environmental factors that decisively control biomass production. Addition of Pb-inhibited biomass growth of *C. tetragonoloba* and *S. indicum*. Pb contamination showed significant ($p < 0.05$) negative impacts on both fresh and dry biomass of *C. tetragonoloba* and *S. indicum* (Table 5). Compared to control treatments, Pb stress at 1000 mg kg$^{-1}$ reduced root fresh weight (8.15 g plant$^{-1}$ and 5.92 g plant$^{-1}$) and shoot fresh weight (21.13 g plant$^{-1}$ and 9.15 g plant$^{-1}$) in *C. tetragonoloba* and *S. indicum*, respectively. The dry biomass follows the same trend as fresh weight. Compared to control treatments, Pb stress at 1000 mg kg$^{-1}$ reduced root dry weight from (5.09 g plant$^{-1}$ and 3.70 g plant$^{-1}$) and shoot dry weight (10.90 g plant$^{-1}$ and 5.87 g plant$^{-1}$) in *C. tetragonoloba* and *S. indicum*, respectively. Plant biomass is a significant indicator for characterizing the growth performance of plants in the presence of heavy metal. Under severe lead toxicity stress, plants displayed obvious symptoms of growth inhibition. Plant biomass can be restricted by high doses

### Table 5. Phytotoxicity of Pb on germination and growth parameters of Cyamopsis tetragonoloba L. and Sesamum indicum L.

| Plant species | Pb applied mg kg$^{-1}$ | Germination % | Root length cm | Shoot length cm | Root fresh weight g plant$^{-1}$ | Shoot fresh weight g plant$^{-1}$ | Root dry weight g plant$^{-1}$ | Shoot dry weight g plant$^{-1}$ |
|---------------|-------------------------|--------------|----------------|----------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| *C. tetragonoloba* | 0                       | 88.57 ± 2.86 | 17.85 ± 0.34 | 134.30 ± 1.70 | 14.29 ± 0.18                  | 53.74 ± 3.52                  | 8.93 ± 0.12                    | 20.33 ± 2.08                    |
|                | 100                     | 81.90 ± 1.66 | 16.83 ± 0.85 | 129.20 ± 1.70 | 12.58 ± 1.04                  | 41.19 ± 9.22                  | 7.83 ± 0.56                    | 18.30 ± 1.47                    |
|                | 200                     | 80.00 ± 17.84| 16.49 ± 0.17 | 122.57 ± 3.23 | 11.36 ± 0.42                  | 35.01 ± 6.16                  | 7.10 ± 0.26                    | 17.30 ± 1.47                    |
| *S. indicum*  | 0                       | 94.48 ± 5.58 | 13.76 ± 0.48 | 119.04 ± 1.60 | 12.27 ± 0.33                  | 33.13 ± 1.01                  | 8.33 ± 0.12                    | 18.33 ± 1.20                    |
|                | 100                     | 90.00 ± 5.00 | 12.64 ± 0.32 | 116.48 ± 1.60 | 10.24 ± 1.95                  | 34.42 ± 6.12                  | 7.33 ± 0.42                    | 16.27 ± 1.10                    |
|                | 200                     | 88.33 ± 4.41 | 12.16 ± 1.60 | 116.00 ± 1.60 | 8.66 ± 2.19                   | 36.92 ± 3.31                  | 6.24 ± 0.90                    | 14.03 ± 1.00                    |
|                | 400                     | 87.22 ± 6.31 | 11.31 ± 0.72 | 108.16 ± 1.60 | 7.84 ± 1.31                   | 31.29 ± 1.49                  | 4.90 ± 0.82                    | 11.56 ± 0.51                    |
|                | 600                     | 85.56 ± 8.22 | 11.04 ± 0.32 | 107.84 ± 1.60 | 7.51 ± 0.63                   | 25.80 ± 3.59                  | 4.70 ± 0.40                    | 9.67 ± 1.15                     |
|                | 800                     | 83.89 ± 2.55 | 10.72 ± 0.16 | 103.04 ± 1.60 | 7.30 ± 1.48                   | 17.42 ± 1.49                  | 4.53 ± 0.92                    | 7.59 ± 0.72                     |
|                | 1000                    | 80.00 ± 10.93| 10.67 ± 0.56 | 85.33 ± 9.92  | 5.92 ± 0.97                   | 9.15 ± 1.18                   | 3.70 ± 0.61                    | 5.87 ± 0.81                     |

Notes: Similar letters in same column are statistically non-significant according to Duncan’s Multiple Range Test ($p < 0.05$). Data are means ($n = 3 ± SD$), *in superscript represent significantly highest followed by later alphabets for lower means.
of lead exposure. Decrease in plant biomass may be associated with disturbed metabolic activities due to reduced uptake of essential nutrients when grown under Pb stress (Gopal & Rizvi, 2008; Kopittke et al., 2007).

Lead, if present at high levels, can interfere with normal enzyme functions in plants and especially photosynthesis which is one of the plant processes most drastically affected by Pb toxicity. It is evident that chlorophyll content in leaves of *C. tetragonoloba* and *S. indicum* were influenced by Pb treatments. Chlorophyll contents decreased significantly (p < 0.05) with gradual increase in Pb concentration from 0 to 1000 mg kg⁻¹ (Figure 1). In *C. tetragonoloba* and *S. indicum*, the maximum amount of chlorophyll contents were measured at control, while the lowest concentration of chlorophyll a (0.63 and 0.51 mg g⁻¹), chlorophyll b (0.35 and 0.21 mg g⁻¹), and total chlorophyll (0.98 and 0.72 mg g⁻¹) was at 1000 mg Pb kg⁻¹, respectively. A decreased rate of photosynthetic pigment accumulation in association with Pb treatment may be the consequence of peroxidation of chloroplast membranes due to increased level of ROS generation (Srinivasan, Sahi, Paulo, & Venkatachalam, 2014). Lead may inhibit chlorophyll biosynthesis by impairing the uptake of essential photosynthetic pigment elements, such as magnesium, potassium, calcium, and iron (Gopal & Rizvi, 2008) which disturbed photosynthesis with the substitution of divalent cations by lead.

Tolerance indices (TIs) were also affected by Pb toxicity. Plant tolerance to heavy metal stress is estimated based on their root and/or shoot growth inhibition by the metal present in a medium (Srinivasan et al., 2014). According to Audet and Charest (2007), if TI values less than 1, this indicates that the plant suffered a stress due to metal pollution with a net decrease in biomass. By contrast, if TI values greater than 1, suggest that plants have developed tolerance with a net increase in biomass (hyper accumulator). If TI values equal to 1, the plant is unaffected by metal pollution, indicate no difference relative to control treatments. In this study, both the plant species (*C. tetragonoloba* and *S. indicum*) had

**Figure 1.** Effect of Pb stress on photosynthetic pigments chlorophyll-a (a) chlorophyll-b (b), and total chlorophyll (a + b) (c), on *C. tetragonoloba* and *S. indicum* after 12-week growth in soil medium with varying concentrations of Pb.

Notes: Similar letters are statistically non-significant according to Duncan’s Multiple Range Test (p < 0.05), Data are means (n = 3 ± SD), *in superscript represent significantly highest followed by later alphabets for lower means.

**Table 6.** Effect of Pb stress on the growth tolerance indices (TIs) of *Cyamopsis tetragonoloba* L. and *Sesamum indicum* L.

| Plant species       | Pb applied (mg kg⁻¹) | Root length | Shoot length | Root fresh weight | Shoot fresh weight | Root dry weight | Shoot dry weight |
|---------------------|----------------------|-------------|--------------|-------------------|-------------------|----------------|-----------------|
| *C. tetragonoloba*  | 100                  | 94.25 ± 2.97| 96.20 ± 0.05| 88.01 ± 6.32      | 76.97 ± 18.18     | 88.01 ± 6.32   | 91.19 ± 17.30   |
|                     | 200                  | 92.39 ± 0.81| 91.26 ± 1.25| 79.48 ± 2.78      | 64.89 ± 6.02      | 79.48 ± 2.78   | 85.40 ± 7.06    |
|                     | 400                  | 84.79 ± 2.57| 88.60 ± 0.93| 75.85 ± 1.80      | 59.13 ± 6.87      | 75.85 ± 1.80   | 77.43 ± 5.40    |
|                     | 600                  | 81.94 ± 2.51| 75.97 ± 2.80| 66.84 ± 6.47      | 56.22 ± 6.54      | 66.84 ± 6.47   | 71.54 ± 15.45   |
|                     | 800                  | 76.09 ± 8.08| 75.22 ± 3.48| 59.36 ± 4.06      | 50.16 ± 3.18      | 59.36 ± 4.06   | 62.64 ± 7.01    |
|                     | 1000                 | 76.09 ± 8.08| 74.81 ± 0.19| 57.01 ± 1.74      | 39.24 ± 11.38     | 57.01 ± 1.74   | 53.63 ± 4.13    |
| *S. indicum*        | 100                  | 91.99 ± 5.54| 97.85 ± 0.29| 83.34 ± 14.76     | 83.13 ± 13.52     | 88.06 ± 6.23   | 89.23 ± 11.27   |
|                     | 200                  | 88.71 ± 14.73| 97.45 ± 0.03| 76.54 ± 17.63     | 76.35 ± 8.75      | 74.83 ± 9.98   | 76.92 ± 9.40    |
|                     | 400                  | 82.36 ± 8.10| 90.88 ± 2.57| 63.86 ± 10.19     | 57.52 ± 3.14      | 58.72 ± 9.12   | 63.20 ± 3.68    |
|                     | 600                  | 80.35 ± 5.13| 90.59 ± 0.13| 61.20 ± 3.59      | 62.78 ± 10.24     | 56.33 ± 4.09   | 52.94 ± 7.72    |
|                     | 800                  | 77.99 ± 3.89| 86.56 ± 0.18| 59.22 ± 12.60     | 42.22 ± 4.52      | 54.36 ± 10.68  | 41.38 ± 2.78    |
|                     | 1000                 | 77.49 ± 1.42| 71.73 ± 8.94| 48.18 ± 7.09      | 22.13 ± 2.69      | 44.34 ± 6.74   | 32.28 ± 6.39    |

Notes: Similar letters in same column are statistically non-significant according to Duncan’s Multiple Range Test (p < 0.05), Data are means (n = 3 ± SD), *in superscript represent significantly highest followed by later alphabets for lower means."
different tolerance indices (TIs) under Pb stress (Table 6). At 1000 mg kg\(^{-1}\) Pb treatment, *C. tetragonoloba* and *S. indicum* had the TIs for root lengths (76.09% and 77.49%) and shoot lengths (74.81% and 71.73%), root fresh weights (57.01% and 48.18%) and shoot fresh weights (39.24% and 22.13%), root dry weights (57.01% and 44.34%) and shoot dry weights (53.63% and 32.28%) respectively. Srinivasan et al. (2014) reported that growth inhibition is a common response to heavy metal stress and is also one of the most important agricultural indices of heavy metal tolerance.

### 3.3. Pb concentration in plant tissues

Concentration trends of Pb uptake among the different plant tissues (root, stem, leaf, and pod) in both *C. tetragonoloba* and *S. indicum* are presented in Table 7. In *C. tetragonoloba*, the highest concentration of Pb found in the root: 626.67 mg kg\(^{-1}\) followed by stem: 299.67 mg kg\(^{-1}\), leaf: 89.33 mg kg\(^{-1}\), and pod: 10.40 mg kg\(^{-1}\) at 1000 mg Pb kg\(^{-1}\). However, in *S. indicum* Pb concentration primarily in the root: 525.67 mg kg\(^{-1}\), with small amount being transferred to leaf: 75.33 mg kg\(^{-1}\) followed by stem: 64.89 mg kg\(^{-1}\) and pod: 8.13 mg kg\(^{-1}\) at 1000 mg Pb kg\(^{-1}\). The high Pb contents in the plant tissues are clearly related to the concentration of metal in the growing environment. Because of strong Pb binding with organic and/or colloidal materials, it is believed that only small amounts of the lead in soil are soluble, and thereby available for plant uptake (Kopittke, Asher, Kopittke, & Menzies, 2008). The Pb accumulation capacity, based on their availabilities in the soil, varies greatly among different plant species and cultivars, and is also affected by various soil conditions reviewed by Bertrand, Muhammad, Camille, Peter, and Eric (2011).

### 3.4. Pb accumulation in root and shoot

Beside concentrations, a total amount of metals accumulated in the shoots is considered as the most important parameter to evaluate the potential of phytoextraction in plants (Hanen et al., 2010). A significant rise in Pb accumulation per plant in root and shoot of both the plant species varied with respect to Pb treatments are expressed in (Figure 2). The maximum Pb accumulation in root and shoot of both *C. tetragonoloba* and *S. indicum* were observed at 600 and 800 mg Pb kg\(^{-1}\) treatment. The mean Pb accumulation in *C. tetragonoloba* root were ranged from 856.17 to 3585.17 μg plant\(^{-1}\), while the accumulation of Pb by shoot were ranged from 1749.84 to 4655.07 μg plant\(^{-1}\). On the other hand, in *S. indicum* root accumulation was ranged from 747.2 to 2280.53 μg plant\(^{-1}\) while, the accumulation of Pb by shoot ranged from 622.99 to 1177.7 μg plant\(^{-1}\). Studies have shown the uptake of metals; their partition and translocation to different plant parts, as well as the degree of tolerance

| Plant species | Pb applied (mg kg\(^{-1}\)) | Root concentration mg kg\(^{-1}\) | Stem concentration mg kg\(^{-1}\) | Leaf concentration mg kg\(^{-1}\) | Pod concentration mg kg\(^{-1}\) | BCF | BAC | TF |
|---------------|-----------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|-----|-----|----|
| *C. tetragonoloba* | 100 | 108.67 d ± 3.32 | 46.33 d ± 1.20 | 30.00 e ± 3.61 | 3.00 f ± 0.55 | 39.00 e ± 3.61 | 1.70 c ± 1.15 | 0.95 a ± 0.05 |
| | 200 | 244.33 c ± 4.19 | 66.33 d ± 1.20 | 54.67 d ± 3.32 | 5.40 d ± 0.75 | 50.00 e ± 3.61 | 2.70 bc ± 1.13 | 0.60 b ± 0.02 |
| | 400 | 433.00 b ± 19.93 | 128.67 c ± 16.30 | 59.67 cd ± 5.51 | 4.43 b ± 1.46 | 61.33 cd ± 5.51 | 4.43 b ± 1.46 | 0.48 c ± 0.05 |
| | 600 | 603.67 a ± 16.20 | 202.33 b ± 7.51 | 69.33 bc ± 6.03 | 5.10 b ± 0.90 | 78.67 d ± 1.13 | 5.10 b ± 0.90 | 0.50 c ± 0.03 |
| | 800 | 626.67 a ± 5.51 | 299.67 a ± 20.50 | 89.33 a ± 8.02 | 8.03 a ± 0.95 | 89.33 a ± 8.02 | 8.03 a ± 0.95 | 0.64 c ± 0.02 |
| | 1000 | 626.67 a ± 5.51 | 299.67 a ± 20.50 | 89.33 a ± 8.02 | 8.03 a ± 0.95 | 89.33 a ± 8.02 | 8.03 a ± 0.95 | 0.64 c ± 0.02 |
| *S. indicum* | 100 | 102.00 d ± 3.00 | 17.17 e ± 1.11 | 20.33 f ± 2.31 | 0.91 f ± 0.20 | 20.33 f ± 2.31 | 0.91 f ± 0.20 | 0.38 b ± 0.01 |
| | 200 | 209.00 c ± 11.30 | 31.13 d ± 1.79 | 34.33 e ± 0.58 | 2.00 e ± 0.10 | 34.33 e ± 0.58 | 2.00 e ± 0.10 | 0.27 ± 0.02 |
| | 400 | 411.33 b ± 9.50 | 41.67 c ± 2.89 | 43.67 d ± 1.53 | 3.20 d ± 0.95 | 43.67 d ± 1.53 | 3.20 d ± 0.95 | 0.27 ± 0.02 |
| | 600 | 443.00 b ± 38.11 | 56.07 b ± 5.66 | 61.33 c ± 1.53 | 4.17 c ± 0.55 | 61.33 c ± 1.53 | 4.17 c ± 0.55 | 0.27 ± 0.02 |
| | 800 | 500.67 a ± 17.62 | 59.97 ab ± 4.95 | 72.67 b ± 1.16 | 7.24 b ± 0.16 | 72.67 b ± 1.16 | 7.24 b ± 0.16 | 0.38 b ± 0.01 |
| | 1000 | 525.67 a ± 6.43 | 64.89 a ± 3.37 | 75.33 a ± 0.58 | 8.13 b ± 0.06 | 75.33 a ± 0.58 | 8.13 b ± 0.06 | 0.38 b ± 0.01 |
indicating that *S. indicum* can also be categorized as Pb phytostabilizer.

### 4. Conclusions

From this study, it has been concluded that none of the plant species were identified as metal hyperaccumulators and not suitable for phytoextraction. Considering the rapid growth, biomass, accumulation efficiency, adaptive properties, tolerance, restoration potential towards Pb, and being leguminous and fast growing crop, *C. tetragonoloba* can be used as an effective tool to decontaminate Pb-polluted soils in quick and successive flushes than *S. indicum*. Furthermore, the value of BCFs, BACs, and TFs suggests that both the plant species were suitable for Pb phytostabilization but *C. tetragonoloba* was more potential candidates for phytoremediation than *S. indicum*. Moreover, both plants species have economic and ecological values. These plants can both remediate metal-contaminated sites and produce valuable biomass, which can bring income for the owners of the sites. The harvested biomass could then be incinerated and disposed off or the accumulated metal could also be recovered for commercial uses and thus reused as biofuel. Further work is needed to understand the mechanisms of metal absorption and tolerance in plants.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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