International Journal of Phytoremediation

ISSN: 1522-6514 (Print) 1549-7879 (Online) Journal homepage: https://www.tandfonline.com/loi/bijp20

Evaluation of lead and chromium tolerance and accumulation level in *Gomphrena celosoides*: a novel metal accumulator from lead acid battery waste contaminated site in Nigeria

Sifau A. Adejumo, Sarita Tiwari, Sanjay Thul & Bijaya Ketan Sarangi

To cite this article: Sifau A. Adejumo, Sarita Tiwari, Sanjay Thul & Bijaya Ketan Sarangi (2019): Evaluation of lead and chromium tolerance and accumulation level in *Gomphrena celosoides*: a novel metal accumulator from lead acid battery waste contaminated site in Nigeria, International Journal of Phytoremediation, DOI: 10.1080/15226514.2019.1633258

To link to this article: https://doi.org/10.1080/15226514.2019.1633258

Published online: 09 Jul 2019.

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
https://doi.org/10.1080/15226514.2019.1633258

Evaluation of lead and chromium tolerance and accumulation level in *Gomphrena celosoides*: a novel metal accumulator from lead acid battery waste contaminated site in Nigeria

Sifau A. Adejumo[a], Sarita Tiwari[b], Sanjay Thul[b], and Bijaya Ketan Sarangi[b]

[a]Environmental Biology Unit, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria; [b]Department of Environmental Biotechnology Division, CSIR-NEERI, Nagpur, India

ABSTRACT

Biology, tolerance, and metal (Pb and Cr) accumulating ability of *Gomphrena celosoides* were studied under hydroponic conditions. The seedlings were raised in Hoagland's solution containing different concentrations of Pb (0, 500, 1000, 1500, 2000, 3000, 4000, and 5000 mg l^{-1}) and Cr (0, 50, 100, 150, 200, 300, and 400 mg l^{-1}). Biomass and metal accumulation in different plant parts were determined at seven (7) and fourteen (14) days after stress. Antioxidant enzyme activities, protein, and proline contents were estimated in stressed and unstressed plants. *Gomphrena celosoides* was able to tolerate Pb and Cr concentrations up to 4000 and 100 mg l^{-1}, respectively in hydroponic solution. Metal accumulation was concentration and duration dependent with the highest Pb (21,127.90 and 117,985.29 mg kg^{-1}) and Cr (3130.85 and 2428.90 mg kg^{-1}) in shoot and root, respectively found in the plants exposed to 5000 mg l^{-1} Pb and 400 mg l^{-1} Cr for 14 days. Proline, antioxidant enzyme activities, and protein contents were the highest in plant exposed to higher Pb and Cr concentrations for 7 and 14 days. *Gomphrena celosoides* could be considered as Pb and Cr accumulator with proline and increase in antioxidant enzyme activities being the tolerance mechanisms.

KEYWORDS

Tolerance; hyperaccumulation; osmolytes; abiotic stress; metallophytes; heavy metals; antioxidants

Introduction

Increase in industrialization and urbanization has been blamed for the contamination of the environment with toxic heavy metals like Pb, Cr, As, Cd, and Cr (Peterson and Girling 1981; Zayed and Terry 2003; Morsy et al. 2012). The most worrisome of this is the contamination of agricultural land with heavy metals. It reduces the size of arable lands, reduces crop yield by disrupting the physiological and biochemical processes and it poses threats to human health through food poisoning (Shanker et al. 2005: Rizwan et al. 2018). To restore contaminated soil and enhance crop yield, different methods have been proposed for the remediation of metal contaminated soil; chemo-remediation (Ogundiran 2007), excavation and landfilling, compost remediation (Rennevan et al. 2007; Bolan et al. 2010; Adejumo et al. 2011), soil washing and flushing (Iturbe et al. 2003; Udovic and Lestan 2009; Moon et al. 2012), and phytoremediation (Brooks 1998; Li et al. 2003; Shah and Nongkynrih 2007). Among these, phytoremediation approach is currently gaining much attention because of its eco-friendliness and cost effectiveness (Cunningham and Berti 2000; Reeves 2006). It is a green technology that is self-sustainable compared to the conventional physical and chemical remediation procedures (USEPA 2000; Pokhrel and Dubey 2012).

Phytoremediation comprises of phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization (Pulford and Watson 2003; LeDuc et al. 2004; Padmavathiamma and Li 2007). The phytoextraction process which involves the use of plants to absorb metals from contaminated matrices is commonly employed (Brooks 1998; Li et al. 2003; Shah and Nongkynrih 2007). The plants for phytoextraction process must, however, be tolerant and be able to accumulate high concentration of metals in their above-ground tissue (Clemens 2006). These plant species are called metal accumulators or hyperaccumulators (Brooks et al. 1977; Kramer 2010) and are categorized as plants that can accumulate metals in the shoot from 100 to 1000 fold compared to non-accumulators without showing any toxicity symptom (Baker 1987; McGrath et al. 2002; van der Ent et al. 2013). They achieve this through the help of several stress tolerance markers which could be enzymatic or non-enzymatic strategies (Clemens 2001). The non-enzymatic include, production of stress-related osmolytes and amino-acids like proline, gluthathione, phytochelatin, glycine betaine, cysteine, etc.), while, the enzymatic strategy involves the up or down regulation in the activities of the enzymes associated with stress such as aminotransferases or ureases, superoxide dismutase and catalase (Clemens 2001; Candan and Tarhan 2003; Hossain et al. 2012). The enzymatic and non-enzymatic strategies help in increasing stress tolerance and preventing oxidative stress by scavenging the reactive oxygen species

CONTACT Sifau A. Adejumo nikade_05@yahoo.com Environmental Biology Unit, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan 200005, Nigeria

(ROS) which are produced in response to metal toxicity (Hossain et al. 2012). Increase in proline production has been reported to be related to stress tolerance in some metallophytes (Adejumo et al. 2015). Heavy metal tolerance in *A. thaliana* is also controlled by chelation with metallothioneins, phytochelatins, and glutathione (Clemens et al. 1999; Gupta et al. 2010; Auguy et al. 2013).

Many of these plant species have been identified and reported in about 45 families but, majority belong to Brassicaceae family (e.g. *Hirschfeldia incana, Thlaspi caerulescens, Thlaspi praecox*, and *Arabdopsis halleri*), whereas few were reported for other families (Blaylock et al. 1997; Reeves and Baker 2000; Abdul et al. 2001; Delorme et al. 2001; Prasad and Freitas 2003; Auguy et al. 2013). Besides, several of these metal accumulators are metal and location specific (Zhao et al. 2003). For instance, in the Brassicaceae family, many hyperaccumulators have been reported for metals like Ni, Cd, and Zn (Whiting et al. 2000; Roosens et al. 2003; Wang et al. 2006). This results in dearth of information on the molecular mechanisms, especially for Pb tolerance and accumulation in plants. To expand the scope of phytotechnology, there is need for the identification of other metal accumulators and tolerant plants that can be applied to different contaminated environments and for different metals (polymetallic hyperaccumulators). New species are now emerging in other families and among these is the anthraceae family where Gomphrena genus belongs (Carvalho et al. 2013). Under this genus, *Gomphrena claussenni* has been reported to be extremely tolerant to high zinc and cadmium concentration (Carvalho et al. 2013), whereas *G. celosoides* is another metallophyte that has been found inhabiting highly contaminated metalliferous sites and reportedly accumulated high concentration of Pb from battery waste contaminated sites (Adeosun et al. 2017; Adejumo et al. 2018).

However, apart from identification of naturally occurring metal tolerant plants, the understanding and investigation of their tolerance levels have also been described as effective process for phytoextraction of heavy metals (McDonald 2006; Mudgal et al. 2010). Limited number of species which can tolerate and accumulate metal in aboveground tissue hinders application of phytotechnology. Similarly, for the plant to be effective for phytoextraction outside its natural environment, the tolerance level and biology of such identified plant in response to different metals need to be studied and determined (Memon et al. 2001, Chaney et al. 2005; Verbruggen et al. 2009). This is because plants to be used for phytoextraction must display combined traits of high capacity for metal absorption, root to shoot translocation, detoxification, rapid growth, and high biomass accumulation as there are no correlations between accumulation and tolerance (Verbruggen et al. 2009). A metal tolerant plant does not necessarily mean an hyperacumulator. The level of tolerance of different hyperaccumulators and mechanisms being employed for detoxification have also been reported to depend on the magnitude of contamination and the metal involved (Yang et al. 2005; Awaad et al. 2010; Álvarez et al. 2012; Auguy et al. 2013).

Understanding the biology of hyperaccumulator, tolerance level, mechanisms involved in metal uptake, detoxification and sequestration will, therefore, help in determining the efficiency and ability of such plant for phytoextraction and successful field application. It is believed that plant species that are suitable for phytoremediation should have evolved biological mechanisms to tolerate and survive metal contamination under different conditions including their natural habitat (Sun et al. 2006). In our previous studies, different plants growing on the sites that are highly contaminated with Pb were analyzed for their ability to accumulate heavy metals most especially in the shoot (Adeosun et al. 2017; Adejumo et al. 2018). Among the identified plant species, *G. celosoides* was found to accumulate high concentration of Pb in its tissue and thrives well in metal enriched environment (Adeosun et al. 2017). This results demonstrated that *G. celosoides* is a Pb accumulator species. In this study, the Pb and Cr tolerance level and accumulation ability of this identified plant species was compared to evaluate its dose-dependent response in hydroponic experiment for different duration. It was assumed that the hydroponic metal exposure experiments would subsequently provide an excellent way to evaluate the maximum levels of Pb and Cr tolerance and accumulation in this plant. Its biology, mechanisms of tolerance, proline production and enzymatic activity in response to metal treatments were also focally studied to be able to develop it further for phytoremediation of metal contaminated sites, especially Pb and Cr and for the purpose of genetic engineering and possible development of transgenic plant with improved metal uptake and biomass production.

Methodology

Experimental

Source of plant materials and treatments for hydroponic experiment

The experiment involved laboratory and hydroponic studies. The seeds of the identified Pb tolerant and hyperaccumulator plant (*G. celosoides*) collected from the abandoned lead acid battery waste contaminated site in Ibadan, Nigeria were used for the studies. The hydroponic solution was prepared using the half strength 20% Hoagland solution to provide nutrients for the plant during the experimental period under hydroponic conditions. To induce metal stress in the solution, Pb was introduced as lead acetate and Cr as Cr (Vl) from potassium dichromate salt ($K_2Cr_2O_7$). Two trials were conducted with the first trial lasting for 7 days and the second trial for 14 days using lower Pb (0, 500, 1000, 1500, and 2000 mg l^{-1}) and Cr (0, 50, 100, and 150 mg l^{-1}) concentrations as well as higher Pb (2000, 3000, 4000, and 5000 mg l^{-1}) and Cr (200, 300, and 400 mg l^{-1}) concentrations. The choice of the metal concentrations was based on the previous study where, *G. celosoides* in its natural environment was found growing on Pb contaminated soil containing 42,230 mg/kg Pb and accumulated high Pb concentrations of 6800 and 4644 mg/g in the shoot and root, respectively. Meanwhile, to ensure survival, rather than simulating this in the hydroponic experiment, the

concentration was initially reduced to 500–2000 mg/l. When the plants survived in Pb concentration up to 2000 mg/l for 7 days, another set of seedlings were exposed to higher concentrations of 2000–5000 mg/l Pb for 7 days. Second trial was carried out using the same lower and higher concentrations of Pb and Cr but the seedlings were stressed continuously for 14 days duration.

Procedure for the hydroponic experiment

The seeds were first pre-germinated in the nursery (Plate 1a) using soil-compost mixture in the greenhouse of the Environmental Biotechnology Division, CSIR-NEERI, Nagpur, India. Experiments commenced when the seedlings were 2 weeks old. Seedlings with heights ranging between 10 and 15 cm, number of leaves between 4.5 and 8.5 and fresh

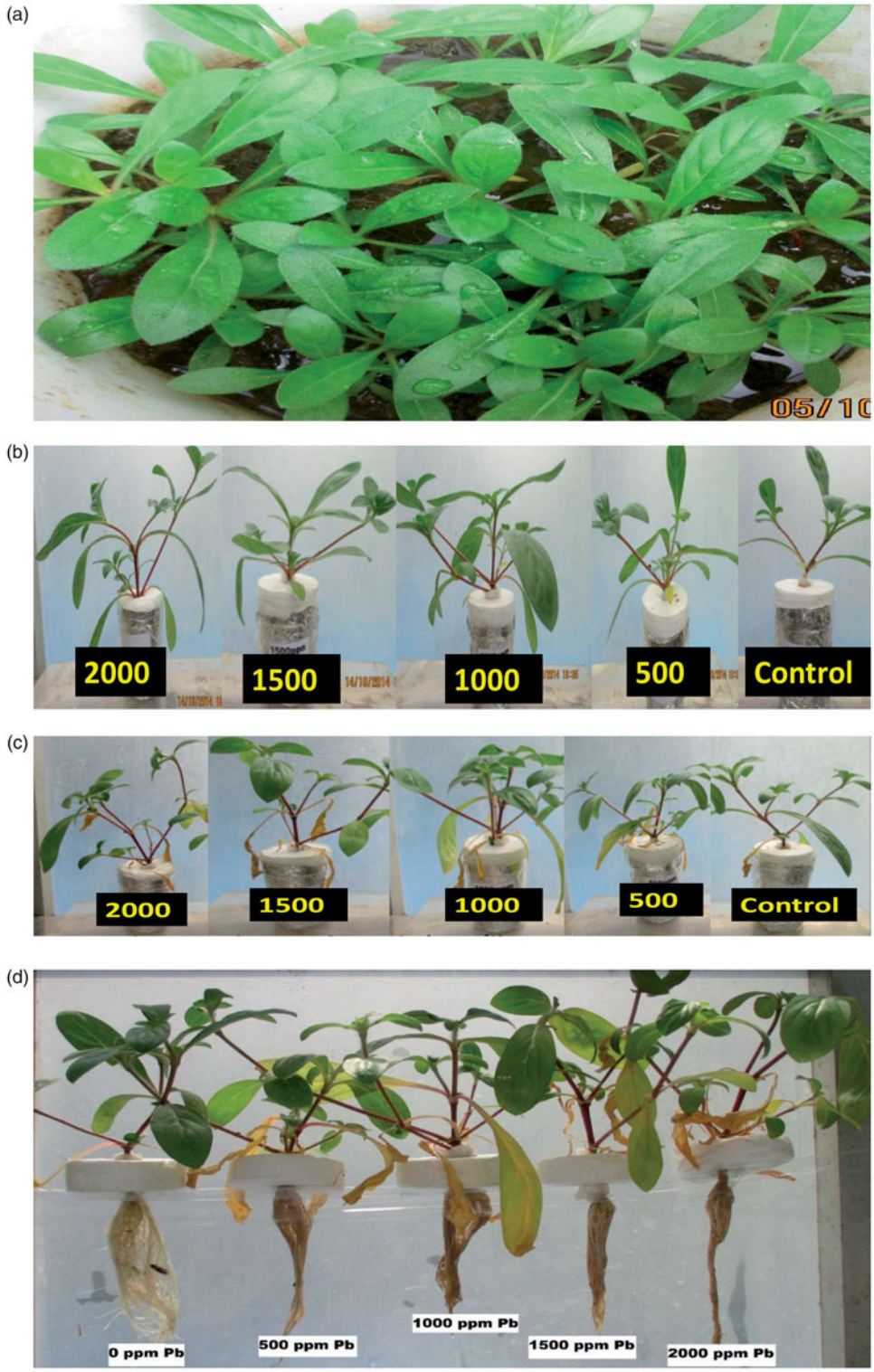

Plate 1. (a) *Gomphrena celosoides* during nursery stage before hydroponic studies. (b) *Gomphrena celosoides* during hydroponic studies before Pb treatment. (c) *Gomphrena celosoides* 7 days after Pb treatment. (d) *Gomphrena celosoides* 14 days after Pb treatment.

weight of average 4.5 g were selected and used for the tolerance study in lower metal concentrations. The seedlings were uprooted gently to preserve their roots, washed with tap water, and later distilled water before transferring them randomly into the Hoagland's solution. The plants were first acclimatized for 1 week in the Hoagland's solution before imposing stress treatment. The seedlings were allowed to grow in the lower metal solutions for 7 days. The experiment was terminated at 7 days after stress. Fresh solution was prepared for higher metal concentration trial for 7 days. This time around, 4 weeks old plants were used and seedlings with number of leaves ranges from 7 to 13 and plant height ranges from 13 to 17 cm were selected, uprooted gently to preserve their roots, washed with tap water, and later distilled water before transferring them randomly to the Hoagland solution, acclimatized in the Hoagland's solution for 1 week before exposing them to higher metal concentrations. For the second trial, fresh seedlings were raised following the same procedure and at 4 weeks after planting, seedlings of relatively the same height and number of leaves were selected and raised in hydroponic solution containing lower and higher metal concentrations continuously for 14 days following the same procedure described in the first trial. Under each trial, the treatments were replicated three times and arranged in the growing chamber using completely randomized design.

Data collection

Data were collected on growth parameters, biomass, and metal accumulation in plant. Growth performances were monitored at acclimatization, before and after stress. Fresh weight was determined after each stress. At the end of the experiment, fresh leaf samples were also taken, kept at −80 °C for molecular and biochemical analysis. Leaf and root biomass of the plants were determined after oven drying for the determination of dry matter accumulation. The oven dried plant samples (root and shoot) were then ground carefully and separately into powder using electronic blender. Thereafter, 1 g each of the plant materials was weighed into the conical flask and 10 ml of conc. HNO_3 was added following the procedure described by Adejumo et al. (2018) but slightly modified. Metal accumulation (Pb and Cr concentrations) in different plant parts was determined from the extract using inductively coupled plasma emission spectrometry (ICP–OES, Thermo-Fisher, iCAP, 6300 series).

Laboratory procedures

DNA extraction

To determine the effect of the metal concentration on DNA quality, total genomic DNA was extracted from the experimental plants following standard CTAB protocol as described by Doyle and Doyle (1990) with slight modifications. Briefly, about 200 mg of leaf tissue were ground with a sterilized mortar and pestle (while keeping them frozen with liquid nitrogen) to break cells and homogenize tissue. Extraction buffer (2 ml) containing 1 M Tris-Hcl (pH = 7.5), 5 M NaCl, 0.5 M EDTA, 2.5% CTAB, 1% PVP, and 0.2% b-mercaptoethanol was added to each tube and shaken to dissolve macerated tissues in buffer completely and this was followed by incubation overnight at 65 °C. The tubes were then allowed to cool at room temperature before adding equal volume of chloroform/phenol/isoamyl alcohol (25:24:1). Mixed gently by inversion for 10–15 min and then centrifuged at 10,000 rpm for 10 min. Supernatant was taken out in a separate tube. This step was repeated by mixing the supernatant with equal volume of Chloroform: Isopropanylalcohol (24:1). After that, aqueous upper phase was removed and mixed with 1/25 of 5 M NaCl and 0.6 vol of isopropanol and allowing them to precipitate overnight. The mixtures were again centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was discarded by gentle inversion and the pellet was washed twice with 70% alcohol. Pellets were air dried and diluted using 100 µl of prechilled autoclaved water and kept at −20 °C for analysis. The extracted DNA concentration was quantified using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Rockland, DE, USA).

Determination of proline contents

Proline content was estimated according to Bates et al (1973), by homogenizing leaf (0.5 g) in 3.0% (w/v) sulphosalycylic acid and the homogenate was filtered. From the filtrate, 2 ml was taken and mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin. The mixture was boiled for 60 min in water bath, and then the reaction was stopped by placing the tubes in an ice bath. The mixture was separated by adding 4 ml of toluene, and the absorbance of the fraction with toluene separated from liquid phase was read at 520 nm. Proline concentration was calculated from a standard curve ranging from 0.0 to 100 µg proline. Proline content was expressed as µg/g FW.

Determination of antioxidant enzyme activities and protein content

The antioxidant enzyme activities were determined following the procedure described by Zhang et al (2007). Fresh leaves (250 mg) and root of *Gomphrena* stressed for 7 and 14 days, respectively, were homogenized in a chilled mortar using extraction buffer which consisted of 50 mM N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES), 0.4 mM EDTA, 5 mM $MgCl_2$, 1% Polyvinylpyrrolidone, 2 mM Dithiothreitol, 10% glycerol, and 1 nM Phenylmethylsulfonyl fluoride (PMSF). Homogenates were centrifuged at 14,000 g at 4 °C for 20 min, and the supernatant was used for enzyme activities. The activities of Catalase (CAT) and Superoxide Dismutase (SOD) were determined from the supernatant using a method described by Zhang et al. (2007). For SOD the reaction mixture contained 3.6 ml millique water, 0.1 ml of enzyme extract, 5.5 ml of 50 mM potassium-phosphate buffer, pH 7.8 and 0.8 ml of 3 mM pyrogallol (dissolved in 10 mM HCl). The rate of pyrogallol reduction was measured at 325 nm using Cary 60 UV-VIS Spectrophotometer (Agilent Technologies). CAT was determined by adding 40 µl of enzyme extract to 9.96 ml of H_2O_2 phosphate buffer

pH 7.0 (0.16 ml of 30% H_2O_2 to 100 ml of 50 mM potassium phosphate buffer pH 7). The rate of change of H_2O_2 was measured at 250 nm and one unit of the enzyme activity was defined as the amount of enzyme that reduced 50% of the H_2O_2 in 60 s. The enzyme activity was expressed as unit/mg protein. Bradford assay was used to determine the total amount of protein present in each sample/enzyme extract.

Statistical analyses

Analysis of variance (ANOVA) was used for the statistical analysis and this was carried out using SPSS statistics software version 17.0 (Statistical Package for Social Science for Windows, SPSS, Inc., Chicago, IL, USA). The significant differences among mean values were determined by post-hoc test using DMRT and Tukey HSD tests at $p < 0.05$. All values reported are the means of three replicates.

Translocation Factor (TF) was determined to evaluate metal translocation from root to shoot. It was calculated as the ratio of metal in the shoot/metal in the root. Similarly, **Bioaccumulation Factor** (BF) was determined to assess the ability of *G. celosoides* in taking up metals from growing medium and this was calculated for shoot and root as the ratio of metal in the different plant parts to the metal concentration in the hydroponic solution (metal concentration in the plant root or shoot/metal concentration in the solution).

Results

Growth response of G. celosoides in lower Pb concentrations under hydroponic experiment for 7 days

The number of leaves and plant height generally increased in all the treatments starting from the day of acclimatization to 7 days after acclimatization (i.e., before stress). At 7 days after Pb treatments, 13.3, 32.3, and 3% increase in the number of leaves of the plants exposed to 500, 1000, and 2000 mg l^{-1} Pb, respectively, were recorded, but these values were low compared to 52% increase recorded in the control treatment. Similarly, the increase observed in the plant height of unstressed plants (Control) was more than that of the stressed plants. Whereas, 100% increase was recorded for control plant, 54.54, 14.70, 28.00, and 9.38% increase were recorded for plants exposed to 500, 1000, 1500, and 2000 mg l^{-1} Pb, respectively. Appreciable increase was recorded in 500 mg l^{-1} Pb, but as Pb concentration increases, there was reduction in the plant height. Compared to the initial weight before hydroponic, an increase was also observed in fresh weight of all the plants at 7 days after treatments except for those exposed to 2000 mg l^{-1} Pb treatment (Figure 3). The increase was more in the control than the stressed plants and was about 20.00–37.73% more than the stressed plants. On the total dry weight, contrary to what was observed for the fresh weight, the dry matter was conversely, higher in the stressed plants than the unstressed plants except in 1500 mg l^{-1} Pb treatment (Table 1).

Growth response of G. celosoides to Pb treatment under hydroponic experiment for 7 days in higher Pb concentration

Surprisingly, in the experiment with higher Pb concentrations (2000, 3000, 4000, and 5000 mg l^{-1} Pb), the growth of *G. celosoides* was remarkable at 7 days after stress compared to what was observed under lower Pb concentrations (500, 1000, 1500, and 2000 mg l^{-1} Pb). The number of leaves increased by 88.24, 285.71, 66.67, and 43.48% in 0, 2000, 3000, and 4000 mg l^{-1} Pb concentration, respectively when the initial number of leaves at the day of acclimatization was put into consideration. Reduction of 38.23% was, however, recorded in the highest Pb concentration of 5000 mg l^{-1} Pb where the number of leaf reduced from 13.0 to 10.5. Conversely, plant height unlike the number of leaves was reduced after stress in all the Pb treatments (2000–5000 mg l^{-1} Pb) compared to control. There was a decrease in fresh weight of all the plants exposed to Pb treatments at 7 days after treatments except for those exposed to 2000 mg l^{-1} Pb treatment. The dry matter/biomass accumulation was however higher in the stressed plants than unstressed (Table 2).

Growth response of G. celosoides to Pb treatment under hydroponic experiment for 14 days in lower and higher Pb concentration

As observed under the 7-day duration, the number of leaves and plant height generally increased in all the treatments starting from the day of acclimatization to 14 days after Pb treatments except in 2000 mg l^{-1} Pb, treatment. Meanwhile, on the plant height, remarkable increase was observed in all the treatment at 14 days after Pb exposure compared to the value at acclimatization and before exposure. Similar to what was observed for 7-day stress, the dry matter yield in the treated plants was more than that of control plant (Table 3). Growth response of *G. celosoides* to Pb treatment

Table 1. Growth response of *G. celosoides* to Pb treatment under hydroponic experiment for 7 days in lower Pb concentration.

Pb conc (mg/l)	NOLA1	NOLBS	NOLAS7	PHA1 (cm)	PHBS (cm)	PHAS7 (cm)	SDW (g/plant)	RDW (g/plant)	TDW (g/plant)
0	11.50c	15.00c	17.50b	4.50a	7.00b	10.00	7.50a	1.82a	0.34a
500	15.00a	19.00a	17.00b	5.50b	8.00b	10.00	6.00b	1.79a	0.30a
1000	15.50a	18.00a	20.5.00a	8.50a	9.00a	3.50	5.99b	2.30a	0.26ab
1500	13.00b	18.00a	13.00c	6.25b	7.50b	4.00	4.50c	1.17a	0.20b
2000	14.50ab	17.00b	15.00c	8.00a	8.50ab	2.50	4.67c	2.02a	0.18b

NOLA1: Number of leaves at acclimatization day 1; NOLBS: number of leaf before stress; NOLAS7: number of leaf 7 days after stress; PHA1: plant height at acclimatization day (cm); PHBS: plant height before stress; PHAS7: plant height 7 days after stress; FWBS: fresh weight before stress; FWAS7: fresh weight 7 days after stress; WG: weight gain, SDW: shoot dry weight; RDW: root dry weight; TDW: total dry weight.
Means followed by the same letter in the same column are not significantly different from each other.

Table 2. Growth response of *G. celosoides* to Pb treatment under hydroponic experiment for 7 days in higher Pb concentration.

Pb conc (mg/l)	NOLA1	NOLBS	NOLAS7	PHA1 (cm)	PHBS (cm)	PHAS7 (cm)	TFWAS (g)	SDW (g/plant)	RDW (g/plant)	TDW (g/plant)
0	8.50c	10.00c	16.00c	16.00a	18.50a	19.00a	4.15a	0.56c	0.11c	0.67c
2000	7.00d	9.00d	27.00a	13.50b	17.00b	15.50c	4.76a	0.75a	0.14b	0.89a
3000	7.50d	9.00d	12.50d	17.50a	19.50a	16.50b	2.43c	0.56c	0.12c	0.68c
4000	11.50b	14.50b	19.00b	16.50a	19.50a	15.50c	3.31b	0.62b	0.18a	0.80b
5000	13.00a	17.00a	10.50e	13.00b	16.50b	15.00c	2.74b	0.60b	0.10d	0.70c

NOLA1: Number of leaves at acclimatization day 1; NOLBS: number of leaf before stress; NOLAS7: number of leaf 7 days after stress; PHA1: plant height at acclimatization day (cm); PHBS: plant height before stress; PHAS7: plant height 7 days after stress; FWBS: fresh weight before stress; FWAS7: fresh weight 7 days after stress; WG: weight gain; SDW: shoot dry weight; RDW: root dry weight; TDW: total dry weight.
Means followed by the same letter in the same column are not significantly different from each other.

Table 3. Growth response of *G. celosoides* to Pb treatment under hydroponic experiment for 14 days in lower Pb concentration.

	NOL				PLH							
Treatments	BA	BS	AS7	AS14	BA	BS	AS7	AS14	FWAS14	SDW	RDW	TDW
0	14.00b	16.00b	23.00a	24.50a	17.50b	21.00c	20.50c	25.50ab	4.30a	1.18a	0.22a	1.40a
500	11.50c	13.00c	12.00b	13.50c	14.50c	17.00d	16.50d	17.50c	2.02b	0.79b	0.16b	0.95a
1000	8.00d	10.00d	8.00c	18.00b	21.50a	23.50b	24.00b	25.00ab	2.83b	1.31a	0.19a	1.50a
1500	7.50d	9.00d	9.00c	15.00c	25.00a	27.00a	27.50a	27.50a	3.17a	1.34a	0.20a	1.54a
2000	16.00a	19.00a	11.50b	10.50d	15.00c	18.50d	22.00c	17.50c	2.26b	0.90b	0.15b	1.05b

NOLBA: Number of leaves at acclimatization day 1; NOLBS: number of leaf before stress; NOLAS7: number of leaf 7 days after stress; NOLAS14: number of leaf 14 days after stress; PHBA: plant height at acclimatization day (cm): PHBS: plant height before stress; PHAS7: plant height 7 days after stress; FWBS: fresh weight before stress; FWAS14: fresh weight 14 days after stress; WG: weight gain; SDW: shoot dry weight; RDW: root dry weight; TDW: total dry weight.
Means followed by the same letter in the same column are not significantly different from each other.

Table 4. Growth response of *G. celosoides* to Pb treatment under hydroponic experiment for 14 days in higher Pb concentration.

	NOL				PLH							
Treatments	BA	BS	AS7	AS14	BA	BS	AS7	AS14	FWAS14	SDW	RDW	TDW
0	21.00a	24.00a	19.50a	39.00a	19.00a	21.50a	22.50a	25.00a	4.20a	0.96a	0.22b	1.18a
2000	14.50b	17.00b	10.00c	17.00b	17.50b	20.00a	17.00b	23.00a	2.28b	0.59b	0.50a	1.09b
3000	10.00c	13.00c	12.50b	14.00c	17.50b	20.50a	23.50a	22.00a	2.45b	0.85a	0.21b	1.06b
4000	12.00b	15.00bc	9.00c	9.00d	14.50d	16.00b	19.00ab	16.00b	1.95b	0.71a	0.20b	0.90b
5000	7.00c	8.50d	8.00c	0.00d	16.00c	19.00a	17.00b	0.00c	2.24b	0.86a	0.34b	1.20a

NOLBA: Number of leaves at acclimatization day 1; NOLBS: number of leaf before stress; NOLAS7: number of leaf 7 days after stress; NOLAS14: number of leaf 14 days after stress; PHBA: plant height at acclimatization day (cm); PHBS: plant height before stress; PHAS7: plant height 7 days after stress; FWBS: fresh weight before stress; FWAS14: fresh weight 14 days after stress; WG: weight gain, SDW: shoot dry weight, RDW: root dry weight, TDW: total dry weight.
Means followed by the same letter in the same column are not significantly different from each other.

under hydroponic experiment for 14 days in higher Pb concentration followed the same trend in term of number of leaves but there was a reduction in the case of higher Pb concentration of 4000 mg l^{-1} Pb and there was no data for the highest Pb concentration due to the death of the plant exposed to this treatment. In the case of plant height, an increase was recorded in all the treatments up till 14 days after exposure and 4000 mg l^{-1} Pb. The dry matter content of the plant exposed to the highest Pb concentration of 5000 mg l^{-1} Pb was not significantly different from that of control (Table 4).

Pb accumulation in the shoot and root of G. celosoides*, bioaccumulation factor for shoot (BFS) and root (BFR), and translocation factor under 7-days stress in lower Pb concentration*

The Pb concentrations in the shoot and root of *G. celosoides* were found to depend on the concentration in the medium. There was a correlation in the concentration of Pb in the solution and accumulation in plant. The plants exposed to 500 mg l^{-1} Pb had the lowest (601 mg l^{-1} Pb) while the highest concentration of Pb was recorded in the shoot of the plant exposed to 1500 mg l^{-1} Pb followed by that of 2000 mg l^{-1} Pb. The plants exposed to these treatments were able to survive and accumulated 16361.5 and 15149.8 mg kg^{-1} Pb, respectively in their shoots (Figure 1a). The accumulation in the shoot of *G. celosoides* exposed to 1500 mg kg^{-1} Pb was 122% mg kg^{-1} Pb greater than that of 1000 and 2618.76% and 27 times greater than that of 500 mg kg^{-1} Pb. Generally, the highest Pb concentration was found in the root compared to the shoot. The plants exposed to 2000 mg l^{-1} Pb accumulated the highest value of 155.791 mg kg^{-1} Pb, in the root while the plants grown in 1500 mg l^{-1}Pb had the lowest (Figure 1b). The bioaccumulation factor for the root of the plant exposed to 500 mg l^{-1} Pb was the highest (158.16) while that of the plant exposed to 1500 mg l^{-1} Pb was the lowest. The bioaccumulation factor for the shoot showed highest value (10.91) in plant exposed to 1500 mg l^{-1} Pb followed by those of 2000 and 1000 mg l^{-1} treatments and the lowest was recorded in the 500 mg l^{-1} Pb treatment (Figure 2a). The translocation factor was less than 1 in all the Pb treatments but was the highest in the plants exposed to 1500 mg l^{-1} Pb, followed by that of 2000 mg l^{-1} treatment and the lowest value was recorded for 500 mg l^{-1} Pb (Figure 2b).

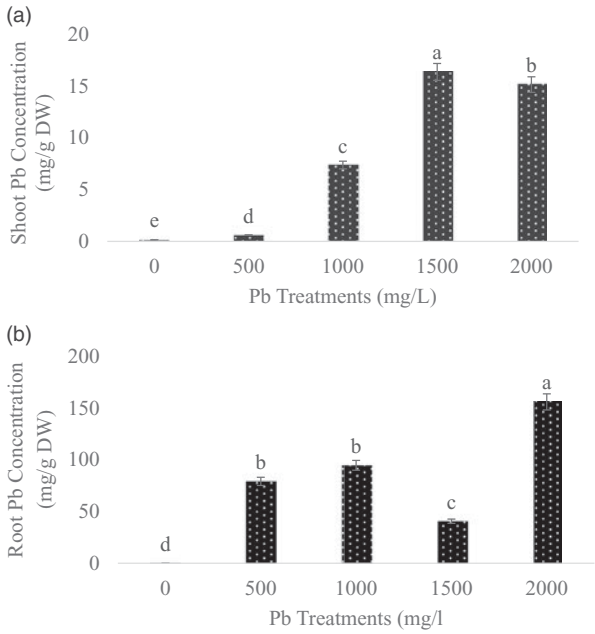

Figure 1. (a) Pb accumulation in shoot under 7-day stress in lower Pb concentrations. (b) Pb accumulation in root under 7-day stress in lower Pb concentrations. The letter on each bar indicates the level of statistical difference between the treatments as separated by DMRT at 0.05% level of probability

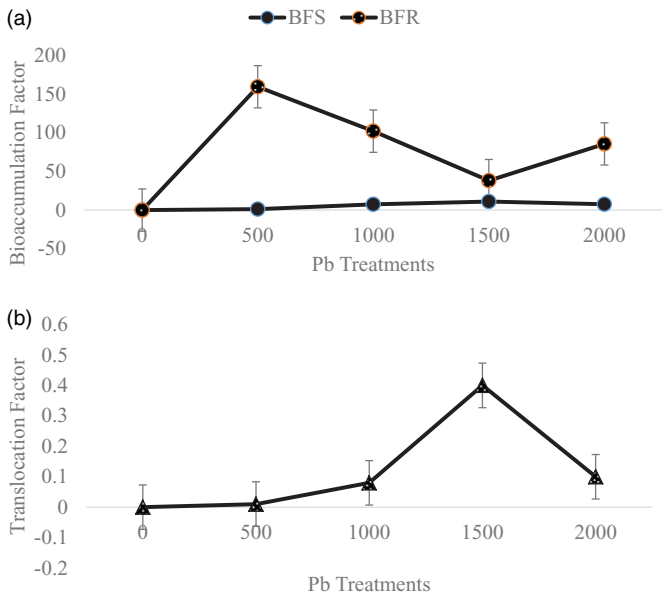

Figure 2. (a) Bioaccumulation factor for shoot (BFS) and root (BFR) under 7-day stress in lower Pb concentrations. (b) Translocation factor (TF) under 7-day stress in lower Pb concentrations.

Pb accumulation in shoot and root, bioaccumulation factor for shoot (BFS) and root (BFR), and translocation factor (TF) in higher Pb concentration 7 days after stress

As observed for lower Pb concentrations trial, the same trend was recorded with higher concentrations of Pb. As the concentration increases in the growing medium, the uptake by the plant also increases and highest Pb concentration in the shoot was found in plant exposed to 5000 mg l^{-1} after stress treatment (Figure 3a). Higher concentration was also found in the root compared to the shoot (Figure 3b). The bioaccumulation factors for both the root and shoot were,

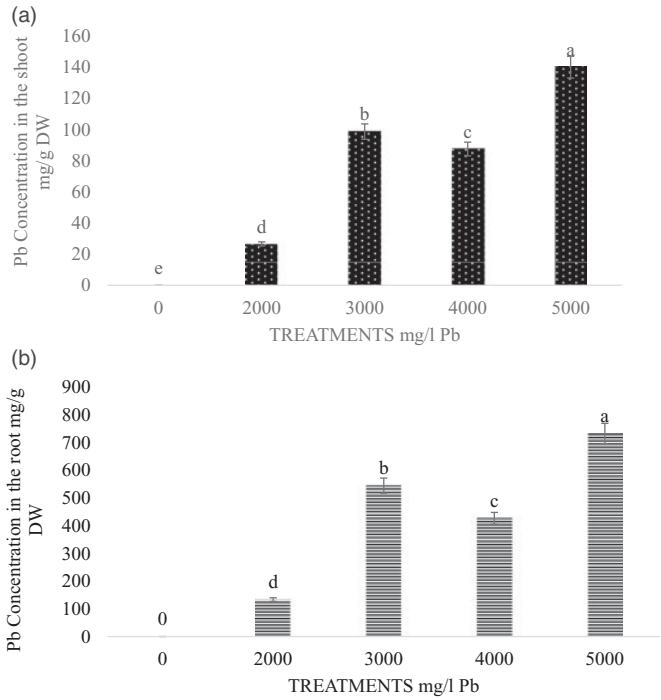

Figure 3. (a) Pb accumulation in shoot under 7-day stress in higher Pb concentrations. (b) Pb accumulation in root under 7-day stress in higher Pb concentrations. The letter on each bar indicates the level of statistical difference between the treatments as separated by DMRT at 0.05% level of probability

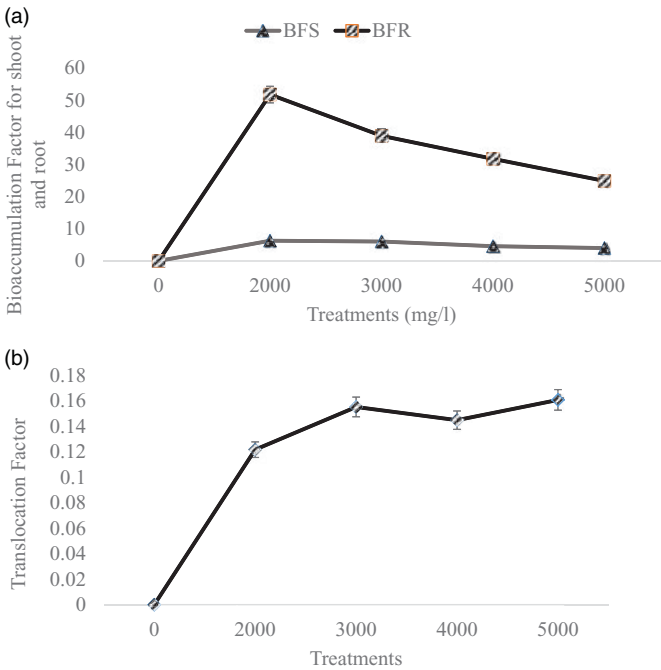

Figure 4. (a) Bioaccumulation factor for shoot (BFS) and root (BFR) under 7-day stress in higher Pb concentrations. (b) Translocation factor (TF) under 7-day stress in higher Pb concentrations.

however, higher in the plant treated with 2000 mg l^{-1} Pb than other treatments and the lowest was recorded in 5000 mg l^{-1} treatment (Figure 4a). The translocation factor without reference to control was more in the plants exposed to 3000, 5000, and 4000 mg l^{-1} than that of 2000 mg l^{-1} (Figure 4b).

Comparism between Pb concentration in the shoot and root of G. celosoides *after Pb stress under hydroponic experiment for 7 and 14 days*

Though with less variation, comparatively, the Pb accumulation in the plant was found to be concentration and duration dependent. The concentration of lead in the *G. celosoides* shoot at 14 days after stress was more than that of the 7 days in plants exposed to 500, 2000, 4000, and 5000 mg l^{-1} Pb while those plants exposed to 1000, 1500, and 3000 mg l^{-1} Pb accumulated more Pb in the shoot at 7 days than 14 days after stress. In all the Pb treatments, more Pb was accumulated in the plant root at 14 days after stress than 7 days after stress except in those treated with 5000 mg l^{-1} Pb whereas there was no difference in the root Pb concentrations both at 7 and 14 days in Plants treated with 1000 mg l^{-1} (Figure 5). At 14 days after stress, the BFS of the plants treated with 1500 and 2000 mg l^{-1} were higher than that of 1000 mg l^{-1} treatment. The BFS was generally more than the BFR and in higher Pb concentrations, more of the accumulated Pb as reflected by BFS and BFR values were translocated to the shoot (Figure 6a). The BFR in plants exposed to 500 mg l^{-1} was more than those of the other treatments followed by that of 2000 mg l^{-1} while the smallest value was recorded for the plant treated with 5000 mg l^{-1} (Figure 6b). In all the treatments, the TF was less than one. At 14 days after stress, the highest TF value was recorded for the plant exposed to lowest Pb concentration (500 mg l^{-1}) while other Pb treatments had reduced values and was slightly more in the plant exposed to highest Pb concentration (5000 mg l^{-1}) (Figure 6c).

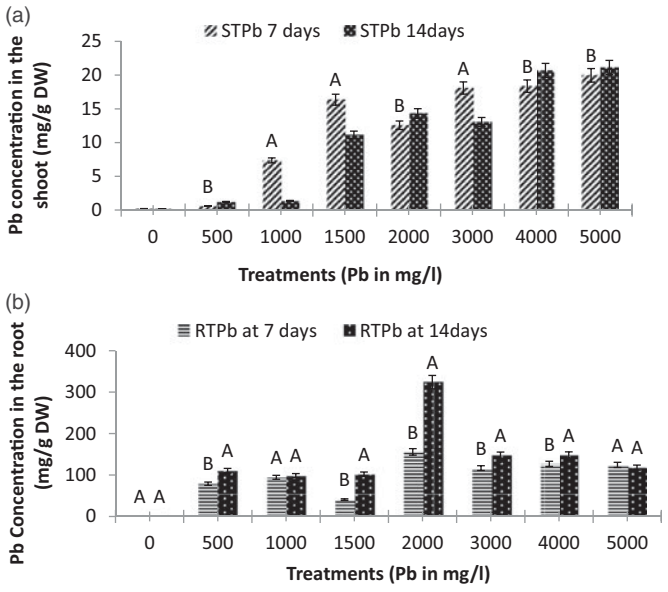

Figure 5. (a) Comparative Pb concentration in the shoot (A) and root (B) of *G. celosoides* after Pb stress under hydroponic experiment for 7 and 14 days. (b) Comparative Pb concentration in the shoot (A) and root (B) of *G. celosoides* after Pb stress under hydroponic experiment for 7 and 14 days. The letters indicate the difference between the two bars under the same Pb treatment as separated by DMRT at 0.05% level of probability

Chromium uptake and biomass accumulation by gomphrena celosoides *at 7 days after stress under lower Cr concentration in hydroponic solution*

Compared to control, there was a general reduction in the biomass accumulation 7 days after exposure to Cr. Similar to what was observed in Pb treatments, *G. celosoides* was able to accumulate Cr both in the shoot and root depending on the concentration gradients and accumulation increased with the increase in Cr concentration. However, the concentration in the shoot unlike that of Pb was more than that of the root and this was reflected in higher translocation factors (>1) in higher concentrations. The BFS (bioaccumulation factor for the shoot) and BFR (Bioaccumulation factor for the root) also showed the ability of *G. celosoides* for chromium uptake (Table 5).

Proline accumulation in the G. celosoides *exposed to Pb and Cr treatments for 7 and 14 days*

The result of proline estimation in stressed and unstressed plants showed a concentration-dependent trend as well as stress durations. The highest proline content was found in Gomphrena plants exposed to highest Pb concentration at 7 days of stress duration while the smallest was found in control. As expected, compared to control, the proline content was high in plants exposed to Pb treatments. More proline was found in plants exposed to 500, 1000, 1500, 2000, and 5000 mg l^{-1} Pb and the concentration was relatively high in plants treated with 5000 mg l^{-1} Pb as reflected by deep reddish/bloody color (Plate 3). An increase in the

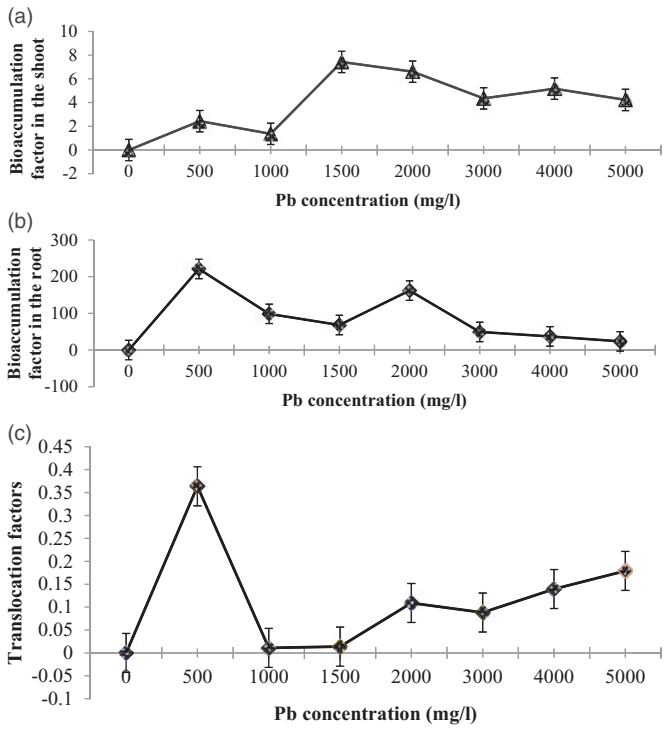

Figure 6. (a) Bioaccumulation Factor for shoot (BFS) of *G. celosoides* after Pb stress under hydroponic experiment for 14 days. (b) Bioaccumulation Factor for root (BFR) of *G. celosoides* after Pb stress under hydroponic experiment for 14 days. (c) Translocation Factor (TF) of *G. celosoides* after Pb stress under hydroponic experiment for 14 days.

Table 5. Chromium uptake by *Gomphrena celosoides* 7days after stress under lower Cr concentration in hydroponic solution.

Treatments (mg/l Cr)	Shoot Cr (mg/g)	Root Cr (mg/g)	BFS	BFR	TF	SDW (mg)	RDW (mg)
0	12.91d	29.70e	0	0	0.43	513.50e	336.60a
50	1060.77c	1342.20d	21.22	26.84	0.79	109.00d	77.30e
100	2355.50b	1522.30c	23.56	15.22	1.55	312.00a	112.00d
150	2781.70ab	2226.80b	18.55	14.85	1.25	197.00c	144.60c
200	3130.85a	2428.90a	15.65	12.15	1.29	235.50b	271.00b

Means followed by the same letter in the same column are not significantly different from each other

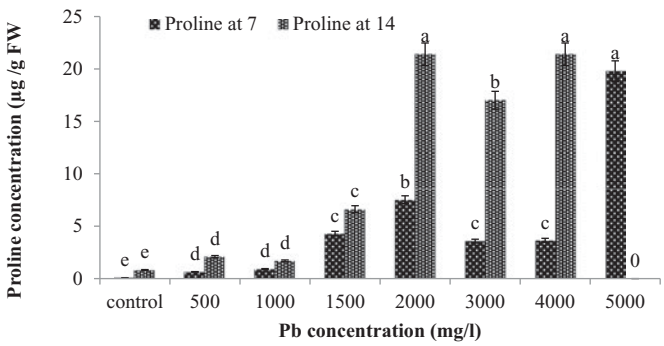

Figure 7. Proline concentration in the control and lead (Pb) stressed plants under 7 and 14 days treatments. The letter on each bar indicates the level of statistical difference between the treatments for 7 and 14 days duration as separated by DMRT at 0.05% level of probability

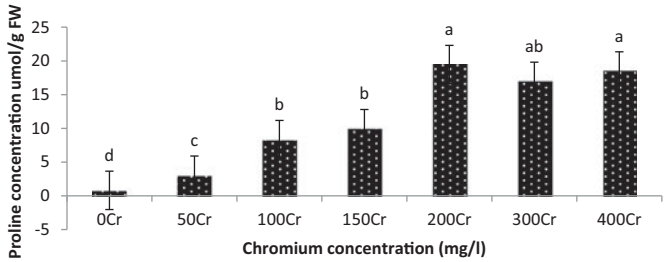

Figure 8. Proline concentration in the control and chromium stressed plants under 7 days treatment. The letter on each bar indicates the level of statistical difference between the treatments as separated by DMRT at 0.05% level of probability.

proline contents was observed in all the treatments under 14 days stress with the highest recorded in plants exposed to 2000 and 4000 mg l^{-1} Pb treatments. Control plants had the lowest value of proline (Figure 7). Similar trend was observed in the case of chromium with the highest proline content found in plants exposed to 200 mg l^{-1} and highest chromium concentration (400 mg l^{-1} Cr) and the lowest in control (Figure 8).

Effects of Pb treatments on the quantity and quality of DNA of G. celosoides *exposed to 7 days Pb stress*

The DNA extraction was only carried out on the *G. celosoides* plants exposed to lower metal concentrations for 7 days under hydroponic conditions. The quantification and gel electrophoresis of the extracted DNA showed some variations. The DNA extracted from plant exposed to 2000 mg l^{-1} Pb was the highest followed by those of control and 1500 mg l^{-1} treatments. The lowest DNA was extracted from plants exposed to 1000 mg l^{-1} Pb treatment (Table 6 and Plate 2).

Protein contents in G. celosoides *exposed to Pb treatments for 7 and 14 days*

Total protein extracted from the leaf of the *G. celosoides* exposed to Pb treatments under hydroponic conditions for 7 and 14 days treatments showed variations with regards to different Pb concentrations and stress durations. At 7 days duration, increase was observed in leaf protein contents of the plant exposed to 500, 1000, 1500, 2000, 3000, and 5000 mg l^{-1} Pb compared to that of control and the highest was recorded in the plant exposed to 5000 mg l^{-1}. After 14 days treatment, there was a reverse in the leaf protein contents of the treated and untreated plants. The control

Table 6. Results of DNA extraction and quantification of *G. celosoides* exposed to lower Pb concentrations.

Pb concentration (mg/l)	Dilution factor	Nanogram/microlitre	Total (ng)
0	100	747.3	74730.0
500	100	533.8	53380.0
1000	100	352.0	35200.0
1500	100	673.0	67300.0
2000	100	797.0	79700.0

leaf had the highest protein content compared to those of treated plants. This was followed by that of 2000 mg l^{-1} treatment and the lowest was found in the leaf of the plants exposed to 3000 mg l^{-1} Pb. Protein determination was only positive in the control root at 14 days after treatment, while negative results were obtained in the case of the Pb treated roots (Figure 9).

Antioxidant enzymes activities in G. celosoides *exposed to Pb treatments*

In the G. celosoides, SOD activity increased significantly in a concentration-dependent manner, up to 4000 mg/L Pb in the leaf tissues. At 7 days after exposure, superoxide enzymes activity was found to increase as Pb concentration increased with the highest recorded in the plant exposed to 4000 mg l^{-1} Pb and the lowest was found in the control (without Pb). This shows a positive correlation between the SOD activity and the Pb dose However, the SOD activity was slightly decreased at 5000 mg/L Pb concentration (Figure 10). The observation was different in the case of CAT. The level of CAT activity in plant exposed to 5000 mg l^{-1} Pb for 7 days was lower than that of the plant treated with 4000 mg l^{-1} Pb and at 14 days, these plants had withered. At 7 days after Pb exposure, there was no remarkable difference between the activities of CAT in the control and Pb treated plants except in plant exposed to 4000 mg l^{-1} Pb that had reduced CAT activity. The lowest activity was recorded in plant exposed to 4000 mg l^{-1} Pb at 7 days after stress (Figure 11a) while at 14 days after stress the

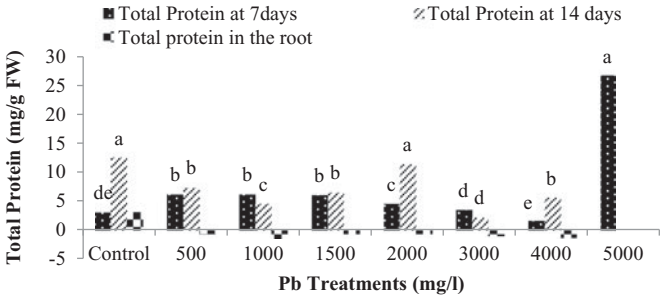

Figure 9. Total protein contents in the leaf at 7 and 14 days after stress under different Pb concentrations. Bars representing the same treatment and carrying the same alphabet are not significantly different from each other using Duncan Multiple Range Test (DMRT).

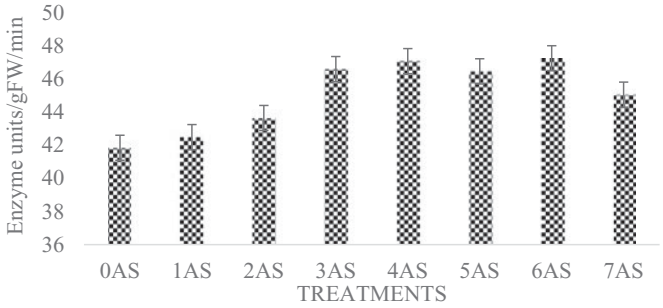

Figure 10. Superoxide activities in different Pb concentrations at 7 days after exposure to Pb treatments. NB: 0AS = Control, 1AS = 500 mg/l Pb, 2AS = 1000 mg/l Pb, 3AS = 1500 mg/l Pb, 4AS = 2000 mg/l Pb, 5AS = 3000 mg/l Pb, 6AS = 4000 mg/l Pb, 7AS = 5000 mg/l Pb.

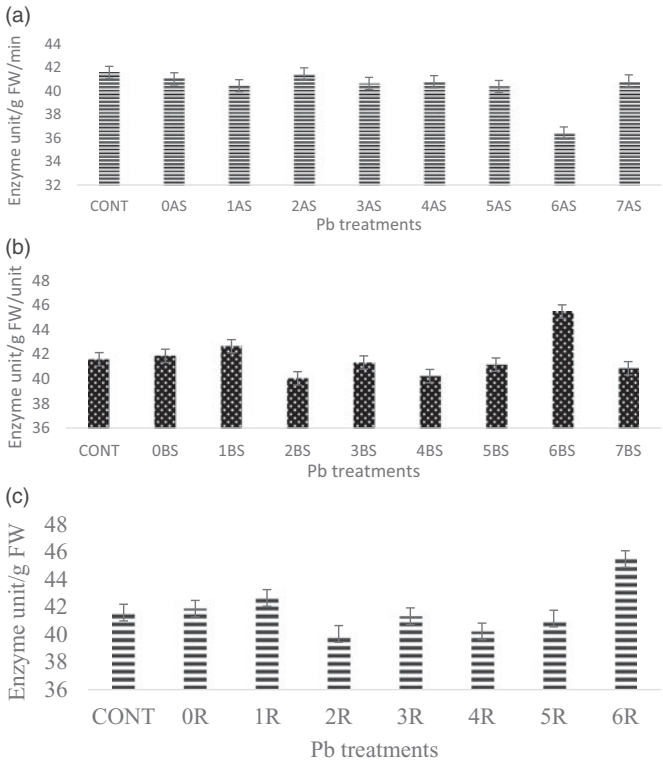

Figure 11. (a) Catalase enzyme activities in Gomphrena leaf exposed to different Pb concentrations at 7 days after exposure to Pb treatments. NB: 0AS = Control, 1AS = 500 mg/l Pb, 2AS = 1000 mg/l Pb, 3AS = 1500 mg/l Pb, 4AS = 2000 mg/l Pb, 5AS = 3000 mg/l Pb, 6AS = 4000 mg/l Pb, 7AS = 5000 mg/l Pb for leaf at 7 days after stress. (b) Catalase enzyme activities in Gomphrena leaf exposed to different Pb concentrations at 14 days after exposure to Pb treatments. NB: 0BS = Control, 1BS = 500 mg/l Pb, 2BS = 1000 mg/l Pb, 3BS = 1500 mg/l Pb, 4BS = 2000 mg/l Pb, 5BS = 3000 mg/l Pb, 6BS = 4000 mg/l Pb, 7BS = 5000 mg/l Pb at 14 days after stress. (c) Catalase enzyme activities in Gomphrena root exposed to different Pb concentrations at 14 days after exposure to Pb treatments. NB: 0R = Control, 1R = 500, 2R =1000, 3R = 1500, 4R = 2000, 5R = 3000, 6R = 4000 mg/l

same treatment gave the highest value of CAT activity (Figure 11b). Meanwhile, when the CAT activity was tested in the root 14 days after Pb exposure, it was also found to be high in the root of plant exposed to 4000 mg l^{-1} Pb (Figure 11c).

Discussion

The performance of *G. celosoides* in term of growth parameters showed the ability of this plant to survive and tolerate high metal concentration. The emergence of new leaves within the 7 and 14 days of stress shows that *G. celosoides* was a model metallophyte that is able to tolerate/withstand Pb stress up till 4000 mg l^{-1}. Though, the seeds of the G. celosoides was collected from the wild where the plant was growing on the highly contaminated site but only the tolerant plant like *G. celosoides* with presumed inherent tolerant ability can survive the shock of metal stress from the hydroponic condition where the heavy metals were introduced in their readily available/toxic forms and in higher concentrations. The plant was not only growing in height but the number of leaves were also increasing under metal stress, though, the response was found to be concentration and time dependent. The dry weight was more in the stressed plant than unstressed plant. This could simply be attributed to high metal accumulation in the metal-treated plants. Dry weight is a factor of dry matter accumulation which also depends on nutrient uptake. The higher Pb accumulation in stressed plant could have therefore contributed to high dry matter content of the stressed plants. However, morphologically, as days of metal exposure increased as well as concentration, the lower leaves of the Pb stressed plants were already drying up at this stage (Plate 1b,c) and root growth was more rapidly affected than that of other plants parts under metal exposure (Plate 1d). The root response was due to the fact that root is the plant organ that has direct contact with the toxic metal (Fahr et al. 2013). Under tolerable level, plant root can only protect itself by storing the metal in the non-sensitive tissue, sequester it its vacuole or transport it to the shoot as it is found in the hyperaccumulators (Fahr et al. 2013).

Gomphrena celosoides can be described as Pb and Cr accumulator as high concentrations of these metals were accumulated both in the root and shoot of this plant species under this experiment and the accumulation was dose and time dependent. In all the Pb treatments, however, *G. celosoides* accumulated more than 0.1% DW of Pb (Figure 5) and this qualified it as lead hyperaccumulator (Auguy et al. 2013). Similarly, as reported for metal accumulators, the concentration of metals in the plant tissue of *G. celosoides* was higher than the metal concentration in the growing medium due to absorption against the concentration gradient. It was expected that high concentration of Pb in the solution could have increased the osmotic potential of the solution thereby reducing the water and ion absorption capacity of the stressed plants, as well as growth but rather,

metal accumulators, have developed different strategies that enable them to absorb metal in excess of what is present in the solution (Baker 1981; Peer et al. 2003). The studied plant was also able to survive in higher concentrations up to 4000 mg l^{-1} Pb and 200 mg l^{-1} Cr, respectively for the duration of 2 weeks under hydroponic trials and accumulated both Pb and Cr in higher concentrations. The concentration in shoot was however lower than that of the root. These findings were similar to what has been previously reported in other Pb accumulators (Baker et al. 1994; Reeves 2006; Auguy et al. 2013). The variation and increase in metal uptake in response to metal concentration in the medium could be explained using passive absorption principles which is defined as the movement of ions from region of higher concentration to the region of lower concentration. Though this is not always applicable in some cases but tolerant plants, have been reported to use this process to accumulate and store toxic metals in their tissues (Baker and Brooks 1989). The lower Pb translocation to the shoot compared to the concentration in the root could be attributed to poor mobility of Pb (Ogundiran and Osibanjo 2008). Since Pb is not an essential element, it is believed that plants do not have specific channels for its uptake and upward movement except through the help of different chelators (Morel et al. 1986; Sharma and Dubey 2005; Brunet et al. 2009). In some plants, it has been reported that Pb can be transported *via* vascular tissues to aerial parts through complexation with organic molecules (Huang and Cunningham 1996; Clemens 2001; Antosiewicz 2005; Hanc et al. 2009). *Sesbania drummondii* transported Pb to the leaves after complexation with acetate, nitrate, and sulfide (Sharma et al. 2004), and a *cyclic nucleotide gated channel* (*NtCBP4*) was used in the case of tobacco (Sunkar et al. 2000) or chelation with Phytochelatins (Zhang et al. 2008; Fahr et al. 2013). These, however, cannot be established in this study but the highest Pb concentration that was found in the root and shoot of this plant could probably due to any of these assertions.

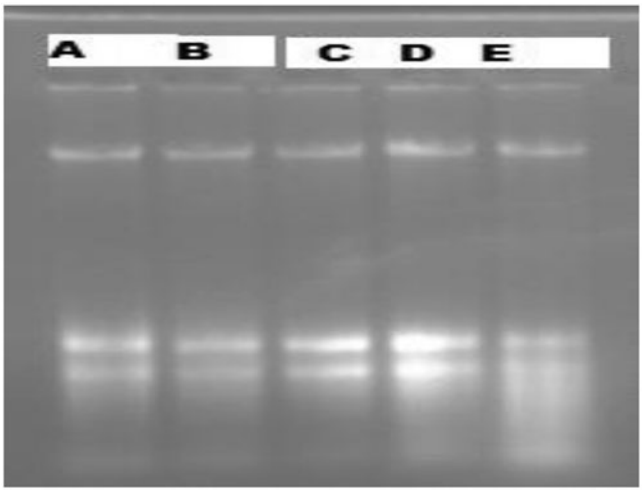

Plate 2. DNA isolation form *G. celosoides* exposed to 7 days Pb stress Legends: A = Control, B = 500 mg/l, C = 1000 mg/l, D = 1500 mg/l and E = 2000 mg/l treatments.

However, there was a fluctuation in bioaccumulation and translocation factors. The TF, which is a strong indication of the hyperaccumulating ability of *G. celosoides* to translocate metal to the shoot, is also concentration and duration dependent. The TF was more under 1500 mg l^{-1} Pb at 7 days after stress in lower Pb treatments but as stress duration progressed the high value was recorded under 500 mg l^{-1} Pb. The value was relatively low in higher Pb treatments but increasing up till 5000 mg l^{-1} Pb treatment at 14 days after exposure. This reduction in the TF in higher treatments might be attributed to the disruption in the metabolic processes taking place in the root of the plants exposed to higher Pb concentration as reflected in the root morphological features at 14 days after stress. Ion translocation involves the participation of many enzymes and energy in form of ATP. In this study, there was a reduction in the total protein content of the root at 14 days after exposure which has been attributed to protein degradation in the root (Fahr et al. 2013). This might have contributed to limited translocation of Pb in highly concentrated solution. The remarkable growth and biomass accumulation and TF in 500 mg l^{-1} Pb at 14 stage could indicate that this concentration was probably more tolerable than higher concentrations.

The DNA extracted from plant exposed to 2000 mg l^{-1} Pb was also higher than that of control. This could be due

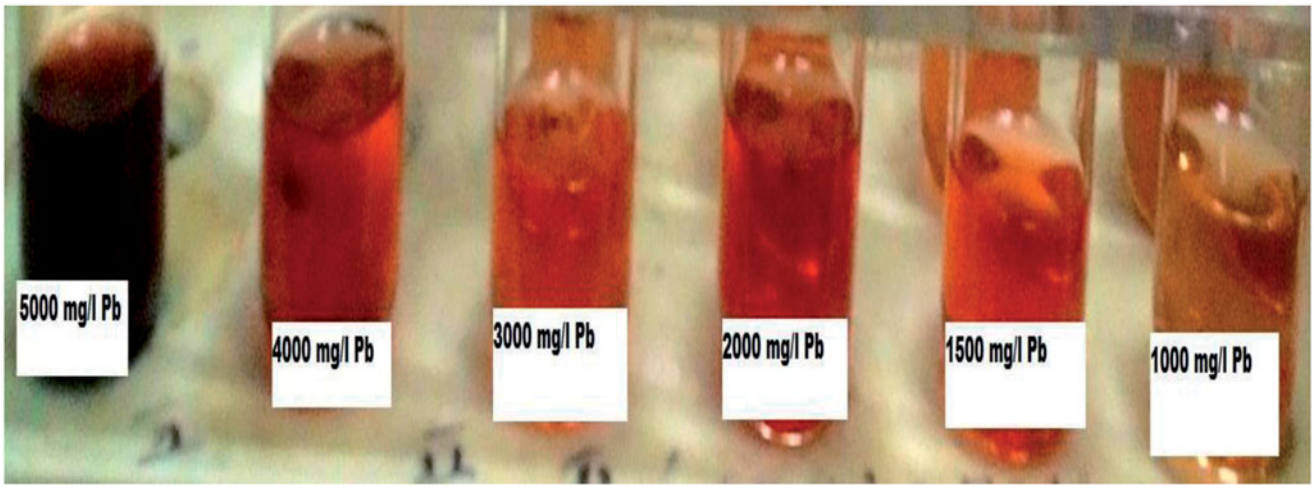

Plate 3. Different color shades showing the expression of proline in response to different Pb treatments.

to the initiation of different DNA under higher Pb stress as previously reported that stress is capable of inducing or repressing the nucleic acids production and expression in the stressed plant (Clemens 2006; Awaad et al. 2010). Similarly, there was an increase in protein concentration in response to heavy metal stress and the accumulation was also concentration and time dependent. Increase was recorded in metal-treated plants at the initial 7-day stress in excess of control plant but the trend was reversed at 14 days after metal exposure. Increase in protein contents of the stressed plant compared to the unstressed could be due to the production of different stress responsive and transport proteins like heat shock proteins which are reported to accumulate in response to different stresses (Krystofova et al. 2009; Bondino et al. 2012). There was a reverse as the stress duration increased as was also observed by Krystofova et al. (2009). This probably could be due to cellular and metabolic breakdown under high metal concentration which was pronounced in 5000 mg l^{-1} Pb. At 7 days after exposure the level of protein in this treatment was the highest which indicates an increase in the production of stress responsive proteins in this treatment more than other treatments but this was reversed as stress duration increased.

A number of non-protein thiols have also been reported to contribute to plant stress tolerance and metal transport from root to shoot (Zagorchev et al. 2013). Among these, proline is most widely reported (Hossain et al. 2012). Different types of osmolytes like proline have been reported to be produced for chelation and sequestration of metals. From this study, high concentration of proline was found in the stressed plant and this showed that proline production is one of the strategies employed by *G. celosoides* for tolerance (Adejumo et al. 2015). Three-twenty times higher contents of proline in the leaf of the plant exposed to Pb in comparison with control was observed and production was concentration and time dependent. Their induced production in metal-stressed plants and which was concentration dependent could be providing protection against oxidative stress (Hossain et al. 2012; Freeman et al. 2004; van de Mortel et al. 2008). Proline production was also induced by the exposure to Cr and this result was confirmed by the findings of Bluskov et al. (2005).

Antioxidant enzymes are described as biochemical markers of stress. Increase in their activities could connotes an increase in metabolic activity of treated plant (Zhang et al. 2007; Zembala et al. 2010). The increase in the activities of antioxidant enzymes has also been reported to correspond to the detoxification reaction in the treated plants but varies with the plant species, metal concentration and duration of exposure (Malar et al. 2014; Khan et al. 2016). These were confirmed with the increase in the activities of SOD in the shoot that was increasing with increase in Pb concentration. The activity of SOD and CAT was more pronounced in the shoot of plant raised in 4000 mg $l^{-1}$of Pb solution. At this concentration, the scavenging ability of these enzymes on the reactive oxygen species (ROS) produced under heavy metal stress was said to be highly induced (Rucinska et al. 1999; Candan and Tarhan 2003; Zembala et al. 2010). This could be one of the anti-oxidative defense systems being employed by *G. celosoides* to increase tolerance to heavy metal toxicity. Increase in CAT activity in *Triticum aestivum* upon Pb exposure has also been reported (Kaur et al. 2013). The level of enzyme activity in plant exposed to 5000 mg l^{-1} Pb was, however, lower than that of the plant treated with 4000 mg l^{-1} Pb probably due to the breakdown or degradation of different biomolecules and plant cells in the highest Pb concentration, the event that manifested in the death of these plants before 14 days after stress. Higher level of heavy metals above the tolerable limit by the plant has been reported to overcome the defense system thereby causing oxidative stress through lipid peroxidation and overproduction of reactive oxygen species (Rizwan et al. 2018). Islam et al. (2007) and Kaur et al. (2013) reported a decline in the activity of POXs in *Elsholtzia argyi and Triticum aestivum* roots upon Pb exposure. Higher concentration of Pb or longer treatment might have inhibited cell metabolism and ROS production, resulting in the decrease in the activity of some antioxidant enzymes like CAT (Verma and Dubey 2003).

Conclusion

The findings from this study coupled with the initial results clearly demonstrated that *G. celosoides* is a novel metallophyte and metal accumulator that can be promoted for phytoextraction. Unlike the field condition, hydroponic study using Pb^{2+} in its readily available/toxic form for monitoring Pb uptake gives a clear picture of the ability of *G. celosoides* to tolerate and accumulate Pb in its tissue and translocating it to the shoot. Under hydroponic conditions, lead accumulation in roots and shoots of *G. celosoides* was found to be dose and time dependent. Though roots accumulated more than shoots at the same concentration and exposure, but the studied plant was extremely tolerant to Pb and Cr and was able to accumulate high concentration of these metals in its tissues. For field application of phytoremediation especially where the contaminant is more than one, the use of *G. celosoides* can be promoted. *Gomphrena celosoides* was able to tolerate 4000 mg l^{-1} Pb and 200 mg l^{-1} Cr in hydroponic solution for 7 days. There was increase in Proline and protein concentration in response to heavy metal stress and the accumulation was also concentration and time dependent. In conclusion, this study shows that *G. celosoides is* a model plant and can serve as a tool for identifying genes involved in Pb tolerance and accumulation.

Acknowledgement

The authors appreciate immensely the CV Raman Fellowship for granting the unique opportunity of benefitting from its prestigious post-doctoral fellowship. Our gratitude also goes to the Director (CSIR-NEERI, Nagpur, India) for his approval.

References

Adejumo SA, Togun AO, Adediran JA, Ogundiran MB. 2011. Field assessment of progressive remediation of soil contaminated with

lead-acid battery waste in response to compost application. Pedologist. 54(3):182–193.
Adejumo SA, Adeosun AA, Olaniyan AB, Awodoyin RO. 2015. Seasonal variations in distribution, heavy metal uptake and proline production of native plants growing on Pb-contaminated site in Ibadan, South-Western, Nigeria. Nigerian J Ecol. 14:37–47.
Adejumo SA, Tiwari S, Shinde V, Sarangi BK. 2018. Heavy metal (Pb) accumulation in metallophytes as influenced by the variations in rhizospheric and non-rhizospheric soils physico-chemical characteristics. Int J Phytoremediation. 20(3):237–248. doi:10.1080/15226514.2017.1374333.
Adeosun A, Adejumo SA, Srivastava P. 2017. Lead accumulation and distribution at cellular level in native plants growing on battery wastes contaminated sites in Ibadan, Nigeria. IJPSS. 16(5):1–15. doi:10.9734/IJPSS/2017/32252.
Álvarez R, del Hoyo A, García-Breijo F, Reig-Armiñana J, del Campo EM, Guéra A, Barreno E, Casano LM. 2012. Different strategies to achieve Pb-tolerance by the two *Trebouxia algae* coexisting in the lichen *Ramalina farinacea*. J Plant Physiol. 169(18):1797–1806. doi:10.1016/j.jplph.2012.07.005.
Antosiewicz DM. 2005. Study of calcium-dependent lead-tolerance on plants differing in their level of Ca-deficiency tolerance. Environ Pollut. 134(1):23–34. doi:10.1016/j.envpol.2004.07.019.
Auguy F, Fahr M, Moulin P, Brugel A, Laplaze L, Mzibri ME, Filali-Maltouf A, Doumas P, Smouni A. 2013. Lead tolerance and accumulation in *Hirschfeldia incana*, a Mediterranean Brassicaceae from metalliferous mine spoils. PLoS One. 8(5):e61932. doi:10.1371/journal.pone.0061932.
Awaad HA, Youssef MAH, Moustafa E. 2010. Identification of genetic variation among bread wheat genotypes for lead tolerance using morpho – physiological and molecular markers. J Am Sci. 6(10):1142–1153.
Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and phytochemistry. Biorecovery. 1:81–126.
Baker A. 1981. Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr. 3(1–4):643–654. doi:10.1080/01904168109362867.
Baker AJ. 1987. Metal tolerance. New Phytol. 106:93–111. doi:10.1111/j.1469-8137.1987.tb04685.x.
Baker AJM, Reeves RD, Hajar A. 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). New Phytol. 127:61–68. doi:10.1111/j.1469-8137.1994.tb04259.x.
Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39(1):205–207. doi:10.1007/BF00018060.
Blaylock M, Salt DE, Dushenkov S, Zakharova O, Gussman C, Kapulnik Y, Ensley BD, Raskin I. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol. 31(3):860–865. doi:10.1021/es960552a.
Bluskov S, Arocena J, Omotoso O, Young J. 2005. Uptake, distribution, and speciation of chromium in *Brassica juncea*. Int J Phytoremediation. 7(2):153–165. doi:10.1080/16226510590950441.
Bolan N, Naidu R, Choppala G, Park J, Mora ML, Budianta D, Panneerselvam P. 2010. Solute interactions in soils in relation to the bioavailability and environmental remediation of heavy metals and metalloids. Pedologist. 53(3):1–18.
Bondino HG, Valle EM, Ten HA. 2012. Evolution and functional diversification of the small heat shock protein/alpha-crystallin family in higher plants. Planta. 235(6):1299–1313. doi:10.1007/s00425-011-1575-9.
Brooks R, Lee J, Reeves R, Jaffre T. 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicators plants. J Geochem Explor 7:49–57. doi:10.1016/0375-6742(77)90074-7.
Brooks RR. 1998. Geobotany and hyperaccumulator. In: Brooks RR, editor. Plants that hyperaccumulate heavy metals, their role in phytoremediation, microbiology, archaeology, mineral exploration and phytomining. Wallingford, UK: CAB, International; Cambridge: Cambridge University Press. p. 55–94.
Brunet J, Varrault G, Zuily-Fodil Y, Repellin A. 2009. Accumulation of lead in the roots of grass pea (*Lathyrus sativus* L.) plants triggers systemic variation in gene expression in the shoots. Chemosphere. 77(8):1113–1120. doi:10.1016/j.chemosphere.2009.07.058.
Candan N, Tarhan L. 2003. The correlation between antioxidant enzyme activities and lipid peroxidation levels in *Mentha pulegium* organs grown in Ca^{2+}, Mg^{2+}, Cu^{2+}, $Zn^{2+}$and Mn^{2+}stress conditions. Plant Sci. 163:769–779.
Carvalho VMT, Amaral DC, Guilherme LRG, Aarts M. 2013. *Gomphrena claussenii,* the first South American metallophyte species with indicator-like Zn and Cd accumulation and extreme metal tolerance. Front Plant Sci. 4:180. doi:10.3389/fpls.2013.00180. PMID:23750166.
Chaney RL, Angle JS, McIntosh MS, Reeves RD, Li YM, Brewer EP, Chen KY, Roseberg RJ, Perner H, Synkowski EC. 2005. Using hyperaccumulator plants to phytoextract soil Ni and Cd. Z Naturforsch. 60:190–198.
Clemens S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. Planta. 212(4):475–486. doi:10.1007/s004250000458.
Clemens S. 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie. 88(11):1707–1719. doi:10.1016/j.biochi.2006.07.003.
Clemens S, Kim EJ, Neumann D, Schroeder JI. 1999. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. Embo J. 18:3325–3333.
Cunningham SD, Berti WR. 2000. Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-Pb issue. In: Terry N, Banuelos G, editors. Phytoremediation of contaminated soil and water. New York: Lewis Publishers. p. 359–376
Delorme TA, Gagliardi JV, Angle JS, Chaney RL. 2001. Influence of the zinc hyperaccumulator *Thlaspi caerulescens* J. & C. Presl. and the nonmetal accumulator *Trifolium pratense* L. on soil microbial populations. Can J Microbiol. 47(8):773–776. doi:10.1139/w01-067.
Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus. 12(1990):13–15.
Fahr M, Laplaze L, Bendaou N, Hocher V, ElMzibri M, Bogusz D, Smouni A. 2013. Effect of lead on root growth. Front Plant Sci. 4:175. doi:10.3389/fpls.2013.00175.
Freeman C, Fenner N, Ostle NJ, Kang H, Dowrick DJ, Reynolds B, Lock MA, Sleep D, Hughes S, Hudson J. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature. 430(6996):195–198. ¯doi:10.1038/nature02707.
Gupta DK, Huang HG, Yang XE, Razafindrabe BH, Inouhe M. 2010. The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione. J Hazard Mater. 177(1–3):437–444. doi:10.1016/j.jhazmat.2009.12.052.
Hanc A, Baralkiewicz D, Piechalak A, Tomaszewska B, Wagner B, Bulska E. 2009. An analysis of long-distance root to leaf transport of lead in *Pisum sativum* plants by laser blation-ICP-MS. Int J Envi-Ron Anal Chem. 89:651–659. doi:10.1080/03067310802559366.
Hossain MA, Piyatida P, Teixeirada Silva JA, Fujita M. 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot. 2012:37. doi:10.1155/2012/872875.
Huang JV, Cunningham SD. 1996. Lead phytoextraction: species variation in lead uptake and translocation. New Phytol. 134:73–84.
Islam E, Yang X, Li T, Liu D, Jin X, Meng F. 2007. Effect of Pb toxicity on root morphology, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*. J Hazard Mater. 147(3):806–816. doi:10.1016/j.jhazmat.2007.01.117.
Iturbe R, Flores RM, Torres LG. 2003. Subsoil contaminated by hydrocarbons in an out of service oil distribution and storage station in Zacatecas, Mexico. Env Geol. 44:608–620. doi:10.1007/s00254-003-0799-9.
Kaur G, Singh HP, Batish DR, Kohli RK. 2013. Lead (Pb)-induced biochemical and ultrastructural changes in wheat (*Triticum aestivum*) roots. Protoplasma. 1:53–62. doi:10.1007/s00709-011-0372-4.
Khan I, Iqba M, Ashraf MY, Ashraf MA, Ali S. 2016. Organic chelants-mediated enhanced lead (Pb) uptake and accumulation is associated

with higher activity of enzymatic antioxidants in spinach (*Spinacea oleracea* L.). J Hazard Mater. 317:352–361. doi:10.1016/j.jhazmat.2016.06.007.

Kramer U. 2010. Metal hyperaccumulation in plants. Ann Rev Plant Biol. 61:517–534.

Krystofova O, Shestivska V, Galiova M, Novotny K, Kaiser Zehnalek JJ, Babula P, Opatrilova R, Adam V, Kizek R. 2009. Sunflower plants as bioindicators of environmental pollution with lead (II) ions. Sensors. 2009(9):5040–5058. doi:10.3390/s90705040.

LeDuc DL, Tarun AS, Montes-Bayon M, Meija J, Malit MF, Wu CP, AbdelSamie M, Chiang CY, Tagmount A, DeSouza M. 2004. Overexpression of selenocysteine methyltransferase in Arabidopsis and Indian mustard increases selenium tolerance and accumulation. Plant Physiol. 135(1):377–383. doi:10.1104/pp.103.026989.

Li YM, Chaney R, Brewer E, Roseberg R, Angle JS, Baker A, Reeves R, Nelkin J. 2003. Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. Plant Soil. 249(1):107–115.

Malar S, Vikram SS, Favas PJ, Peruma V. 2014. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart)]. Bot Stud. 55:54.

McDonald S. 2006. Phytoremediation of Pb-contaminated soil using *Typha latifolia* (Broadleaf Cattail) [master's thesis]. Humboldt State University.

McGrath SP, Zhao FJ, Lombi E. 2002. Phytoremediation of metals, metalloids and radionuclutide. Adv Agron. 75:1–56.

Memon AR, Aktoprakligil D, Ozdemir A, Vertii A. 2001. Heavy metal accumulation and detoxification mechanisms in plants. Turk J Bot. 25:111–121.

Moon DH, Lee J-R, Wazne M, Park J. 2012. Assessment of soil washing for Zn contaminated soils using various washing solutions. J Ind Eng Chem. 18(2):822–825. Pp doi:10.1016/j.jiec.2011.11.137.

Morel JL, Mench M, Guckert A. 1986. Measurement of Pb^{2+}, Cu^{2+} and Cd^{2+} binding with mucilage exudates from maize (*Zea mays* L.) roots. Biol Fert Soils. 2(1):29–34. doi:10.1007/BF00638958.

Morsy AA, Salama HHA, Kamel HA, Mansour MMF. 2012. Effect of heavy metals on plasma membrane lipids and antioxidant enzymes of *Zygophyllum species*. Eurasia J Biosci. 6:1–10. doi:10.5053/ejobios.2012.6.0.1.

Mudgal V, Madaan N, Mudgal A. 2010. Heavy metals in plants: phytoremediation: plants used to remediate heavy metal pollution. Agric Biol J North Am. 1:40–46.

Ogundiran MB. 2007. Assessment and chemical remediation of soil contaminated by lead acid battery wastes in Lalupon village Oyo State, Nigeria [Ph.D thesis]. University of Ibadan. x + 205pp.

Ogundiran MB, Osibanjo O. 2008. Heavy metal concentrations in soils and accumulation in plants growing in a deserted slag dumpsite in Nigeria. Afr J Biotechnol. 7(17):3053–3060.

Padmavathiamma PK, Li LY. 2007. Phytoremediation technology: hyper-accumulation metals in plants. Water Air Soil Pollut. 184(1–4):105–126. doi:10.1007/s11270-007-9401-5.

Peer WA, Mamoudian M, Lahner B, Reeves RD, Murphy AS, Salt DE. 2003. Identifying model metal hyperaccumulating plants: germplasm analysis of 20 Brassicaceae accessions from a wide geographic area. New Phytol. 159(2):421–430. doi:10.1046/j.1469-8137.2003.00822.x.

Peterson PJ, Girling CA. 1981. Other trace metals; Chromium P. In: Lepp NW, editor. Effect of heavy metal pollution on plants. Vol. 1. London; Englewood, NJ: Applied Science Publishers. p. 222–229.

Pokhrel LR, Dubey B. 2012. Global scenarios of metal mining, environmental repercussions, public policies, and sustainability: a review. Crit Rev Environ Sci Technol. 43:2352–2388. doi:10.1080/10643389.2012.672086.

Prasad MNV, Freitas H. 2003. Metal hyperaccumulation in plants – biodiversity prospecting for phytoremediation technology. Electron J Biotechnol. 6(3):285–321.

Pulford ID, Watson C. 2003. Phytoremediation of heavy metal-contaminated land by trees-a review. Environ Int. 29(4):529–540. doi:10.1016/S0160-4120(02)00152-6.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, editors. Phytoremediation of toxic metals: using plants to clean up the environment. New York: John Wiley & Sons, Inc. p. 193–229.

Reeves RD. 2006. Hyperaccumulation of trace elements by plants. In: Morel JL, Echevarria G, Goncharova N, editors. Phytoremediation of metal-contaminated soils. NATO Sciences Series 68. New York: Springer. p. 25–52.

Rennevan H, Tony RH, Abir A, Andy JM, Mike LJ, Sabeha KO. 2007. Remediation of metal contaminated soil with mineral amended composts. Environ Pollut. 150:347–354.

Rizwan M, Ali S, Rehman MZ, Javed MR, Bashir A. 2018. Lead toxicity in cereals and its management strategies: a critical review. Water Air Soil Pollut. 229:211. doi:10.1007/s11270-018-3865-3.

Roosens N, Verbruggen N, Meerts P, Ximénez-Embú NP, Smith J. 2003. Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from Western Europe. Plant Cell Environ. 26(10):1657–1672. doi:10.1046/j.1365-3040.2003.01084.x.

Rucinska R, Waplak S, Gwozdz AE. 1999. Free radical formation and activity of antioxidant enzymes in lupine roots exposed to lead. Plant Physiol Biochem. 37:187–194. doi:10.1016/S0981-9428(99)80033-3.

Shah K, Nongkynrih JM. 2007. Metal hyperaccumulation and bioremediation. Biol Plant. 51(4):618–634. doi:10.1007/s10535-007-0134-5.

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S. 2005. Chromium toxicity in plants. Environ Int. 31(5):739–753. doi:10.1016/j.envint.2005.02.003.

Sharma M, Dubey RS. 2005. Lead toxicity in plants. Braz J Plant Physiol. 17(1):No Print ISSN 1677–2420.

Sharma NC, Gardea-Torresdey JL, Parsons J, Sahi SV. 2004. Chemical speciation and cellular deposition of lead in *Sesbania drummondii*. Environ Toxicol Chem. 23(9):2068–2073. doi:10.1897/03-540.

Sun R, Zhou Q, Jin C. 2006. Cadmium accumulation in relation to organic acids in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator. Plant Soil. 285(1–2):125–134. doi:10.1007/s11104-006-0064-6.

Sunkar R, Kaplan B, Bouche N, Arazi T, Dolev D, Talke IN, Maathuis FJM, Sanders D, Bouchez D, Fromm H, et al. 2000. Expression of atrun-cated tobacco NtCBP4 channel in transgenic plants and disruption of the homologous *Arabidopsis* CNGC1 gene confer Pb^{2+} tolerance. Plant J. 24(4):533–542. doi:10.1046/j.1365-313x.2000.00901.x.

Udovic M, Lestan D. 2009. Pb, Zn and Cd mobility, availability and fractionation in aged soil remediated by EDTA leaching. Chemosphere. 74(10):1367–1373. doi:10.1016/j.chemosphere.2008.11.013.

USEPA. 2000. Introduction to phytoremediation. EPA. No. EPA/600/R-99/107; USEPA.

van de Mortel JE, Schat H, Moerland PD, van Themaat EVL, van der Ent S, Blankestijn H, Ghandilyan A, Tsiatsiani S, Aarts MGM. 2008. Expression differences for genes involved in lignin, glutathione and sulphate metabolism in response to cadmium in *Arabidopsis thaliana* and the related Zn/Cd- hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ. 31(3):301–324. doi:10.1111/j.1365-3040.2007.01764.x.

van der Ent A, Baker AJM, Reeves RD, Pollard AJ, Schat H. 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil. 362(1–2):319–334. doi:10.1007/s11104-012-1287-3.

Verbruggen N, Hermans C, Schat H. 2009. Molecular mechanisms of metal hyperaccumulation in plants. New Phytol. 181(4):759–776. doi:10.1111/j.1469-8137.2008.02748.x.

Verma S, Dubey RS. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci. 164(4):645–655. doi:10.1016/S0168-9452(03)00022-0.

Wang AS, Angle JJS, Chaney RL, Delorme TA, Reeves RD. 2006. Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. Plant Soil. 281(1–2):325–337. doi:10.1007/s11104-005-4642-9.

Whiting SN, Leake JR, McGrath SP, Baker A. 2000. Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi*

caerulescens. New Phytol. 145(2):199–210. doi:10.1046/j.1469-8137.2000.00570.x.

Yang X, Jin X, Feng Y, Islam E. 2005. Molecular mechanisms and genetic basis of heavy metal tolerance/hyperaccumulation in plants. J Integrative Plant Biol. 47(9):1025–1035. doi:10.1111/j.1744-7909.2005.00144.x.

Zagorchev L, Seal CE, Kranner I, Odjakova M. 2013. A central role for thiols in plant tolerance to abiotic stress. IJMS. 14:7405–7432. doi:10.3390/ijms14047405.

Zayed AM, Terry N. 2003. Chromium in the environment: factors affecting biological remediation. Plant and Soil. 249(1):139–156.

Zembala M, Filek M, Walas S, Mrowiec H, Kornaś A, Miszalski Z, Hartikainen H. 2010. Effect of selenium on macro-and microelement distribution and physiological parameters of rape and wheat seedlings exposed to cadmium stress. Plant Soil. 329(1–2):457–468. doi:10.1007/s11104-009-0171-2.

Zhang F, Wang Y, Lou Z, Dong J. 2007. Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere. 67(1):44–50. doi:10.1016/j.chemosphere.2006.10.007.

Zhang Z, Gao X, Qiu B. 2008. Detection of phytochelatins in the hyperaccumulator *Sedum alfredii* exposed to cadmium and lead. Phytochemistry. 69(4):911–918. doi:10.1016/j.phytochem.2007.10.012.

Zhao FJ, Lombi E, McGrath SP. 2003. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator. *Thlaspi caerulescens*. Plant Soil. 249(1):37–43.