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RELATIVE TOLERANCE AND PHYTOREMEDIATION CAPACITY OF DIFFERENT *Brassica species* UNDER LEAD STRESS

J. A. Mahmud[1], S. M. A. Mahmud[2], M. Kabir[3]

ABSTRACT

The present study was carried out to investigate the metal accumulation and tolerance abilities of five *Brassica* species (*B. campestris, B. napus, B. juncea, R. sativus and B. oleracea*) seedlings exposed to four levels of lead (Pb) stress [0 mM, 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$] for six weeks. Of the *Brassica* species studied, *B. juncea* accumulated the highest amount of Pb in a dose-dependent manner and in every case, the Pb content was higher in the roots than the shoots. Lead toxicity causes a severe impact on plant growth and biomass. All level of Pb stress reduced seedlings height fresh weight and dry weight in each varieties at 2, 4 and 6 weeks of age. In addition, leaf relative water content and SPAD value were also decreased with increase of stress concentration. But among the varieties, *B. juncea* showed lowest reduction of abovementioned parameters. Considering the growth and physiological performance *B. juncea* is relatively tolerant species though it accumulated highest Pb in roots and shoots. So, *B. juncea* might be used as a tool of phytoremediation in Pb polluted soil.

Keywords: *Brassica* species, heavy metal, lead, phytoremediation

INTRODUCTION

Lead (Pb) is a metallic pollutant emanating from various environmental sources including industrial wastes, combustion of fossil fuels and use of agrochemicals. Lead may exist in the atmosphere as dusts, fumes, mists, vapors and in soil as a mineral (Hasanuzzaman *et al.*, 2012a). Soils along roadsides are rich in lead because vehicles burn leaded gasoline, which contributes to environmental lead pollution. Other important sources of Pb pollution are geological weathering, industrial processing of ores and minerals, leaching of Pb from solid wastes, and animal and human excreta (Fahr *et al.*, 2013). Lead is non-degradable, readily enters the food chain, and can subsequently endanger human and animal health. In Bangladesh, Pb pollution is increasing day by day specially in heavy traffic and industrial area. So, Pb is one of the most important abiotic stressor and deserves the increasing attention it has received in recent decades.

Lead is absorbed by plants mainly through the root system and in minor amounts through the leaves. Within the plants, Pb accumulates primarily in roots, but some is translocated to aerial plant parts. Soil pH, soil particle size, cation-exchange capacity, as well as root surface area, root exudation, and mycorrhizal transpiration rate affect the availability and uptake of lead by plants. Only a limited amount of Pb is translocated from roots to other organs because there are natural plant barriers in the root endodermis. At lethal concentrations, this barrier is broken and lead may enter vascular tissues (Hasanuzzaman *et al*., 2012a). Lead in plants may form deposits of various sizes, present mainly in intercellular spaces, cell walls, and vacuoles. Small deposits of this Pb can inhibits activities of many enzymes, upsets mineral nutrition and water balance, changes the hormonal status, and affects membrane structure and permeability (Gill and Tuteja, 2010; Hasanuzzaman *et al*., 2012b). So, remediation of lead from the soil is very important. Plant-based remediation system or phytoremediation is well-known all over the globe, and considered one of the low cost, novel, green technology. To find out suitable plants for Pb removal from the contaminated soil, a broader understanding is needed of the physiological and biochemical features of potentially useful species. Members of the Brasicaceae family are promising candidates for phytoextraction of heavy metals such as Cd, Pb, Zn and Ni (Prasad and Freitas, 2003; Robinson *et al*., 2009), but the degrees of tolerance and

[1]Assoc. Professor, [2&3]MS student, Dept. of Agroforestry and Environmental Science, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

remediation capacity of metal of different *Brassica* species are varies greatly. Identification of more tolerant and phytoremediator species of metal is very urgent task for plant scientists. Therefore, this study was designed to examine the relative tolerance of different *Brassica* species (*B. campestris, B. napus, B. juncea, R. sativus and B. oleracea*) under various Pb concentrations and to identify the phytoremediation capacity of different *Brassica* species (*B. campestris, B. napus, B. juncea, R. sativus and B. oleracea*) under various Pb concentrations.

MATERIALS AND METHODS

Uniform sized seeds of five *Brassica* species (*Brassica campestris* L. cv. BARI Sharisha 9, *Brassica napus* L. cv. BARI Sharisha 13, *Brassica juncea* L. cv. BARI Sharisha 16, Raphanus *sativus* L. cv. BARI Mula 2 and *Brassica oleracea* L. cv. BARI Fulcopi 2) were selected and surface sterilized with 70% ethanol followed by washing several times with sterile distilled water. Then sterilized seeds were sown in plastic pot containing Pb contaminated soil in Sher-e-Bangla Agricultural University during December 2017 to January 2018. The soil of different pots contained 0 mM, 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$, in where each variety was cultivated. Forty (40) seeds were placed in every plastic pot and standard fertilization was maintained for the all treatments. The experiment was conducted following Completely Randomized Design (CRD) with 20 treatments and it was replicated thrice. The following data were taken at 2, 4 and 6 weeks after sowing.

Determination of growth parameters

Plant height was taken from each treatment and expressed as cm. Five randomly selected fresh seedlings from each treatment were weighted, recorded and considered as fresh weight (FW). Dry weight (DW) was determined after drying the seedlings at 70°C in oven for 48 h. Both DW and FW were expressed in gram (g).

Determination of relative water content

Relative water content (RWC) was measured according to Barrs and Weatherly (1962). The whole leaf discs were weighed as FW then those were floated on distilled water in petri dish and kept in dark place. After 8 hours those were weighed again after drying excess surface water as turgid weight (TW). Finally DW was measured after drying at 70°C for 48 h. Leaf RWC was calculated by following formula:

$$RWC (\%) = [(FW–DW)/(TW–DW)]\times 100$$

Determination of SPAD value

Chlorophyll (Chl) content in terms of SPAD (soil plant analysis development) values was recorded using a portable SPAD 502 Plus meter (Konica-Minolta, Tokyo, Japan). In each measurement, the SPAD reading was repeated 5 times from the leaf tip to base, and the average was used for analysis.

Estimation of root and shoot Pb content

Lead content was determined by using an atomic absorption spectrophotometer. The plant samples were oven dried at 70°C for 72 h. The dried samples from root and shoot (0.1 g) were ground and digested separately with acid mixture (HNO_3:$HClO_4$ = 5:1 v/v) for 48 h at 80°C. Then absorbance of sample was recorded from atomic absorption spectrophotometer and Pb content of shoot and root was calculated using standard curve of known concentration.

Statistical analysis

All the obtained data were subjected to analysis of variance (ANOVA) and the mean differences will be compared by Fisher's LSD test using XLSTAT v.2017 (Addinsoft, 2017).

RESULTS AND DISCUSSION

Results

Plant height and biomass production

All *Brassica* plants exposed to increasing concentration of Pb demonstrated a reduction in plant height, fresh weight and dry weight at 2, 4 and 6 weeks after sowing (WAS). The reduction of plant growth and biomass followed the dose-dependent fashion and the maximum plant height, fresh weight and dry weight reduction was observed in all *Brassica* seedlings upon exposure to 1.0 mM Pb $(NO_3)_2$ (Fig. 1).

The maximum plant height was observed in *R. sativus* (7.43 cm) at 2 WAS but at 4 and 6 WAS highest plant height was observed in *B. napus* (15.8 and 38.97 cm respectively). However the minimum plant height was observed in *B. oleracea* in every case (Fig. 1). In contrast to control seedlings the plant height reduction was 15.4, 14.4, 11.6, 20.3 and 13.9% at 2 WAS; 17.8, 17.6, 10.0, 23.3 and 13.9% at 4 WAS; 17.7, 17.6, 10.0, 23.4 and 13.8% at 6 WAS under 1.0 mM $Pb(NO_3)_2$ stress in *B. campestris, B. napus, B. juncea, R. sativus and B. oleracea,* respectively (Fig. 1). So, from the data it is clear that in all date the minimum reduction of plant height was observed in *B. juncea* and maximum reduction was observed in *R. sativus* (Fig. 1).

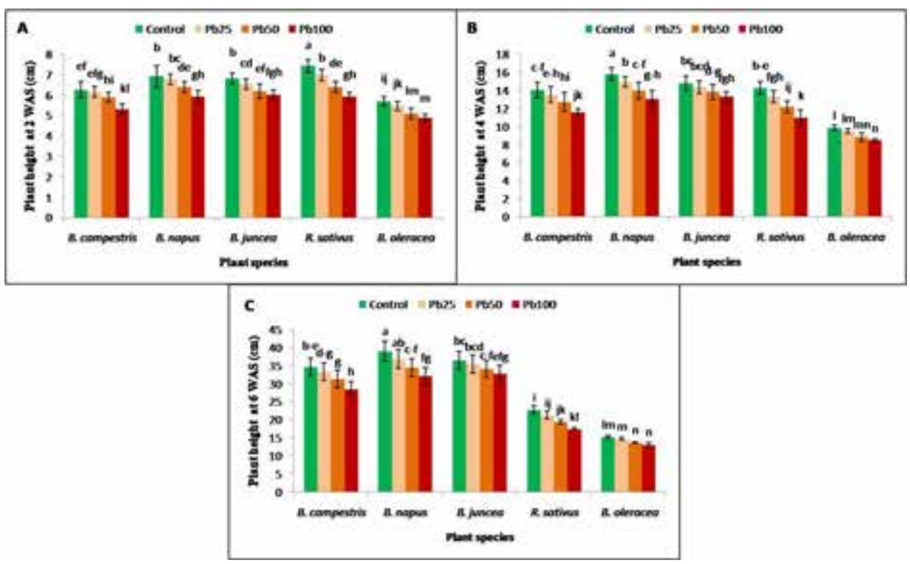

Fig. 1. Plant height (cm) of different *Brassica* species under Pb stress at 2 (A), 4 (B) and 6 (C) WAS (weeks after sowing). Pb25, Pb50 and Pb100 indicate 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ respectively. Bars (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

The maximum plant fresh weight was observed in *B. oleracea* in all cases (2.7, 6.9 and 23.8 g at 2, 4 and 6 WAS, respectively) whereas minimum plant fresh weight was observed in *B. campestris* (1.9, 4.8 and 13.8 g at 2, 4 and 6 WAS, respectively, Fig. 2). Reduction of fresh weight of tested species was recorded by 20.7, 23.5, 17.6, 21.2 and 25.9% at 2 WAS; 15.8, 23.9, 10.0, 21.9 and 23.0% at 4 WAS; 18.2, 19.5, 10.0, 20.3 and 21.5% at 6 WAS under 1.0 mM Pb $(NO_3)_2$ stress in *B. campestris, B. napus, B. juncea, R. sativus and B. oleracea* respectively, in comparison with control seedlings (Fig. 2). Therefore, above data demonstrate that in all date the least fresh weight reduction was observed in *B. juncea* and highest reduction was observed in *B. oleracea* (Fig. 2). Same as fresh weight the maximum plant dry weight was observed in *B. oleracea* in all cases (0.27, 0.70 and 2.40 g at 2, 4 and 6 WAS respectively) whereas minimum plant dry weight was observed in *B. campestris* (0.20, 0.47 and 1.40 g at 2, 4 and 6 WAS respectively, Fig. 3). In contrast to control seedlings dry weight declined by 17.3, 18.9, 11.3, 21.7 and 23.4% at 2 WAS; 13.5, 19.3, 8.6, 20.6 and 18.5% at 4 WAS; 13.6, 15.3, 5.5, 17.6 and 18.8% at 6 WAS under 1.0 mM Pb $(NO_3)_2$ stress in *B. campestris, B. napus, B. juncea, R. sativus and B.*

oleracea respectively (Fig. 3). So, from the data it is obvious that in each every observation date the minimum reduction of plant height was observed in *B. juncea* and maximum reduction was observed in *B. oleracea* (Fig. 3).

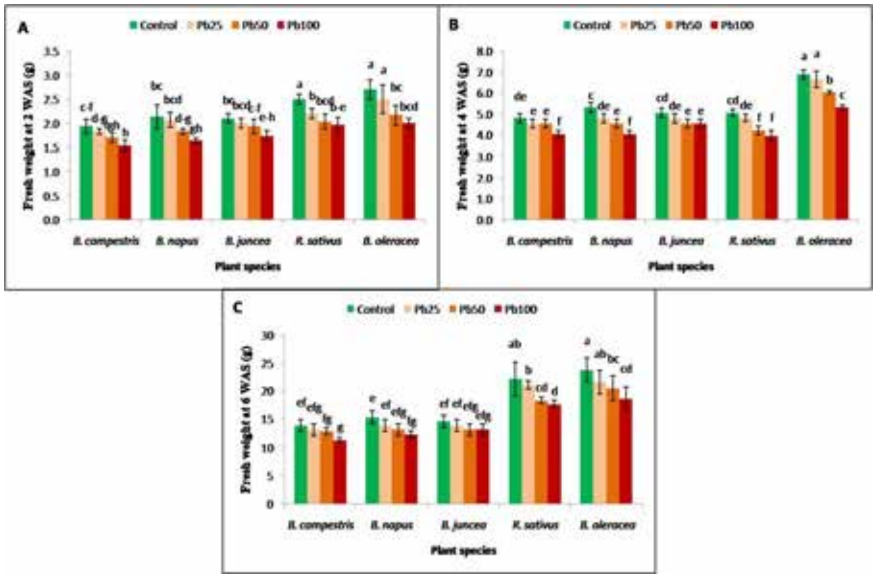

Fig. 2. Fresh weight (g) of different *Brassica* species under Pb stress at 2 (A), 4 (B) and 6 (C) WAS (weeks after sowing). Pb25, Pb50 and Pb100 indicate 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ respectively. Bars (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

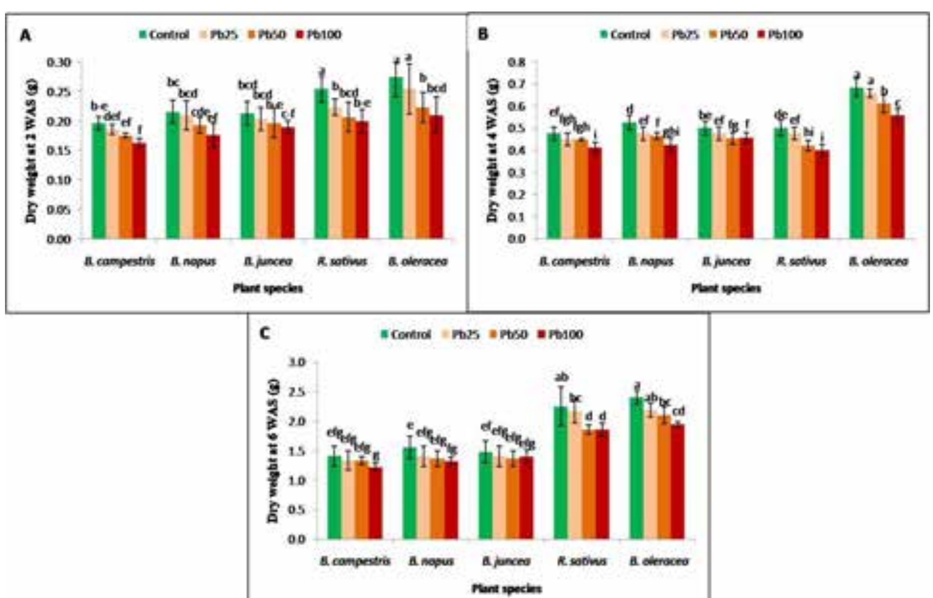

Fig. 3. Dry weight (g) of different *Brassica* species under Pb stress at 2 (A), 4 (B) and 6 (C) WAS (weeks after sowing). Pb25, Pb50 and Pb100 indicate 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ respectively. Bars (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

Chlorophyll content

Lead stress diminished the photosynthetic pigments content as indicated by SPAD values in a dose-dependent manner. The maximum SPAD value was found in *B. juncea* (61.3) *and B. oleracea* (61.3) whereas minimum was observed in B. *campestris* (57.3, Fig. 4). Under 0.25 mM Pb $(NO_3)_2$, the SPAD value reduction in all species was slight except *R. sativus.* However, 0.5 and 1 mM Pb $(NO_3)_2$ stress substantially decreased the SPAD value (Fig. 4). Compared to control seedlings reduction of SPAD value of tested species was recorded by 8.0, 9.1, 10.3, 11.8 and 9.8% under 0.5 mM $Pb(NO_3)_2$ and 18.5, 17.7, 13.5, 21.2 and 19.6% under 1.0 mM $Pb(NO_3)_2$ stress in *B. campestris, B. napus, B. juncea, R. sativus and B. oleracea* respectively. Therefore, above findings reveal that in all date the least SPAD value reduction was observed in *B. juncea* and maximum decline was observed in *R. sativus* (Fig. 4).

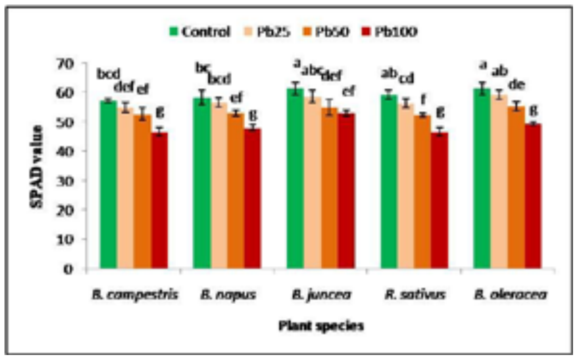

Fig. 4. SPAD value of different *Brassica* species under Pb stress at 6 WAS (weeks after sowing). Pb25, Pb50 and Pb100 indicate 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ respectively. Bars (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

Leaf relative water content (%)

The highest leaf relative water content was found in *B. campestris* (91.7%) which is statistically similar with *B. napus, B. juncea and B. oleracea* while minimum leaf relative water content was observed in *B. sativus* (89.3%; Fig. 5). Leaf relative water content decreased by dose-dependent manner where maximum reduction was recorded under 1.0 mM Pb $(NO_3)_2$. In contrast to control reduction of leaf relative water content was 8.7, 5.9, 6.2, 6.7 and 8.0% under 1.0 mM $Pb(NO_3)_2$ stress in *B. campestris, B. napus, B. juncea, R. sativus and B. oleracea* respectively, at 6 WAS. Maximum drop of leaf relative content was recorded in *B. oleracea* and minimum in *B. juncea* (Fig. 5).

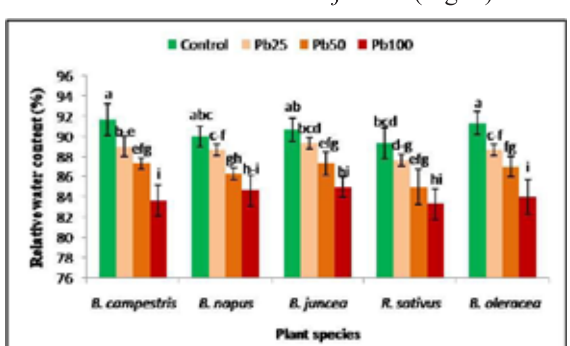

Fig. 5. Leaf relative water content value of different *Brassica* species under Pb stress at 6 WAS (weeks after sowing). Pb25, Pb50 and Pb100 indicate 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ respectively. Bars (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

Lead accumulation in the shoots and roots

All *Brassica* species accumulated Pb in their shoots and roots as a result of Pb exposure, and the accumulation increased with the increase in stress level (Fig. 6). Lead accumulation was higher in the roots than in the shoots in all species. *Brassica juncea* accumulated more Pb in its shoots and roots (3.0, 5.2 and 9.6 mg g^{-1} dry weight in shoots and 6.8, 11.6 and 21.4 mg g^{-1} dry weight in roots under 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ stress respectively) than *B. campestris, B. napus, R. sativus and B. oleracea* (Fig. 6). Under 0.25 mM $Pb(NO_3)_2$, the shoots of *B. juncea* accumulated 36.4, 25.0, 66.7 and 42.9% higher Pb than *B. campestris*, *B. napus*, *R. sativus and B. oleracea* respectively, whereas under 0.5 mM $Pb(NO_3)_2$ stress, *B. juncea* accumulated 44.4, 40.5, 73.3 and 44.4% higher Pb than *B. campestris*, *B. napus*, *R. sativus and B. oleracea* respectively, and under 1.0 mM $Pb(NO_3)_2$ stress, the shoots of *B. juncea* accumulated 45.5, 28.0, 68.4 and 47.7% higher Pb than *B. campestris*, *B. napus*, *R. sativus and B. oleracea*, respectively (Fig. 6). Similarly, under 0.25 mM $Pb(NO_3)_2$ stress, the roots of *B. juncea* accumulated 38.2, 27.7, 72.3 and 31.0% higher Pb than *B. campestris*, *B. napus*, *R. sativus and B. oleracea* respectively, whereas under 0.5 mM $Pb(NO_3)_2$ stress, *B. juncea* accumulated 43.2, 40.3, 75.0 and 43.7% higher Pb than *B. campestris*, *B. napus*, *R. sativus and B. oleracea* respectively, and under 1.0 mM $Pb(NO_3)_2$ stress, the shoots of *B. juncea* accumulated 44.9, 28.4, 67.2 and 48.6% higher Pb than *B. campestris*, *B. napus*, *R. sativus and B. oleracea* respectively (Fig. 6).

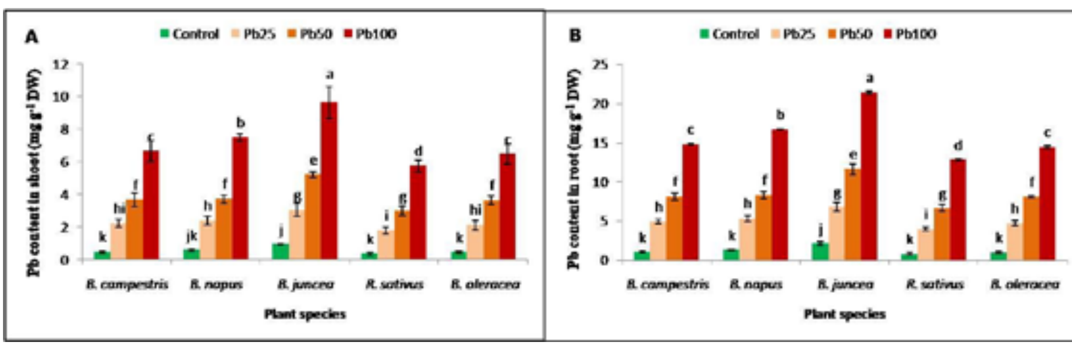

Fig. 6. Pb content in shoot (A) and root (B) of different *Brassica* species under Pb stress at 6 WAS (weeks after sowing). Pb25, Pb50 and Pb100 indicate 0.25 mM, 0.5 mM and 1 mM $Pb(NO_3)_2$ respectively. DW means dry weight. Means (±SD) were calculated from three replications for each treatment. Bars with different letters are significantly different at $P \leq 0.05$ applying Fisher's LSD test.

DISCUSSION

It is well recognized that plant genotypes show difference in accumulation and tolerance against heavy metal contamination (Gill *et al*., 2011; Ansari *et al*., 2015; Irfan *et al*., 2014). Member of Brassicaceae family are recognized as hyperaccumulators of heavy metal, but their metal accumulation capacities and tolerance ability are not similar (Prasad and Freitas, 2003; Mahmud *et al*., 2017). Lead stress reduces the growth, development, and productivity of plants by disrupting different physiological processes (Hasanuzzaman *et al*., 2019; Soares *et al*., 2020), which were reflected in the findings of our study. In the present study, plant height, fresh and dry weight of all five *Brassica* species decreased significantly under Pb stress in a dose-dependent manner except few cases. The lower reduction of above mentioned parameters were observed in *B. juncea* than the other tested species. Therefore, in terms of growth, *B. juncea* was more tolerant to Pb stress. *Brassica juncea* also proved its higher accumulation capacity and tolerance ability than *B. campestris* and *B. napus* under Cd stress (Mahmud *et al*., 2017). Growth reduction in response to Pb stress has also been reported in *B. campestris* (Hasanuzzaman *et al*., 2019), *B. juncea* (Soares *et al*., 2020) and in response to Cd in mustard (Iqbal *et al*., 2010), mung bean (Nahar *et al*., 2016) and rice (Rahman *et al*., 2016) and for various *Brassica* species after exposure to excess Zn or Cu (Ebbs and Kochian, 1997).

Different heavy metal toxicity reduce leaf relative water content in *B. campestris* (Hasanuzzaman *et al*., 2019), wheat (Hasanuzzaman and Fujita, 2013), mung bean (Nahar *et al*., 2016) and rice (Rahman *et al*., 2016). For tracing the tolerance level of plants under any stress, leaf relative water content is very important factors (Mahmud *et al*., 2017). In our study, leaf leaf relative water content decreased in all five *Brassica* seedlings with the increase in stress level, but the reduction of leaf leaf relative water content was lower in *B. juncea*, suggesting that *B. juncea* is more tolerant to Pb stress than the other four species.

Chlorophyll content declines due to inhibition of protochlorophyllide reduction and aminolevulinic acid synthesis (Stobart *et al*., 1985). Heavy metal stress adversely affect photosynthesis by altering the photosystem II (Baszynski, 1986), decreasing amount of plastoquinone in the chloroplast (Krupa *et al*., 1992) and unsettling the calvin cycle (Weigel, 1985). In the present study, a significant reduction in the SPAD value (indicate photosynthetic pigments) was noticed in the leaves of all the five *Brassica* species, but the rate of reduction was lower in *B. juncea*. A similar result was found by Nouairi *et al*. (2006) and Mahmud *et al*. (2017) in which the total chl content reduction of *B. juncea* was significantly lower than *B. napus* or *B. campestris* under Cd toxicity.

Plants grown in heavy metal-contaminated medium showed higher metal accumulation in the roots than the shoots (Srivastava *et al*., 2014; Ahmad *et al*., 2015; Nahar *et al*., 2016; Rahman *et al*., 2016). *Brassica juncea* is extensively studied plant famous for its hyperaccumulation capacity to various heavy metals like Cd, Cr, Cu, Ni etc (Naaz and Chauhan, 2019). Nouairi *et al*. (2006 and 2009) and Mahmud *et al*. (2017) observed B. *juncea* possesses a greater potential for Cd accumulation than *B. napus* under Cd toxicity. They reported that levels of non-protein thiols and phytochelatins increased greatly in leaves of *B. juncea* by increasing Cd supply, but no change was observed in *B. napus*. In our study, Pb exposure resulted in the accumulation of Pb in the root and shoot tissues of all tested seedlings, with the highest accumulation in *B. juncea*. The root tissues accumulated higher Cd content than the shoot tissues of all *Brassica* species.

CONCLUSION

The findings of our study illustrate that Pb exposure to different *Brassica* species resulted in differences in Pb accumulation. Due to Pb toxicity growth and physiological performances of all *Brassica* species hampered seriously. Plant height and biomass reduction due to Pb accumulation in roots and shoots was observed in all five *Brassica* species with increasing Pb concentration in the growing medium, but the lowest decrease in plant height, fresh weight and dry weight was observed in *B. juncea* even though it had the highest level of Pd of the tested species. Lead treatment induced a reduction in leaf water status, and chlorophyll content which consequently inhibits photosynthesis, resulting in reduced plant height and growth. Chlorophyll degradation and leaf RWC reduction were lower in *B. juncea* even though it accumulated the highest level of Pb in its roots and shoots. Considering Pb accumulation capacity, growth and physiological attributes, we might conclude that among the studied member of Brassicaceae family, *B. juncea* is a hyper-accumulator and relatively tolerant species to Pb toxicity.

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