Asian Australas. J. Biosci. Biotechnol. 2019, 4 (2), 80-87

Asian-Australasian Journal of Bioscience and Biotechnology
ISSN 2414-1283 (Print) 2414-6293 (Online)
www.ebupress.com/journal/aajbb

Article

Phytoremediation potential of mustard (*Brassica juncea*) varieties exposed to lead stress

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Received: 18 July 2019/Accepted: 20 August 2019/ Published: 31 August 2019

Abstract: While the growth of most plants is severely restricted by the toxic effects lead (Pb), some (plants) can cope with the heavy metal stress. These hyperaccumulators are used to extract (lead) from contaminated soil in a process called phytoremediation. Although some species of Brassica are widely being used as hyperaccumulators, the phytoremediation potential of many varieties of *Brassica juncea* (mustard) is not well understood. The present study was conducted to assess the phytoremediation potentials of 11 mustard genotypes under Pb stress. Twenty-day-old seedlings were exposed to 200 µM Pb nitrate under hydroponic conditions and grown for 60 days. The experiment was conducted following a completely randomized design with three replications. In response to Pb stress, a significant reduction in growth of the studied traits was observed in all of the varieties. The lowest reduction for all of the studied traits including Pb accumulation was recorded in BJ DH 17. Nevertheless, the translocation of Pb from root to shoot and shoot to grain was highest in Sambal. Bioaccumulation co-efficient was highest in Sambal whereas it was lowest in BJ DH 17. Based on the results of the present study, the variety Sambal is recognized as the most suitable genotype that can be used for Pb phytoextraction.

Keywords: *B. juncea*; bioaccumulation; lead; phytoextraction; root; shoot

1. Introduction

The pollution of the biosphere with toxic metal or metalloids poses a serious threat to human and animal health. Heavy metal (HM) concentration is rising in the soil environment due to various natural sources such as forest fires, wind-blown dust, decaying vegetation and sea spray, as well as due to certain anthropogenic activities like use of phosphate fertilizer, metal production, mining and wood production (Mirzaei *et al*., 2014). Among metal contaminants, lead (Pb) is one of the major concerns because of its extensive distribution in the soil environment (Yang *et al.*, 2016), representing a risk of bio-accumulation through the food chain. Pb contamination of agricultural lands is also in part responsible for limiting the crop productivity. High level of Pb restricts plant root and shoot growth and development through the impairment of biomass production by preventing the synthesis of chlorophyll (Malar *et al.*, 2016) alteration of water balance and restricting the absorbance of plant nutrients (Nagajyoti *et al.*, 2010), prolongation of the cell cycle (Wierzbicka, 1994), induction of a lot of metabolic disturbances (Lamhamdi *et al.*, 2011) and through permanent damage of DNA (Pourrut *et al*., 2011). However, there are some plant species which can act as hyperaccumulators due to different defensive strategies against lead toxicity (Gupta *et al.*, 2013). These hyperaccumulators, grow on contaminated soils and are capable of accumulating huge amounts of HM in their tissues, without obvious toxicity symptoms. Their vigorous growth and high biomass production are capable to remove a considerable amount of Pb and other toxic elements from the soil which provide a promising future in ecological restoration of mine area and remediation of polluted soil (Mourato *et al.,* 2015). These plant species survive, grow and reproduce in environments polluted with heavy metals (HMs), indicating that they developed certain mechanisms to cope with such adverse environmental conditions. The use of such hyperaccumulators for the cleanup of a contaminated environment is called phytoremediation. However, phytoremediation, the technique of using the plant species to reduce toxicity from soil, is a cost-effective and ecofriendly approach of environmental cleanup.

In some species, application of hyperaccumulators is limited by their slow growth rate, small biomass and long period of time, required for cleaning up HMs from soil. For phytoremediation, it requires plants that maintain good fitness on contaminated soils in parallel with the highest possible accumulation of pollutants in aerial parts. Countless studies in various scientific disciplines, dealing with different plant species in different conditions, have focused on HM phytoremediation. Importantly, in Brassicaceae family, different species of *Brassica* have been considered as one of the most promising oil seed crop which can be used as a hyperaccumulator due to its phytoextraction potential of different metals or metalloids (Goswami and Das, 2015, Diwan *et al.,* 2008, Purakayastha *et al.,* 2008, Bryson and Barker, 2007, Mourato *et al.,* 2015). Phytoremediation potential varied among the species, varieties and even genotypes (Baker, 2008, Mourato *et al.,* 2015). Same species also have different level of phytoremediation potential to different HM toxicity. Although, some of the *Brassica* species are reported to be suitable for this environmentally attractive technique, however, its full potential has yet to be met. Therefore, it is important to assess the different germplasm of geographical origin under various environmental conditions to reveal novel insights or to find the suitable ecotypes that can be used for phytoremediation. *B. juncea* is one of the most potential hyperaccumulators and widely regarded as a good plant for phytoremediation purposes (Mourato *et al.*, 2015, Goswami and Das, 2015). For these reasons, the present study was conducted to assess the phytoremediation potentiality of 5 commercially cultivated verity and 6 advance line of *Brassica juncea* grown under Pb stress to identify the genotypes, suitable for phytoremediation program, through the analysis of plant growth and yield-attributing traits including phytoextraction capacity.

2. Materials and Methods

2.1. Plant materials

The study was carried out in the growth chamber of the Department of Genetics and Plant Breeding in Bangladesh Agricultural University, Mymensingh, during the period from July, 2014 to June, 2015. Eleven mustard (*Brassica juncea*) genotypes, (5 varieties (Sambal, BARI Sarisha 10, BARI Sarisha 11, BARI Sarisha 16 and Daulat) and 6 advanced lines (BJDH 05, BJDH 12, BJDH 17, BJDH 20, BJ 1111536, RAI 5)) were used as plant materials. One of them (Sambal) was collected from the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh and the other 10 (BJDH 05, BJDH 12, BJDH 17, BJDH 20, BJ 1111536, RAI 5, BARI Sarisha 10, BARI Sarisha 11, BARI Sarisha 16 and Daulat) were collected from Regional Agricultural Research Station, Bangladesh Agricultural Research Institute, Jamalpur.

2.2. Experimental design and stress treatments

The experiment was conducted following a completely randomized design (CRD**)** with three replications. Seeds of the selected genotypes were surface sterilized with 0.1 % $HgCl_2$ and grown on plastic pots filled with sterilized sand and Hoagland Solution (HS) (Hoagland and Arnon, 1950) under control conditions in a growth chamber (50-54 PPFD light, 65-70 % relative humidity, 25 °C). After 20 days of seedling growth, one group of equal size seedlings was subjected to Pb stress (200 µM lead nitrate ($Pb(NO_3)_2$) in HS and grown under same control conditions. Control seedlings were grown only in nutrient solution under the same experimental conditions. Seedlings were placed carefully so that roots were in touch with HS. Oxygen supply to the root zone was ensured by putting a bubbler into the solution. The pH of the nutrient solution was 5.6. The nutrient solution was changed when it turned into opaque. At the ripening stage, the temperature of the growth chamber was increased to 30 °C.

2.3. Data collection

Data on plant height, leaf number and leaf greenness were recorded after 20 days of Pb stress treatment whereas the data on root length, root and shoot dry weight, number of pods per plant, and seed yield per plant were recorded after 60 days of Pb stress treatment. Leaf greenness was determined at the first 2-3 leafs of randomly selected plants at different heights by comparing to a leaf color chart. Pb contents in the root, shoot and seeds were determined following the method described by Ramesar *et al.* (2014).

Translocation (TF) of Pb from root to shoot and shoot to root was measured by the following equation:

$$TF = \frac{\text{Concentration of metals on shoot}}{\text{Concentration of metals on root}}$$

Bioaccumulation coefficient (BC) of Pb was measured by the following equation:

$$BC = \frac{\text{Pb content in per gram dry plant tissue}}{\text{Pb content in per ml nutrient solutions}}$$

2.4. Statistical analysis

The data of the experiment were analyzed by suing MSTATC and MINITAB software package program. The comparison of different treatment means was made by DMAR at 5% level of probability.

3. Results and Discussion

3.1. Impact of lead on morphological traits

Roots have the ability to take up considerable amounts of Pb which creates an adverse cell environment and impairs enzymatic activities (Sharma and Dubey, 2005). It has been reported that inorganic salts of lead (chlorides and nitrates) induces numerous c-mitoses together with strong inhibition of root growth and lowering of mitotic activity (Patra *et al.,* 2004). At higher concentration of Pb, shoot and root growth is inhibited due to prolongation of the cell cycle (Wierzbicka, 1994), subsequently, the mitotic activity of meristematic tissue in root and shoot is reduced which results in the decrease in the length of root and shoot (Sen *et al.*, 2013, Heidari and Sarani, 2011). In our present study a similar result was observed. A significant reduction in the leaf number and length of root and shoot was observed after Pb treatment (Table 1). The lowest length of shoot and root was found in Sambal and Daulat (Table 2) compared to other varieties. Probably Pb was more rapidly absorbed in these two varieties compared to the other varieties and showed a maximum difference in root and shoot length, supported by Sharma and Dubey (2005).

The photosynthetic activity is also adversely affected by Pb toxicity due to inhibition of chlorophyll synthesis. The absorbed Pb binds to the cell wall of root and restrikes the entry of different essential cations (Burzynski *et al.,* 1987) which are required for plastid and chlorophyll synthesis process. At the same time, HM uptakes stimulates chlorophyllase activity thereby enhancing the degradation process of chlorophyll (Drazkiewicz, 1994) and results in the destruction of chloroplast ultrastructure (Sharma and Dubey, 2005). Therefore, in this study, leaf greenness was significantly reduced by Pb treatment (Table 1). Moreover, Pb induces accumulation of reactive oxygen species (ROS). A rather common consequence of heavy metal poisoning is the enhanced production of reactive oxygen species (ROS) due to interference with electron transport activities, especially that of chloroplast membranes which may interfere in chlorophyll synthesis. In addition, the restricted cell cycle results in the significant reduction of leaf and pod number, yield, shoot dry weight and root dry weight (Table 1). It was observed that all traits were significantly different among genotypes regarding mean performance after Pb treatment. BJ DH 17 was identified as superior genotype in respect to all traits recorded in this study (Table 2). Conversely, 'Sambal', was noticed as poor-performing genotype in respect to all of the traits studied.

Plant height, leaf number, root length, pod number, yield per plant, shoot dry weight and root dry weight exhibited highly significant differences due to Pb-variety interactions (Table 3). 'BJDJ 17' was identified as the best genotype in respect to highest plant height, leaf number, leaf greenness, root length, pods number and yield. Highest shoot dry weight and root dry weight were noticed in 'BJDH 12' in Pb-variety interactions. 'Sambal' was found as poor-performing genotype due to having lowest plant height, leaf greenness, root length, pod number and yield. In case of 'Daulat', the leaf number, shoot dry weight and root dry weight were lowest. Reduction values of all traits after Pb treatment were highest in 'Daulat' and 'Sambal' genotypes. In contrast, it was lowest in 'BJDH 17' (Figure 1A and B).

3.2. Lead concentration in roots, shoots and grains

The root of *B. juncea* has the ability to take up considerable amounts of HM (Anamika et al. 2009). Once HMs are absorbed, they can be accumulated in the roots or be exported to the shoots, and even to the seeds via the transpiration stream (Ximénez et al. 2001, Palizban et al. 2015). However, Pb is greatly restricted in its translocation from root towards above ground parts. Plant species and cultivars differ widely in their efficiency to absorb, transport and accumulate the Pb in different tissues. It was reported that, *B. juncea*, can accumulate significant amounts of Pb in both siliques and seeds (Ramesar et al. 2014). It was always observed that most of the Pb, absorbed by the root system, is sequestered in the roots and less was available for translocation to the shoot tissues (Ramesar et al. 2014). Because, one reason can be that Pb has an affinity to bind strongly to the carboxyl group of polysaccharides in the cell wall (Krzesłowska 2011). The unbound Pb moves from the root to other parts of the plant. Therefore, our results of the Pb concentration in roots > shoots > grains (Figure 2A and B) was consistent with the result of Ramesar et al. (2014), in *B. juncea*.

It was reported that, *B. juncea*, can accumulate significant amounts of Pb in both siliques and seeds. However, plant species and cultivar differ widely in their efficiency to absorb, transport and accumulate the Pb in different tissues (Ramesar et al. 2014). Pb accumulation in the dry mass of roots, shoots and grains of *B juncea* cultivated in hydroponics are shown in Figure 2. Daulat was identified as superior genotype in performance of Pb accumulation in roots, followed by Sambal (Figure 2A). Both of these varieties accumulated the highest content of Pb in the shoot and grain compared to other genotypes (Figure 2A and B). However, there was no remarkable difference between Sambal and Daulat in these cases. In contrast, BJ DH 17 was selected as inferior in performance of lead accumulation in roots, shoot and grains followed by BARI Sarisha 11.

In context of phytoremediation, Sambal was identified as the best genotype among all in performance of translocation factor ($TF_{root\ to\ shoot}$) followed by Daulat. In context of TF of shoot to grain, Sambal' was identified as the best genotype in performance followed by Daulat. However, Sambal and Daulat, were identified as statistically identical. Conversely, BARI Sarisha played poor performance in $TF_{shoot\ to\ grain}$ followed by BJ DH 12. In conclusion, BARI Sarisha 11 was identified as best genotype for its low $TF_{shoots\ to\ grain}$ (Figure 3B). A previous study reported that TF>1 indicates that translocation of metals is made effectively to the shoot from root (Ng et al. 2016). Here, no genotypes showed its $TF_{root\ to\ shoot}$ and $TF_{shoot\ to\ grain}$ was more than 1. So, translocation of metals was not efficient in those genotypes.

A plant's ability to accumulate metals from the medium can be estimated using the bioaccumulation coefficient (BC), which was defined as the ratio of metal concentration in the plant to that in medium. Sambal was identified as the best genotype among all in respect to BC followed by Daulat. However, in this case also Sambal and Daulat were identified as statistically identical. In contrast, BJ DH 17 was found as poor performer in BC followed by BARI Sarisha 11 (Figure 3C).

In conclusion, the possibility of using a crop as a phytoremediants depends on the efficacy of accumulation and distribution of metals among their above ground morphological organs. In respect of Pb concentration in shoots, Sambal had the best genotype among all followed by Daulat. Sambal was also identified as the best genotype in performance of Pb accumulation in grains followed by Daulat. Although BJ DH 17 was identified as superior genotype in respect to all morphological traits, it was owned not suitable for lead accumulation in above ground parts of the plant. Therefore, Sambal can be use for removing the Pd contaminated toxic soil clean up.

Table 1. Mean effect of Pb on plant morphological characteristics of 10 cultivars during vegetative and reproductive growth after 20 and 60 days of treatment.

	20 days after Pb treatment			60 days after Pb treatment				
Treatment	**Plant height (cm)**	**Leaf number**	**Leaf greenness**	**Root length (cm)**	**Pod number**	**Yield (g)**	**Shoot dry weight (g)**	**Root dry weight (g)**
Control	42.51[a]	23.75[a]	3.69[a]	27.63[a]	23.21[a]	1.06[a]	7.50[a]	4.61[a]
Pb treatment	30.24[b]	19.18[b]	1.90[b]	22.27[b]	16.21[b]	0.81[b]	6.36[b]	3.88[b]
Reduction (%)	28.90	19.30	48.40	19.40	30.20	22.90	15.10	15.70

Different letters within the column indicate statistical differences between the treatments (DMRT, $p<0.05$, n=3). For greenness scale, 0-1= yellow, 1-2= light green, 2-3= green and 3-4=dark green.

Table 2. Varietal responses of mustard genotypes in terms of morphological parameters after Pb treatment.

Variety	Plant height (cm)	Leaf number	Leaf greenness	Root length (cm)	Pod number	Yield (g)/plant	Shoot dry weight (g)	Root dry weight (g)
BJ DH 5	33.50^{g}	20.67^{f}	2.83^{b-e}	23.17^{e}	19.17^{e}	0.91^{d}	6.84^{g}	3.93^{g}
BJ DH 12	41.17^{d}	24.67^{c}	3.17^{ab}	26.33^{c}	20.6^{d}	0.98^{c}	7.44^{d}	4.80^{d}
BJ DH 17	49.83^{a}	27.00^{a}	3.50^{a}	33.50^{a}	23^{a}	1.09^{a}	7.80^{a}	5.46^{a}
BJ DH 20	43.83^{c}	25.50^{b}	3.17^{a-c}	30.33^{b}	21.67^{c}	1.03^{b}	7.51^{b}	5.12^{b}
BJ 1111536	35.50^{f}	21.83^{e}	2.68^{c-f}	23.33^{e}	20.17^{d}	0.96^{c}	7.22^{f}	4.38^{f}
RAI 5	32.50^{h}	20.50^{f}	2.35^{gh}	21.17^{f}	19.17^{e}	0.92^{d}	6.83^{g}	3.65^{h}
BARI Sarisha 10	29.50^{i}	17.50^{g}	2.33^{h}	21.17^{f}	19.00^{e}	0.9^{d}	6.56^{h}	3.60^{h}
BARI Sarisha 11	44.67^{b}	26.67^{a}	3.17^{a-d}	33.17^{a}	22.67^{b}	1.08^{a}	7.50^{c}	4.96^{c}
BARI Sarisha 16	36.83^{e}	22.83^{d}	2.67^{d-g}	24.83^{d}	18.83^{e}	0.89^{d}	7.37^{e}	4.59^{e}
Sambal	25.67^{k}	15.50^{h}	2.52^{e-h}	18.17^{h}	15.83^{g}	0.78^{f}	5.40^{j}	3.12^{i}
Daulat	27.17^{k}	13.50i	2.50^{f-h}	19.33^{g}	16.67^{f}	0.82^{e}	5.82^{i}	3.14^{i}

Different letters within the column indicate statistical differences among the cultivars (DMRT, $p<0.05$). For greenness scale, 0-1= Yellow, 1-2= light green, 2-3= green and 3-4= very green.

Table 3. Mean Performance of different varieties for different plant characters in treatment (Pb)-variety interactions.

Treatment	Variety	Plant height (cm)	Leaf number	Leaf greenness	Root length (cm)	Pod number	Yield (g)	Shoot dry weight (g)	Root dry weight (g)
1	**BJ DH 5**	39.67^{g}	23.00^{f}	4.00^{a}	25.67^{f}	22.67^{e}	1.03^{de}	7.77^{e}	5.11^{e}
2	**BJ DH 5**	27.33^{m}	18.33^{i}	1.67^{de}	20.67^{ij}	15.67^{k}	0.78^{k}	7.38^{h}	4.31^{k}
1	**BJ DH 12**	46.67^{c}	26.33^{bc}	4.00^{a}	29.00^{d}	24.33^{bc}	1.12^{c}	8.08^{a}	5.66^{a}
2	**BJ DH 12**	35.67^{i}	23.00^{f}	2.33^{cd}	23.67^{g}	17.00^{j}	0.85^{j}	7.85^{b}	5.33^{b}
1	**BJ DH 17**	52.67^{a}	28.00^{a}	3.67^{a}	35.33^{a}	25.33^{ab}	1.17^{ab}	7.69^{f}	4.75^{h}
2	**BJ DH 17**	47.00^{c}	26.00^{bc}	3.33^{ab}	31.67^{bc}	20.67^{gh}	1.02^{d-f}	7.38^{i}	4.08^{m}
1	**BJ DH 20**	48.33^{b}	26.67^{b}	3.67^{a}	32.33^{b}	24.67^{a-c}	1.13^{bc}	7.82^{d}	5.22^{d}
2	**BJ DH 20**	39.33^{g}	24.33^{d}	2.67^{bc}	28.33^{de}	18.67^{i}	0.93^{hi}	7.18^{j}	3.91^{o}
1	**BJ 1111536**	42.33^{e}	24^{de}	3.67^{a}	26.33^{f}	23.00^{de}	1.05^{d}	7.85^{c}	4.92^{f}
2	**BJ 1111536**	28.67^{l}	19.67^{h}	1.67^{de}	20.33^{j}	17.33^{j}	0.87^{j}	6.47^{q}	3.62^{q}
1	**RAI 5**	39.67^{g}	23.33^{e}	3.33^{ab}	24.33^{g}	24.00^{cd}	1.12^{c}	7.03^{m}	3.80^{p}
2	**RAI 5**	25.33^{n}	17.67^{i}	1.33^{e}	18.00^{k}	14.33^{l}	0.72^{l}	6.29^{r}	3.54^{r}
1	**BARI Sarisha 10**	37.67^{h}	20.67^{g}	3.33^{ab}	23.67^{g}	22.33^{e}	1.02^{d-f}	7.53^{g}	5.25^{c}
2	**BARI Sarisha 10**	21.33^{o}	14.33^{j}	1.33^{e}	18.67^{k}	15.67^{k}	0.78^{k}	7.10^{m}	4.50^{j}
1	**BARI Sarisha 11**	48.33^{b}	27.67^{a}	4.00^{a}	35.33^{a}	25.67^{a}	1.18^{a}	7.18^{k}	4.91^{fg}
2	**BARI Sarisha 11**	41.00^{f}	25.67^{c}	2.33^{cd}	31.00^{c}	19.67^{hi}	0.98^{e-g}	6.74^{p}	4.02^{n}
1	**BARI Sarisha 16**	43.33^{d}	24.67^{d}	3.67^{a}	28.00^{e}	22.00^{ef}	1.00^{e-g}	6.27^{s}	3.29^{s}
2	**BARI Sarisha 16**	30.33^{k}	21.00^{g}	1.67^{de}	21.67^{gh}	15.67^{k}	0.78^{k}	5.94^{t}	3.22^{t}
1	**Sambal**	33.33^{j}	19.33^{h}	3.67^{a}	21.33^{hi}	20.33^{gh}	0.92^{i}	7.17^{l}	4.70^{i}
2	**Sambal**	18.00^{p}	11.67^{k}	1.33^{e}	15.00^{m}	11.33^{m}	0.63^{m}	6.88^{o}	4.26^{l}
1	**Daulat**	35.67^{i}	17.67^{i}	3.67^{a}	22.67^{g}	21.00^{fg}	0.97^{gh}	4.61^{u}	2.61^{u}
2	**Daulat**	18.67^{p}	9.33^{l}	1.33^{e}	16.00^{l}	12.33^{m}	0.67^{m}	4.32^{v}	2.48^{v}

Data shown are means of 11 genotypes. Different letters within the column indicate statistical differences among the treatments and cultivars (DMRT, $p<0.05$). 1 and 2 indicate control and Pb treatment, respectively.

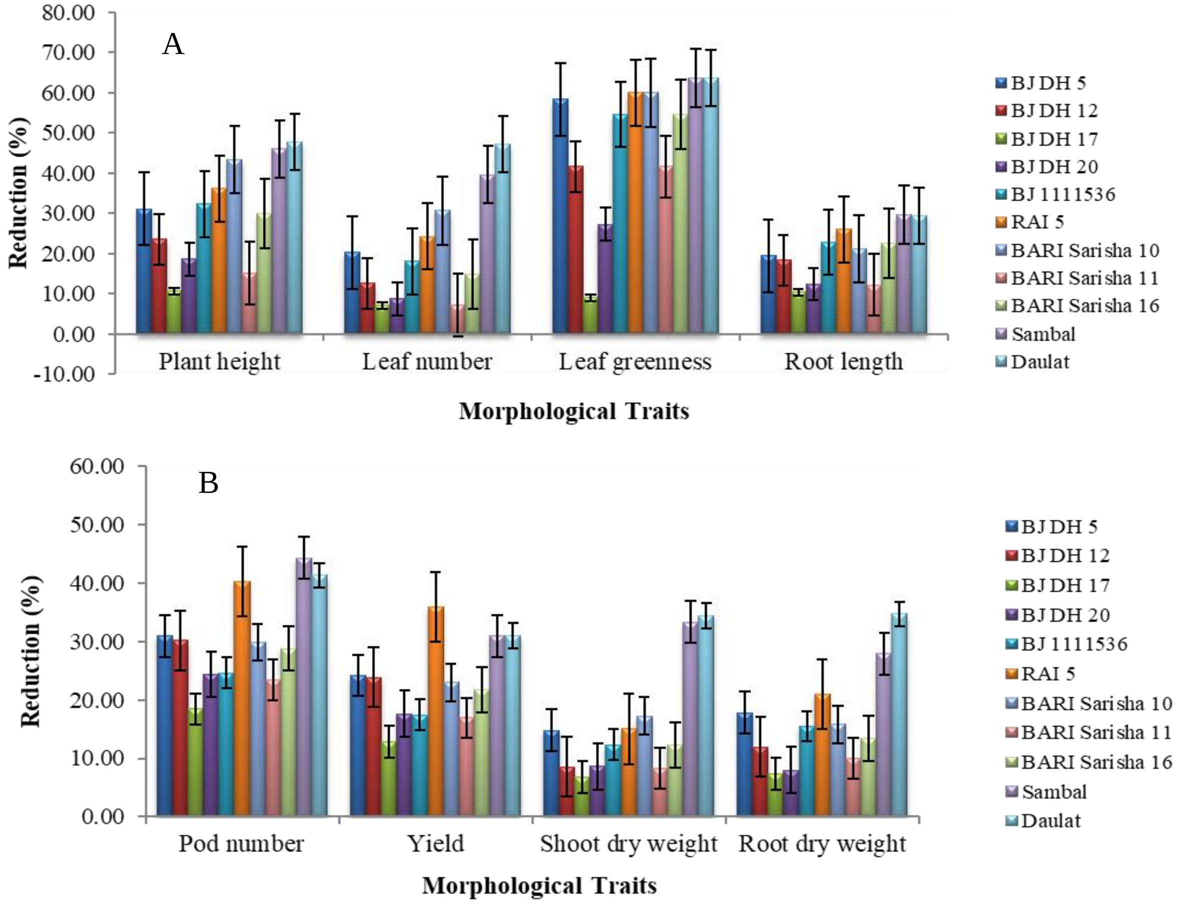

Figure 1. Reduction percentage of different morphological characters due to treatment (Pb)-variety interactions. (A) for plant height (cm), leaf number, leaf greenness, root length (cm). (B) for pod number, yield (g), shoot dry wetght (g), root dry weight (g). Vertical lines in the bars indicates standard error.

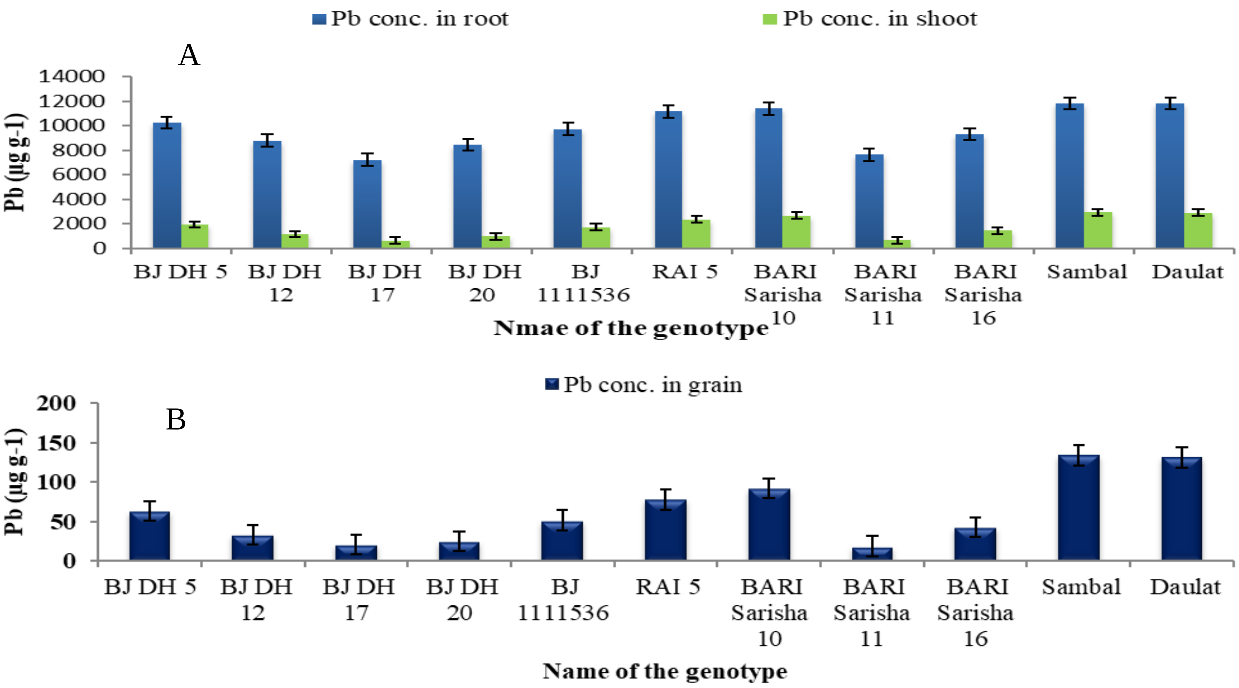

Figure 2. Accumulation of lead in the dry biomass of root, shoot and grain of 11 *Brassica juncea* varieties after 60 days of Pb treatment (200ppm). Bar data in the graph indicates (A) mean Pb content in root and shoot, (B) mean Pb content in grain and vertical lines in the bars indicates standard error.

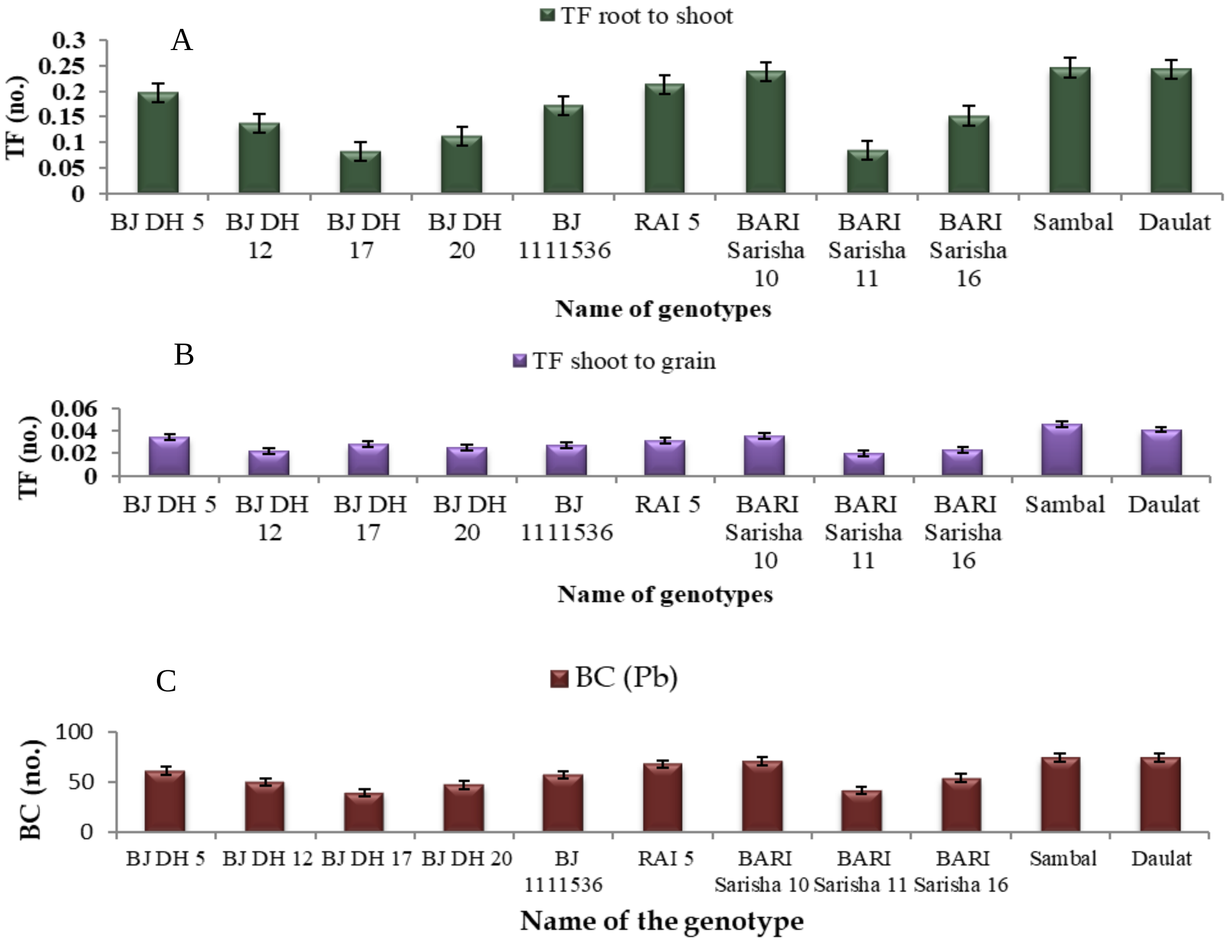

Figure 3. Translocation factor (TF of (A) root to shoot, (B) shoot to grain) and (C) bioaccumulation coefficient (BC, no unit) of 11 *Brassica juncea* varieties at lead treatment (200ppm). Bar data in the graph indicates vatietial mean and vertical line in the graph indicates standard error.

Acknowledgements

Authors are grateful to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for the assignation of National Science and Technology fellowship which has partly supported the research work.

Conflict of interest

None to declare.

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