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LEAD TOLERANCE AND ACCUMULATION POTENTIAL OF *BRASSICA JUNCEA* L. VARIETIES IN IMITATIVELY CONTAMINATED SOIL

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ABSTRACT: *Brassica juncea* is extensively studied plant famous for its hyperaccumulation capacity to various heavy metals like Cd, Cr, Cu, Ni etc. However, plants within species differ in their genetic capabilities to accumulate heavy metals. In present study 10 different genotypes of *B. juncea* were investigated for their potential to tolerate and accumulate Lead (Pb) which is notorious for its low mobility in soil and great hazards to living beings. Screening was done at five different levels of Pb (125, 250, 500, 700, 1000 mg Pb/Kg soil). Plants were evaluated for their growth responses, Pb concentration in root and shoot and Pb accumulation per plant. Among all the tested varieties Pusa Vijay proved to be best having lowest reduction in growth and highest Pb accumulation per plant (307.9 µg/plant) and therefore considered as a suitable candidate for phytoremediation of Pb contaminated soil.

KEYWORDS: Phytoremediation, Indian mustard, lead accumulation, varietal screening.

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1. INTRODUCTION

Unsustainable development all over the world owes ecology an apology. Increased amount of contaminants into the soil is one of the menaces caused by unhesitating industrialization. Organic pollutants may degrade over a period of time into less damaging components but inorganic pollutants, particularly heavy metals remain for a long time into the soil. This buildup of heavy metals into the soil takes soil pollution to more hazardous levels. Thus, increased availability of heavy metals to living organism leads to biomagnification. Heavy metals like Pb, Cd, Hg, As etc are main threats to organisms because these are non-essential and have no known role in biological processes [1]. According to ATSDR (Agency for Toxic Substances and Disease Registry) Lead is on the second rank in the 2017 priority list of hazardous substances that will be the candidates for toxicological profile [2]. Lead is also stated as "the chemical of great concern" by the new European Registration, Evaluation and Authorization of Chemical Substances (REACH) regulations [3]. Lead is dispersed throughout the environment for the most part as the consequence of anthropogenic behavior. Presence of lead in atmosphere is in the form of particles and is settle down and contaminate the soil by precipitation and gravitation where it is strongly bind with soil particles therefore its major part persist in the soil and very less amount percolates into ground water. Hence soil and deposits of atmospheric fallouts are the main source of lead. Nowadays several parts of world are experiencing lead contamination like Lead contaminated water samples were obtained from Newark School district, New Jersey at the concentration of 15ppb [4]; lead contaminated noodle samples were collected from UP, India at the concentration of 17.2 ppm [5]; lead in soil samples at the concentration of 151-222 ppm was reported in Flint, Michigan [6]; also in a blood samples study of the children from Mumbai and Delhi 76% samples showed the presence of lead at 5-20 µg/dL [7] etc. The toxicity levels of lead in the soil for plants are not easy to ascertain however, soil which contains 100-500 ppm of lead could be consider as highly contaminated soil [8]. Toxic Heavy metals such as Cd, Cr, As, Pb, Ni etc. accumulate in different parts of the plant and interfere in their metabolic and physiological pathways which results to heavy metal stress in plant system [9]. Indian mustard is reported to potentially accumulate heavy metals like Cd [10] and Pb [11] at high concentration and produce reasonable biomass. According to a finding 500mg/l lead is not phytotxic to *Brassica* species [12] however plant species and cultivars within species differs in the uptake and distribution of trace elements [13,14,15]. Therefore, it is a smart strategy to screen out comparatively more tolerant variety which accumulates high metal content in addition to biomass production. Hence in present investigation 10 varieties of *B. juncea* has been tested on the basis of growth (biomass) and accumulation attributes at varying soil lead concentrations (0, 125, 250, 500, 700, 1000 mg Pb/kg soil).

2. MATERIALS AND METHODS

Experimentation

Authentic seeds of 10 varieties of Indian mustard (*B. juncea* L. Czern) viz. Pusa Jai kisan, Pusa Jagannath, Pusa Vijay, Pusa Tarak, Pusa Bold, Pusa Mahak, Pusa Agrani, PM 26, PM 27 and PM 25 were obtained from Genetics division of Indian Agriculture Research Institute (IARI), New Delhi, India. Healthy seeds were washed repeatedly with double distilled water and soaked overnight to germinate uniformly. Soaked seeds were sown in earthen pots of 23 cm diameter filled with 3 kg (dried) of sandy loam soil and farmyard manure mixture (6:1) under natural environment during Rabi (winter) season. Lead (Pb) concentrations in soil (0, 125, 250, 500, 700, 1000 mg Pb/kg soil) were maintained by adding Lead nitrate [$Pb(NO_3)_2$] to the soil prior to sowing. After successful establishment of seedlings three plants per pot were maintained. Pots were arranged in complete randomized block design in the net house of Department of Botany, Aligarh, Muslim, University, Aligarh, India with three replicates per treatment. Watering and weeding was done when required. Plants were harvested 60 days after sowing.

Growth measurements

Plant samples were uprooted, washed thoroughly in running tap water and blotted gently. Plant length was measured on a meter scale. Plant dry weight was measured after drying the samples in an oven at 80 ^{0}C till no further reduction in weight was observed. Leaf Area was calculated by Graph paper.

Estimation of Pb concentration and accumulation

Soil Pb concentration was measured by digesting dried soil sample in aqua regia (HNO_3:HCl 3:1 v/v). In case of plant tissue dried samples of root and shoot were powdered, weighed and digested in HNO_3:$HClO_4$ (3:1 v/v) mixture until colorless [16] using a digestion assembly. After maintaining final volume with deionized water Pb content was measured using AAS (Atomic Absorption Spectrophotometer) (Perkin-Elmer A, Analyst, 300; Norwalk, CT) using appropriate cathode lamp and standard. Pb concentration (µg/g) and accumulation (µg/plant) were calculated using the following formula [17].

$$\text{Pb concentration } (\mu g/g) = \frac{\text{AAS reading x volume of sample (ml)}}{\text{weight of dried sample (g)}}$$

$$\text{Pb accumulation } (\mu g/\text{plant}) = (\text{Pb conc. in shoot x DW of shoot}) + (\text{Pb conc. in root x DW of root})$$

Soil characteristics

Soil characteristics were analyzed by Quarsi Agriculture Farm, Aligarh, UP, India except for Pb content.

Soil properties	Content/Values (Units)
pH	7.1
EC	0.83 (mmhos/cm)
Organic Carbon	0.37 (%)
Available Nitrogen	404.86 (kg/hectare)
Available Phosphorus	40.5 (kg/hectare)
Available Potassium	407.25(kg/hectare)
Sulfur	5.83 (ppm)
Zinc	7.25 (ppm)
Fe	4.27 (ppm)
Mn	3.57 (ppm)
Cu	0.63 (ppm)
Pb	0.79 (ppm)

Statistical Analysis

Statistical analysis of data was performed using SPSS Software ver.16. Test of significance was carried out by two ways ANOVA (Analysis Of Variance) followed by DMRT (Duncan's Multiple Range Test) at 0.05 α level. One way ANOVA was applied where two ways interaction was not significant. Error was also calculated with three replicates represented as error bars in figures.

3. RESULTS AND DISCUSSION

It is well documented that plant genotypes differ in accumulation and tolerance against heavy metal contamination [10,18,19]. Therefore in this investigation ten different genotypes of Indian mustard were investigated for their capacity to tolerate different levels of lead contamination. For this purpose different parameters were studied viz. shoot and root length, shoot and root dry weight, leaf dry weight and leaf area (Fig. 1). All the growth parameters were responded in a dose dependent manner. Plant growth was increased slightly at the lower Pb concentrations; this increase was most prominent in cv. Pusa Vijay and least prominent in cv. PM-25. At higher Pb concentrations significant reduction in growth was observed in all the genotypes. Percentage of variation of test plant from control plant changes along with varying concentrations of Pb for all the varieties. In cv. Pusa Jai Kisan 15.17% and 12.16% increase was observed in root length at 125 and 250 mg Pb/Kg soil followed by 5.11-31.53% reduction at 500-1000 mg Pb/Kg soil. Similarly, in cv. Pusa Jagannath 14.55% and 10.30% increase, followed by 5.61-36.36% reduction; in cv. Pusa Vijay 17.01% and 13.76% increase, followed by 4.59-20.12% reduction; in cv. Pusa Tarak 13.58% and 5.73% increase, followed by 6.22-40.92% reduction; in cv. Pusa Bold 13.22% and 8.43% increase, followed by 5.79-39.34% reduction; in cv. Pusa Mahak 10.73% and 0.63% increase, followed by 7.76-42.76% reduction; in cv. Pusa Agrani 6.4% increase followed by 1.91-42.98% reduction; in cv. PM-26 5.39% increase followed by 3.19-43.91% reduction; in cv. PM-27 1.21-44.33% reduction and in cv. PM-25 1.45-44.83% reduction in root length was observed at the range of 125-1000 mg Pb/Kg soil with respect to control (Fig. 1A). For root dry weight, cv. Pusa Jai kisan showed 14.29% and 11.94% increase at 125 and 250 mg Pb/Kg soil, respectively followed by 1.92-17.70% reduction at 500-1000 mg Pb/Kg soil. In the same way, cv. Pusa Jagannath showed 13.54% and 19.39% increase followed by 2.62-20.09% reduction; cv. Pusa Vijay showed 16.90% and 12.33% increase followed by 1.19-12.52% reduction; cv. Pusa Tarak showed 12.78% and 6.50% increase followed by 2.91-25.11% reduction; cv. Pusa Bold showed 13.01% and 7.76% increase followed by 2.74-22.15% reduction; cv. Pusa Mahak showed 9.43% and 4.48% increase followed by 3.07-27.36% reduction; cv. Pusa Agrani showed 7.04% and 3.88% increase followed by 3.40-28.16% reduction; cv. PM-26 showed 4.79% and 1.01% increase followed by 3.53-30.23% reduction; cv. PM-27 showed 1.54-31.28% reduction and PM 25 showed 1.84-32.37% reduction in root dry weight at the range of 125-1000 mg Pb/Kg soil with respect to control (Fig.1B).When shoot length is concern 13.77% and 11.65% increase was observed in Pusa Jai Kisan at 125 and

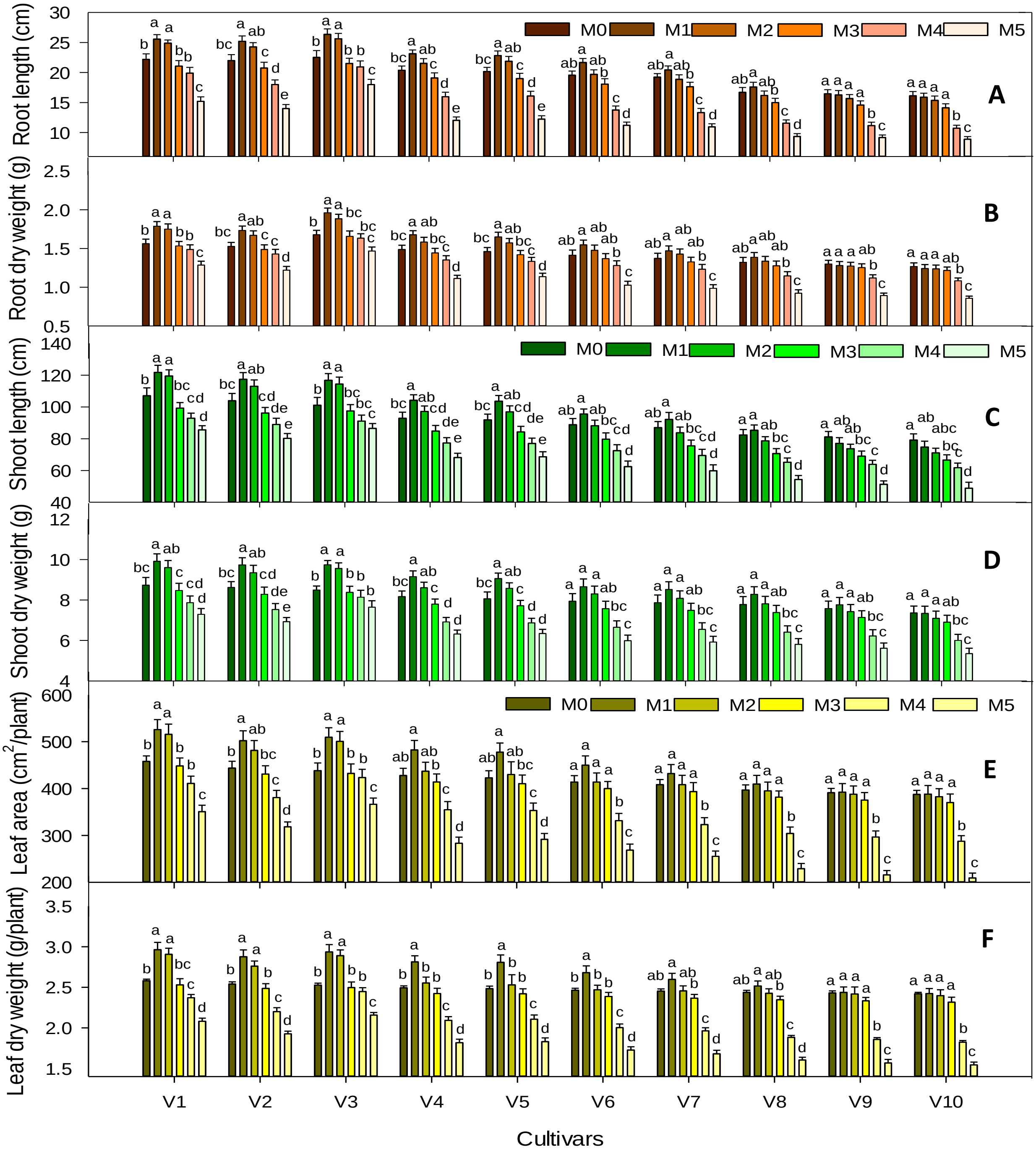

Fig. 1. Effect of varying concentrations of Pb [0(M0), 125(M1), 250(M2), 500(M3), 700(M4), 1000(M5) mg/kg soil] on root length (A), root dry weight (B), shoot length (C), shoot dry weight (D), leaf area (E) and leaf dry weight (F) of *Brassica juncea* cultivars viz. V1 (Pusa Jai kisan), V2 (Pusa Jagannath), V3 (Pusa Vijay), V4 (Pusa Tarak), V5 (Pusa Bold), V6 (Pusa Mahak), V7 (Pusa Agrani), V8 (PM-26), V9 (PM-27) and V10 (PM-25) after 60 days of sowing. Values are means of three replicates. SE represented as capped bars. Significant difference at $p<0.05$ was determined by one way ANOVA to compare the effect of varying Pb concentrations on a particular cultivar. Bars bearing same alphabets do not differ significantly (DMRT was applied separately to each cultivar).

250 mg Pb/Kg soil followed by 7.29-20.06% reduction at 500-1000 mg Pb/Kg soil. Likewise, in cv. Pusa Jagannath 12.99% and 8.79% increase, followed by 7.44-22.81% reduction; in cv. Pusa Vijay 15.50% and 13.16% increase followed by 3.56-14.44% reduction; in cv. Pusa Tarak 12.16% and 4.59% increase followed by 8.72-26.59% reduction; in cv. Pusa Bold 12.73% and 5.48% increase followed by 8.31-25.28% reduction; in cv. Pusa Mahak 7.62% increase followed by 0.71-29.65% reduction; in cv. Pusa Agrani 5.94% increase followed by 3.72-31.26% reduction; in cv. PM-26 3.64% increase followed by 4.45-34.12% reduction; in cv. PM-27 5.05-36.80% reduction and in cv. PM-25 5.56-38.27% reduction in shoot length was observed at the range of 125-1000 mg Pb/Kg soil with respect to control (Fig. 1C). In case of shoot dry weight cv. Pusa Jai Kisan showed 13.51% and 9.88% increase at 125 and 250 mg Pb/Kg soil, followed by 3.09-16.60% reduction at 500-1000 mg Pb/Kg soil. Also, in cv. Pusa Jagannath 12.87% and 8.43% increase followed by 3.94-19.64% reduction; in cv. Pusa Vijay 14.64% and 12.56% increase followed by 1.33-9.97% reduction; in cv. Pusa Tarak 12.12% and 5.39% increase, followed by 4.57-22.61% reduction; in cv. Pusa Bold 12.41% and 6.41% increase, followed by 4.22-21.26% reduction; in cv. Pusa Mahak 9.07% and 4.62% increase followed by 4.62-24.57% reduction; in cv. Pusa Agrani 8.31% and 2.75% increase followed by 4.83-24.70% reduction; in cv. PM-26 6.47% and 0.43% increase, followed by 5.18-25.36% reduction; in cv. PM-27 2.46% increase, followed by 1.98-25.87% reduction and in PM 25 0.27-27.26% reduction in shoot dry weight was observed at the range of 125-1000 mg Pb/Kg soil with respect to control (Fig. 1D). The leaf area of cv. Pusa Jai Kisan was increased upto 14.82% and 12.60% at 125 and 250 mg Pb/Kg soil respectively and reduced upto 2.07-23.43% at 500-1000 mg Pb/Kg soil. While, in cv. Pusa Jagannath 13.19% and 8.49% increase, followed by 2.88-28.24% reduction; in cv. Pusa Vijay 16.23% and 14.24% increase, followed by 1.35-16.38% reduction; in cv. Pusa Tarak 12.78% and 2.14% increase followed by 3.22-33.78% reduction; in cv. Pusa Bold 12.98% and 1.70% increase followed by 2.95-31.02% reduction; in cv. Pusa Mahak 8.77% and 0.04% increase followed by 3.27-35.11% reduction; in cv. Pusa Agrani 5.80% increase followed by 0.02-37.52% reduction; in cv. PM-26 3.20% increase followed by 0.48-42.32% reduction; in cv. PM-27 0.27% increase followed by 0.83-44.87% reduction and in cv. PM-25 0.13% increase followed by 1.24-45.97% reduction in leaf area was observed at the range of 125-1000 mg Pb/Kg soil with respect to control (Fig.1E). The cv. Pusa Jai kisan showed increase in leaf dry weight upto 14.86% and 12.66% at 125 and 250 mg Pb/Kg soil and decrease upto 1.94-19.38% when the level of Pb increased from 500-1000 mg Pb/Kg soil. In cv. Pusa Jagannath 13.25% and 8.66% increase followed by 2.10-24.15% reduction; in cv. Pusa Vijay 16.23% and 14.38% increase followed by 1.19-14.64% reduction; in cv. Pusa Tarak 12.83% and 2.41% increase followed by 2.81-27.14% reduction; in cv. Pusa Bold 13.02% and 1.88% increase followed by 2.55-26.31% reduction; in cv. Pusa Mahak 8.80% and 0.27% increase followed by 3.11-29.91% reduction; in cv. Pusa Agrani 5.84% and 0.14% increase followed by 3.53-31.52% reduction; in cv. PM-26 3.28% increase

followed by 0.41-34.20% reduction; in cv. PM-27 0.27% increase, followed by 0.55-35.53% reduction and in cv. PM-25 0.14% increase followed by 0.96-36.23% reduction in leaf dry weight was observed at the range of 125-1000 mg Pb/Kg soil with respect to control (Fig. 1F). In this study *B. juncea* cultivars exhibits differential response against Pb contamination and hence portraying that the cultivars possess different genetic ability to combat against Pb. A similar study was done by against Ni contamination in which *B. juncea* cultivars showed different responses to applied Ni doses [18]. Pb is a non-essential toxic heavy metal but at lower applied doses a slight increase in growth was observed. This could be a "preconditioning" or "adaptive stress response" also termed as "Hormesis" [20]. At higher doses significant reduction in growth was observed. A study conducted by Sikka [21] revealed that reduced growth of *B. juncea* in Pb contaminated soil may be associated with reduced uptake of micronutrients (viz. Fe, Cu, Mn and Zn). It suggests that Pb compete for root absorption sites with micronutrients and this leads to the deficiency of micronutrients in the plants, which later on becomes visible symptoms as stunted growth and reduced biomass. According to Bhattacharya and Chaudhuri [22] application of Pb can hype the catabolic enzyme activity like RNAse activity. Increased chlorophyllase and protease activity were also reported by Drazkiewicz [23] and Palma [24], respectively. This could also be a reason behind reduced growth in plants. Other possible reason behind disturbed morphological traits includes adverse effects on cell division, structure and growth of cells [25]; interruption in plant's metabolic pathways, damaged plant roots [26]. Lead induced microtubule disorganization is a major problem seeking attention of researchers [27,28]. However, for phytoremediation the plant should also accumulate significant amount of metal in addition to metal tolerance. Thus, concentration of Pb in root and shoot were also measured in all the genotypes (Fig. 2). Root metal accumulation was significantly increased at each treatment except for cv. PM-26, cv. PM-27 and cv. PM-25 (non-significant increase at 125 mg Pb/Kg soil). At highest Pb treatment cv. Pusa Jai kisan ended up with 54.4 mg Pb/Kg DW, cv. Pusa Jagannath with 51 mg Pb/Kg DW, cv. Pusa Vijay with 58.2 mg Pb/Kg DW, Pusa Tarak with 50.6 mg Pb/Kg DW, Pusa Bold with 50.8 mg Pb/Kg DW, Pusa Mahak with 50.1 mg Pb/Kg DW, Pusa Agrani with 49.8 mg Pb/Kg DW, cv. PM-26 with 49.4 mg Pb/Kg DW, cv. PM-27 with 49.2 mg Pb/Kg DW and cv. PM-25 ended up with 48.7 mg Pb/Kg DW as their root Pb concentrations (Fig. 2A). As far as shoot metal accumulation is concerned Pb concentrations were below detectable limit in control. At 125 mg Pb/Kg soil highest concentration of Pb was found in shoots of cv. PM-25 (1.13 mg Pb/Kg DW) and lowest Pb was found in Pusa Jagannath (0.47 mg Pb/Kg DW). Similarly highest concentrations noted as 3.80 (V10), 6.67 (V10), 15.1 (V9), 28.4 mg Pb/Kg DW (V9) and lowest concentrations noted as 1.37, 2.45, 7.1, 14.9 mg Pb/Kg DW (V2) at 250, 500,

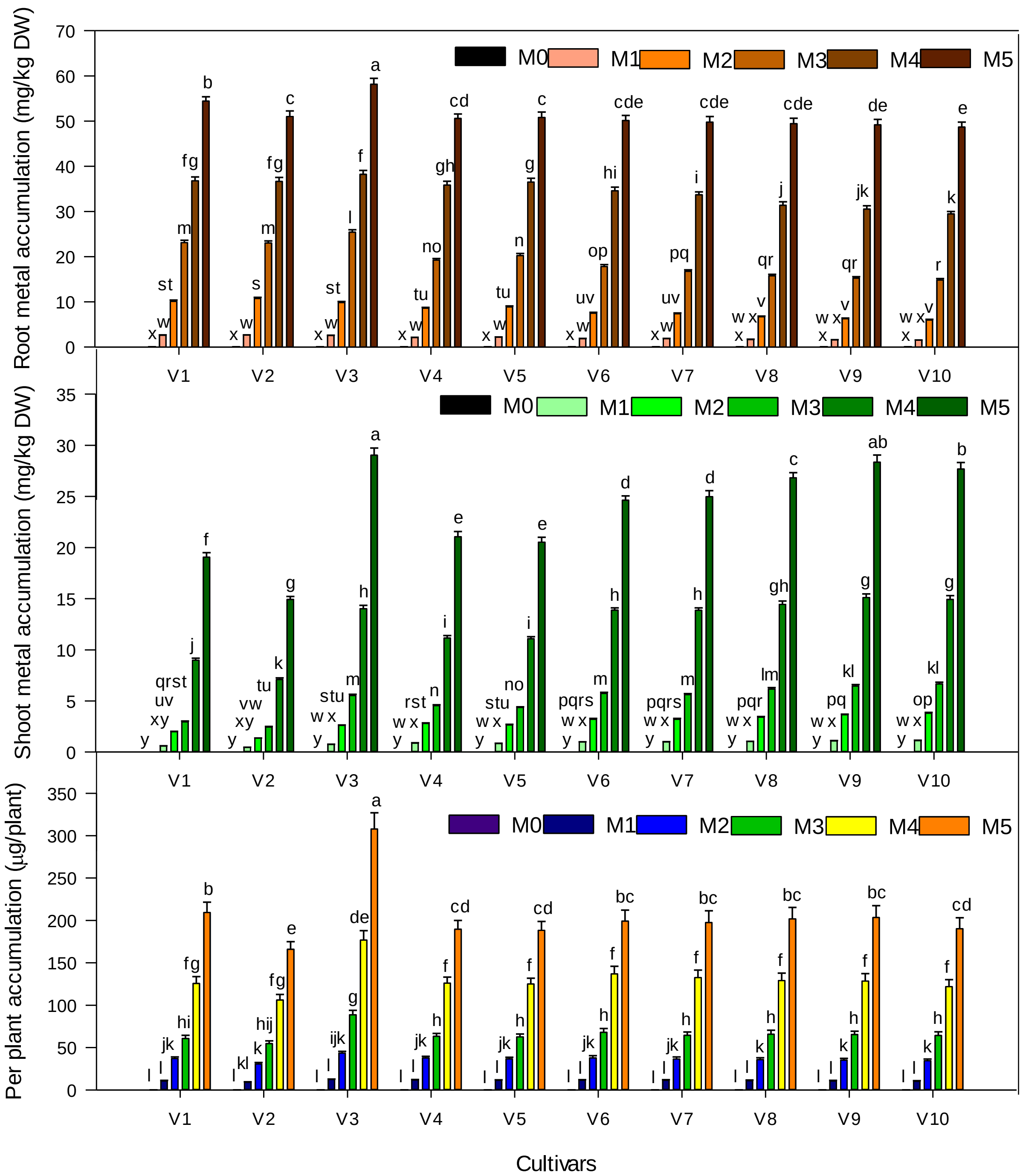

Fig. 2. Accumulation potential (root metal accumulation-A, shoot metal accumulation-B and per plant accumulation-C) of *Brassica juncea* cultivars viz. V1 (Pusa Jai kisan), V2 (Pusa Jagannath), V3 (Pusa Vijay), V4 (Pusa Tarak), V5 (Pusa Bold), V6 (Pusa Mahak), V7 (Pusa Agrani), V8 (PM-26), V9 (PM-27) and V10 (PM-25) against varying Pb doses [0(M0), 125(M1), 250(M2), 500(M3), 700(M4), 1000(M5) mg/kg soil] after 60 days of sowing. Values are means of three replicates. SE represented as capped bars. Significant difference at $p<0.05$ was determined by two way ANOVA to compare the means. Bars bearing same alphabets do not differ significantly (alphabets were applied using M X V interaction LSD).

700 and 1000 mg Pb/Kg soil respectively (Fig. 2B). Pb accumulation per plant (Fig. 2C) was also calculated. Maximum accumulation per plant was achieved in Pusa Vijay (12.35, 43.51, 88.62, 176.9 and 307.9 µg/plant at 125, 250, 500, 700 and 1000 mg Pb/Kg soil, respectively) and minimum accumulation per plant was observed in Pusa Jagannath (9.15, 30.77, 54.62, 106.1 and 166µg/plant at 125, 250, 500, 700 and 1000 mg Pb/Kg soil respectively). On the ground of Pb accumulation per plant (DW basis) V3 proved to be best and V2 accumulates least amount of Pb among all the tested varieties (Fig. 2C). Considering Pb accumulation in all the experimental cultivars, Pb concentration was always far greater in roots than the shoots. This finding was in agreement with Wierzbicka [29] and Geebelen [30]. Exclusion, accumulation and sequestration in vacuoles are the strategies of plants to tolerate metal contamination [31]. Pb ions retain in the roots because of its ability to strongly bind with the cell wall ion exchangeable sites i.e. carboxyl group of glucuronic and galacturonic acids at the cell wall, hence blocking its apoplastic movement [32]. In addition Pb ions could also precipitate extracellularly by forming carbonate salts [27]. Casparian strips restrict the movement of Pb across endodermis leaving other parts of the plant unaffected [33]. In plants metals are also sequestered in vacuoles by binding with phytochelatins (PCs) and metallothioneins (MTs) [34]. Ghnaya [35] suggested that PCs and MTs form complex with metals and mainly sequester in root cell vacuole while in translocation to xylem, organic acids such as citric acid etc. are involved binding with metals. Pb uptake is a factor, indicating towards the cumulative accumulation of Pb by a single plant in terms of dry weight. On the basis of Pb uptake our study suggest that V3 proved to be best followed by V1,V9,V8,V6,V7,V10,V4,V5 and then V2 sequentially.

4. CONCLUSION

Although it is well studied that *B. juncea* is a hyperaccumulator plant, but, in our study none of the cultivars of *B. juncea* accumulated Pb more than 1000mg/Kg DW [36, 37]. However, all the cultivars successfully tolerate high amount of Pb without any prominent toxicity symptom. The present findings concluded that in case of Pb phytoremediation *B. juncea* is good tool for Phytostabilization and not for hyperaccumulation. However, among all the tested varieties, if we consider both biomass production and Pb accumulation *B. juncea* Pusa Vijay is best suitable candidate for phytoremediation.

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CONFLICT OF INTEREST

There is no conflict of interests among authors.

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