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Rhizospheric supplements of lead (Pb) as nutrient or pollutant in Mash [*Vigna mungo(*L.) Hepper]: Diagnostic integration by photosynthetic pigments, physiological growth and enzyme assay

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An experiment using four mash bean genotypes was conducted to find out the essentiality or toxicity of escalating lead (Pb) concentrations and variations in the sensitivity range of these genotypes. Four genotypes were sown in pots and positioned with complete randomization. Pots by number were replicated four times for each concentration of lead in every genotype. Lead (Pb) was added in soil as Pb $(NO_3)_2$ solutions after twenty days of germination to raise lead concentration of 10.0, 20.0, 30.0, 40.0, 50.0, and 60.0 mg kg^{-1} soil. Photosynthetic pigments and leaf area index was measured in leave of plant on expiry of twenty five days after lead addition. Increments in lead concentration affected negatively by lowering photosynthetic pigments and Leaf Area Index. A significantly conceivable proportion of reduction was evident at 20 mg kg^{-1} lead (Pb) and escalating levels. In this regard, the least effective significant dose was 20 mg kg^{-1} while the most effective proven dose was 60 mg kg^{-1} for each attribute. Lead (Pb) concentration of 10 mg kg^{-1} established an opposite index of an increase in studied characteristics. MASH 80 was the least sensitive while MASH 88 and MASH 97 were the most sensitive genotypes. In term of nitrate reductase activity, MASH 88 was the most sensitive while MASH 80 the least for nitrate reductase Activity.

Keywords: Chlorophyll; Carotenoids; Lead ; Leaf area index; Nitrate Reductase, Mash

INTRODUCTION

Soil is the sink and source of heavy metals (both geogenic and anthropogenic) and plants being the ecosystem regulators, balance the chemistry of life on earth. Roots of plants are the only connection between soil and plants which are the real engineers of ecosystem dynamics responsible for environmental balance and stability. The plant-soil interface termed as 'rhizosphere' is a typical zone of soil where the physical, chemical and biological characteristics are different from bulk soil outside the rhizosphere (seshadri et al., 2015).

In recent years heavy metal pollutants have increased in environment due to anthropogenic activities causing a serious problem not only for human but for whole ecosystem (Asad et al., 2019; Maleki et al., 2017). These metals contaminate the food chain and become a source of toxicity for ecosystem functioning (Budijono, 2017; Ali and Khan, 2019). Air, soil, and water are contaminated by heavy metals which affect the plants, animals and humans in the food chain (Aendo et al., 2020; Biswas et al., 2019)

Lead (Pb) being a non-degradable and long-lived metal is a source of strong

rhizospherictoxicity (Bahri et al., 2015; Yang et al., 2020). Being highly toxic it pose a threat to plants growth and health [Rizwan et al., 2018; Shi et al., 2014].

Lead (Pb) is one of the potentially toxic heavy metal pollutants of the environment and its concentrations are rapidly increasing in agricultural soils (Hamid et al., 2010). The most significant factors which can distribute lead as a pollutant in the environment are burning of fossil fuels, agricultural manufacturing, mining, pesticides and fertilizers (Ross, 1994). After absorption, it may show some benificial effects (Samiullah and Nazar, 1983) or phytotoxicity as diverse biochemical changes in green plants such as: reduced nitrate reductase activity (Singh et al., 1997), reduction in proteins (Kevresan et al., 2001), decreases in chlorophyll content (Ewais, 1997); increases in chlorophyll *a*/*b* ratio and decrease in carotenoids (Fargasova, 2001).These biochemical changes in metal contaminated plants results in senescence and other symptoms like chlorosis, nacrosis and lesions.

Chlorophylls are important pigments of the photosynthetic activity for primary productivity and its degradation contributes significantly to these morphological symptoms. Many studies have demonstrated influences of heavy metals on chlorophyll and protein contents in higher plants (Prasad et al., 2001). Chlorophyll a, b and total chlorophyll are reported to drastically reduce in metal treated plants (Somashekaraiah et al., 1992). Besides chlorophylls, carotenoids are natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. There are several dozen carotenoids in the foods that we eat, and most of these carotenoids have antioxidant activity. β-carotene has been best studied since, in most countries it is the most common carotenoid in fruits and vegetables. An increase in carotenoid content is suggested a defense strategy of the plants to combat metal stress (Sinha et al., 2007). Reduction in photosynthetic pigments, altered physiological processes and biochemical phenomena are integrated in the form of changes in morphology and yield contributing factors. Among these morphological traits, leaf growth, development and total leaf number decisively determine the yield of a plant (Sharma and Sharma, 1993).

Mash bean [*Vignamungo*(L.) Hepper] is among the most important pulse crops of the world. It has great value as food, fodder and green manure. In addition to improving the soil fertility, it is a cheap source of protein for direct human consumption. Chemical analysis of mash bean seed indicates that it contains protein (20-24%), oil (2.1%), Fats (1-2%), carbohydrates and a fair amount of vitamin A and B (James, 1981). By focusing on the importance of mash bean and considering the toxicity of gradually increasing lead (Pb) concentration in environment, the present experiment was devised to find out the extent of crop sensitivity to various concentrations of lead (Pb).

MATERIALS AND METHODS

Under adverse rhizospheric conditions such as presence of heavy metals, plants are reported to show suppression or promotion in growth, development and physiological characters. Hence, a pot experiment was devised to find out Lead (Pb) toxicity extent for four mash bean [*Vigna mungo*(L.) Hepper] genotypes. The plant characteristics selected for evaluation were Chlorophyll a, Chlorophyll b, Carotenoids contents (mg g^{-1} leaf F. wt), leaf area index (LAI) and Nitrate Reductase (EC 1.6.6.1) Activity (NRA).

Materials

After the initial survey, soil free from effluents hazards, was selected for the experiment. After drying soil was mixed well and passed through 2mm sieve. Seeds of four mash genotypes i-e MASH 80, MASH 88, MASH 97 and MASH ES-1 were used in the experiment. For imposing metal pollution in soil, chloride of lead (Pb) of Sigma Aldrich, Japan was used.

Experimental design and methodologies

Experiment was designed with complete randomization of treatments and genotypes to avoid unequal exposure of environmental resources. Each treatment was repeated four times by pots. Soil physical and chemical analysis was carried out by methods of Richards, (1954). Earthen pots of 30 cm diameter were filled with 10 kg sandy loam soils and lined with polyethylene bags ensuring seepage prevention and were arranged in completely randomized design. Seeds, similar in size and weight, sterilized with 10% (v/v) hydrogen peroxide, were germinated and thinning was performed to maintain one seedling in each pot in order to avoid the imbalanced uptake of nutrients by plants. Pests and insects were control by spray of insecticides. To develop the metal toxicity, calculated amounts of nitrate of lead (Pb) was added in soil accordingly to raise the lead (Pb) levels of 10.0,

20.0, 30.0, 40.0, 50.0, and 60.0 mg kg^{-1} soil. Metals salt was applied in soil as a water solution of $Pb(NO_3)_2$ (method similar to that used by Stoeva and Bineva ,2003) after twenty days of sowing. Pots without the addition of metals salts acted as control.

Data recordings

Chlorophyll (a, b) and Carotenoids Contents (mg g^{-1} leaf F. wt)

Chlorophyll contents were measured by using the method of Arnon (1949) after twenty five days of metal treatment. The leaves were ground and extracted with 80% acetone. The absorbance was measured at 645nm and 663nm for Chla and b respectively and at 480nm for carotenoids by using spectrophotometer (Hitachi Model-U 2001 Japan). Chlorophyll contents were calculated according to the Lichtenthaler (1987) formulae and carotenoids contents were calculated after Davies, (1976).

Chl a (mg g^{-1} leaf fresh weight) = [12.7(OD663)-2.69 (OD645)]× V/1000 ×W.

Chl b (mg g^{-1} leaf fresh weight) = [22.9(OD645) - 4.68(OD663)]×V/1000 ×W.

Carotenoids (mg g^{-1} leaf fresh weight) = [Acar/EM] × 1000.

Where

Acar = OD480+0.114(OD663)-0.638(OD645); EM (100%) =2500; OD =Optical density;

V=Volume of samples=Weight of sample.

Physiological Growth

Physiological Growth in the form of leaf area index (LAI) was measured and calculated by using the formula given by Puttaswamy et al., (1976).

LAI = L × W × N × K

Where,

L= length of the leaf in cm; W= maximum width of the leaf in cm; N= number of leaves per plant; K= constant (0.65 for legume crops).

Nitrate Reductase Activity (NRA)

Nitrate Reductase Activity (NRA) was determined after twenty five days of metal treatment using the method of Sym (1984). For this purpose, the reagents used were

A) Phosphate buffer (0.2 M) which was prepared using following chemicals

NaH_2PO_4 1M solution(156.01 g/l) was prepared as stock

Na_2HPO_4 (177.99 g/l) M solution was prepared as the stock

B) 0.01M phosphate buffer (pH=7.0) containing 0.02M KNO_3

1% sulphanilamide in 3N HCL

0.02% N(1-Naphthyl-ethylene diaminedihydrogenchloride)

Leaf material (5 g) was chopped (4 to 5 mm slices) in a 20 ml test tube containing 5 ml of medium, comprising 0.01 M phosphate buffer (pH 7.0), 0.02 M KNO_3 and 0.01% Triton x-100. These test tubes were incubated in dark at 32° C for one hour. The assay of enzyme was done through nitrite analysis as the NO_3 of the medium was converted into NO_2 by nitrate reductase. After one hour of incubation period, 1 ml of medium was drawn and mixed with 0.5 ml of sulphanilamide (10 g/l) in 3 ml HCl. Immediately, after shaking 0.5 ml of N-1-naphthyl ethylene diamine dihydrochloride (0.1 g/l) was added to produce pink diazo complex with NO_2. After 20 minutes the dye solution was diluted with 5 ml of distilled water and centrifuged for three minutes at 2050 x g to remove turbidity, if present. A correction blank consisting of all chemicals mentioned above without sample was used. The standard curve from $NaNO_2$ was developed to estimate NO_2 present in media. Optical density was noted at 542nm on spectrophotometer (Hitachi-220).Nitrate reductase activity was calculated as:

Nitrate Reductase Activity=Graph reading × Dilution factor × O.D of sample (µmol NO_2/h/g FW)

Statistical analysis

The data collected were analyzed for analysis of variance for all the parameters using COSTAT computer package (CoHort Software, Berkeley, CA). Duncan's New Multiple Range test at 5% level of probability (Duncan, 1955) was used to compare means. Where significant F values were obtained in analysis, differences between individual means were tested by LSD tests at 0.05% significance level, by using MSTAT-C Computer Statistical Programme (MSTAT Development Team, 1989)

RESULTS

Chlorophyll a Contents (mg g^{-1} leaf F. wt)

According to Duncan's Multiple Range test (Table: 1), chlorophyll a contents were decreased when lead (Pb) was supplied in the nutrient medium. The effectiveness of lead (Pb) in reducing the pigment was significant at all levels of its concentration. The most promising decrease appeared to occur at 60mg kg^{-1} in all genotypes.

Table 1: Chlorophyll a contents (mg g^{-1} leaf F. wt) of 45 days old mash [*Vigna mungo*(L.) Hepper] grown in lead (Pb) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) [Values represent means ± SE].

Lead(Pb) (mg kg^{-1} soil)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENT SMEANS (LSD=0.096 ;n=16)
	(LSD=0.188 ;n=4)				
Control	1.448±0.196 [B]	1.707±1.164 [A]	1.426±0.378 [B]	1.080±0.169 [C]	1.415 a±0.316
10	1.313±0.174 (9.323) [B]	1.329±0.164 (22.144) [B]	0.983±0.149 (31.065) [CD]	0.945±0.147 (12.500) [CD]	1.142 b±0.233 (19.293)
20	0.839±0.028 (42.058) [DE]	1.043±0.307 (-19.683) [C]	0.737±0.106 (48.316) [E]	0.443±0.123 (58.981) [F]	0.765 c±0.272 (45.936)
30	0.365±0.067 (74.792) [FG]	0.675±0.164 (60.456) [E]	0.459±0.090 (67.812) [F]	0.325±0.040 (69.907) [FGH]	0.456 d±0.167 (67.773)
40	0.245±0.021 (83.080) [GHIJK]	0.304±0.017 (82.190) [FGHIJ]	0.318±0.125 (77.699) [FGHI]	0.180±0.042 (83.333) [HIJKL]	0.262 e±0.082 (81.484)
50	0.099±0.047 (93.162) [KL]	0.123±0.030 (92.794) [JKL]	0.135±0.047 (19.532) [IJKL]	0.056±0.012 (94.814) [L]	0.103 f±0.045 (92.720)
60	0.047±0.014 (96.754) [L]	0.084±0.048 (95.079) [KL]	0.034±0.012 (97.616) [L]	0.030±0.008 (97.222) [L]	0.049 f±0.032 (96.537)
GENOTYPES MEANS →	0.622b±0.554	0.752 a±0.610 (-20.900)	0.584b±0.492 (6.109)	0.437 c±0.405 (29.742)	0.599±0.536
	(LSD=0.073 ; n=28)				

Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).

Deviating from the general trend, lead (Pb) added 19.683% more to the chlorophyll a contents when 20mg kg^{-1} concentration was imposed to the plants of MASH 88. Among the genotypes, MASH 88 revealed maximum (0.752) and MASH ES-1 revealed minimum (0.437) value for chlorophyll a concentration.

Chlorophyll b Contents (mg g^{-1} leaf F. wt)

Chlorophyll b contents decreased under the influence of lead (Pb) toxicity (Table2). Statistically remarkable variations from control were assessed for more than 10mg kg^{-1} lead (Pb) concentration. Exposure to 60mg kg^{-1} lead revealed its strongest impact in reducing the chlorophyll b contents in all genotypes. An additive actions of 10mg kg^{-1} lead (Pb) was noted for chlorophyll b contents of the plants in MASH 80 by 18.450% and in MASH 88 by 36.164%. Of the genotypes, MASH 97 revealed maximum (0.484) and MASH 80 revealed minimum (0.269) value for chlorophyll b contents.

Total Carotenoids Contents (mg g^{-1} leaf F. wt)

Reduction in total carotenoids contents were conducive to supplied lead (Pb) toxicity revealing that lead (Pb) toxicity changed the carotenoids contents toward the descending trend (Table: 3). A precise relationship occurred between carotenoids reduction and metal toxicity level, the significant factor of which was consistent under all treatments. Maximum effect of lead (Pb), in all the genotypes, was by 60mg kg^{-1} but in MASH 80 the same was achieved by 40mg kg^{-1} concentration. Among the genotypes, MASH 88 revealed maximum (0.374) and MASH 80 revealed minimum (0.189) value.

Leaf area index (LAI)

From the data for mean values shown in Table 4, it could be inferred that the lead (Pb) supply reduced the leaf area index (LAI). lead (Pb) induced reduction was significant in plants subjected to all levels of lead (Pb) toxicity maximum (34.132%) being by 60mg kg^{-1} and

minimum (6.153%) by 10mg kg^{-1} concentration. All the genotypes responded in a similar fashion to escalating levels of lead (Pb) toxicity. Of the genotypes, MASH 88 yielded maximum (347.051) and MASH 80 revealed minimum (145.871) values for leaf area index (LAI) while the remaining genotypes lied between these two.

Nitrate Reductase (EC 1.6.6.1) Activity (NRA)

Lead (Pb) stress imposition induced a gradual reduction in Nitrate Reductase Activity (Table:5). Recorded and documented data for mean performance of NRA reflected the significant role metal played in checking Nitrate Reductase Activity (NRA) when supplied above than 10mg kg-1 concentrations. Lower Nitrate Reductase Activity (NRA) was detected and documented against 10mg kg-1. The most marked decrease in Nitrate Reductase Activity (NRA) was in plants subjected to 50mg kg-1 except in MASH 97 for which the same was true at 60mg kg-1. Although not statistically justified, the role of supplemented lead (Pb) deviated from the logical expectation of reduction when applied in concentrations of 10mg kg-1 in MASH 80, MASH 88 and MASH 97 where plants got an increase of 17.462%, 3.911% and 1.889% respectively in nitrate reductase activity. Among the genotypes, MASH 80 revealed maximum (0.615) and MASH 88 revealed minimum (0.404) values.

Table 2: Chlorophyll b contents (mg g^{-1} leaf F. wt) of 45 days old mash [*Vigna mungo*(L.) Hepper] grown in lead (Pb) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) [Values represent means ± SE].

Lead(Pb) (mg kg^{-1} soil)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTSMEANS (LSD=0.059 ;n=16)
	(LSD=0.117 ;n=4)				
Control	0.493±0.037 [EF]	0.777±0.210 [B]	1.025±0.105 [A]	0.953±0.106 [A]	0.812 a±0.241
10	0.584±0.040 (-18.458) [DE]	1.058±0.054 (-36.164) [A]	0.980±0.127 (4.390) [A]	0.707±0.061 (25.813) [BC]	0.832 a±0.212 (-2.463)
20	0.371±0.023 (24.746) [G]	0.749±0.066 (36.03) [BC]	0.663±0.131 (35.317) [CD]	0.391±0.138 (58.971) [FG]	0.543 b±0.193 (33.128)
30	0.193±0.026 (60.851) [IJK]	0.409±0.041 (4.736) [FG]	0.360±0.123 (64.878) [GH]	0.247±0.039 (74.081) [HI]	0.303 c±0.108 (62.684)
40	0.082±0.020 (83.367) [KL]	0.183±0.043 (76.447) [IJK]	0.209±0.065 (79.609) [IJ]	0.100±0.025 (89.506) [JKL]	0.143d±0.067 (82.389)
50	0.135±0.191 (72.616) [IJKL]	0.112±0.032 (85.585) [JKL]	0.120±0.041 (88.292) [JKL]	0.053±0.021 (94.438) [L]	0.105 d±0.094 (87.068)
60	0.028±0.003 (94.320) [L]	0.052±0.036 (93.307) [L]	0.034±0.015 (96.682) [L]	0.025±0.009 (97.376) [L]	0.035 e±0.021 (95.689)
GENOTYPES MEANS →	0.269 c±0.213	0.477 a±0.375 (-77.323)	0.484 a±0.393 (79.925)	0.354 b±0.341 (-31.598)	0.396±0.345
	(LSD=0.045 ; n=28)				

Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters)

Table 3: Total Carotenoids contents (mg g^{-1} leaf F. wt) of 45 days old mash [*Vignamungo*(L.) Hepper] grown in lead (Pb) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) [Values represent means ± SE].

Lead(Pb) (mg kg^{-1} soil)	MASH 80	MASH 88	MASH 97	MASH ES-1	Treatment Smeans (LSD=0.053 ;n=16)
	(LSD=0.099 ;n=4)				
Control	0.496±0.055 [E]	0.701±0.093 [AB]	0.765±0.033 [A]	0.724±0.183 [AB]	0.671a±0.144
10	0.437±0.119 (11.895) [E]	0.687±0.058 (1.997) [ABC]	0.595±0.087 (22.222) [CD]	0.532±0.095 (26.519) [DE]	0.563b±0.125 (16.095)
20	0.179±0.009 (63.911) [GH]	0.664±0.213 (5.278) [BC]	0.444±0.051 (41.960) [E]	0.229±0.068 (68.370) [FG]	0.379c±0.223 (43.517)
30	0.091±0.026 (81.653) [HIJ]	0.320±0.110 (54.350) [F]	0.298±0.061 (61.045) [F]	0.153±0.031 (78.867) [GHI]	0.215 d±0.116 (67.958)
40	0.053±0.006 (98.314) [J]	0.166±0.012 (76.319) [GH]	0.189±0.090 (36.078) [G]	0.066±0.010 (90.883) [IJ]	0.118e±0.074 (82.414)
50	0.037±0.019 (92.540) [J]	0.053±0.016 (92.439) [J]	0.065±0.020 (91.403) [IJ]	0.020±0.005 (97.237) [J]	0.044f±0.022 (93.442)
60	0.029±0.042 (94.153) [J]	0.030±0.018 (95.720) [J]	0.015±0.007 (98.039) [J]	0.015±0.003 (97.928) [J]	0.022f±0.022 (96.721)
GENOTYPES MEANS →	0.189c±0.191	0.374a±0.300 (-97.883)	0.339a±0.266 (-79.365)	0.248b±0.270 (-31.216)	0.287±0.267
	(LSD=0.040 ; n=28)				

Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).

Table 4: Leaf Area Index (LAI) of 45 days old mash [*Vigna mungo*(L.)Hepper] grown in lead (Pb) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil)

Lead (Pb) (mg kg^{-1} soil)	MASH 80	MASH 88	MASH 97	MASH ES-1	Treatment Smeans (LSD=14.443 ;n=16)
	(LSD=28.89 ;n=4)				
Control	505.672±6.284 [A]	402.495±14.413 [BC]	334.667±31.663 [EFGH]	390.457±20.716 [BCDE]	408.323a±66.361
10	421.025±27.642 (16.739) [B]	390.372±10.060 (3.011) [BCDE]	328.275±19.923 (1.909) [FGHI]	393.115±10.819 (-0.680) [BCD]	383.196b±38.753 (6.153)
20	337.987±26.805 (33.160) [DEFGH]	379.967±10.806 (5.597) [BCDEF]	316.125±27.216 (5.540) [GHIJ]	351.507±9.433 (9.975) [GHIJ]	346.396c±30.062 (15.166)
30	309.400±18.792 (38.814) [GHIJ]	345.905±34.275 (14.059) [CDEFG]	325.375±23.995 (2.776) [FGHI]	310.565±28.519 (20.461) [GHIJ]	322.811d±28.554 (20.942)
40	292.537±34.968 (42.148) [GHIJK]	316.845±13.955 (21.279) [GHIJ]	299.430±31.831 (10.528) [GHIJ]	300.957±8.855 (22.921) [GHIJ]	302.443e±24.214 (25.930)
50	287.062±16.530 (43.231) [HIJK]	301.457±17.981 (25.102) [GHIJ]	272.117±15.951 (18.690) [IJK]	281.335±13.475 (27.947) [HIJK]	285.493f±18.095 (30.081)
60	267.412±8.085 (47.117) [JK]	292.317±8.406 (27.373) [GHIJK]	241.280±8.519 (27.904) [K]	274.807±20.627 (29.619) [IJK]	268.954g±22.054 (34.132)
GENOTYPES MEANS →	245.871a±83.986	347.051a±44.992 (-41.151)	302.467c±38.740 (-23.018)	328.963b±49.185 (-33.794)	331.088±59.067
	(LSD=10.918 ; n=28)				

[Values represent means ± SE].Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (upper case letters).

Table 5: Nitrate Reductase (EC 1.6.6.1) Activity (NRA) of 45 days old mash [*Vigna mungo*(L.)Hepper] grown in lead (Pb) supplemented soil (0, 10, 20, 30, 40 50 and 60mg/kg soil) [Values represent means ± SE].

Lead(Pb) (mg kg^{-1} soil)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTSMEANS (LSD=0.079 ;n=16)
	(LSD=0.154 ;n=4)				
Control	0.796±0.146 [ABCD]	0.818±0.216 [ABC]	0.688±0.031 [CDE]	0.660±0.070 [DEF]	0.741 a±0.140
10	0.935±0.082 (-17.462) [A]	0.850±0.069 (-3.911) [AB]	0.701±0.115 (-1.889) [BCDE]	0.61±0.063 (7.575) [EFG]	0.776 a±0.148 (-4.723)
20	0.679±0.072 (14.698) [CDE]	0.722±0.133 (11.735) [BCDE]	0.583±0.074 (15.261) [EFGH]	0.402±0.077 (39.090) [IJ]	0.597 b±0.152 (19.433)
30	0.490±0.010 (38.442) [GHIJ]	0.580±0.080 (29.095) [EFGH]	0.587±0.106 (14.680) [EFG]	0.414±0.062 (37.272) [IJ]	0.518 b±0.098 (30.094)
40	0.491±0.076 (38.316) [GHIJ]	0.380±0.057 (53.545) [IJ]	0.521±0.090 (24.273) [FGHI]	0.343±0.108 (48.030) [JK]	0.434 c±0.107 (41.430)
50	0.416±0.224 (47.738) [IJ]	0.373±0.086 (54.400) [IJ]	0.430±0.121 (37.500) [HIJ]	0.189±0.030 (71.363) [KL]	0.352 d±0.157 (52.496)
60	0.499±0.108 (37.311) [GHI]	0.407±0.264 (50.244) [IJ]	0.166±0.079 (75.872) [L]	0.199±0.063 (69.848) [KL]	0.318 d±0.198 (57.085)
GENOTYPES MEANS →	0.615 a±0.210	0.590 a±0.237 (4.065)	0.525 b±0.191 (14.634)	0.404 c±0.184 (34.030)	0.533±0.220
	(LSD=0.059 ; n=28)				

Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments and genotypes (lower case letter) as well as among interactions (uppercase letters).

DISCUSSION

In the experiment, chlorophyll a and b concentration decreased with increasing metal stress (Table 1and 2).Chlorophyll a, b and total chlorophyll are reported to be reduced in metal treated plants (Somashekaraiah et al., 1992; Siedlecka and Krupa, 1996; Zhang et al 2018). Reduced contents of chlorophyll might be resulted by inhibition of uptake and transportation of other essential metal elements such as Fe^{2+}, Zn^{2+}, Mg^{2+} and Mn^{2+} by antagonistic effects and competition of heavy metals with these elements (Liu et al., 2004). Reduction in chlorophyll contents under metal stress can also be due to its decomposition by increase in chlorophyllase activity (Hegedus et al., 2001). The reduced in chlorophyll contents in plants exposed to heavy metal stress are believed to be due to reduced activity of protochlorophyl lidereductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; impairment in the supply of Mg^{2+}, Fe^{2+}, Zn^{2+} and Mn^{2+} (Shanker et al., 2005) and inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA-dehydratase) (Padmaja et al., 1990) because synthesis of δ-aminolevulinic acid (ALA) is the first identified step in tetrapyrrole biosynthesis, leading to the formation of hemes and chlorophyll (Garnick and Sassa, 1971). Depleted chlorophyll contents of heavy metals treated plants might be also due to reduced ALAD activity, which results in lesser availability of porphobilinogen (PBG) required for chlorophyll biosynthesis (Prasad and Prasad, 1987).

Metal stress reduced carotenoids contents (Table 3). Reduction in carotenoids contents might be attributed to activation of osmotic stress which, in turn, activates the biosynthesis of abscisic acid (ABA). One major step in ABA biosynthesis is the carotenoid cleavage catalyzed by a 9-*cis*epoxycarotenoiddioxygenase (NCED). Carotenoids biosynthesis might be regulated at transnscriptional level as the genes controlling carotenoids biosynthesis are sensitive to the external environmental factors. However, little is known about the mechanisms of the enzyme(s) involved in carotenoids biosynthesis in plants

(Bartley and Scolnik, 1995). Another possible reason for reduction in carotenoids contents might be biosynthesis of anthocyanins. The anthocyanins are synthesized during stress and interfere with carotenoids resulting in reduced rate of photosynthesis. Such interference might be due to binding of anthocyanin with carotenoids making it unavailable for assay (Burger and Edwards, 1996).

The experimental results revealed a gradual reduction in leaf area index with increasing concentration of metal (Table 4). Leaf area reduction can be due to growth inhibition in metal treated plants (Ouariti and Ghorbal, 1997). Leaf growth reduction might be the result of low water potential due to very negative solute potential in the soil solution (Hayward and Spurr, 1944). The reduction in leaf area could be assumed due to reduction of chlorophyll contents and inhibition of photosynthetic activity; Decreased activities of many enzymes involved in the fixation of CO_2 (Barcelo, 1988) and changes in the thylakoid organization, (Fodor *et al*., 1996) may contribute to reduction in photosynthesis and ultimately growth. Reduction in growth perhaps is conducive to ROS production by stress also. Because lower osmotic potential by metal toxicity which contributed to growth reduction may be conducive to low concentration of osmotica such as carbohydrates and amino acids (Zhang *et al*., 1999). Reduced cytokinin contents by metal might be responsible for growth reduction by inhibition of cell division and cell elongation. Decreased cytokinin levels might cause a decrease in nitrate reductase activity (Bueno et al., 1994). This reduction in nitrate reductase activity might be ascribed to nutrients limitations (Andrews *et al*., 1999; Pilipovic et al., 2019).

The experimental results revealed, as a general trend, reduction in nitrate reductase activity by lead (Pb) stress (Table 5). Inhibition of NRA by metal might be caused either by reduction of enzyme biosynthesis or by suppression of activity of existing enzyme. Inhibition of NRA may happen due to destabilization of the NR thiolgroups by metal (Jones and Mhuimhneachain 1995). It has been suggested that metal binding to –SH groups of the enzyme triggers conformational changes in the enzyme causing its inactivation. Reduced NRA may be attributed to reduced N contents availability to plant either due to shortage in soil or consumption by plant (Campbell 1999). Lead (Pb) binds with sulfhydryl group of ATPase inactivating the enzyme (Kennedy and Gonsalves 1989). As a result, ATPase related processes such as plasmalemma polarization and H+ efflux may be inhibited under metal stress (Liamas et al 2000). Stress mediated decreased cytokinin levels might cause a decrease in nitrate reductase activity (Bueno et al 1994). Reduction in nitrate reductase activity owes to Phosphorus limited plants created by metal stress through reduction of kinetin (Gniazdowska and Rychter 2000). Reduction in nitrate reductase activity might be due to decreased chlorophyll contents or reduced rate of photosynthesis (Rai et al., 1992; Li et al., 2012; Zhang et al., 2018). It has been suggested that NR activity depends upon active photosynthesis or production of photosynthesis. It requires photosynthetically generated reductant (NADH) and energy (Raghuram and Sopory 1995).

The experimental results revealed some exceptions to the expected augmentation where photosynthetic pigments and leaf growth were increased by metal imposition (Table 1-4). This is in agreement with the reports of earlier workers who reported that plants tend to adapt themselves to cope-up with metal toxicity (Mangi et al.,, 1978 and Garg and Chandra, 1994). According to Oliver and Naidu (2003) plants show different reactions against Pb toxicity. Some of them are sensitive and the others have more tolerance. Plant sensitivity to heavy metals depends on a network of physiological and molecular mechanisms such as; accumulation and binding of metal to extracellular exudates and cell wall constituents during uptake; transport of heavy metals from cytoplasm to vacuoles, metal complexes formation inside the cell by various substances, osmoprotectants and osmolytes accumulation with induction of antioxidative enzymes and alteration in plant metabolism for proper functioning and repairing of stress injured cell components (Cho et al., 2003). A few cases of increase in the plant biomass due to low metal pollutants have been reported (Breckle, 1991). The absence of decline in photosynthetic pigments could be attributed to the fact that low dose of metal accumulates in roots than in the shoot and the effect is restricted to the root but not in the shoot (Selvam and Wong, 2008).

An increase in carotenoids content is suggested a defense strategy of the plants to combat metal stress (Sinha et al., 2007). Increased carotenoids contents by metal stress might be ascribed as cellular antioxidants (thiols, carotenoids, ascorbate, etc.) which may play an important role in inducing resistance against free

radicals formed during various metabolic reactions leading to oxidative stress (Kumar et al., 2002).

The increase in LAI could be due to the presence of the phenomenon of hormesis, a dose dependent response of the seedlings where the low dose stimulates the growth while high dose suppresses the growth (Peralta-Videa et al., 2001; Calabrese, 2002; Shah et al., 2008). Leaf NRA in *Vigna radiate* increased with increasing Pb (Singh et al., 1997)

Plant tolerance mechanism by osmoprotactant and antioxidants formation at low concentration of metal stress might be a reason for deviations from expected results. High level of proline, especially in roots, can eliminate hydroxyl radicals, maintain osmoregulation, prevent enzyme destruction (Kuznetsov and Shevyakova, 1997) and decrease toxicity of heavy metals (Alia and Saradhi, 1991). It has been shown lead can cause oxidative stress in plants (Verma and Dubey, 2003).

CONCLUSION

Lead concentration of 10 mg kg^{-1} was proven as nutrient while the concentrations above 10 mg kg^{-1} were proven as pollutants

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

GY and SF designed and performed the experiments. AS and SS wrote the manuscript. and analysed the data. GN and MN reviewed the manuscript. All authors read and approved the final version.

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