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Research article

Oxidative stress induced by lead in *Vigna radiata* L. seedling attenuated by exogenous nitric oxide

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Abstract: The present study deals with the effectiveness of nitric oxide (NO) on some biophysical and biochemical variables in *Vigna radiata* subjected to lead (Pb) toxicity. Pb adversely affected seedling growth and biochemical parameters of the test crop. The seedlings were grown in soil supplemented with graded concentrations of Pb. Pb toxicity caused a marked decrease in growth. Seedlings growth reduced maximum at the highest concentrations of Pb. The effect of Pb on seedlings was mitigated by NO donor sodium nitroprusside (SNP). Pre-treatment of seeds with SNP caused amelioration of detrimental effects of heavy metal stress on growth. The oxidative stress induced by Pb elevated malondialdehyde (MDA) content in the seedlings. Nitric oxide decreased MDA content in Pb treated seedlings. Exposure of seedlings to Pb enhanced antioxidant enzyme activities of the seedlings, but exogenous SNP decreased the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) in single (SNP) and SNP combined with Pb (Pb+SNP) treatment. Pb induced oxidative stress by enhancing the reactive oxygen species. SNP demonstrated a positive role against Pb toxicity which was evident from decreased activities of antioxidant enzymes. The SNP treatment enhanced plant tolerance against Pb toxicity.

Keywords: Antioxidants - Heavy metal - Stress - Lead - MDA - *Vigna radiata*.

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INTRODUCTION

Plants absorb most of the elements from the soil. Some of the engrossed elements are essential because they are required by plants to complete their life cycle. Plants also absorb heavy metals like Pb (Sharma & Dubey 2005), chromium (Hayat *et al.* 2012), mercury (Chen & Yang 2012), cadmium (Yadav & Singh 2013, Singh *et al.* 2005), and arsenic (Kumar *et al.* 2015), which show toxicity at very low concentrations. Heavy metals are present in waste water and runoff in agricultural fields which affect crop metabolism and growth of plants. Heavy metals are defined on the basis of size, density and number of atoms or position in the periodic table. Pb is a highly toxic element that occurs in soil in low concentration. Pb is not found in native form, but it forms complex with other elements in the soil. In soil, Pb contamination increased because of its wide application in gasoline, paints, water pipes, fertilizers, paper making and many other industrial processes (Pallavi & Rama 2005). Pb is highly immobile and persists for a long duration in the soil. Some plants can tolerate or avoid Pb, but others experience toxicity because it easily affects some metabolic pathways (Wierzbicka 1999). Pb has low solubility and accessibility for plant uptake (Blaylock & Huang 2000). It dissolves in water slowly. It precipitates as phosphates and sulphates and is usually transported to different plant parts from soil-rhizosphere (Blaylock & Huang 2006). Pb has a detrimental effect on plant physiological processes and modifies growth parameters, thus affects growth and productivity. Pb is known to affect the contents of photosynthetic pigments, sugar and protein (Bhardwaj *et al.* 2009). Alteration in pigments influences the biosynthesis of photo-assimilates. The detrimental expressions of Pb are in forms of chlorotic spots, senescence, necrotic lesions on the leaf surface, and stunted growth of plants. The various enzymes are inactivated by Pb binding with their SH-groups (Pinho *et al.* 2012). Sugars and proteins are energy rich compounds which provide energy for the biosynthesis of compounds required by the plants. Contents of sugar and protein are altered in plant subjected to

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unfavourable conditions. The plant membrane is the target site of heavy metals. Membrane lipids are oxidised to peroxides and reactive oxygen species are produced. Thus, permeability of the membrane is altered. Reactive oxygen species induces activities of antioxidant enzymes. Nitrate is one of the important forms of nitrogen required in the biosynthesis of amino acids, the building block of protein. The nitrate reductase enzyme which converts NO_3^- into NH_3 or NH_4^+ is induced by the substrate. Metabolic changes influence overall plant growth and health. Thus shoot length, pigments *viz*. chlorophyll and carotenoids, sugar, protein, and activities of nitrate reductase and antioxidant enzymes were taken into consideration in the present study.

SNP issued as a source of NO which acts as a signalling molecule. SNP alleviates the drastic effects of metal stress in various plant species. NO is a labile gaseous messenger molecule which involves in various physiological responses, biotic and abiotic stresses like salinity, pathogen attack, drought, herbicides, temperature and heavy metal toxicity (Wendehenne *et al.* 2005, Singh *et al.* 2017). NO has both detrimental and positive effects on plants depending on its concentration and location in the cells (Leitner *et al.* 2009).

In plant cells *in vivo* production of NO is nitrate reductase dependent in cytosol (Gill *et al.* 2013). NO production, *in vivo* is due to nitrate reductase enzyme, which converts nitrate into nitrite (Yamasaki & Sakihama 2000). The NO formation through nitrate reductase has been reported in several plant species (Xu & Zhao 2003). Plants have several enzymes *viz*. cytosolic nitrate reductase, nitric oxide synthase and xanthine dehydrogenase involved in NO production (Gill *et al.* 2013).

SNP alleviates oxidative stress in *Solanum* (Singh *et al.* 2012) and other in crops (Gill *et al.* 2013). SNP directs various signalling cascade pathways in plant cell (Neill *et al.* 2003) and initiates diverse hormone signalling pathways (Pagnussat *et al.* 2004), Ca^{++} dependent stomata closing (Garcia-Mata *et al.* 2003) and change in gene expression (Hao & Zhang 2010, Hasanuzzaman *et al.* 2010). Exogenous application of SNP mitigates the toxicity of heavy metals like cadmium in rice (Hsu & Kao 2004), copper (Yu *et al.* 2005), and aluminium (Zhang *et al.* 2008) and allelochemicals (Singh *et al.* 2012).

The survey of the literature reveals that there are comprehensive studies on the effect of SNP on *Vigna radiata* L. seedlings stressed by Pb. The study was undertaken to observe alteration of plant biophysical processes by lead and amelioration of toxic effects by SNP as a growth regulator.

MATERIALS AND METHODS

Seeds of *Vigna radiata* L. (mungbean) were procured from certified seed agency of Allahabad, Uttar Pradesh, India. Lead in form of lead acetate [$(CH_3.COO)_2Pb3H_2O$] (Molecular weight 379.33g.mol^{-1}) was purchased from LobaChemie and sodium nitroprusside ($Na_2[Fe(CN)_5NO].2H_2O$) [sodium pentacyanonitrosyl ferrate (II)](molecular weight 297.95g.mol^{-1}) from Merck.

Growth and pot culture

The experiment was conducted in May, 2014 in the glasshouse in the Department of Botany, University of Allahabad, Allahabad (24^{o}47’ and 50^o 47’N latitude; 81^o 91’ and 82^o 21’E longitude; 78 m above sea level). Healthy seeds of *Vigna radiata* L. were selected and surface sterilized with 0.1% $HgCl_2$ for 5 min and then washed thoroughly with double distilled water (DDW). One set of the seeds was soaked in DDW and other set was soaked in SNP (250 µM) for 3 hours. The fire clay pots (height 7 cm and diameter 21 cm) each filled with 1 kg of garden soil, were divided into two sets. One set of pots was without Pb and other contained varying concentrations of Pb, 0.5g (Pb_1), 1g (Pb_2) and 2g (Pb_3) per kg of soil according to the treatments. The first set was divided into two subsets; in one subset of pots seed soaked in distilled water were sown (C) while in other subset seeds soaked in SNP were sown (SNP). The other set of pots with Pb was divided into two subsets. In one subset of pots DDW soaked seeds were sown and in other subset SNP treated seeds were sown. The **e**xperiment was conducted in triplicate in glass house condition. After 14 days first fully expanded leaves of the seedlings were sampled for measurement of biophysical and analyses of biochemical parameters.

Determination of growth

The seedling growth was measured as shoot length of the plants. The seedlings were uprooted from the pots and washed gently with tap water to remove soil particles. The plants were blotted with filter papers to remove excess of water and measured the length of shoots by using a meter scale.

Photosynthetic pigments

The amount of photosynthetic pigments was quantified following the method of Lichtenthaler (1987). Fresh leaves (100 mg) of the seedlings were homogenised in 80% (v/v) acetone. The extracts were centrifuged until

the supernatant became clear. The absorbance of the resulting solutions was recorded on 663, 645, and 470 nm, spectrophotometrically (Shimadzu single beam UV–visible spectrophotometer-1700).
The pigments were quantified by the following formulae:

Chlorophyll a ($\mu g.ml^{-1}$) = $12.21xA_{663}$-$2.81xA_{645}$

Chlorophyll b ($\mu g.ml^{-1}$) = $20.13xA_{645}$-$5.03xA_{663}$

Total chlorophyll ($\mu g.ml^{-1}$) = ($20.2x\ A_{645}$+$8.02x\ A_{663}$)

Carotenoids ($\mu g.ml^{-1}$) = [$1000xA_{470}$-3.27(Chl. a)-104(Chl. b)]/198

Where A is the observed OD

Sugar and Protein content

The quantification of total soluble sugar (TSS) was done following Hedge& Hofreiter (1962). About 50 mg of the sample was homogenized in 3 ml 95% ethanol. After centrifugation 0.1 ml of supernatant was mixed with 4 ml anthrone reagent and heated in water bath at 95ºC temperature and cooled. Absorbance was recorded at 620 nm. The amount of sugar was determined by the standard curve prepared from glucose.

Protein content was determined according to the method of Lowry *et al.* (1951). Ten mg of first fully expanded fresh leaves of the plants were homogenized with 1 ml 1 N NaOH for 5 minutes at 100^{o}C. Five ml of alkaline copper reagent were added to it and the mixture was allowed to stand at room temperature for 10 minutes followed by addition of 0.5 ml of Folin-Ciocalteau reagent immediately in the tube. The absorbance of the solution was measured at 650 nm after 30 minutes. The standard curve of bovine serum albumin was used to calculate the amount of protein.

Lipid peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) content determined by thiobarbituric acid reactive substance as described by Heath & Packer (1968). Fresh leaf (200 mg) was homogenized in 0.1% (w/v) trichloroacetic acid and centrifuged at 10,000g for 10 min. Malondialdehyde level was used as index of lipid peroxidation and was expressed as nmol.g-^{1} FW. One millilitre supernatant was mixed with 4 ml 0.5% thiobarbituric acid. The mixture was heated at 95°C for 30 min and centrifuged after cooling. The absorbance of the supernatant was recorded at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction co-efficient of 155 $mM^{-1}.cm^{-1}$ and expressed as n $mol.g^{-1}$ FW.

Nitrate reductase activity

Nitrate reductase (NR) activity was measured following the p procedure of Jaworski (1971). Fresh leaf tissue (250 mg) was incubated in 4.5 ml medium which contained 100 mM phosphate buffer (pH 7.5), 3% KNO_3 and 5% propanol. About 0.4 ml aliquot was treated with 0.3 ml 3% sulphanilamide in 3N HCl and 0.3 ml 0.02% N-1-naphthyl ethylene diamine dihydrochloride. The absorbance was measured at 540 nm. NRactivity was calculated with a standard curve prepared from $NaNO_2$ and expressed in µmol NO_2g^{-1} FW h^{-1}.

Extraction and assay of activities of antioxidant enzymes

Enzyme extract was prepared by homogenizing leaves with 0.1 M sodium phosphate buffer (pH 7.0) containing polyvinyl pyrrolidone. The homogenate was centrifuged at 4^{o}C at 15000 g for 30 min in cooling centrifuge (Remi instruments C 24). The supernatant was used for the assay of superoxide dismutase, catalase and peroxidase.

Superoxide dismutase assay

The activity of superoxide dismutase (SOD) was determined by the nitroblue tetrazolium (NBT) photochemical assay following Beyer & Fridovich (1987). The reaction mixture (4 ml) consisted of 20mM methionine, 0.15mM ethylene diamine-tetra-acetic acid, 0.12 mM NBT, 1.3 M riboflavin, 0.05 M sodium carbonate (pH 10.2) and enzyme extract. Test tubes were exposed to fluorescent lamp for 30 min and identical unilluminated assay mixture served as blank. The photo reduction of NBT (formation of purple formazone) was recorded spectrophotometricallyat 560 nm and compared with blank sample having no enzyme extract. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT.

Catalase assay

Catalase (CAT) activity was assayed following Cakmak & Marschner (1992). Assay mixture contained 25 mM potassium phosphate buffer (pH 7.0), (0.2 ml) 10 mM H_2O_2 and 0.5 ml enzyme extract. The rate of H_2O_2 decomposition for 1 min was monitored at 240 nm and calculated using extinction coefficient of 39.4 $mM^{-1}.cm^{-1}$ and expressed as enzyme unit g^{-1} FW. One unit of CAT was determined as the amount of enzyme required to oxidize 1 mM H_2O_2 min^{-1}.

Peroxidase assay

Peroxidase (EC 1.11.1.7) activity was assayed following McCune & Galston (1959). Fresh leaf tissue (0.2 g) was homogenized in 0.1 M potassium phosphate buffer (pH 6.0) and centrifuged at 10,0009 g for 20 min at 4ºC. The reaction mixture contained 2.0 ml enzyme extract, 2 ml potassium phosphate buffer, 1.0 ml 0.1 N pyrogallol and 0.2 ml 0.02% H_2O_2 and determined spectrophotometrically at 430 nm. One unit of enzyme activity was defined as the amount which produced an increase of 0.1 OD per minute.

Statistical analysis

Data represent the mean ± standard errors of results from three data were statistically analysed using analysis of variance (ANOVA) by using SPSS software (Version 16 SPSS Inc., Chicago, IL, USA). The appropriate standard error of means was calculated for presentation with tables and graphs. The treatment means were analysed by Duncan's multiple range test (DMRT) at level of $P < 0.05$.

RESULTS

Growth

One time pre-soaking of seeds was effective as it caused the significant modification in biophysical and biochemical parameters of *Vigna.* Hayat *et al.* (2012) also recorded the effects of SNP on tomato seeds soaked in SNP for eight hours. Pre- soaking of tomato seeds with SNP solution of 250 µM concentration was effective to cause maximum growth during preliminary experiments (Singh *et al.* 2012).

The growth of seedlings was measured in terms of shoot length. Shoot length decreased significantly (P<.05) in treatments with Pb. The decrease of 6, 23 and 34% in shoot length, was recorded in the seedlings with Pb1, Pb2 and Pb3 treatments respectively. Pre-soaking with SNP without Pb enhanced the shoot length of the seedlings by 12.5% as compared with control. The seedlings pre-soaked with SNP and treated with Pb_1, Pb_2 and Pb_3 showed an alleviation of 3, 14 and 14% in shoot length respectively (Fig. 1A).

Photosynthetic pigments

The amounts of photosynthetic pigments *viz.* Chl *a*, Chl *b*, total Chl and carotenoids are shown in table 1. The contents of Chl *a*, Chl *b*, total Chl and carotenoids declined with graded doses of Pb in Pb_1, Pb_2 and Pb_3 treatments. The detrimental effect of Pb is evident in Chl *a*, Chl *b*, total Chl and carotenoids and decrease of 72, 86, 76 and 88% was recorded in the highest dose of Pb. However in the seedlings pre-soaked with SNP without Pb photosynthetic pigments were greater than that of Pb and control. The Pb treated seedlings pre-soaked with SNP caused significant improvement in Chl *a*, Chl *b*, total Chl and carotenoids contents but were always lower than that of control.

Table 1. Effect of SNP on photosynthetic pigments in seedlings of *Vigna radiata* grown under lead stress.

Treatment	Chl a ($mg.g^{-1}FW$)		Chl b ($mg.g^{-1}FW$)		Total Chl ($mg.g^{-1}FW$)		Carotenoids ($mg.g^{-1}FW$)	
	-SNP	+SNP	-SNP	+SNP	-SNP	+SNP	-SNP	+SNP
C	7.17±0.34b	9.31±0.47a	2.53±0.18b	4.05±0.14a	9.70±0.51b	13.37±0.34a	1.84±0.31b	2.71±0.35a
Pb_1	5.07±0.56c	7.05±0.47b	1.05±0.10d	1.42±0.16c	6.13±0.652c	8.47±0.61b	0.90±0.12d	1.57±0.14bc
Pb_2	4.04±0.56e	5.44±0.53cd	0.56±0.29e	0.95±0.22d	4.60±0.27d	6.39±0.30c	0.61±0.14de	1.07±0.28c
Pb_3	1.96±0.58f	3.35±0.69e	0.36±0.08f	0.46±0.16e	2.33±0.49e	3.82±.53d	0.21±0.14f	0.70±0.14de

Note: Data are means ± standard error of three replicates. Bars followed by different letters show significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test.

Sugar and Protein

Total soluble sugar (TSS) declined by 15, 23 and 36% and protein 20, 48 and 75% in Pb_1, Pb_2 and Pb_3 treatments respectively as compared with control. Exposure to SNP enhanced TSS and protein contents to 13 and 28% respectively as compared with control (Fig. 1B–C).

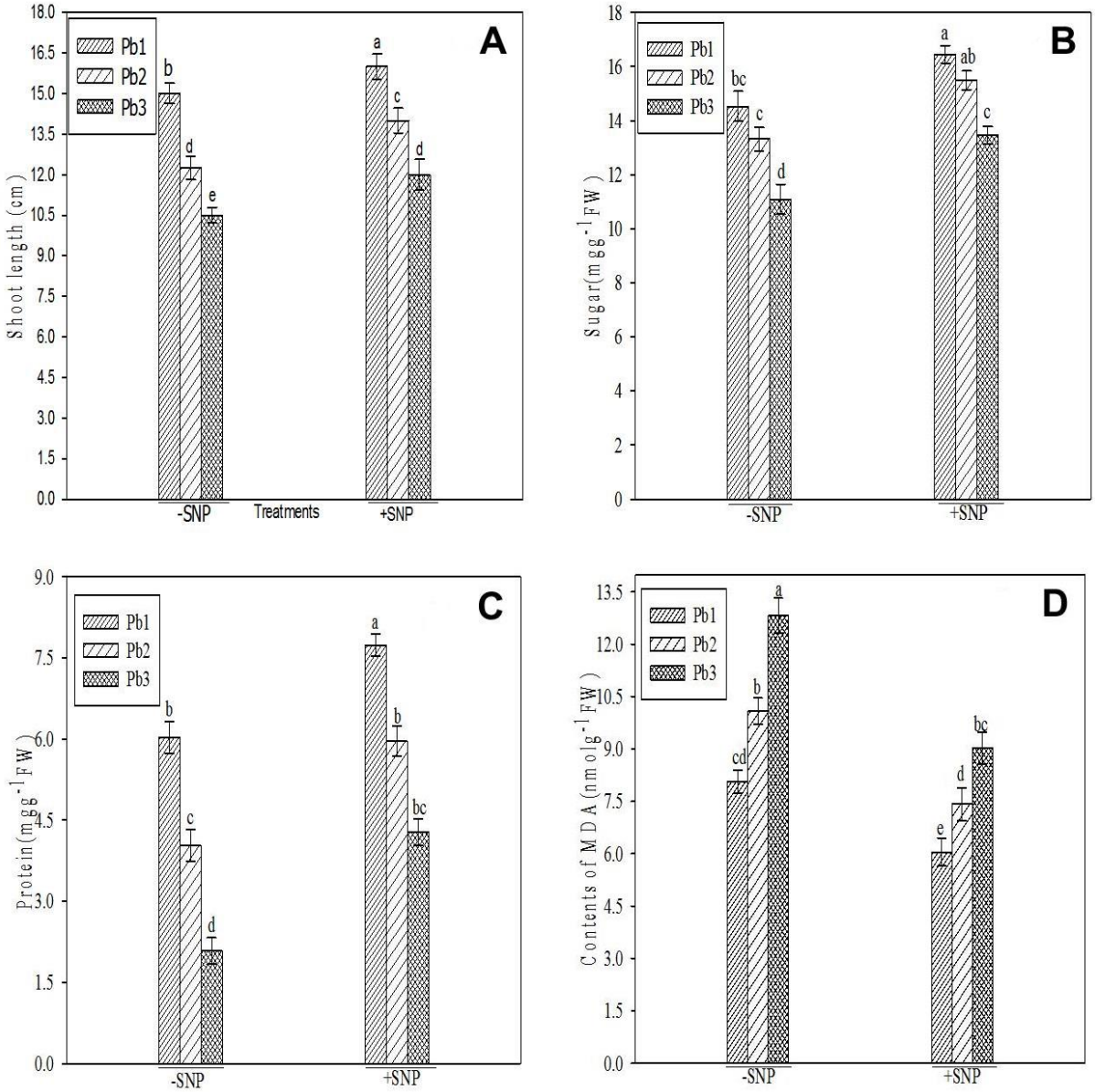

Figure 1. Effect of NO in seedlings of *Vigna radiata* under lead stress: **A,** Shoot length; **B,** Sugar; **C,** Protein; **D,** Lipid peroxidation. [Data are means± standard error of three replicates. Bars followed by different letters show significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test]

Lipid peroxidation and nitrate reductase activities

The content of MDA increased significantly in the seedlings treated with graded concentrations of Pb. The lipid peroxidation (LP) increased by 72, 115 and 174% in the seedlings under Pb_1, Pb_2 and Pb_3 treatments respectively. MDA content in SNP treated seedlings decreased to 53% as compared with control. SNP caused a decrease in the level of MDA content in the seedlings under the influence of Pb as compared with treatments with the respective concentration of Pb but was always higher than that of control (Fig. 1D).

The nitrate reductase activity in the leaves of *Vigna* seedlings was affected by Pb. The activity of NR decreased in dose-dependent manner. The maximum 45% inhibition of NR was recorded in the seedlings treated with Pb_3. Maximum 15% increase in NR activitywas recorded in the seedlings preexposed to SNP. The activities of NR enhanced by 8.7,18 and 20% in the seedlings pre exposed to SNP in Pb_1, Pb_2 and Pb_3 treatments respectively (Fig. 2a).

Activities of antioxidative enzymes

The result pertaining to the activities of antioxidative enzymes *viz*. superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in leaves are presented in figure 2B–D. The activities of SOD, CAT and POD increased in treatments with Pb in concentration dependent manner. The activities of SOD increased by 25, 67

and 87% CAT by 49, 76 and 145% and POD by 17, 34 and 64%, in the seedlings exposed to Pb_1, Pb_2 and Pb_3 treatments respectively. The activities of antioxidant enzymes recorded at the lowest level in the seedlings, pre treated with SNP. The activities of antioxidative enzymes exhibited a significant reduction in the seedlings grown from the seeds pre-treated with SNP and exposed to Pb. The exposure of seedlings to SNP declined the activities of SOD, CAT and POX by 9, 20 and 12% respectively.

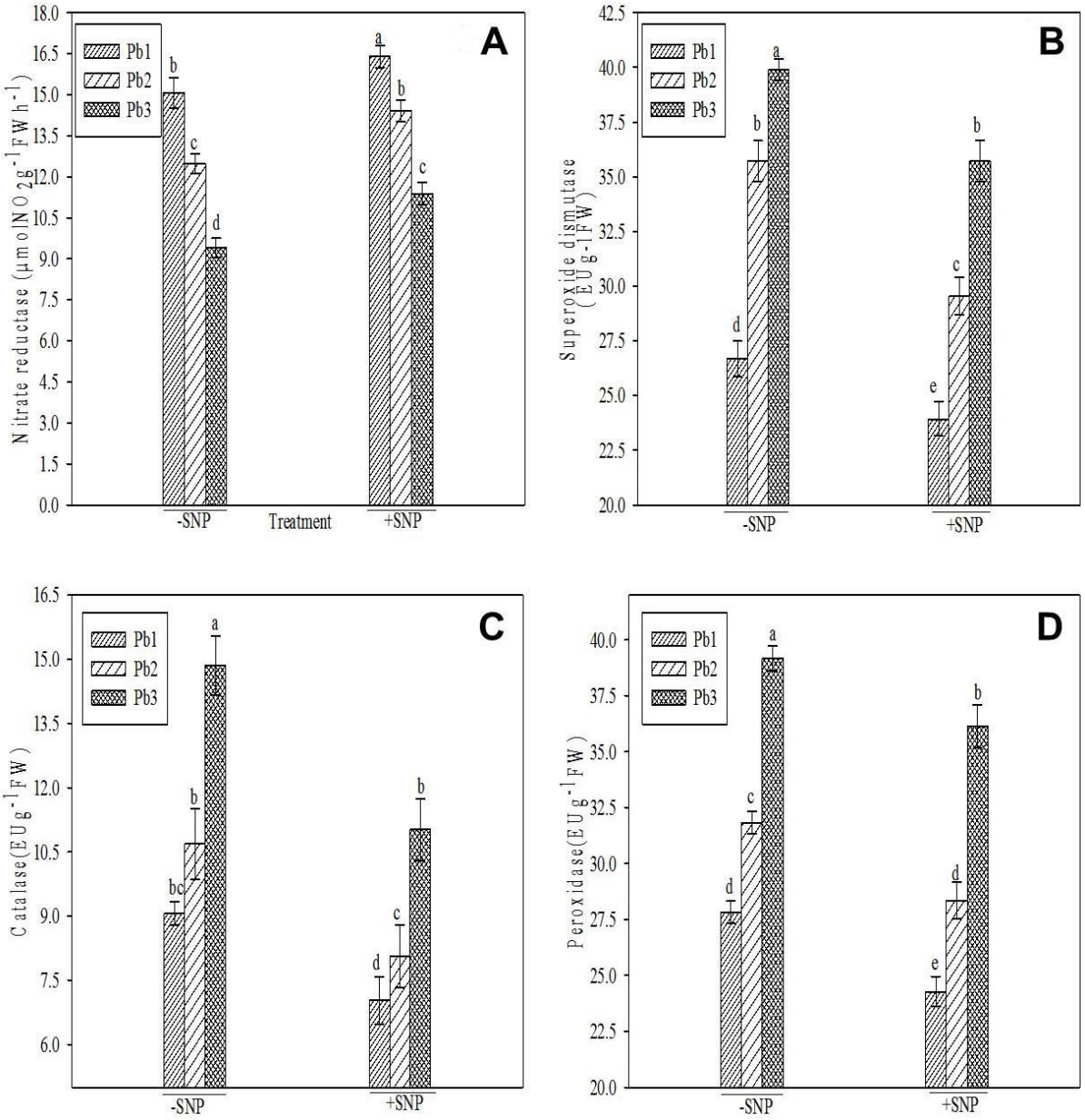

Figure 2. Effect of NO in seedlings of *Vigna radiata* under lead stress: **A,** Nitrate reductase; **B,** Superoxide dismutase; **C,** Catalase; **D,** Peroxidase. [Data are means± standard error of three replicates. Bars followed by different letters show significant differences at $P < 0.05$ significance level between treatments according to the Duncan's multiple range test]

DISCUSSION

Growth is the key factor for evaluating plant responses to various biotic and abiotic environmental stresses. The growth of the seedlings decreased significantly as the concentration of Pb increased. The growth reduction may be due to decrease in photosynthetic pigments and increased lipid peroxidation. Singh *et al.* (2003) also reported decrease in growth parameters of *Vigna radiata* under lead stress. The inhibition in growth parameters by Pb stress has been observed in various plant species as a consequence of deregulation of various primary physiological and metabolic processes (Singh *et al.* 1997a, b). Exogenous SNP appears to provide protection to plant against Pb toxicity which is evident from increased plant growth. The application of exogenous SNP significantly ($P<0.05$) mitigated Pb toxicity.

The photosynthetic pigments in Pb treated seedlings decreased progressively with increased concentration of Pb. Reduction in the content of Chl and carotenoids in the seedlings under stress induced by Pb may be due to the inhibitory effect of Pb on the enzyme which involved in pigment biosynthesis. Pb stress, either inhibited biosynthesis or stimulated degradation of photosynthetic pigments. The decrease in chlorophyll contents resulted in decreased biomass production and inhibition of seedling growth. The Pb toxicity affected various biochemicals, morphological and physiological processes which caused stunted plant growth (Wang *et al.* 2003). According to Pinho *et al.* (2012) Pb deactivates several enzymatic processes by binding with SH-groups causing impairment of metabolic activities which may be responsible for reduced plant growth.

The present study showed that Pb decreased protein content. The decrease of protein content may be due to inhibition of protein synthesis or degradation of protein (Palma *et al.* 2002). Costa & Spitz (1997) recorded decrease in protein content in *Lupinus albus* under heavy metal stress. Oxidative stress caused by Pb may be another possible reason for the decrease of protein content (Bharwana *et al.* 2013). Exogenous supply of SNP increased protein concentration (Hsu & Kao 2004). The heavy metal in plants caused impairment of metabolic activities which gradually decreased sugar contents. The decreased photosynthetic pigments inhibited photosynthetic activity which reduced sugar content (Hussain *et al.* 2013). According to Stiborova *et al.* (1987) heavy metals interact with ribulose biphosphate carboxylase reactive centre and show the negative impact by inhibiting the carboxylation. Our results reveal that photosynthetic pigments, total soluble sugar and protein content decreased in presence of Pb. These findings are in agreement with Bhardwaj *et al.* (2009).

Heavy metal stress elevated the level of MDA. Impaired metabolic activities caused by Pb resulted in the generation of reactive oxygen species which enhanced oxidative stress leading to lipid peroxidation and membrane damage. In the present study higher concentration of Pb ($2g.kg^{-1}$) increased MDA content to the maximum. Increase in MDA content was also recorded by Mroczek-Zdgrka & Wojick (2012) in Pb treated *Vicia faba* seedlings. Differential reduction in MDA content is evident in treatment with SNP. The decreased MDA content was also observed in wheat seedlings treated with SNP (Singh *et al.* 2008). NO is known to inhibit ion leakage and protect plants tissue against membrane damage by lipid peroxidation (Beligini *et al.* 2002). This appears that SNP protects membrane from damage caused by Pb stress.

Nitrate reductase activity decreased in higher concentration of Pb. NR activity may also be inhibited by the NADH regulated supply of NO_3^- at the site of enzyme synthesis (Burzynski & Grabowski 1984, Dabas 1992). Nitrate reductase is the substrate induced enzyme. Decreasein NR activity may be due to restricted supply of $NO_3^-$to the roots. Pb appears to interfere with absorption of NO_3^- from the soil. It may ensure the absorption of NO_3^- to the roots and increases NR activity to the maximum.

Plants have antioxidant defence systems to maintain their growth and health. Plants face oxidative damage when exposed to Pb and various heavy metals (Bharwana *et al.* 2013). Plants develop a machinery of antioxidant system to prevent the oxidative damage. In the present study activities of antioxidative enzymes like SOD, CAT and POD increased in the seedlings under Pb stress. Heavy metals increase the activities of SOD in *Avena sativa* L. (Luna *et al.* 1994) and *Oryza sativa* L. (Verma & Dubey 2003). This evinced over production of ROS in the seedlings under the influence of Pb stress.

The SNP is known to increase or decrease the activities of antioxidant enzymes. According to Zhang *et al.* (2008) SNP enhanced the activities of antioxidants. Plants have antioxidant potential to scavenge ROS and have greater stress tolerance (Ashraf & Akram 2009). Exogenous NO decreased the activities of antioxidant enzymes, levels of H_2O_2 and MDA in the maize plants exposed to saline stress (Kaya *et al.* 2015). In the present study decreased activities of antioxidant enzymes on the application of SNP showed that plant mitigated the Pb stress to maintain plant health. Thus NO by decreasing the activities of antioxidant enzymes prevented the energy diversion from the biosynthesis of antioxidative enzymes and thus supported plant growth. Decreased activity of SOD may be correlated with decreased level of lipid peroxidation and enhanced level of photosynthetic pigments. These alterations protected plants against oxidative stress in various stress conditions (Laspina *et al.* 2005, Hung & Kao 2003). The decreased activity of SOD reflects that the production of ROS (O_2^-) is low due to the action of NO (Caro & Puntraulo 1998). Plants pre-treated with SNP in different concentrations (100 and 200 µM) decreased POD activity (Ferreir *et al.* 2010). SNP inhibits activities of CAT and POD in *Nicotiana tabacum* (Clark *et al.* 2000). Xiong *et al.* (2010) reported that rice seedlings pre-treated with NO decreased ROS production and prevented Pb dependent negative impacts. It is evident that SNP inhibits production of ROS and regulates the activities of antioxidant enzymes. In plants, the growing in normal environmental condition the production of ROS corresponds to the activities of antioxidant enzymes. SNP buttresses the defence system of

Vigna seedlings treated with Pb. Concentration dependent increase in SOD activities was recorded in Pb. POD and CAT detoxify H_2O_2 produced by the activities of SOD. CAT appears to play a major role in detoxification of H_2O_2 produced in response to Pb treatment of *Vigna* seedlings. SNP decreased the activities of antioxidant enzymes in Pb treated seedlings. Thus, SNP prevents the energy diversion required to increase the activities of antioxidant enzymes. The SNP appears to mitigate the toxic effect of Pb on the *Vigna* seedlings.

CONCLUSION

In the present study, SNP appears to play a significant role to improve growth and development of the *Vigna* seedlings exposed to the Pb stress. It is evident that Pb decreased growth, pigments, sugar, protein and nitrate reductase activity and adversely affected the metabolism of the seedlings. The increase in MDA content is an index of oxidative stress caused by Pb. The activities of antioxidant enzymes SOD, CAT and POD were stimulated to cope with the oxidative stress caused by Pb. SNP mitigated the Pb toxicity and played important role to buttress antioxidant defence system. Exogenous application of SNP protected *Vigna radiata* L. seedlings against Pb toxicity.

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REFERENCES

Akram MS, Ashraf M & Akram NA (2009) Effectiveness of potassium sulfate in mitigating salt-induced adverse effects on different physio-biochemical attributes in sunflower (*Helianthus annuus* L.). *Flora* 204: 471–483.

Beligini MV & Lamattina L (2002) Nitric oxide interferes with plant photoxidative stress by detoxifying reactive oxygen species. *Plant, Cell & Environment* 25: 737–748.

Beyer WF & Fridovich I (1987) Assaying for superoxide dismutase activity some large consequences of minor changes in conditions. *Analytical Biochemistry* 161: 559–566.

Bhardwaj P, Chaturvedi1 AK & Prasad P (2009) Effect of Enhanced Lead and Cadmium in soil on Physiological andBiochemical attributes of *Phaseolus vulgaris* L. *Nature and Science* 7: 8.

Bharwana SA, Ali S, Farooq MA, Iqbal, Abbas F & Ahmad MSA (2013) Alleviation of Lead Toxicity by Silicon is Related to Elevated Photosynthesis, Antioxidant Enzymes Suppressed Lead Uptake and Oxidative Stress in Cotton. *Journal of Bioremediaton & Biodegradation* 4: 4.

Blaylock MJ & Huang JW (2000) Phytoextraction of metals. In: Raskin I & Ensley BD (eds) *Phytoremediation of Toxic Metals: Using Plants To Clean Upthe Environment*. John Wiley & Sons, New York, NY, USA, pp. 53–71.

Burzynski M & Grabowski A (1984) Influence of lead onnitrate uptake and reduction in cucumber seedlings. *Acta Societatis Botanicorum Poloniaet* 53: 77–86.

Cakmak I & Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. *Plant Physiology* 98: 1222–1227.

Caro A & Puntarulo S (1998) Nitric oxide decreases superoxide anion generation by microsomes from soybean embryonic axes. *Physiologia Plantarum* 104: 357–364.

Chen J & Yang ZM (2012) Mercury toxicity, molecular response and tolerance in higher plants. *Biometals* 25: 847–857.

Yu CC, Hung KT & Kao CH (2005) Nitric oxide reduces Cu toxicity and Cu-induced NH^{4+}accumulation in rice leaves. *Journal of Plant Physiology* 162: 1319–1330.

Clark D, Dunar, Navarre J & Klessig DA (2000) Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. *Molecular Plant Microbe- Interactions* 13: 1380–1384

Costa G & Spitz E (1997) Influence of cadmium onsoluble carbohydrates, free amino acids, proteincontent of *in vitro* cultured *Lupinus albus*. *Plant Science* 128: 131–140.

McCune DC & Galston AW (1959) Inverse effects of gibberellin on peroxidase activity and growth in dwarf strains of peas and corn. *Plant Physiology* 34: 416–418

Dabas S (1992) *To study the effect of lead on efficiency of nitrogen fixation and nitrogen assimilation in Vigna*

radiata L. Wilczek, *Ph.D. Thesis*. M.D. University, Rohtak, India.

Ferreira LC, Cataneo AC, Ramazzini LM, Corniani RN, Fumis T de F, de Souza YA, Scavroni J & Soares BJA (2010) Nitric oxide reduces oxidative stress generated by lactofen in soybean plants. *Pesticide Biochemistry and Physiology* 97: 47–54.

Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L & Blatt MR (2003) Nitricoxide regulated K and Cl channels in guard cells through a subset of abscisic acid-evoked signallingpathways. *Proceedingsof the National Academy of Sciences U.S.* 100: 11116–11121.

Gill SS, Hasanuzzaman M, Kamrun N, Macovei A & Tuteja N (2013) Importence of nitric oxide in cadmium stress tolerance in crop plants. *Plant Physiology and Biochemistry* 63: 254–261.

Hao GP & Zhang JH (2010) The role of nitric oxide as a bioactive signalling moleculein plants under abiotic stress. In: Hayat S, Mori M, Pichtel J & Ahmad A (eds) Nitric Oxide in Plant Physiology.Wiley-VCH Verlag, Weinheim, pp. 115–138.

Hasanuzzaman M, Hossain MA & Fujita M (2010) Physiological and biochemical mechanisms of nitric oxide induced abiotic stress tolerance in plants. *Journal of Plant Physiology* 5: 295–324

Hayat S, Khalique G, Irfan M, Wani AS, Tripathi BN & Ahmad A (2012) Physiological changes induced by chromium stress in plants: an overview. *Protoplasma* 249(3): 599–611.

Hayat S, Yadav S, Wani AS, Irfan M, Alyemini MN & Ahmad A (2012) Impact of Sodium Nitruprusside on nitrate reductsae, proline content and antioxidant system in tomato under salinity stress. *Horticulture, Environment and Biotechnology* 53(5): 362–367.

Heath RL & Packer L (1968) Photo-peroxidation in isolatedchloroplasts 1. Kinetics and stoichiometry of fatty acidperoxidation. *Archives Biochemistry Biophysics* 125: 189–198.

Hedg JE & Hofreiter BT (1962) *Estimation of carbohydrate - Methods in carbohydrate chemistry*. Academic Press, New York, USA, pp. 17–22.

Hsu YT & Kao CH (2004) Cadmium toxicity is reduced by nitric oxide in rice leaves. *Plant Growth Regulation* 42: 227–238.

Hussain A, Abbas N, Arshad F, Akram M, Iqbal Khan Z, Ahmad K, Mansha M & Mirzaei F (2013) Effects of diverse doses of Lead (Pb) on different growth attributes of *Zea-Mays* L. *Agricultural Sciences* 4: 262–265.

Jaworski E (1971) Nitrate reductase assay in intact planttissue. *Biochemical and Biophysical Research Communication* 43: 1274–1279.

Kaya C, Ashraf M, Sonmez O, Tuna AL & AydemirS (2015) Exogenously applied nitric oxide confers tolerance to salinity-induced oxidative stress in two maize (*Zea mays* L.) cultivars differing in salinity tolerance. *Turkish Journal of Agriculture and Forestry* 39: 1411–1426.

Kumar S, Dubey RS, Tripathi RD, Chakrabarty D & Trivedi PK (2015) Omics and biotechnology of arsenic stress and detoxification in plants: *Current Updates and Prospective* 74: 221–230.

Laspina NV, Groppa MD, Tomaro ML & Benavides MP (2005) Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant Science* 169: 323–330.

Leitner M, Vandelle E, Gaupels F, Bellin D & Delledonne M (2009) NO signals in the haze nitric oxide signalling in plant defence. *Current opinions in Plant Biology* 12:451–458.

Lichtenthaler HK (1987) Chlorophyll and carotenoids, pigments of photosynthetic bio-membranes. In: Packer L & Douce R (eds) *Methods in Enzymology*. Academic Press, Washington, pp.350–382.

Lowary OH, Rosebrough NJ, FanAL & Randall RI (1951) Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193: 265–275.

Luna CM, Gonzalez CA & Trippi VS (1994) Oxidative damage caused by an excess of copper in oat leaves. *Plant Cell Physiology* 35: 11–15.

Mroczek-Zdyrska M & Wojcik M (2012) The influence of seleniumon root growth and oxidative stress induced by lead in*Vicia faba* L. minor plants. *Biological Trace Element Research* 147: 320–328.

Neill SJ, Desikan R & Hancock JT (2003) Nitric oxide signalling in plants. *New Phytologist* 159: 11–35.

Nwugol CC & Huerta AJ (2008) Silicon-induced cadmium resistance in rice (*Oryza sativa*). *Journal of Plant Nutrition and Soil Sciences* 171: 841–848.

Pagnussat GC, Lanteri ML, Lombardo MC & Lamattina L (2004) Nitric oxidemediates the indole-acetic acid activation of a mitogen-activated proteinkinase cascade involved in adventitious root formation. *Plant Physiology* 135: 279–286.

Palma JM, Sandalio LM, Javier Corpas F, Romero-Puertas MC, Mccarthy I & del Rio LA (2002) Plantproteases protein degradation and oxidative stress: role of peroxisomes. *Plant Physiology and Biochemistry* 40: 521–530.

Sharma P & Dubey RS (2005) Lead toxicity in plants. *Brazilian Journal of Plant Physiology* 17(1): 35–52.

Pinho S & Ladeiro B (2012) Phytotoxicity by Lead as Heavy Metal Focus on Oxidative Stress. *Journal of Botany* 2012: Article ID 369572.

Singh H, NB Singh, Singh A, Hussain I & Yadav V (2017) Physiological and biochemical roles of nitric oxide against toxicity produced by glyphosate herbicide in *Pisum sativum. Russian Journal of Plant Physiology* 64: No. 4.

Singh HP, Batish DR, Kaur G, Arora K & Kohali RK (2008) Nitric oxide (as sodium nitroprusside) supplementation ameliorates Cd toxicity in hydroponically grown wheat roots. *Environmental and Experimental Botany* 63: 158–167.

Singh NB, Kavita Y & Nimisha A (2012) Positive effects of nitric oxide on *Solanum lycopersicum*. *Journal of Plant Interactions* 9: 10–18.

Singh NB, Singh H & Sunaina (2015) Protective role of exogenous SNP against heavy metal toxicity in *Brassica oleracea* (var. Capitata). *Iranian Journal of Plant Physiology* 5 (4): 1449–1456.

Singh RP, Choudhary A, Gulati A, Dahiya HC, Jaiwal PK & Sengar RS (1997a) Response of plant to salinity in interaction with other Abiotic and biotic factors. In: Jaiwal PK, Singh RP & Gulati A (eds) *Strategies for Improving Salt Tolerance in Higher Plants*. Science Publishers, En. Field, N. H., pp. 25–39.

Singh RP, Tripathi RD, Dabas S, Rizvi SMH, Ali MB, Sinha SK, Gupta DK, Mishra S & Rai UN (2003) Effect of lead on growth and nitrate assimilation of *Vigna radiata* (L.) Wilczek seedlings in a salt affected environment. *Chemosphere* 52: 1245–1250.

Singh RP, Tripathi RD, Sinha SK, Maheshwari R & Srivastava HS (1997b) Responses of higher plants to lead contaminated environment. *Chemosphere* 34: 2467–2493.

Stiborova M, Ditrichova M & Brezinova A (1987) Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley and maize seedlings. *Biologia Plantarum* 29: 453–467.

Verma S & Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science* 164: 645–655.

Wang W, Vinocur B & Altman A (2003) Plant responses todrought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218(1): 1–14.

Wendehenne D, Gould K, Lamotte O, Durner J, Vandelle E, Lecourieux D, Courtois C, Barnavon L, Bentejac M & Pugin A (2005) NO signalling functions in the biotic and abiotic stress responses. *BMC Plant Biology* 5: S35.

Wierzbicka M (1999) Comparison of lead tolerance in *Allium cepa* with other plant species. *Environtal Pollution* 104:41–52.

Xiong J, Fu G, Tao L & Zhu C (2010) Roles of nitric oxide in alleviating heavy metal toxicity in plants. *Archives Biochemistry and Biophysics* 497: 13–20.

Xu YC & Zhao BL (2003) The main origin of endogenous NO in higher non-leguminous plants. *Plant Physiology and Biochemistry* 41: 833–838.

Yadav K & Singh NB (2013) Effect of benzoic acid and cadmium toxicity on wheat seedlings. *Chlilan Journal of agricultural research* 3(2): 168–174.

Yamasaki H & Sakihama Y (2000) Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR dependent formation. *FEBS Letters* 468: 89–92.

Yu CC, Hung KT & Kao CH (2005) Nitric oxide reduces Cu toxicity and Cu-induced NH^{4+} accumulation in rice leaves. *Journal of Plant Physiology* 162: 1319–1330.

Zhang H, Li YH, Hu LY, Wang SH, Zhang FQ & Hu KD (2008) Effect of exogenous nitric oxide donor on antioxidant metabolism in wheat leaves under aluminium stress. *Russian Journal of Plant Physiology* 55: 469–474.