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CADMIUM AND LEAD BEHAVIOUR AT THE PLANT-SOIL INTERFACE: IMPLICATIONS IN PHYTOREMEDIATION

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Abstract

Heavy metals are natural elements of the world's crust, yet their geochemical and biochemical balance has changed drastically through indiscriminate human activities. When treatment T4 was compared to T0 the EC was increased significantly with 15.10% and 13.75% on the proposed date of intervals. It is evident that in the case of cadmium metal stress (T1), the average OP was substantially increased by 16.8 and 39.79 in comparison with control (T0) at dates of 60 and 90 DAS. In comparison to control (T0) at the dates of 60 and 90 DAS of the interval, it is clear that the average MSI decreased substantially with 60.41 and 84.54% when exposed. In comparison to control (T0) at intervals 60 and 90 DAS, the average CAM was significantly increased with 32.56 percent and 34.12 percent.

***Keywords*:** Cadmium, Dose, Forage, Gap, Higher, Lead.

Introduction

Cadmium one of the most toxic heavy metals having an upper limit is 14.157 µg/g. Effects of Cd, according to Sharmila *et al*. 2017, when mustard exposed to Cd_2^+affects the growth of the plant and reduces the activity of photosystem II with a rise in the level of proline. Affect the oxidative phosphorylation in mitochondria and water uptake (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al*. (2018b), Kumar, P., Misao, L., *et al*., 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al*., 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al*., 2019g, Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i); Linear increase in amount and production of MDA and H_2O_2 during stress in roots of chickpea; inhibits the plant growth by stimulating ROS; affects the leaves, shoot, Significant reduction in amount of nitrogen, phosphorus and chlorophyll were observed with an increase in concentration of Cadmium; affects the translocation and storage of sugar in sweet sorghum; reduces the internodal space and internodes number in maize (Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j. Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L, Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n, Kumar, P. 2013o., Kumar, P., Dwivedi, P. 2015p, Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q, Kumar, P., 2014r, Kumar, P., Dwivedi, P., Singh, P., 2012s).

Lead (Pb) is one of the non – essential trace elements that mainly accumulate due to anthropogenic activities in agricultural soils. The upper limits of leads are 61.87 µg/g. The increased levels of Pb in the soil increase the concentration of Pb in plants growing in these soils and ultimately increases the risk of Pb toxicity in food crops. Lead toxicity induces the effects chlorophyll, affects concentration and catabolism of IAA, and stimulates ROS production and also POD activity, reduced total nitrogen and total phosphorus in the plant reduction in germination. Also, the reduction in the relative water content (RWC) and net photosynthetic rate. Legumes were known for their important source of balanced protein food for vegetarians and poor peoples, which makes a major part of the population. Production of the pulses varies with the crop density and adaption, prevailing agro-climatic condition. Globally, the total area under pulses has 851.91 lakh ha having a production of 774.73 lakh tonnes. Pulses are grown in about 198 countries globally, but dry beans cultivated only in 152 countries, which consist of 35.95 percent area of the total world area. Chickpea consists of 139.81 lakh ha of the area with the production of 137.31 lakh tons. Lentil consists of 45.24 lakh ha of the area with the production of 48.27 lakh tons. Pigeon pea consists of 70.33 lakh ha of the area with the production of 48.27 lakh tons. Pea consists of 69.32 lakh ha of the area with the production of 48.90 lakh tons. Beans consist of 306.13 lakh ha of the area with the production of 245.16 lakh tons. Chickpea also is known as Bengal gram, the most important pulse crop of India. India having ranks first for the chickpea production of 98.80 lakh tons with an area of 99.27 lakh ha. Pakistan stood second having 6.29 lakh tons of production in 9.50 lakh ha area. Iran stood third having 2.62 lakh tons of production in 5.94 lakh ha area. Australia stood fourth having 6.29 lakh tons of production in 5.08 lakh ha area. Turkey stood fifth having 4.50 lakh tons of production in 3.88 lakh ha area. In India, the highest chickpea production recorded in Madhya Pradesh, 40.62 lakh tons production and 34.46 lakh ha of the area of the total. In terms of area 15.41 lakh ha Maharashtra stood second but for production has third 11.98 lakh tons. As Rajasthan second in production 14.47 lakh tons but area wise third 15.37 lakh ha. Highest yield recorded in Telangana 1459 kg/ha, followed by Gujrat 1201 kg/ha, West Bengal 1163 kg/ha and lowest in Karnataka 578 kg/ha (Kumar, P., Pandey, A.K., *et al*., 2018aa, Kumar, P., Kumar, S. *et al*., 2018bb, Kumar, P., Krishna, V., *et al*., 2018cc, Singh *et al* 2020a., Singh *et al*., 2020b., Sood, *et al*., 2020., Bhadrecha *et al* 2020, Singh *et al*., 2020c, Sharma *et al*., 2020, Singh *et al*., 2020d, Bhati *et al*., 2020, Singh *et al*., 2019, Sharma *et al*., 2019).

The soil the primary recipient came on contact with a waste of from all the industries, a chemical used in agriculture. Density criteria for the heavy metals range from above 3.5 g/cm3 to above 7 g/cm3. Any substance added into the soil which can cause an adverse effect on the soil functioning and ability to yield a crop knows as soil contamination. Due to its toxicity and capacity to accumulate, they are considered as an important source of

environmental contamination .Polyamines (Pas) are those compounds which consist of two or more primary amine group, have low molecular mass and present in free form; i.e. putrescine, spermidine, and spermine. Polyamines are present in almost all living organisms and also in the plant). Polyamines are helpful in growth and development, also respond during abiotic or biotic stress, the Pas are present in trace amounts like putrescine but in mammal's spermidine and spermine are present.The symbiosis of plant roots with fungi occurs in various forms known as mycorrhiza. Arbuscular mycorrhizal fungi (AMFs) are major soil microorganisms that are key to enabling plant nutrient uptake, particularly in low-input farming, vegetation, and rhizoremediation processes, in various agroecosystems.Salicylic acid (SA) a compound which has been used to reduce the heavy metals toxicity in plants, which helps in the regulation of plant growth. Reduces the heavy metals uptake, protects the membrane integrity and provides stability and by scavenging the reactive oxygen species which activates the antioxidant defenses mechanism and improves the photosynthesis.

Materials and Methods

This was the pot for the experiment with a 30 cm diameter and a 25 cm height and ten kg of soil each along witha small hole underneath it. Under the work plan, targeted pots with Endomycorrhiza have been inoculated.The exogenous use of cadmium (100 ppm) by Cadmium sulfate and Lead (100 ppm) by Lead chloride on the plant creates heavy metal stresses.Fifteen days interval application with Putrescine (1ppm) and Salicylic Acid (1ppm). Two phases such as 60 DAS and 90 DAS were measured in the respective pots. (Table 1).

Table 1 : Name of the Treatments and symbol used respectively

Name of Treatments	Symbol Used For Respective Treatments
Control	T-0
Cadmium(100 ppm)	T-1
Lead(100 ppm)	T-2
Cadmium + Mycorrhiza	T-3
Lead + Mycorrhiza	T-4
Cadmium + Putrescine	T-5
Lead + Putrescine	T-6
Cadmium + Salicylic Acid	T-7
Lead + Salicylic Acid	T-8

Design and Layout of Experiment

In a completely randomized (CRD) design, the experiment was developed. Eight treatments were available, including control. Three times every treatment has been replicated.

Observation Recorded

The observations were recorded two stages such as 60 DAS, and 90 DAS. The recorded observations of biochemical parameters and the standard procedure adopted during study are given below:

Electrical conductivity

The electrical conductivity of fresh tissue was measured according to the protocol given by Zhu *et al*. (2000). Electrical conductivity was based on the electrolyte leakage from the fresh plant tissue into the solution.

Procedure

One hundred mg plant material (roots) was taken in test tubes containing 10 ml of double-distilled deionized water. This set was kept at room temperature for 30 minutes. After 30 minutes the EC of deionized water was measured with the help of EC meter. The EC of deionized water before use was treated as a control. The EC of fresh tissue was calculated as the formula given below

EC [dSm-1] of fresh tissue=EC (Water + Sample) -EC (water)

Osmotic Potential

The estimation of the osmotic potential of the leaf was measured according to the protocol given by Metwali *et al*. (2013). Osmotic potential: the changes in free energy of water produced by the addition of a solute are called the osmotic potential of the solution.

Procedure

The fresh samples of leaves were collected from each cultivar to determine electric conductivity (EC). One hundred mg plant leaves were taken in test tubes containing 10 ml of double-distilled deionized water. This set was kept at room temperature for 30 minutes. After 30 minutes the EC of deionized water was measured with the help of EC meter. The EC of deionized water before use was treated as a control. The EC of fresh tissue was calculated as the formula given below

EC [dSm-1] of the fresh tissue = EC (Water + Sample) - EC (water)

The EC multiplied by factor 0.36 to present OP (-bar).

Osmotic Potential (-Bar) = 0.36 EC [dSm^{-1}] of fresh tissue

Estimation of Membrane Stability Index (MSI) and Injury Index

The MSI was calculated using the formula described by Premchandra *et al* (1990). Membrane damage can be evaluated indirectly by measuring solute leakage (electrolyte leakage) from cells and the MSI. The stimulation effect of stress on Electro Leyte leakage might be attributed to the injury of the plasma membrane.

Procedure

Leaves were taken from the youngest fully-grown leaf. The membrane stability index (MSI) and Membrane Injury Index was estimated by placing 200 mg of leaves in 10 ml double-distilled water in two sets. One set was heated at 40°C for 30min in a water bath and the electrical conductivity (C1) was measured. The second set was boiled at100°C in a boiling water bath for 10 min and the conductivity (C2) was measured; both conductivities were measured using a conductivity meter (ME977- C, Max Electronics, India). The MSI and MII was calculated using the formula described below

MSI= 100 □ – $\frac{\square\square}{\square\square}$ **Or**

MSI = 100 – MI

MII = 100$\frac{\square\square}{\square\square}$

Results and Discussion

Electrical Conductivity (dSm^{-1})

In chickpea variety GPF-1, cadmium stress and lead stress have investigated the outcome of polyamine (putrescine), mycorrhizal, salicylic acid and their combination on EC at 60 and 90 days after sowing (DAS) data were recorded (Table 2 & Fig. a).It is clear to me that, concerning control (T0) on dates of 60 and 90 DAS of an interval, the mean EC was significantly improved at 76.9% and 51.9% when exposed to cadmium metal stress (T1). Similarly, the EC was substantially increased with 5.03 percent, compared to control (t0) at the dates of the proposed interval when the plant is exposed to a high dose of lead (T2).A mitigating effect was shown by an increased EC of 8.63% and 11.94% compared to T0 in the proposed interval dates of exogenous application of endomycorrhiza in soil (T3). Similarly, the EC was increased significantly with 15.10% and 13.75% as compared to T0 at the proposed interval date when treatment T4 was compared to T0.The exogenous application of putrescine (T5), compared to T0, demonstrated EC mitigation at the proposed interval with 24.24% and 17.82%. The EC average was considerably improved with a higher dose of putrescine (T6) compared with T0 at 21.58% and 26.26%. Likewise, when T7 was compared to T0, the EC was significantly increased by 32.37 percent at the proposed interval date and by 34.40 percent at the same time.The average EC was lower as compared to T8 with 33.09% and 42.70% when treated with a higher dose of salicylic acid (T0). The salicylic acid showed the best mitigation effect against the cadmium and lead by increasing the EC on the proposed date of interval. Pirasteh-Anosheh *et al*., (2016)conducted a field trial for three years in a row to study the effect of different concentration of SA (0, 0.2, 1.0, 1.2 and 2.0 mM) on grain and biological yield, also the Na^+, Cl^-, Ca^{2+}, Mg^{2+} and K^+ on barely under salinity stress. It was found that there was a reduction in storage factor (SF), grain and biological yield under salt stress. Application of SA at higher concentration increases the SF for Cl^- and Na^+ but reduces the K^+.this This showed that the SA helped in the storage of Cl^- and Na^+ ions in the root system. This suggested that SA improves ions transport is bare during the salt stress. Ahmad *et al*., (2018) conducted a pot experiment on faba beans to investigate the effect of SA on NaCl stress (50mM and 100mM). The 100mM causes maximum reduction in shoot and root length, also reduction in lead pigments and leaf relative water content (LRWC) with an increase in NaCl concentration.

Table 2 : EC (dSm^{-1}) of chickpea during *Rabi*

Treatments	Electrical conductivity (60 DAS)	Electrical conductivity (90 DAS)
T0	10.667^{g}±0.667	53.133^{h}±0.593
T1	46.333^{a}±1.333	110.533^{a}±0.867
T2	44.000^{ab}±1.155	99.167^{b}±0.601
T3	42.333^{bc}±0.882	97.333^{b}±0.726
T4	39.333^{cd}±0.882	95.333^{c}±0. 441
T5	35.000^{e}±1.528	90.833^{d}±0.928
T6	36.333^{de}±0.882	81.500^{e}±0.764
T7	31.333^{f}±1.202	72.500^{f}±0.289
T8	31.000^{f}±1.155	63.333^{g}±0.601

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

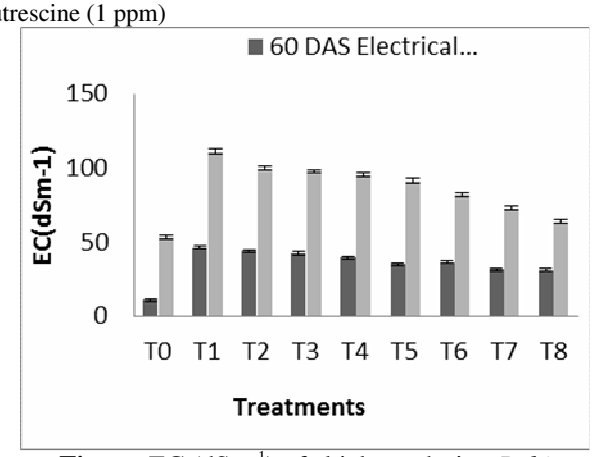

Fig. a : EC (dSm^{-1}) of chickpea during *Rabi*

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

Osmotic Potential (-Bar)

In the cadmium and lead stress chickpea variety GPF-2, polyamine (putrescine), mycorrhiza, salicylic acid, and their mixture on OP were studied. Sixty and 90 days after sowing (DAS) (Table 3 & Fig. b) were recorded. It is apparent that in exposure to cadmium metal stress (T1) the average OP was significantly increased to 16.8 and 39.79 compared to control (T0) at intervals of 60 and 90 DAS. Similarly, the plant was significantly enhanced with 15.84 and 35.70 when exposed to the highest dose of lead (T2) compared to the control (T0) on the interval dates of the proposed plant. A mitigation effect by an increase of the OP with 15.24 and 35.04 was shown by the Exogenous application of endomycorrhiza in soil (T3), compared to T0 on the proposed interval date. Similarly, when compared to T0 treatment T4, the OP was significantly increased at the proposed interval date with 14.16 and 34.32 respectively. Compared with T0, exogenous putrescin (T5) application demonstrated OP mitigation at the proposed interval date with 12.6 and 32.7.When treated with a high dose of putrescine (T6), the average OP was significantly improved, compared to T0 with 13.08 or 29.34. Also, the OP increased considerably less with 11.28 and 26.1 on the proposed interval date when treatment T7 was compared with T0.In treatments with a higher dose of salicylic acid (T0), the average OP was significantly reduced compared to T8 at 11.16 and 22.8. The best effect on cadmium has been shown with salicylic acid by increasing the operation on the proposed interval date. Rehman *et al*., (2019) experimented to study the effect of salinity on Cadmium uptake, tolerance, and phytoremediation potential of Conocarpus. The one-month-old plant was exposed to Cd (0, 8.9, 44.5, 89 and 178µM) alone or combined with NaCl (0, 100, 200mM) in Hoagland's nutrient solution. It was found that the reduction in shoot and root biomasses, Low water content and chlorophyll content more in a combination of Cd and Saline stress compared to Cd alone (Mishra, P.K., Maurya, B.R., Kumar, Pp. 2012t, Kumar, P., Mandal, B., Dwivedi, P. 2011u. Kumar, P., Mandal, B., Dwivedi, P. 2011v, Kumar, P., Pathak, S. 2016w. Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x, Kumar, P., Harsavardhn, M. *et al*., 2018y.

Kumar, P., Yumnam, J. *et al*., 2018z). Uptake of potassium ion reduced in Cd combined with saline or alone Cd. The uptake was increased in the presence of salinity; oxidative stress increased the production of H_2O_2 and MDA content. The tolerance of Conocarpus during the Cd stress reduced in the presence of salinity because of increased uptake of toxic ions and due to an infestation of oxidative stress.

Table 3 : Osmotic Potential (-Bar) of chickpea during *Rabi*

Treatments	Osmotic Potential (60 DAS)	Osmotic Potential (90 DAS)
T0	$3.840^{g} \pm 0.240$	$19.128^{h} \pm 0.213$
T1	$16.680^{a} \pm 0.480$	$39.792^{a} \pm 0.312$
T2	$15.840^{ab} \pm 0.416$	$35.700^{b} \pm 0.216$
T3	$15.240^{bc} \pm 0.317$	$35.040^{b} \pm 0.262$
T4	$14.160^{cd} \pm 0.317$	$34.320^{c} \pm 0.159$
T5	$12.600^{e} \pm 0.550$	$32.700^{d} \pm 0.334$
T6	$13.080^{de} \pm 0.317$	$29.340^{e} \pm 0.275$
T7	$11.280^{f} \pm 0.433$	$26.100^{f} \pm 0.104$
T8	$11.160^{f} \pm 0.416$	$22.800^{g} \pm 0.216$

Where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid (1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

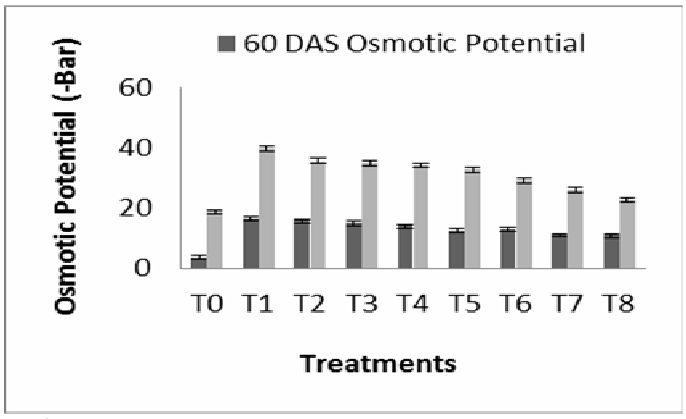

Fig b : Osmotic Potential (-Bar) of chickpea during *Rabi*

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid (1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

Membrane Stability Index (%)

In chickpea variety, GPF-2 under cadmium and lead stress, the effect of polyamine (putrescine), mycorrhiza, salicyl acid (percent) and its combination on MSI was investigated. 60 and 90 days after seeding (DAS) data were recorded (Table 4 & Fig. c).In exposed to cadmium metal stress (T1) on dates 60 and 90 DAS, it is obvious that the mean MSI decreased significantly with 60.41 and 84.54 percent, compared to control (T0). Similarly, the MSI of the plant was substantially reduced at 60.71 and 79.44 percent when compared to control (T0) at the date of the proposed interval when it was being exposed to a greater dose of plum (T2).The mitigating effect by increasing the MSI with 58.89 and 80.80% in comparison with T0 on suggested dates of the interval was shown by the Exodus application of endomycorrhiza in the soil (T3). Similarly, MSI was significantly increased by 54.14 and 59.30 percent as at the proposed interval date when treatment T4 was compared with T0. The MSI attenuation was 48,69 and 58,78 percent at the date of interval proposed compared with T0 for the exogenous application of putrescine (T5).In the case of a higher dose of putrescine, the average MSI was significantly improved compared to T0 with 40,91% and 42,33% (T6). Similarly, the MSI was significantly lower at the proposed date of the interval when T7 was compared with T0 with 41.54% and 63.44%. The average MSI in treatment with higher doses of salicylic acid (T0) was significantly reduced compared to 35.23 and 37.01% of T8.The salicylic acid showed the best mitigation effect against the cadmium and lead by increasing the MSI on the proposed date of interval. Rady *et al*., (2019) experimented to analyze the effect of exogenous application of polyamine under lead (2.0mM) stress on growth and productivity of wheat. The seeds of wheat were soaked in 0.25mM Spm, 0.50 Spd or 1.mM put, showed bettergrowth and yield attributes, RWC, MSI, leaf pigment and nutrient uptake compared to seeds soaked in water under 2.0mM lead stress. Among the polyamines, put showed the best result and thus it was recommended the soaking of wheat seed under lead stress.Amin *et al*., (2018) reported that both crop sesame (*Sesamum indicum* L.) and guar (*Cyamopsis tetragonoloba* L.) able to tolerate the 1000mg per kg concentration of Pb. The amount of Pb present in roots was significantly causing the reduction of biomass in both plants.

Table 4 : MSI (%) of chickpea during *Rabi*

Treatments	Membrane Stability Index (60 DAS)	Membrane Stability Index (90 DAS)
T0	$44.417^{a} \pm 0.276$	$37.997^{a} \pm 0.957$
T1	$17.576^{f} \pm 0.403$	$5.873^{d} \pm 1.007$
T2	$17.441^{f} \pm 0.797$	$7.816^{d} \pm 0.108$
T3	$18.251^{f} \pm 0.220$	$7.294^{d} \pm 1.080$
T4	$20.362^{e} \pm 0.335$	$15.461^{c} \pm 0.889$
T5	$22.781^{d} \pm 0.318$	$15.659^{c} \pm 1.208$
T6	$26.237^{c} \pm 0.471$	$21.907^{b} \pm 0.437$
T7	$25.956^{c} \pm 0.528$	$13.887^{c} \pm 0.126$
T8	$28.760^{b} \pm 0.472$	$23.927^{b} \pm 0.186$

Where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid (1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

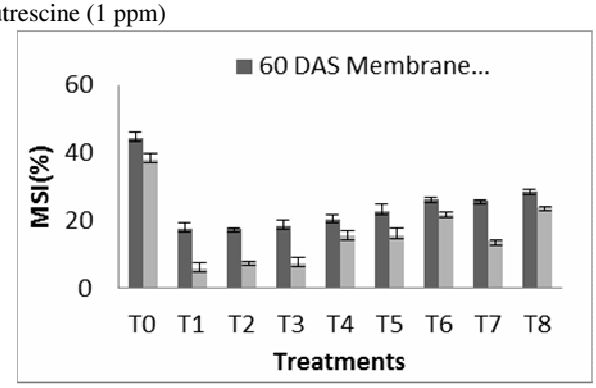

Fig. c : MSI (%) of chickpea during *Rabi*

Where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid (1 ppm); T6-Lead +

Salicylic acid (1 ppm); T7-Cadmium +Putrescine (1 ppm); T8-Lead +Putrescine (1 ppm)

Membrane Injury Index (%)

In chickpeas of the GPF-2 variety, cadmium and lead stress were investigated the effect of polyamine (putrescine), mycorrhizas, salicylic acid, and the MII mixture (percent). Sixty and ninety days after sowing (DAS) data were recorded (Table 5 & Fig. d).In cadmium metal stress (T1) compared with control (T0) at the interval 60 and 90 DAS, MII average was significantly increased by 32.56 and 34.12 percent. Similarly, the MII was substantially enhanced when the plant was exposed to a higher dose of lead (T2) at the proposed interval by 32.72 and 32.73 percent, compared to controls (T0).The mitigation effect was demonstrated by the exogenous use of endomycorrhiza in soil (T3), with the MII being 0.81 and 1.50% lower than T1 on the proposed interval dates. Similarly, MII significantly decreased with 3.53 and 8.49 percent compared to T2 at the proposed interval date when treatment T4 was compared with T2.Compared with T1, there have been decreased MII trends and MII mitigation with 6.31 and 10.39 percent at the proposed interval date compared with the T5 exogenous application of putrescine. The average MII in treatment with a higher dose of putrescine was significantly reduced compared to T2 with 10.65 and 15.28 percent (T6). Similarly, the MII decreased significantly on the proposed interval date with T7 compared to T1 with 10.16 and 8.51 percent.The average MII was significantly reduced as compared to T8 with 13.7 and 17.47% when treated with a higher dose of salicylic acid (T2). The salicylic acid showed the best mitigation effect against the cadmium and lead by increasing the MII on the proposed date of interval. Ahmad *et al*., (2018) conducted a pot experiment on faba beans to investigate the effect of SA on NaCl stress (50mM and 100mM). 100mM causes maximum reduction in shoot and root length, also reduction in lead pigments and leaf relative water content (LRWC) with an increase in NaCl concentration. However, the application of SA on NaCl stressed seedling enhances the length and dry weight of shoot by 57.1% and root by 67.2%), also increase in leaf pigment and LRWC. H_2O_2 and MDA concentration in NaCl stressed seedling was more but in SA+ NaCl the reduction in H_2O_2 and MDA content. Application of SA reduces Na+ accumulation and enhances Ca2+ and k+ uptake during NaCl stress in seedlings of faba bean.Alamri *et al*., (2018) suggested that the application of SA was directly or indirectly involved in improving the physiological process, which helps wheat to overcome from the oxidative damage caused by Pb toxicity.

Table 5 : MII (%) of chickpea during *Rabi*

Treatments	Membrane Injury Index (60 DAS)	Membrane Injury Index (90 DAS)
T0	$55.583^{f} \pm 0.276$	$62.003^{d} \pm 0.957$
T1	$82.424^{a} \pm 0.403$	$94.127^{a} \pm 1.007$
T2	$82.559^{a} \pm 0.797$	$92.184^{a} \pm 0.108$
T3	$81.749^{a} \pm 0.220$	$92.706^{a} \pm 1.080$
T4	$79.638^{b} \pm 0.335$	$84.539^{b} \pm 0.889$
T5	$77.219^{c} \pm 0.318$	$84.341^{b} \pm 1.208$
T6	$73.763^{d} \pm 0.471$	$78.093^{c} \pm 0.437$
T7	$74.044^{d} \pm 0.528$	$86.113^{b} \pm 0.126$
T8	$71.240^{e} \pm 0.472$	$76.073^{c} \pm 0.186$

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

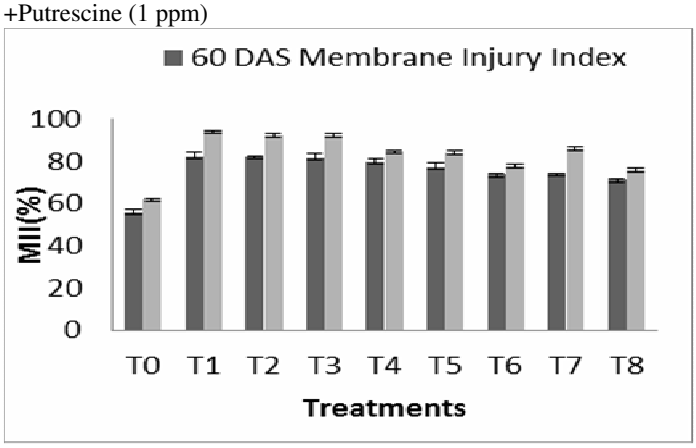

Fig. d : MII (%) of chickpea during *Rabi*

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2-Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid(1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

Conclusion

Polyamines, SA, Mycorrhiza, and Rhizobium provide significant mitigation of cadmium and lead-induced toxicity in chickpea mediated by increasing through their defensive role in plants.

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Author Contributions

The study was designed by P.K. and M.N, the morphological protocolizations were established, experiments were carried out and the data analyzed and interpreted were collected. The paper has been written by P.K., M.N. and T.K.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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