Journal of Elementology ISSN 1644-2296

Yasin G., Saima S., Haq I. , Altaf A., Ahmad A. 2022.
*Exogenous spermidine- and kinetin-mediated changes in the content of heavy metal elements and the biomass of mash bean grown in chromium and lead polluted soils.*
J. Elem., 27(3): 709-726. DOI: 10.5601/jelem.2022.27.2.2235

RECEIVED: 29 December 2021
ACCEPTED: 11 August 2022



**ORIGINAL PAPER**

# EXOGENOUS SPERMIDINE- AND KINETIN-MEDIATED CHANGES IN THE CONTENT OF HEAVY METAL ELEMENTS AND THE BIOMASS OF MASH BEAN GROWN IN CHROMIUM AND LEAD POLLUTED SOILS

**Ghulam Yasin[1], Shahzadi Saima[1], Ikram ul Haq[2], Adeela Altaf[3], Aqsa Ahmad[1]**

**[1] Department of Botany**
**Bahauddin Zakariya University, Multan, Pakistan**
**[2] Institute of Biotechnology and Genetic Engineering**
**Sindh University, Jamshoro, Pakistan**
**[3] Department of Environmental Sciences**
**Bahauddin Zakariya University, Multan, Pakistan**

**Abstract**

The aim of the experiment was to evaluate the role of kinetin and spermidine in modifying the metal uptake and biomass accumulation by mash bean. Four mash bean varieties were grown in pots. In one group of plants, chromium (Cr) was added in doses of 30 and 60 mg $kg^{-1}$ of soil material. In another group, lead (Pb) was added in doses of 20 and 40 mg $kg^{-1}$ of soil material. Plants were sprayed twice, at the age of 20 and 30 days, with 1.0 mM strength of spermidine and 100.0 mM that of kinetin. Each treatment was replicated four times. The pots were arranged in complete randomization. Data of four replicates were taken at the physiological maturity of plants. Concentrations of chromium and lead elements in the root stem and seeds were determined, in addition to dry biomass. Data were subjected to statistical analysis using Costat statistical package software. Significant reductions were recorded in the concentrations of elemental heavy metals in the root, stem and seeds induced by kinetin and spermidine. Furthermore, biomass was increased by kinetin and spermidine. In plants grown under chromium stress, kinetin and spermidine were found more effective in reducing the concentration of chromium in roots and shoots but the analogous effect was insignificant regarding the reduction of chromium concentration in seeds. When lead stress was imposed, spermidine was found to be more effective in reducing concentrations of lead in the root and shoot but kinetin was proven to be less effective in this regard. The effects of both PGRs were found to be pronounced in alleviating the metals' toxicities in terms of plant air dried biomass.

**Keywords:** biomass, chromium, kinetin, lead, spermidine, *Vigna mungo* L. Hepper.

Ghulam Yasin, Department of Botany, Bahauddin Zakariya University, Multan, Pakistan, e-mail: yasingmn_bzu@yahoo.com

## INTRODUCTION

The presence of one metal affects the availability and translocation of another one in the soil and plant. In other words, antagonistic and synergistic behaviors exist among elemental ions (Salgare, Acharekar 1992). The potential of plants for the absorption and accumulation of one metal equally enables them to acquire or outcompete other elemental ions (Djingova, Kuleff 2000, Taiz, Zeiger 2002). Also, a wide range of other phenomena, such as microbial biomass, C and N mineralization and availability, respiration and enzymatic activities, are taken under consideration while studying the bioavailability of ions. Another important factor that affects the metal availability is the degree of their complexation with ligands (Norvell 1984, Marques et al. 2009).

Kinetin belongs to the group of molecules which have low molecular weight and play a role in plant growth and development (Zalabak et al. 2013, Sosnowski et al. 2019). Treatment with kinetin is reported to augment metal stresses in term of K, N, Ca uptake (Haroun et al. 2003). The cell division, growth dominance at the apical region, germination, morphogenesis, nutrient assimilation, development and process of organ senescence are the main aspects controlled by PGRs. Kinetin increases chlorophyll content and photosynthesis (Nahar et al. 2016, Ahanger et al. 2018).

Polyamines (PAs) are also low molecular weight compounds belonging to the class of aliphatic amines. These, like kinetin, perform important functions, especially in stress tolerance (Chen et al. 2019). When applied exoenously, polyamines can provide protection to plants against metal stress (Yu et al. 2018). During stress conditions, polyamines in cells are accumulated and their concentration is regulated (Yu et al. 2019). Free radical scavenging (Drolet et al. 1986), cations balancing pH (Preibe, Jager 1978) or stabilization of membranes by interactions of ions (Ballas et al. 1993) are mediated by polyamines. Increase in the endogenous level of polyamines (putrescine, spermidine and spermine) at the expense of K concentrations was reported (Sarjala et al. 1997). Higher consumption of K ions in the plant necessitates their greater absorption and translocation as compared to metal ions and thereby it decreases the relative accumulation of metal ions.

## MATERIALS AND METHODS

A pot culture experiment was carried under ambient conditions for the purpose of evaluating the effect of kinetin and spermidine on heavy metal uptake and distribution in four genotypes of mash bean (*Vigna mungo* L. Hepper) in chromium (Cr) and lead (Pb) contaminated soil material. Effluents free sandy clay loam soil material was chosen to fill the plastic pots lined

with polyethylene sheet. A batch of 6kg of soil material was out in each pot and watered to obtain the set saturation percentage for one week. Pots were used as the culturing medium of four mash bean genotypes, namely i.e., 80, 88, 97 and ES-1. Five seeds were sown in each pot. Ten days after germination, three seedlings were kept in each pot by manual thinning to ensure the uniformity of nutrients uptake. Three sets of chromium metal levels (0, 30 and 60 mg $kg^{-1}$) were maintained. Similarly, three sets of lead metal levels (0, 20 and 40 mg $kg^{-1}$) were maintained. Chloride salts of both metals in the form of water solution were added to the pots to achieve the set metal levels. One group from each metal was foliarly sprayed with distilled water, kinetin and spermidine at 20, 30 days after germination. Kinetin and spermidine solutions used were of 100.0 mM and 1.0 mM respectively. Complete randomization of treatments with four replications was implemented for the arrangement of pots,. Plants were irrigated and sprayed with insecticides as and when needed. Four plants were harvested from each treatment of every variety at physiological maturity for data collection. After determination of the plants' dry biomass, their roots, stem and seeds were used for elemental analysis.

**Digestion of plant material**

Samples of roots, seeds and stem were ground into fine powder using a Tecator high-speed mill (Foss Tecator AB, Hoganas, Sweden). Material (0.5 g) was digested with concentrated $H_2SO_4$ and $H_2O_2$ (35%).

**Lead and chromium contents determination**

Using a Perkin Elmer 3100 EDS Atomic Absorption Spectrophotometer, digested aliquots which were treated with the respective metal were evaluated for Cr and Pb. Standard setting of the instruments were: air-acetylene flame, head of burner (10 cm), wavelength (357.9 nm), slit (0.7 nm) and lamp current (20 mA). The samples which were found out of calibration range of 0 to 5 mg $L^{-1}$ were diluted accordingly (Yoshida et al. 1976). Air dried biomass was determined by weighing the sun-dried whole plant.

**Statistical analysis**

The results were subjected to evaluation in a Costat computer package (CoHort Software, Berkeley, CA). The Duncan's New Multiple Range test (at 5% level) was applied for comparison of mean values. Significant *F* values were compared in MSTAT-C software (Duncan 1955).

# RESULTS

## Chromium content in root (g kg$^{-1}$)

Varieties revealed significant variability in their responses for chromium content in roots due to differences in the toxicity of the metals and different responses of the plants to PGRs (kinetin and spermidine). The variations in the alleviating roles of PGRs were significant when noted in the different varieties. Soil applied chromium and lead concentrations also showed significant effects on its accumulation (Tables 1, 2, 3).

Table 1

Mean sums of squares for chromium content in root, shoot and seeds (g kg$^{-1}$) of mash bean (80 days age) after exposure to chromium (30, 60 mg kg$^{-1}$ soil)] accompanied with sprays of kinetin (100.0 mM) and spermidine (1.00 mM)

| Source | df | MSS for chromium | | |
|---|---|---|---|---|
| | | root | shoot | seed |
| **Variety (V)** | 3 | 4.5707*** | 7.5352*** | 3.0843*** |
| **Metal (M)** | 2 | 1346.1009*** | 430.7892*** | 0.0055*** |
| **PGRs** | 2 | 4.5726*** | 6.0425*** | 1.0917*** |
| **V ×M** | 6 | 3.5799*** | 2.9041*** | 4.0770*** |
| **V × PGRs** | 6 | 1.8796*** | 0.6741*** | 1.8333*** |
| **M × PGRs** | 4 | 3.2288*** | 1.7418*** | 2.2048*** |
| **V × M ×PGRs** | 12 | 1.0592** | 0.7313*** | 1.0101* |
| **Error** | 108 | 0.3909 | 0.0406 | 5.1763 |

*** $P$<0.005, ** $P$<0.05, * $P$<0.05

Table 2

Mean sums of squares for lead content in root, shoot (g kg$^{-1}$) and seeds (g kg$^{-1}$) of mash bean (80 days age) after exposure to lead (20, 40 mg kg$^{-1}$ soil)] accompanied with sprays of kinetin (100.0 mM) and spermidine (1.00 mM)

| Source | df | MSS for lead | | |
|---|---|---|---|---|
| | | root | shoot | seed |
| **Variety (V)** | 3 | 0.5347*** | 0.1774*** | 3.7045*** |
| **Metal (M)** | 2 | 476.4075*** | 95.7136*** | 0.0054*** |
| **PGRs** | 2 | 0.3621*** | 0.4374*** | 3.9916*** |
| **V ×M** | 6 | 0.1546*** | 0.0690*** | 3.8218*** |
| **V × PGRs** | 6 | 0.0678*** | 0.1125*** | 1.8598** |
| **M × PGRs** | 4 | 0.2497*** | 0.0861*** | 1.9486*** |
| **V × M ×PGRs** | 12 | 0.0908*** | 0.0576*** | 1.1168* |
| **Error** | 108 | 0.0095 | 0.0082 | 5.1763 |

*** $P$<0.005, ** $P$<0.05, * $P$<0.05

Table 3

Mean sums of squares for air dried biomass (g) of mash bean (80 days age) after exposure to chromium (30, 60 mg $kg^{-1}$ soil) and lead (20, 40 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine (1.00 mM)

| Source | df | MSS values for dry biomass |
|---|---|---|
| **Variety (V)** | 3 | 2.300*** |
| **Metal (M)** | 4 | 96.969*** |
| **PGRs** | 2 | 49.440*** |
| **V ×M** | 12 | 1.154*** |
| **V × PGRs** | 6 | 0.389* |
| **M × PGRs** | 8 | 1.547*** |
| **V × M ×PGRs** | 24 | 0.541*** |
| **Error** | 180 | 0.156 |

*** $P<0.001$, * $P<0.05$

A definitive relationship occurred between the soil chromium concentration and its accumulation in the root (Table 4). The concentration of chromium in the root was dependent on its supply to any of the varieties (Table 4*b*). This accumulating behavior of chromium in roots was consistent in all the varieties (Table 4*c*). Exogenous application of PGRs decreased the root metal content (Table 4*c*), and this alleviated the impact of metal stress except the effect of spermidine for higher chromium supplemented plants (Table 4*b*).

In the plants grown in soil with the chromium concentration of 30 mg $kg^{-1}$ and treated with distilled water, the elemental concentration of chromium in the root was 7.28 g $kg^{-1}$. This value dropped to 6.67 g $kg^{-1}$ when the plants were sprayed with kinetin. The plants grown in soil with the chromium concentration of 60 mg $kg^{-1}$ and exposed to distilled water foliar spray, had the elemental concentration of chromium in roots equal 11.51 g $kg^{-1}$. However, the values were lower, 10.36 and 10.14 g $kg^{-1}$, when plants were subjected to sprays of kinetin and spermidine, respectively (Table 4*a*). These effects of PGRs were found in all the varieties (Table 4*c*). Kinetin reduced the chromium content of $V_1$ (Mash 80) and spermidine reduced significantly the chromium content of all the varieties (Table 4*a*). The lowest chromium content was found in $V_1$ (Mash 80), which the other varieties had the same concentration of chromium in their roots (Table 4*c*).

## Chromium content in the shoot (g $kg^{-1}$)

The plant shoot content of chromium differed statistically between the varieties. Different metals and PGRs (kinetin and spermidine) also affected the shoot chromium content to a markedly different degree. Varietal response to soils applied chromium concentrations and to the application of PGRs (kinetin and spermidine) differed when assessed separately and in combination of both treatments (Table 1). Exogenous application of kinetin

Table 4

Chromium content in the root (g $kg^{-1}$) of mash bean (80 days age) after exposure to chromium (30, 60 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, values are as means ± SE

(a) Indices of metal augmentation by kinetin and spermidine ($n$=16, LSD=0.4382)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.2529) |
|---|---|---|---|---|
| No metal input | 0.27±0.03*e* | 0.22±0.02*e* | 0.28±0.02*e* | 0.25±0.03*c* |
| Cr (30 mg $kg^{-1}$) | 7.28±0.45*c* | 6.67±0.59*d* | 7.38±0.77*c* | 7.11±0.63*b* |
| Cr (60 mg $kg^{-1}$) | 11.51±0.38*a* | 10.36±0.57*b* | 10.14±0.19*b* | 10.67±0.50*a* |

(b) Differences of varietal responses to kinetin and spermidine ($n$=12, LSD=0.5059)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 6.15±2.39*ab* | 4.88±1.99*d* | 5.45±2.11*c* |
| $V_2$ | 6.35±2.38*ab* | 6.02±2.26*b* | 6.54±2.32*a* |
| $V_3$ | 6.63±2.56*a* | 6.22±2.36*ab* | 5.49±2.12*c* |
| $V_4$ | 6.29±2.42*ab* | 5.88±2.26*bc* | 6.25±2.25*ab* |
| Mean ($n$=48, LSD=0.2529) | 6.35±2.36*a* | 5.75±2.16*b* | 5.93±2.14*b* |

(c) Differences of varietal responses to metal stress ($n$=12, LSD=0.5059)

| Specification | No metal input | Cr (30 mg $kg^{-1}$) | Cr (60 mg $kg^{-1}$) | Mean ($n$=36, LSD=0.2921) |
|---|---|---|---|---|
| $V_1$ | 0.273±0.03*f* | 5.86±0.52*e* | 10.34±0.48*ab* | 5.49±2.12*b* |
| $V_2$ | 0.260±0.03*f* | 8.12±0.46*c* | 10.54±0.56*ab* | 6.30±2.26*a* |
| $V_3$ | 0.236±0.02*f* | 7.06±0.54*d* | 10.76±0.53*a* | 6.11±2.30*a* |
| $V_4$ | 0.256±0.03*f* | 7.41±0.44*d* | 10.05±0.42*b* | 6.14±2.24*a* |

Different letters following the values show significant differences between values.
$V_1$ – Mash 80, $V_2$ – Mash 88, $V_3$ – Mash 97, $V_4$ – Mash ES-1

and spermidine reduced significantly the chromium content of the shoots in all varieties (Table 5*c*). In this regard, the effect of spermidine was found to be stronger than that of kinetin (Table 5*a*).

However, none of the PGRs was able to have an effect on plants under the higher chromium stress. In plants grown in soil with the chromium concentration of 30 mg $kg^{-1}$ and exposed to distilled water foliar spray, the elemental concentration of chromium in the shoot was 4.66 g $kg^{-1}$. This amount decreased to 4.25 and 3.89 g $kg^{-1}$ when plants were sprayed with kinetin and spermidine.

The plants grown in soil with the higher dose of chromium (60 mg $kg^{-1}$) and treated with distilled water spray had the elemental concentration of chromium in the shoot as 6.53 g $kg^{-1}$. This amount decreased to 5.93 and 5.20 g $kg^{-1}$ when plants were sprayed with kinetin and spermidine (Table 5*a*). Both PGRs were effective in decreasing the shoot chromium content of plants

Table 5

Chromium content in the shoot (g $kg^{-1}$) of mash bean (80 days age) after exposure to chromium (30, 60 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, values are as means ± SE

(a) Indices of metal augmentation by kinetin and spermidine ($n$=16, LSD=0.1412)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.0815) |
|---|---|---|---|---|
| No metal input | 0.10±0.01*g* | 0.07±0.01*g* | 0.08±0.01*g* | 0.08±0.01*c* |
| Cr (30 mg $kg^{-1}$) | 4.66±0.28*d* | 4.25±0.37*e* | 3.89±0.42*f* | 4.27±0.38*b* |
| Cr (60 mg $kg^{-1}$) | 6.53±0.25*a* | 5.93±0.50*b* | 5.20±0.48*c* | 5.88±0.50*a* |

(b) Differences of varietal responses to kinetin and spermidine ($n$=12, LSD=0.1631)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 3.72±1.42*bc* | 3.62±1.32*cd* | 3.34±1.20*fg* |
| $V_2$ | 3.80±1.45*b* | 3.56±1.45*cd* | 3.53±1.32*de* |
| $V_3$ | 3.37±1.24*ef* | 2.66±0.97*h* | 2.18±0.81*i* |
| $V_4$ | 4.17±1.53*a* | 3.83±1.45*b* | 3.18±1.24*g* |
| Mean ($n$=48, LSD=0.0815) | 3.76±1.38*a* | 3.41±1.29*b* | 3.05±1.15*c* |

(c) Differences of varietal responses to metal stress ($n$=12, LSD=0.1631)

| Specification | No metal input | Cr (30 mg $kg^{-1}$) | Cr (60 mg $kg^{-1}$) | Mean ($n$=36, LSD=0.0941) |
|---|---|---|---|---|
| $V_1$ | 0.09±0.01*f* | 4.68±0.14*c* | 5.91±0.35*b* | 3.56±1.28*b* |
| $V_2$ | 0.07±0.01*f* | 4.24±0.22*d* | 6.58±0.18*a* | 3.63±1.37*b* |
| $V_3$ | 0.09±0.01*f* | 3.49±0.36*e* | 4.61±0.43*c* | 2.73±1.02*c* |
| $V_4$ | 0.08±0.01*f* | 4.67±0.43*c* | 6.44±0.27*a* | 3.73±1.39*a* |

Different letters following the values show significant differences between values. Explanations see Table 4.

grown in chromium contaminated soil (Table 5*b*). The content of chromium was found to correlate with its supply in soil (Table 5 b), and this was consistent in plants of all the varieties (Table 5*c*). Variety $V_4$ (Mash ES-1) had the highest and variety $V_3$ (Mash 97) had the lowest Cr content in the shoot (Table 5*c*).

## Chromium content in seeds (g $kg^{-1}$)

The varieties differed in the seed content of chromium. Varietal responses to PGRs were also different. The differences in the effect of PGRs (kinetin and spermidine) were significant as regards the translocation of chromium to seeds. The soil chromium concentration affected its accumulation in seeds (Table 1).

The plants grown in soil with the chromium concentration of 30 mg $kg^{-1}$ and exposed to distilled water foliar spray had an elemental concentration of chromium in seeds equal 0.027 g $kg^{-1}$. This amount decreased to 0.021 and 0.025 g $kg^{-1}$ when plants were sprayed with kinetin and spermidine. In plants treated with distilled water spray and 60 mg $kg^{-1}$ of chromium, the concentration of chromium in seeds was 0.034 g $kg^{-1}$. This amount decreased to 0.031 and 0.033 g $kg^{-1}$ when plants were sprayed with kinetin and spermidine (Table 6*a*). The concentration of chromium in seeds depended on its supply to soil (Table 6*c*) in various varieties (Table 6*b*). Exogenous application of kinetin and spermidine decreased the chromium content of seeds in plants grown in chromium polluted soil (Table 6*b*). The chromium

Table 6

Chromium content in seeds (g $kg^{-1}$) of mash bean (80 days age) after exposure to chromium (30, 60 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, values are as means ± SE

(a) Indices of metal augmentation by kinetin and spermidine ($n$=16, LSD=1.594)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.0013) |
|---|---|---|---|---|
| No metal input | 0.006±0.0008*a* | 0.013±0.0014*a* | 0.015±0.0024*a* | 0.011±0.0025*c* |
| Cr (30 mg $kg^{-1}$) | 0.027±0.0010*a* | 0.021±0.0017*a* | 0.025±0.0009*a* | 0.025±0.0017*b* |
| Cr (60 mg $kg^{-1}$) | 0.034±0.0014*a* | 0.031±0.0013*a* | 0.033±0.0011*a* | 0.032±0.0014*a* |

(b) Differences of varietal responses to kinetin and spermidine ($n$=12, LSD=1.841)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 0.022±0.0067*a* | 0.021±0.0043*a* | 0.023±0.0051*a* |
| $V_2$ | 0.021±0.0062*a* | 0.020±0.0045*a* | 0.025±0.0039*a* |
| $V_3$ | 0.022±0.0062*a* | 0.023±0.0042*a* | 0.024±0.0044*a* |
| $V_4$ | 0.023±0.0059*a* | 0.023±0.0028*a* | 0.026±0.0027*a* |
| Mean ($n$=48, LSD=0.0013) | 0.022±0.0061 | 0.022±0.0039 | 0.024±0.0041 |

(c) Differences of varietal responses to metal stress ($n$=12, LSD=1.841)

| Specification | No metal input | Cr (30 mg $kg^{-1}$) | Cr (60 mg $kg^{-1}$) | Mean ($n$=36, LSD=0.0010) |
|---|---|---|---|---|
| $V_1$ | 0.009±0.0015*a* | 0.025±0.0018*a* | 0.033±0.0014*a* | 0.022±0.0053*b* |
| $V_2$ | 0.010±0.0024*a* | 0.023±0.0024*a* | 0.033±0.0012*a* | 0.022±0.0050*b* |
| $V_3$ | 0.010±0.0019*a* | 0.025±0.0011*a* | 0.033±0.0010*a* | 0.023±0.0049*ab* |
| $V_4$ | 0.015±0.0030*a* | 0.025±0.0012*a* | 0.032±0.0020*a* | 0.024±0.0040*a* |

Different letters following the values show significant differences between values.
Explanations see Table 4.

content was in seeds of $V_4$ (Mash ES- 1) was higher than in seeds of all other varieties (Table 6*c*).

**Lead content in the root (g kg$^{-1}$)**

Plants of the tested varieties differed in their behavior regarding the lead accumulation in the root. The lead concentration in soil and the applied PGRs (kinetin and spermidine) also affected the root content of lead to a markedly different degree. Responses of the varieties to the metal and PGRs (kinetin and spermidine) varied when assessed separately and in combination of both treatments (Table 2).

In plants grown in soil with the lead concentration of 20 mg kg$^{-1}$ and exposed to distilled water foliar spray, the elemental concentration of lead in the root was 4.83 g kg$^{-1}$. This amount decreased to 4.40 and 4.51 g kg$^{-1}$ when plants were sprayed with kinetin and spermidine. The plants grown in soil with the lead concentration of 40 mg kg$^{-1}$ and exposed to distilled water foliar spray had an elemental concentration of lead in the root equal 6.26 g kg$^{-1}$. This amount decreased to 6.24 and 6.15 g kg$^{-1}$ when plants were sprayed with kinetin and spermidine (Table 7*a*). The concentration of lead in the root increased gradually in all varieties after exposure to the increasing levels of lead concentration in soil (Table 7*b*). Exogenous application of kinetin and spermidine decreased the lead content of the root in all varieties (Table 7*a*). However, kinetin was effective for plants grown in soil with low lead concentrations (Table 7*b*). The highest lead content (3.80) was found in Mash ES- 1 and lowest one (3.51) – in Mash 80 (Table 7*c*).

**Lead content in the shoot (g kg$^{-1}$)**

The shoot content of lead varied significantly between the varieties. The metal concentration in soil had a significantly different effect on the shoot lead content. The observed effects of PGRs (kinetin and spermidine) on the metal uptake were consistent when the different varieties were analyzed in the context of interactive effects of the treatments (Table 2).

In plants grown in soil with the lead concentration of 20 mg kg$^{-1}$ and exposed to distilled water foliar spray, the elemental concentration of lead in shoots was 1.43 g kg$^{-1}$. This amount decreased to 1.31 and 1.14 g kg$^{-1}$ when plants were sprayed with kinetin and spermidine. The plants grown in soil with the lead concentration of 40 mg kg$^{-1}$ and exposed to distilled water foliar spray had an elemental concentration of lead in the shoot equal 3.01 g kg$^{-1}$. This amount decreased to 2.83 and 2.76 g kg$^{-1}$ when plants were sprayed with kinetin and spermidine (Table 8*a*). Exogenous application of spermidine and kinetin reduced significantly the lead content of shoots, with the latter substance being more effective than the former (Table 8*c*). This was consistent in all varieties (Table 8*a*) and depended on the metal concentration in soil (Table 8*b*). The content of lead depended on its supply to soil, and was correlated with the higher levels of soil contamination in all

Table 7

Lead content in the root (g $kg^{-1}$) of mash bean (80 days age) after exposure to lead (20, 40 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, (values are as means ± SE)

(a) Indices of metal augmentation by kinetin and spermidine ($n$=16; LSD=0.0683)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.0395) |
|---|---|---|---|---|
| No metal input | 0.14±0.02*f* | 0.13±0.02*f* | 0.12±0.0*f* | 0.13±0.02*c* |
| Pb (20 mg $kg^{-1}$) | 4.83±0.16*c* | 4.40±0.11*e* | 4.51±0.12*d* | 4.58±0.16*b* |
| Pb (40 mg $kg^{-1}$) | 6.26±0.07*a* | 6.24±0.11*a* | 6.15±0.09*b* | 6.22±0.09*a* |

(b) Differences of varietal responses to kinetin and spermidine ($n$=12, LSD=0.07887)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 3.71±1.35*cd* | 3.41±1.28*h* | 3.40±1.26*h* |
| $V_2$ | 3.74±1.38*bc* | 3.54±1.29*g* | 3.67±1.35*cde* |
| $V_3$ | 3.65±1.37*de* | 3.62±1.37*ef* | 3.57±1.33*fg* |
| $V_4$ | 3.88±1.37*a* | 3.79±1.38*b* | 3.73±1.36*bc* |
| Mean ($n$=48, LSD=0.0395) | 3.74±1.32*a* | 3.59±1.29*b* | 3.59±1.28*b* |

(c) Differences of varietal responses to metal stress ($n$=12, LSD=0.07887)

| Specification | No metal input | Pb (20 mg $kg^{-1}$) | Pb (40 mg $kg^{-1}$) | Mean ($n$=36, LSD=0.0456) |
|---|---|---|---|---|
| $V_1$ | 0.10±0.00*h* | 4.43±0.18*f* | 5.99±0.06*c* | 3.51±1.26*c* |
| $V_2$ | 0.13±0.01*gh* | 4.58±0.09*e* | 6.23±0.07*b* | 3.65±1.30*b* |
| $V_3$ | 0.10±0.01*h* | 4.44±0.05*f* | 6.31±0.06*a* | 3.61±1.31*b* |
| $V_4$ | 0.19±0.01*g* | 4.88±0.16*d* | 6.34±0.07*a* | 3.80±1.33*a* |

Different letters following the values show significant differences between values. Explanations see Table 4.

the varieties (Table 8*b*). The maximum lead content (1.46) in bean shoots was in Mash ES- 1 and the minimum one (1.30) was in Mash 80 (Table 8*c*).

## Lead content in seeds (g $kg^{-1}$)

Lead accumulation by plants varied between the varieties. The varieties responded differently to PGRs (kinetin and spermidine). The concentrations of lead applied to soil led to significant differences in the lead accumulation in bean seeds.

The effects of PGRs (kinetin and spermidine) on metal uptake were different between varieties, and there were also evident differences for metal stress interactive effects on the tested varieties (Table 2).

Table 8

Lead content in the shoot (g $kg^{-1}$) of mash bean (80 days age) after exposure to lead (20, 40 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, values are as means ± SE

(a) Indices of metal augmentation by kinetin and spermidine ($n$=16, LSD=0.0634)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.0367) |
|---|---|---|---|---|
| No metal input | 0.06±0.01*g* | 0.06±0.01*g* | 0.03±0.006*g* | 0.05±0.01*c* |
| Pb (20 mg $kg^{-1}$) | 1.43±0.07*d* | 1.31±0.07*e* | 1.14±0.11*f* | 1.30±0.10*b* |
| Pb (40 mg $kg^{-1}$) | 3.01±0.06*a* | 2.83±0.10*b* | 2.76±0.11*c* | 2.87±0.10*a* |

(b) Differences of varietal responses to kinetin and spermidine ($n$=12, LSD=0.0733)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 1.52±0.60*ab* | 1.29±0.56*f* | 1.10±0.51*g* |
| $V_2$ | 1.56±0.66*a* | 1.37±0.58*de* | 1.41±0.60*cd* |
| $V_3$ | 1.46±0.64*bc* | 1.47±0.59*bc* | 1.30±0.60*ef* |
| $V_4$ | 1.47±0.61*bc* | 1.47±0.63*bc* | 1.44±0.62*cd* |
| Mean ($n$=48, LSD=0.0367) | 1.50±0.61*a* | 1.40±0.57*b* | 1.31±0.57*c* |

(c) Differences of varietal responses to metals stress ($n$=12, LSD=0.0733)

| Specification | No metal input | Pb (20 mg $kg^{-1}$) | Pb (40 mg $kg^{-1}$) | Mean ($n$=36, LSD=0.0042) |
|---|---|---|---|---|
| $V_1$ | 0.06±0.014*e* | 1.20±0.17*d* | 2.66±0.10*b* | 1.30±0.55*b* |
| $V_2$ | 0.05±0.010*e* | 1.35±0.06*c* | 2.94±0.09*a* | 1.44±0.60*b* |
| $V_3$ | 0.06±0.013*e* | 1.26±0.09*d* | 2.92±0.06*a* | 1.41±0.59*c* |
| $V_4$ | 0.05±0.014*e* | 1.37±0.03*c* | 2.96±0.09*a* | 1.46±0.60*a* |

Different letters following the values show significant differences between values. Explanations see Table 4.

In plants grown in soil with the lead concentration of 20 mg $kg^{-1}$ and exposed to distilled water foliar spray, the elemental concentration of lead in seeds was 0.025 g $kg^{-1}$. This amount decreased to 0.016 and 0.020 g $kg^{-1}$ when plants were sprayed with kinetin and spermidine. The plants grown in soil with the lead concentration of 40 mg $kg^{-1}$ and exposed to distilled water foliar spray had an elemental concentration of lead in seeds equal 0.032 g $kg^{-1}$. This amount decreased to 0.023 and 0.028 g $kg^{-1}$ when plants were sprayed with kinetin and spermidine (Table 9*a*). Exogenous spermidine and kinetin reduced significantly the lead content in seeds of all varieties, and spermidine was more effective than kinetin (Table 9*c*). The effects of these substances were without varietal discrimination (Table 9*a*) and soil

Table 9

Lead content in seeds (g $kg^{-1}$) of mash bean (80 days age) after exposure to lead (20, 40 mg $kg^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, values are as means ± SE

(a) Indices of metal augmentation by kinetin and spermidine (n=16, LSD=1.594)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.0012) |
|---|---|---|---|---|
| No metal input | 0.004±0.0008 | 0.006±0.0014 | 0.010±0.0024 | 0.007±0.0020*c* |
| Pb (20 mg $kg^{-1}$) | 0.025±0.0010 | 0.016±0.0018 | 0.020±0.0009 | 0.021±0.0022*b* |
| Pb (40 mg $kg^{-1}$) | 0.032±0.0014 | 0.023±0.0013 | 0.028±0.0011 | 0.028±0.0022*a* |

(b) Differences of varietal responses to kinetin and spermidine (n=12, LSD=1.841)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 0.021±0.0067 | 0.014±0.0041 | 0.017±0.0051 |
| $V_2$ | 0.020±0.0062 | 0.013±0.0042 | 0.020±0.0039 |
| $V_3$ | 0.021±0.0061 | 0.017±0.0041 | 0.019±0.0044 |
| $V_4$ | 0.021±0.0058 | 0.016±0.0028 | 0.021±0.0027 |
| Mean ($n$=48, LSD=0.0012) | 0.020±0.0060*a* | 0.015±0.0038*c* | 0.019±0.0041*b* |

(c) Differences of varietal responses to metal stress ($n$=12, LSD=1.841)

| Specification | No metal input | Pb (20 mg $kg^{-1}$) | Pb (40 mg $kg^{-1}$) | Mean ($n$=36, LSD=0.0010) |
|---|---|---|---|---|
| $V_1$ | 0.004±0.0006 | 0.021±0.0027 | 0.028±0.0024 | 0.017±0.0054*c* |
| $V_2$ | 0.006±0.0018 | 0.020±0.0028 | 0.028±0.0020 | 0.018±0.0050*bc* |
| $V_3$ | 0.006±0.0010 | 0.022±0.0014 | 0.028±0.0018 | 0.018±0.0049*ab* |
| $V_4$ | 0.011±0.0023 | 0.022±0.0017 | 0.027±0.0030 | 0.020±0.0041*a* |

Different letters following the values show significant differences between values. Explanations see Table 4.

metal concentration dependent (Table 9*b*). An increasing amount of lead in soil appeared to be responsible for greater lead accumulation in seeds (Table 9*b*). The contents of lead correlated with its soil supply in all varieties (Table 9*c*). The highest lead content (0.020) was found in seeds of Mash ES- 1, and the lowest one (0.017) – in Mash 80 (Table 9*c*).

## Air dried biomass/plant (g)

The varieties revealed significant variation in their responses to the experimental factors regarding air dry mass, and statistically significant differences among the effects of the metals' toxicity were noted. The varietal

response to PGRs (kinetin and spermidine) was not statistically different between the varieties.

The differences in the alleviating effects produced by the PGRs (kinetin and spermidine) were significant. The metals' toxicity also showed significantly different effects on dry biomass (Table 3).

In plants grown in soil with the chromium concentration of 30 mg $kg^{-1}$ and exposed to distilled water foliar spray, the dry biomass was 3.29 g $plant^{-1}$. This amount increased to 4.85 and 3.79 g $plant^{-1}$ when plants were sprayed with kinetin and spermidine. In plants under the chromium stress of 60 mg $kg^{-1}$ concentration and treated with distilled water spray, the dry biomass was 2.19 g $plant^{-1}$. This amount increased to 3.38 and 2.97 g $plant^{-1}$ for plants sprayed with kinetin and spermidine.

The plants grown in soil with the lead concentration of 20 mg $kg^{-1}$ and exposed to distilled water foliar spray achieved the dry biomass af 3.17 g $plant^{-1}$. This amount increased to 5.06 and 4.77 g $plant^{-1}$ in plants sprayed with kinetin and spermidine. In plants grown in soil with the lead concentration of 40 mg $kg^{-1}$ and exposed to distilled water foliar spray, the dry biomass was 2.06 g $plant^{-1}$. This amount increased to 2.64 and 2.87 g $plant^{-1}$ when plants were sprayed with kinetin and spermidine (Table 10*a*). Mean values of plant biomass in varieties were in the order of $V_1$ (80)>$V_3$ (97)> $V_2$ (88), $V_4$ (ES 1) – Table 10*b*. Intensity of high lead stress (40 mg $kg^{-1}$ soil) was more than chromium at higher levels (60 mg $kg^{-1}$ soil) but similar at lower levels and effects of both metals were concentration dependent (Table 10*b*). Exogenous application of spermidine and kinetin increased dry biomass (Table 10*c*) and this was noted in all varieties (Table 10*a*).

## DISCUSSION

In our experiment, the metal content in the root was higher than in the shoot and seeds of stressed plant (Tables 4-9). Vajpayee et al. 2001 reported increased concentrations of chromium in plants with the increase of this element's content in soil. Similarly, Foroughi et al. (1982) reported that less lead was translocated to pods. It has been reported that Pb content of the shoot remained much lower than in the root. Primarily, lead is known to accumulate in root cell walls and a very limited amount of this metal is translocated to the shoot (Huang et al. 1997). In higher plants, roots act as a barrier to the translocation of metals to upper parts of plants (Wallace, Romney 1977), demonstrating the tolerance mechanism towards the metal present in the root cells.

Differences among genotypes might be caused by their varied capability of tolerating metal stresses. The tolerance may be due to solute accumulation

Table 10

Air dried biomass plant$^{-1}$(g) of mash bean (80 days age) after exposure to chromium (30, 60 mg kg$^{-1}$ soil) and lead (20, 40 mg kg$^{-1}$ soil) accompanied with sprays of kinetin (100.0 mM) and spermidine 1.00 mM, values are as means ± SE

(a) Indices of metals augmentation by kinetin and spermidine ($n$=16, LSD=0.2755)

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray | Mean ($n$=48, LSD=0.1593) |
|---|---|---|---|---|
| No metal input | 4.87±0.35*bc* | 6.69±0.23*a* | 6.71±0.36*a* | 6.09±0.53*a* |
| Cr (30 mg kg$^{-1}$) | 3.29±0.16*d* | 4.85±0.30*bc* | 4.79±0.39*bc* | 4.31±0.47*b* |
| Cr (60 mg kg$^{-1}$) | 2.19±0.28*h* | 3.38±0.24*d* | 2.97±0.29*ef* | 2.85±0.37*c* |
| Pb (20 mg kg$^{-1}$) | 3.17±0.29*de* | 5.06±0.15*b* | 4.77±0.20*c* | 4.33±0.47*b* |
| Pb (40 mg kg$^{-1}$) | 2.06±0.15*h* | 2.64±0.27*g* | 2.87±0.14*fg* | 2.52±0.26*b* |

(b) Differences of varietal responses to kinetin and spermidine 0.2465

| Specification | Distilled $H_2O$ spray | Kinetin spray | Spermidine spray |
|---|---|---|---|
| $V_1$ | 3.15±0.55*ef* | 4.88±0.67*a* | 4.81±0.84*ab* |
| $V_2$ | 3.07±0.54*ef* | 4.26±0.79*d* | 4.28±0.81*d* |
| $V_3$ | 3.25±0.42*e* | 4.58±0.70*bc* | 4.37±0.63*cd* |
| $V_4$ | 2.99±0.74*f* | 4.38±0.83*cd* | 4.24±0.79*d* |
| Mean ($n$=80, LSD=0.1234) | 3.11±0.56*b* | 4.52±0.74*a* | 4.42±0.76*a* |

(c) Differences of varietal responses to metals stress ($n$=12, LSD=0.318)

| Specification | No metal input | Cr (30 mg kg$^{-1}$) | Cr (60 mg kg$^{-1}$) | Pb (20 mg kg$^{-1}$) | Pb (40 mg kg$^{-1}$) | Mean ($n$=60, LSD=0.1425) |
|---|---|---|---|---|---|---|
| $V_1$ | 6.31±0.56*a* | 4.93±0.63*c* | 3.28±0.45*I* | 4.25±0.41*efg* | 2.63±0.21*kl* | 4.28±0.79*a* |
| $V_2$ | 6.14±0.52*a* | 4.06±0.26*fgh* | 2.81±0.20*jk* | 3.98±0.62*gh* | 2.37±0.26*lm* | 3.87±0.76*c* |
| $V_3$ | 5.75±0.57*b* | 4.36±0.38*def* | 3.04±0.18*ij* | 4.43±0.37*de* | 2.73±0.26*jk* | 4.06±0.65*b* |
| $V_4$ | 6.16±0.52*a* | 3.89±0.40*h* | 2.25±0.37*m* | 4.67±0.45*cd* | 2.36±0.28*lm* | 3.87±0.83*c* |

Different letters following the values show significant differences between values. Explanations see Table 4.

as a result of the process called osmotic adjustment (Munns 2002). After absorption, ions are compartmentalized in the vacuole or compatible solutes are synthesized for osmotic adjustment (Hare et al. 1998). This osmotic adjustment plays a role in the maintenance of cell turgidity and water uptake. These are essential for many physiological processes (Zhang et al. 1999).

Plant air dried biomass decreased due to the metal application to soil (Table 10). Reduction in dry biomass by heavy metal toxicity might be due

to the inhibition of the plant's growth. Growth inhibition occurs because of the imbalance induced by metals for various enzyme activities and other metabolic processes (Munns 2002, Lacerda et al. 2003). The photosynthetic rate declines in response to chromium (Connell, Al-Hamdani 2001, Joshi et al. 2003) and lead (Parys et al. 1998). Deleterious effects of lead on chlorophyll biosynthesis (Tomas, Singh 1996) and/or chlorophyll degradation stimulate the activity of chlorophyllase, which might be another cause of growth reduction (Abdel Basset et al. 1995).

Dry biomass was increased by kinetin application (Table 10). Kinetin does so by promoting growth via inducing stomatal opening through the activation of the channels (Lemtiri-Chlieh et al. 2000). Growth enhancement might be due to the kinetin-induced increase in the photosynthetic rate by increasing the amount of chlorophyll (Mumtaz et al. 1997), retarding leaf senescence or kinetin-mediated increase in the number of leaves and leaf area (Naqvi 1999). Cytokinin might retard senescence by decreasing the activity of lipoxygenase or by scavenging ROS generated under metal stress (Liu et al. 2000). Kinetin-mediated increase of total yield contributes toward enhanced biomass. This is due to the role of kinetin in controlling flower senescence and promoting flower initiation, floral development, fruit setting, and enhancing cell division and source sink relationship (Rylott, Smith 1990).

Spermidine also increased plant biomass (Table 10). The greater amount of biomass might be due to the growth promotion by spermidine (Krizek et al. 1997). The role of polyamines in plant growth may be attributed to the DNA stabilization as these compounds can bridge minor and major DNA grooves (Matthews 1993); to the stabilization of cell membrane (Kaur-Sawhney, Applewhite 1993), responses to stress (Kakkar et al. 2000) and control of senescence (Del Duca et al. 2000). Polyamines have been reported to play a role in enzyme activation and in the maintenance of ionic balance, as well as in the regulation of growth and development (Aziz et al. 1999). Biomass increase might also be due to the yield increase stimulated by spermidine. Polyamines are important for pollen maturation, germination and flower development (Evans, Malmberg 1989). Polyamines also reduce senescence. Decreased senescence may be due to the control of ethylene production (Nichols, Frost 1985). Increase in yield and its contributing factors by spermidine might be a consequence of the higher uptake of nutrients mediated by spermidine, or because of the increased water potential and sink strength.

Kinetin reduced the metal ion concentrations (Tables 4-9). Kinetin induced enhanced $K^+$ accumulation, which might be the reason for the low uptake of metal ions. The reduced uptake of the metals may be attributed to the growth promotion by kinetin, resulting in the dilution of metal concentrations. Kinetin decreased the accumulation of lead and chromium in various plant organs except for lead in seeds of plants grown under the higher lead level.

Spermidine reduced the content of metal ions (Tables 4-9). This might be due to fact that polyamines regulate the cation channel for ionic movement (Bruggemann et al. 1998). Differences in the varietal response might be a consequence of genetic variability, which in turn is influenced by the nutrient ions, especially nitrogen (Wang et al. 2000).

## CONCLUSION

The study revealed that the application of spermidine and kinetin increased substantially the plant dry biomass and significantly decreased the concentrations of the heavy metal elements in the root, stem and seeds. In plants grown under chromium stress, kinetin and spermidine were found to play roles in reducing metal accumulation in root and shoot but could not significantly reduced metal concentration in seeds. Under lead stress, spermidine was found to be effective in reducing concentration of lead in root and shoot but kinetin was proven to be less effective in this regard. Both PGRs were found to alleviate the effects of metals in term of plant air dried biomass.

**Conflict of interest:** Authors have no conflict of interest and there was no funding body for research and manuscript.

**Authors' contribution:** Ghulam Yasin designed and conducted experiment. Adeela Altaf, Ikram ul Haq and Aqsa Ahmad participated in manuscript drafting. Shahzadi Saima participated in proof reading and correction of manuscript.

## REFERENCES

Abdel-Basset R., Issa A.A., Adam M.S. 1995. *Chlorophyllase activity: Effects of heavy metals and calcium*. Photosynthetica, 31: 421-425.

Ahanger M.A., Alyemeni M.N., Wijaya L., Alamri S.A., Alam P., Ashraf M., Ahmad P. 2018. *Potential of exogenously sourced kinetin in protecting Solanum lycopersicum from NaCl induced oxidative stress through up-regulation of the antioxidant system, ascorbate--glutathione cycle and glyoxalase system*. Plos One., 13.

Aziz A., Martin-Tanguy J., Larher F. 1999. *Salt stress-induced proline accumulation and changes in tyramine and polyamines levels are linked to ionic adjustment in tomato leaf discs.* Plant Sci., 145: 83-91.

Ballas S.K., Mohandas N., Maeion L.J., Shohet S.B. 1993. *Stabilization of erythrocyte membranes by polyamines.* Proc Natl Acad Sci., USA, 80: 1942-1946.

Bruggemann U.I., Pottosin I.I., Schonknecht G. 1998. *Cytoplasmic polyamines block the fast -activating vacuolar cation channel.* Plant J., 16: 101-105.

Chen D., Shao Q., Yin L., Younis A., Zheng B. 2019. *Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses.* Front. Plant Sci., 9: 1945.

Connell S.L., Al-Hamdani S.H. 2001. *Selected physiological responses of kudzu to different chromium concentrations*. Canad. J. Plant. Sci., 81(1): 53-58.

Del Duca S., Dondini L., Della Mea M., Munoz De Rueda P., Seracassini D. 2000. *Factors affecting transglutaminase activity catalysing polyamine conjugation to endogenous substrates in the entire chloroplast*. Plant Physiol. Biochem., 38: 429-39.

Djingova R., Kuleff I. 2000. *Instrumental techniques for trace analysis*. In: *Trace Elements: Their Distribution and Effects in the Environment*. J. P. Vernet., Ed., Elsevier, London, UK.

Drolet G., Dumbrof E.B., Legge R.L., Thompson J.E. 1986. *Radical scavenging of polyamines*. Phytochemistry, 25: 367-371.

Duncan D.B. 1955. *Multiple range and multiple F-test*. Biometrics, 11: 1-42.

Evans P.T., Malmberg R.L. 1989. *Do polyamines have roles in plant development*. Ann. Rev. Plant Physiol., 40: 235-269.

Foroughi M., Venter F., Teicher K. 1982. *Experimental determination of heavy metal accumulation and distribution in bush beans* (*Phaseolus vulgaris* L.). Landwirtschaftliche Forschung, 38: 239-248.

Hare P.D., Cress W.A., Van Staden J. 1998. *Dissecting the roles of osmolyte accumulation during stress*. Plant Cell Env., 21: 535-553.

Haroun S.A., Aldesuquy H.S., Abo-Hamed S.A., El-Said O.P. 2003. *Kinetin-induced modification in growth criteria, ion contents and water relations of sorghum plants treated with cadmium chloride*. Acta Bot Hung, 45(½): 113-126.

Huang J.W., Chen J., Berti W.R., Cunningham S.D. 1997. *Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction*. Env. Sci. Techn., 31: 800-805.

Joshi U.N., Rathori S.S., Arora S.K. 2003. *Chromium induced changes in carbon and nitrogen metabolism in guar*. Adv Arid Legumes Res, 355-360.

Kakkar R.K., Bhadury S., Ray V.K., Kumar S. 2000. *Amelioration of NaCl stress by arginine in rice seedlings: changes in endogenous polyamines*. Biol. Plant., 43: 419-22.

**Kaur-Sawheny R., Applewhite P.B**. 1993. *Endogenous protein bound polyamines: correlation with regions of cell division in tobacco leaves, internodes and ovaries*. Plant Growth Reg., 12: 223-227.

Krizek T., Krame G.F., Mirecki R.M. 1997. *Influence of UV-B radiation and putrescine on shoot and root growth of cucumber seedlings grown in nutrient solution*. J. Plant Nutr., 20: 613-623.

Lacerda C.F., Cambraia J., Cano M.A.O., Ruiz H.A., Prisco J.T. 2003. *Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress*. Env. Exp. Bot., 49: 107-120.

Lemtiri-Chlieh F., Macrobbie E.A.C., Brearly C.A. 2000. *Inositol hexa bisphosphate is a physiological signal regulating the $K^+$ inward rectifying conductance in guard cells*. Proc. Nat. Acad. Sci., USA, 97: 8687-8692.

Liu K., Fu H., Bei O., Luan S. 2000. *Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements*. Plant Physiol., 124(3): 1315-1325.

Marques A.P.G.C., Rangel A.O.S.S., Castro P.M.L. 2009. *Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology*. Crit Rev Environ Sci Technol, 39(8): 622-654.

Matthews H.R. 1993. *Polyamines, chromatin structure and transcription*. BioEssays., 15: 561-567.

Mumtaz S., Naqvi S.S.M., Sheree A., Khanm A. 1997. *Salinity stress and the senescence process in wheat* (*Triticum aestivum* L.). Pak. J. Bot., 29: 299-303.

Munns R. 2002. *Comparative physiology of salt and water stress*. Plant Cell Env., 25: 239-250.

Nahar K., Hasanuzzaman M., Alam M.M., Rahman A., Suzuki T., Fujita M. 2016. *Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in Mung bean plants through upregulating the metal detoxification, antioxidant defense and Methylglyoxal detoxification systems*. Ecotoxicol Environ Saf., 126: 245-255.

Naqvi S.S.M. 1999. *Plant/crop hormones under stressful conditions*. In: *Handbook of plant and crop physiology*. Pessarakli M. (ed.), 645-660.

Nichols R., Frost G. 1985. *Wound induced production of 1-aminocyclopropane-1-carboxylic acid and accelerated senescence of Petunia corollas*. Sci. Hortic., 26: 47-55.

Norvell W.A. 1984. *Comparison of chelating agents as extractants for metals in diverse soil materials*. Soil Sci. Soc Am J., 48(6): 1285-1292,

Parys E., Romanowska E., Siedlecka M., Poskuta J.W. 1998. *The effect of lead on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of Pisum sativum*. Acta Physiol. Plant., 20(3): 313-322.

Preibe A., Jager H.J. 1978. *Effect of NaCl on the level of putrescine and related polyamines in plants differing in salt tolerance*. Plant Sci Lett., 12: 365-369.

Rylott P.D. Smith M.L. 1990. *Effects of applied plant growth substances on pod set in broad beans* (*Vicia faba* var. major). J. Agric. Sci. (Cambridge): 114: 41-47.

Salgare S.A., Acharekar C. 1992. *Effect of industrial pollution on growth and content of certain weeds*. J. Nat. Conserv., 4: 1-6.

Sarjala T., Haggman H., Aronen T. 1997. *Effect of exogenous polyamines and inhibitors of polyamine biosynthesis on growth and free polyamine contents of embryogenic Scots pine callus*. J. Plant Physiol., 150(5): 597-602.

Sosnowski J., Malinowska E., Zankowski K., Krol J.I., Redzik P. 2019. *An estimation of the effects of synthetic auxin and cytokinin and the time of their application on some morphological and physiological characteristics of Medicago x Varia T. Martyn*. Saudi J. Biol Sci., 26: 66-73.

Taiz L., Zeiger E. 1996. *Plant Physiology*. Sinauer Associates, Sunderland, Mass, USA, 2002.

Tomas R.M., Singh V.P. 1996. *Reduction of cadmium induced inhibition of chlorophyll and carotenoid accumulation in Cucumis sativus* L. *by unconazole* (S.3307). Photosynthetica, 32: 145-148.

Vajpayee P., Rai U.N., Ali M.B., Tripathi R.D., Yadav V., Sinha S., Singha S.N. 2001. *Chromium-induced physiologic changes in Vallisneria spiralis* L. *and its role in phytoremediation of tannery effluent*. Bull. Environ. Contam. Toxicol., 67(2): 246-256.

Wallace A., Romney E.M. 1977. *Roots of higher plants as a barrier to translocation of some metals to shoots of plants*. In: *Biological implications of metals in the environment*. Proc of the 15th Annual Hanford Life Science Symposium, Richland, Washington 29 September to 10 October, TIC Conf-750929, 370-379.

Wang L., Xiang D., Zou Q. 2000. *Effect of polyamines on senescence of attached groundnut leaves*. Sci. Agric. Sin., 33(3): 30-35.

Yoshida S., Farno D.A., Cock J.H., Gomez K.A. 1976. *Laboratory manuals of physiological studies of rice*. IRRI. Loss Bonas. Phillipines.

Yu Y., Zhou W.W., Liang X., Zhou K.J., Lin X.Y. 2019. *Increased bound putrescine accumulation contributes to the maintenance of antioxidant enzymes and higher aluminum tolerance in wheat*. Environ. Pollut., 252: 941-949.

Yu Y., Zhou W.W., Zhou K.J., Liu W.J., Liang X., Chen Y., Sun D.S., Lin X.Y. 2018. *Polyamines modulate aluminum-induced oxidative stress differently by inducing or reducing* $H_2O_2$ *production in wheat*. Chemosphere, 212: 645-653.

Zalabak D., Pospisilova H., Smehilova M., Mrizova K., Frebort I., Galuszka P. 2013. *Genetic engineering of cytokinin metabolism: prospective way to improve agricultural traits of crop plants*. Biotechnol Adv., 31: 97-117.

Zhang A., Jennings A., Barlow P.W., Forde B.G. 1999. *Dual pathways for regulation of root branching by nitrate*. Plant Biol, 96: 6529-6534.