Effect of tungsten on growth, biochemical constituents, molybdenum and tungsten contents in wheat

A. Kumar, N.C. Aery

Laboratory of Geobotany and Biogeochemistry, Department of Botany, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India

ABSTRACT

The effect of various concentrations (3, 9, 27, 81, and 243 mg/kg) of tungsten (W) on growth performance, biochemical constituents and tungsten and molybdenum (Mo) contents in wheat was observed. Lower doses (up to 9 mg/kg) of tungsten showed promotory effects whereas higher doses retarded. An increment in growth, biomass, chlorophyll and carbohydrate contents was observed. Tungsten contents in root and shoot showed a very strong linear dependence on the soil applied W contents. Mo contents in plant tissue showed an increase with an increase in the W contents in plant tissue up to a threshold after which it showed an abrupt decrease. The activity of peroxidase enzyme decreased with lower application of W. Higher administration of tungsten (27–243 mg/kg) resulted in increased total phenol, free proline and activity of enzyme peroxidase.

Keywords: oxidative stress; toxic elements; sodium tungstate; plant stress metabolism; pigments; *Triticum aestivum* L.

Tungsten (W) is a transition element of group VIB of the Periodic Table and is the heaviest metal with biological activity. Its biological importance was fully proved in the last decade due to isolation of a number of enzymes containing tungsten at their active site, such as formate dehydrogenase, aldehyde: ferredoxin oxidoreductase, formaldehyde: ferredoxin oxidoreductase etc. (L'vov et al. 2002). Tungsten was observed as growth inducer in *Anabaena doliolum* (Tyagi 1974) and *Trifolium repens* (Quin and Hoglund 1976). Addition of small quantities of tungsten (0.25–1.0 µg/L) to the growth medium was reported to stimulate the nitrate reductase activity of plants (Notton 1983). Adamakis et al. (2010, 2011) observed a reduction in the number and length of cortical microtubules and disruption in their orientation in pea and cotton due to tungsten. Tungsten is not considered as essential mineral nutrient for plants. Very little information is available regarding the role of tungsten in plants. However, the accumulation of tungsten by plants (Aery 2000) and its beneficial effects was observed (Notton 1983). Moreover, both wolframite and scheelite mineralization is found in the nearby areas (Aery 2000). Consequently the present study was carried out to examine the effect of different concentrations of tungsten as sodium tungstate on the growth performance, biochemical constituents and accumulation of molybdenum (Mo) and W in different parts of wheat.

MATERIAL AND METHODS

Experimental set-up. The experiments were set up during the month of November. Three kg soil was filled in the pots of 30 cm height and 25 cm diameter. Five concentrations (3, 9, 27, 81, and 243 mg/kg) of tungsten were applied as sodium tungstate ($Na_2WO_4 \cdot 2\,H_2O$) (E. Merk), with no Mo contents. The concentrations were prepared separately by taking corresponding amount (calculated on the basis of molecular weight) of chemical/kg of air dried soil. The experimental soil was silty-sand type with slightly alkaline pH (7.8). The physico-chemical characteristics of experimental soil are presented in Table 1. No other supplement nutrients were applied. Pots without added W constituted the control. Seeds of uniform size and weight of *Triticum aestivum* L. var. Raj 4037 were

Table 1. Showing the characteristics of the experimental soil

pH	7.8
Organic carbon (%)	0.65
Total nitrogen (%)	1.2
Available phosphorous (mg/kg)	8.5
Available potassium (mg/kg)	22
Available iron (mg/kg)	2.2
Exchangeable calcium (%)	0.53
Tungsten concentration	ND

ND – not detectable

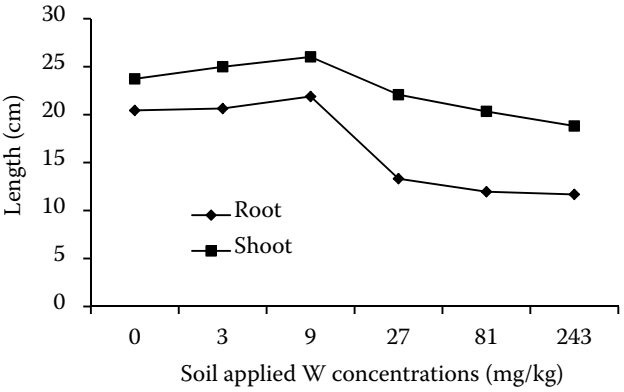

Figure 1. Effect of various concentrations of tungsten on the root and shoot length of *Triticum aestivum* L.

selected. Fifteen seeds of the test crop were sown at 2 cm depth in each pot. The experiment was replicated four times in a completely randomized block design (CRB). Watering (100 mL) was done on alternate days. After 10 days seedlings were thinned to 10 in each pot. After seed setting (after 60 days of sowing), 2nd young leaf was analyzed for biochemical constituents. Plants were harvested after fruiting. The plant samples were dried at 80°C in an oven for 48 h for the measurements of dry weight. After harvest, soil sample for each treatment were collected and analyzed for tungsten contents.

Biochemical analysis. Chlorophyll a, b and total chlorophyll were determined after the method of Arnon (1949). Total phenol contents were estimated by Folin-Ciocalteau method (Bray and Thorpe 1954). Free proline content in leaves was measured using the ninhydrin method (Bates et al. 1973). Estimation of carbohydrate contents was carried out by anthrone and sulphuric acid (Yemm and Willis 1954).

Peroxidase assay. Leaf tissues (0.5 g) were homogenized in 10 mL ice-cold 0.1 mol/L phosphate buffer (pH 6.0). The homogenate was centrifuged for 30 min at 2000 g at 4°C. The supernatant was used for assaying the enzyme activity. 3 mL of 0.05 mol/L pyrogallol and 0.1 mL of supernatant were taken in a clean dry cuvette which was transferred to a spectrophotometer. In order to start reaction 0.5 mL of 1% H_2O_2 was added to the cuvette. Initial absorbance and then change in absorbance was noted after an interval of 30 s for 3 min at 420 nm in a UV-visible spectrophotometer (PHARMASPEC UV 1700, SHIMADZU).

Elemental analysis. The tungsten contents in soil and plant material was determined by the colorimetric method of Quin and Brooks (1972) using toluene-3,4-dithiol. Molybdenum contents in plant tissue were determined using the Atomic Absorption Spectrophotometer (Electronics Corporation of India, Model No. 4141).

Statistical analysis. All the raw data were subjected to the Origin 6.1 Software for the computation of correlation coefficient (r) and regression equation (y).

RESULTS AND DISCUSSION

Growth parameters. Tungsten is considered to be a competitive inhibitor of Mo function in bacteria (Brill et al. 1974), in green alga *Chlorella* (Paneque et al. 1972) and the higher plants such as spinach (Notton and Hewitt 1971). Tyagi (1974) observed that sodium tungstate increases the growth rate and final growth yields of molybdenum-starved cultures of *Anabaena doliolum*. According to Davies and Stockdill (1956) addition of tungstate without molybdate resulted in increased growth. In the present study an increase in shoot and root length was observed at lower applied doses of tungsten (3–9 mg/kg). The increase was by 51% and 23% higher over the control, respectively (Figure 1). Beyond the above level shoot as well as root length decreased.

Lower applied doses of tungsten showed an increment in root-shoot dry weight of wheat. Maximum dry weight of root and shoot was observed at 9 mg/kg applied dose of tungsten (Table 2). Beyond the above level root-shoot dry weight concomitantly decreased. Minimum dry weight was observed at the highest applied dose (243 mg/kg) of tungsten and was 39.35% and 69.63% lower, as compared to control for root and shoot, respectively. An increment in relative yield of wheat was observed at lower application of tungsten. It was observed to be the highest at 9 mg W/kg for both root as well

Table 2. Effect of various concentrations of tungsten on dry matter production and relative dry matter yield of *Triticum aestivum* L.

W: soil concentrations (mg/kg)	Root dry weight (g/plant)	Relative dry matter yield	Shoot dry weight (g/plant)	Relative dry matter yield
Control	0.108 ± 0.02	100	0.299 ± 0.07	100
3	0.146 ± 0.03	135.18	0.334 ± 0.048	111.70
9	0.157 ± 0.03	145.37	0.341 ± 0.04	114.04
27	0.073 ± 0.01	67.59	0.264 ± 0.07	88.29
81	0.056 ± 0.04	51.85	0.238 ± 0.00	79.59
243	0.042 ± 0.01	39.35	0.208 ± 0.09	69.63

as shoot (Table 2). As the W content in plant tissue increased the relative yield initially increased but decreased at higher tissue concentration of W (Figure 2).

The decrement in root-shoot length and dry weight was probably due to reduced cellular turgor (Gabbrielli et al. 1990) which inhibits cell enlargement (Aery and Jagetiya 2000, Mali and Aery 2008) and/or due to inhibition of cell division in the meristematic zone (Powell et al. 1986). Adamakis et al. (2008) showed that tungstate retarded seedling growth rate and stopped root elongation in *Pisum sativum* L. cv. Onmard and *Gossypium hirsutum* L. cv. Campo. Seedling growth recovered when tungstate was removed, but primary roots continued to be stunted, while lateral root initiation and growth were stimulated. Quin and Hoglund (1976) reported that 5 μg/g tungstate slightly depressed the growth and N accumulation in white clover (*Trifolium repens* L.). Adamakis et al. (2011) has reported some toxic effects of W on pea root including retraction of the plasma membrane from the cell wall, shrinkage of the protoplast, disruption of cortical microtubules as well as *F*-actin cytoskeleton and restrain of cell divisions.

Biochemical constituents. Johnson et al. (2009) reported a significant increment in photosynthetic pigment especially chlorophyll a and carotenoids with the application of tungsten in barley. In the present study a remarkable increment was observed in the chlorophyll contents at lower applied doses of tungsten (Figure 3). Highest chlorophyll a, b and total chlorophyll contents was observed at 9 mg W/kg which was respectively, 24.36%, 51.30% and 25.37% higher, over the control. At the higher applied doses of W (27–243 mg/kg) chl a, b and total chlorophyll regularly decreased. At the highest applied dose of W (243 mg/kg) the chlorophyll contents were observed to be the lowest and showed 6.06%, 1.17% and 37.31% reduction over the control (Figure 3), respectively for chl a, chl b and total chlorophyll. The carbohydrate contents in the leaf showed similar trend as that of chlorophyll. Maximum increase in carbohydrate contents was observed at 9 mg W/kg. Beyond this level the carbohydrate contents regularly decreased (Figure 4). Minimum carbohydrate content was observed at 243 mg W/kg and was 20.80% lower as compared to control.

Heavy metal stress results in accumulation in reactive oxygen species. Reactive oxygen species (ROSs) are known to damage cellular membrane by inducing lipid peroxidation (Devi and Prasad 1998). It also can damage DNA, protein, lipid and chlorophyll (Mittova et al. 2000). These deleterious effects are alleviated by the increased accumulation of various compounds such as phenolics and proline. Accumulation of proline (Figure 5) is affected by regulation of proline synthesis from glutamic acid. An increased content of proline inhibits the biosynthesis of proline at the expense of glutamic acid (Pavlíková et al. 2007). Glutamic acid and cysteine are essential for the biosynthesis of phytochelatins which plants need to detoxification of heavy metals – Cd (Pavlíková et al. 2008) and metalloids – As (Pavlík et al. 2010). The induction in accumulation of phenolic compounds and per-

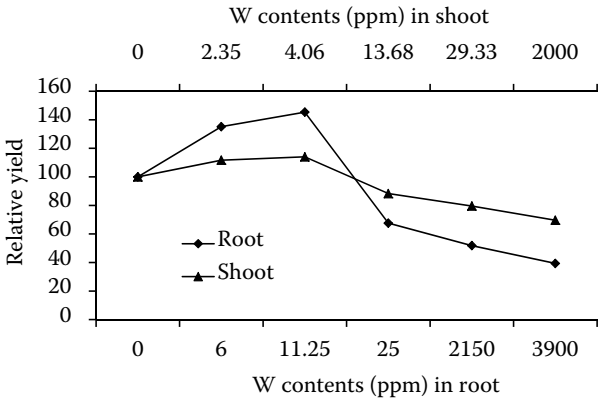

Figure 2. Relative yield as a function of W concentration in *Triticum aestivum* L.

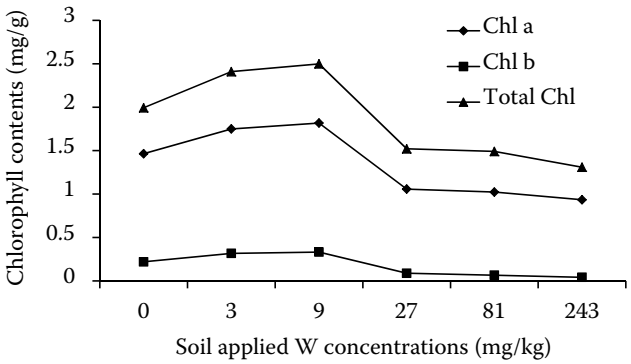

Figure 3. Effect of various concentrations of tungsten on the chlorophyll a, b and total chlorophyll contents (mg/g) in *Triticum aestivum* L.

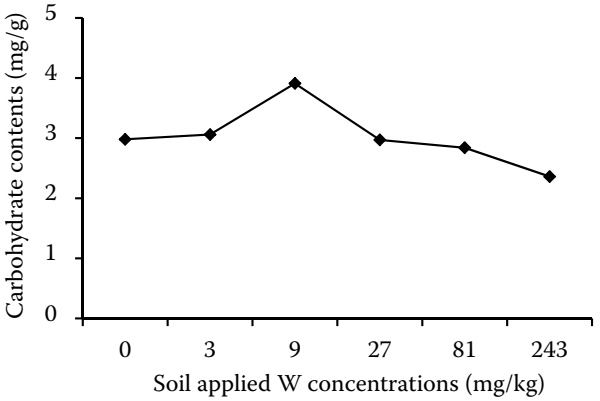

Figure 4. Effect of various concentrations of tungsten on the carbohydrate contents (mg/g) in *Triticum aestivum* L.

oxidase activity in plants with high concentration of metals is a well known fact. Peroxidase activity was observed to be indicator of heavy metal toxicity and subsequent stress situation in plants (Nashikkar and Chakrabarti 1994).

In the present study lower applied doses of W (up to 9 mg/kg) which appear to be suitable to wheat, showed a decrease in stress preventing compounds such as total phenol and free proline. Total phenol and free proline contents were the lowest at 9 mg W/kg and showed a decrease of 13.15% and 71.92%, respectively, over the control. Higher application of W (27–243 mg/kg) resulted in increased free proline contents. The increase in the concentration of these compounds was observed to be maximum at 243 mg/kg and was 26.84% and 6.47%, over the control (Figure 5). Mali and Aery (2009) observed a significant increment in proline contents in cowpea with the application of silicon. At lower applied doses of W (3–9 mg/kg) total phenol contents decreased (Figure 4). As the soil W concentration increased the total phenol contents increased. The induction of phenolic compounds biosynthesis was also observed in wheat in response to U (Jain and Aery 1997), Cr (Mukhopadhyay and Aery 2000) and Si (Mali and Aery 2009). The activity of peroxidase enzyme was minimum at 9 mg W/kg. Beyond this level, peroxidase activity concomitantly increased in higher applied doses of tungsten (27 mg/kg to 243 mg/kg) (Figure 6). The increment in total phenols and peroxidase activity in wheat at higher W concentrations (27–243 mg/kg) reflects the toxicity of W at these doses. Peroxidase activity is a general response to uptake of toxic amount of metals (Van Assche and Clijsters 1990). It enables the plants to protect themselves against the oxidative stresses. An increase in peroxidase activity was observed beyond 9 mg/kg tungsten application indicating that its higher (beyond 9 mg/kg) concentrations are harmful to plants.

Elemental contents. W contents in plant tissues increased with increasing concentration of W in soil. Maximum W contents in plant tissues was observed at 243 mg W/kg and was 3900 ppm and 2000 ppm for root and shoot, respectively (Figure 7). The concentration of accumulated W in plant tissue was directly proportional to the soil-applied tungsten. Roots accumulated almost two

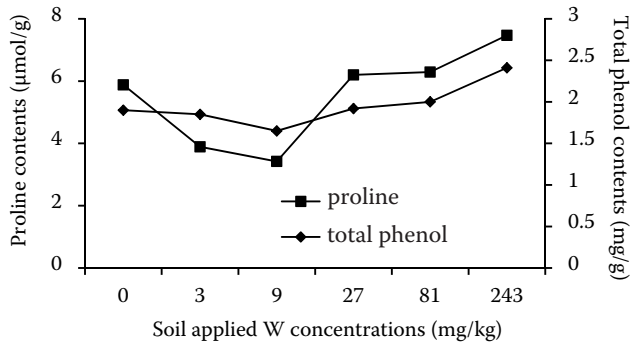

Figure 5. Effect of various concentrations of tungsten on the total phenol (mg/g) and proline contents (µmol/g) in *Triticum aestivum* L.

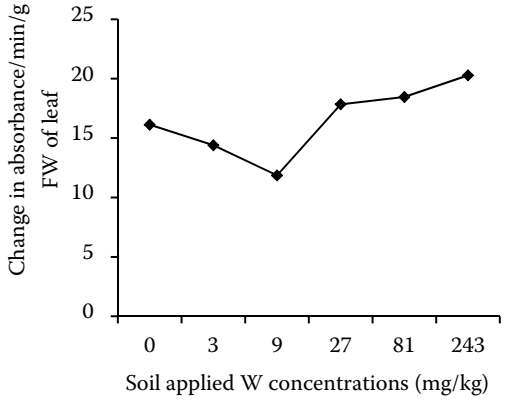

Figure 6. Effect of various concentrations of tungsten on the activity of enzyme peroxidase in *Triticum aestivum* L.

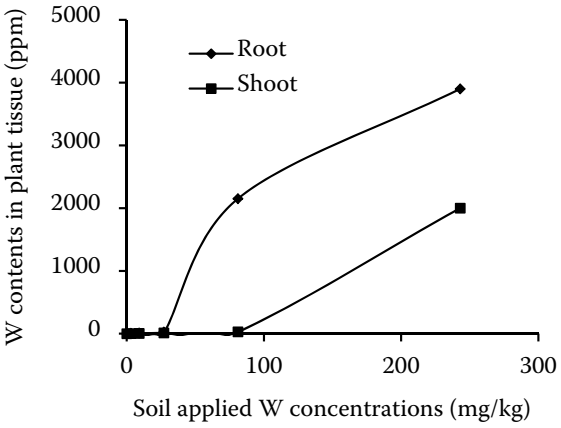

Figure 7. Tungsten contents in plant tissue as affected by soil-applied W concentrations

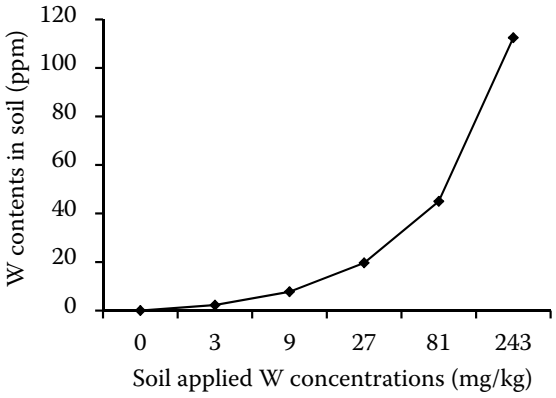

Figure 8. Relation between soil-applied W concentrations and residual W contents in soil

times tungsten than was accumulated by shoots. Similar findings were also observed by Johnson et al. (2009) and Jiang et al. (2007) in sunflower and maize, respectively. Residual W contents in soil showed linear relationship to soil applied W (Figure 8). Application of W to soil resulted in an increase in Mo contents in plant tissue. Maximum Mo contents observed at 81 mg W/kg were 86.57% higher as compared to control (Figure 9).

The Mo contents in the shoot of wheat showed a synergistic relationship with W contents. As the W content in shoot increased, the Mo content also increased (Figure 10). This synergistic relationship was observed up to application of 81 mg/kg W. The results agree with those on white clover plants, which show increment in Mo contents in shoot with the application of W to soil (Quin and Hoglund 1976). Surprisingly, at the dose of 243 mg/kg W applied plant Mo contents decreases although it was still higher than Mo contents in control plants.

Correlation coefficient between residual W contents in soil and W concentration in wheat were also computed. A significant (P = 0.01 for shoot and P = 0.001 for root) positive value of correlation coefficient between these parameters indicates the dependence of plant W on W contents in soil. Regression equations (y) were also computed for these parameters (Table 3).

The results show that tungsten is beneficial for wheat at low concentrations. Results on growth and biochemical parameters indicate that at low concentration tungsten (a) increases the length of root and shoot, (b) induce biomass production, (c) induce the biosynthesis of chlorophyll contents and (d) increase the biosynthesis of carbohydrate contents. At high concentration it becomes toxic

Table 3. Relationship between residual W contents in soil (x) and W contents in plant tissue (y) of *Triticum aestivum* L.

Parameter	Regression equation	Correlation coefficient (r)	P
Shoot	$y = 17.50x - 204.44$	0.9288*	≤ 0.01
Root	$y = 0.88x - 2.66$	0.9716**	≤ 0.001

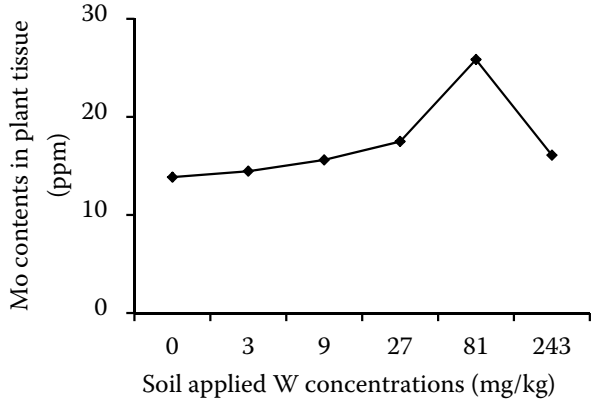

Figure 9. Effect of soil-applied W concentrations on Mo contents in shoot of *Triticum aestivum* L.

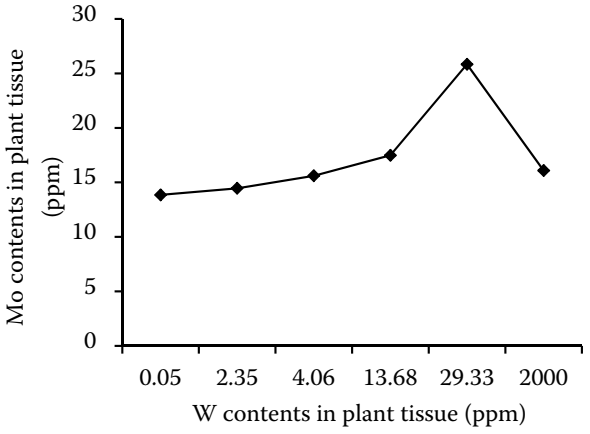

Figure 10. Relation between accumulated W and Mo contents in shoot of *Triticum aestivum* L.

and the toxicity is reflected as decrement in all above parameters. The decrement in total phenol, free proline contents and activity of enzyme peroxidase with low concentration of W exhibits a beneficial role of W in plant metabolism.

Acknowledgement

The authors are thankful to the CSIR, New Delhi for providing financial assistance in the form of SRF to Anil Kumar.

REFERENCES

Adamakis I.D.S., Eleftheriou E.P., Rost T.L. (2008): Effects of sodium tungstate on the ultrastructure and growth of pea (*Pisum sativum*) and cotton (*Gossypium hirsutum*) seedlings. Environmental and Experimental Botany, *63*: 416–425.

Adamakis I.D.S., Panteris E., Eleftheriou E.P. (2010): Tungsten affects the cortical microtubules of *Pisum sativum* root cells: experiments on tungsten-molybdenum antagonism. Plant Biology, *12*: 114–124.

Adamakis I.D., Panteris E., Eleftheriou E.P. (2011): The fatal effect of tungsten on *Pisum sativum* L. root cells: indications for endoplasmic reticulum stress-induced programmed cell death. Planta, *234*: 21–34.

Aery N.C. (2000): Biogeochemical investigations on tungsten deposits of balda, Rajasthan, India. Journal of the Indian Botanical Society, *79*: 67–70.

Aery N.C., Jagetiya B.L. (2000): Effect of cobalt treatments on dry matter production of wheat and DTPA extractable cobalt content in soils. Communications in Soil Science and Plant Analysis, *31*: 1275–1286.

Arnon D.I. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology, *24*: 1–15.

Bates L.S., Waldren R.P., Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant and Soil, *39*: 205–207.

Bray H.G., Thorpe W.V. (1954): Analysis of phenolic compounds of interest in metabolism. Methods of Biochemical Analysis, *1*: 27–52.

Brill W.J., Steiner A.L., Shah V.K. (1974): Effect of molybdenum starvation and tungsten on the synthesis of nitrogenase components in *Klebsiella pneumoniae*. Journal of Bacteriology, *118*: 986–989.

Davies E.B., Stockdill S.M.J. (1956): A pasture response to sodium tungstate on a New Zealand soil. Nature, *178*: 866.

Devi S.R., Prasad M.N.V. (1998): Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: Response of antioxidant enzymes and antioxidants. Plant Science, *138*: 157–165.

Gabbrielli P., Pandolfini T., Vergnano O., Palandri M.R. (1990): Comparison of two serpentine species with different nickel tolerance strategies. Plant and Soil, *122*: 271–277.

Jain G.S., Aery N.C. (1997): Effect of uranium additions on certain biochemical constituents and uranium accumulation in wheat. Biologia, *52*: 599–604.

Jiang F., Heilmeier H., Hartung W. (2007): Abscisic acid relations of plants grown on tungsten enriched substrates. Plant and Soil, *301*: 37–49.

Johnson D.R., Inouye L.S., Bednar A.J., Clarke J.U., Winfield L.E., Boyd R.E., Ang C.Y., Goss J. (2009): Tungsten bioavailability and toxicity in sunflowers (*Helianthus annuus* L.). Land Contamination and Reclamation, *17*: 141–151.

L'vov N.P., Nosikov A.N., Antipov A.N. (2002): Tungsten-containing enzymes. Biochemistry (Moscow), *67*: 196–200.

Mali M., Aery N.C. (2008): Silicon effects on nodule growth, dry-matter production, and mineral nutrition of cowpea (*Vigna unguiculata*). Journal of Plant Nutrition and Soil Science, *171*: 835–840.

Mali M., Aery N.C. (2009): Effect of silicon on growth, biochemical constituents, and mineral nutrition of cowpea. Communications in Soil Science and Plant Analysis, *40*: 1041–1052.

Mittova V., Volokita M., Guy M., Tal M. (2000): Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiologia Plantarum, *110*: 42–51.

Mukhopadhyay N., Aery N.C. (2000): Effect of Cr (III) and Cr (VI) on the growth and physiology of *Triticum aestivum* plants during early seedling growth. Biologia, *55*: 403–408.

Nashikkar V.J., Chakrabarti T. (1994): Catalase and peroxidase activity in plants – an indicator of heavy metal toxicity. Indian Journal of Experimental Biology, *32*: 520–521.

Notton B.A. (1983): Micronutrients and nitrate reductase. In: Robb D.A., Pierpoint W.S. (eds.): Metals and Micronutrients, Uptake and Utilization by Plants. Academic Press, London, 219–239.

Notton B.A., Hewitt E.J. (1971): The role of tungsten in the inhibition of nitrate reductase activity in spinach (*Spinacea oleracea* L.) leaves. Biochemical and Biophysical Research Communications, *44*: 702–710.

Paneque A., Vega J.M., Cárdenas J., Herrera J., Aparicio P.J., Losada M. (1972): ^{185}W-labelled nitrate reductase from Chlorella. Plant and Cell Physiology, *13*: 175–178.

Pavlík M., Pavlíková D., Staszková L., Neuberg M., Kaliszová R., Száková J., Tlustoš P. (2010): The effect of arsenic contamination on amino acids metabolism in *Spinacia oleracea* L. Ecotoxicology and Environmental Safety, *73*: 1309–1313.

Pavlíková D., Pavlík M., Staszková L., Motyka V., Száková J., Tlustoš P., Balík J. (2008): Glutamate kinase as a potential biomarker of heavy metal stress in plants. Ecotoxicology and Environmental Safety, *70*: 223–230.

Pavlíková D., Pavlík M., Staszková L., Tlustoš P., Száková J., Balík J. (2007): The effect of potentially toxic elements and sewage sludge on the activity of regulatory enzyme glutamate kinase. Plant, Soil and Environment, *53*: 201–206.

Powell M.J., Davies M.S., Francis D. (1986): The influence of zinc on the cell cycle in the root meristem of a zinc-tolerant and

a non-tolerant cultivar of *Festuca rubra* L. New Phytologist, *102*: 419–428.

Quin B.F., Brooks R.R. (1972): The rapid determination of tungsten in soils, stream sediments, rocks and vegetation. Analytica Chimica Acta, *58*: 301–309.

Quin B.F., Hoglund J.H. (1976): The effects of tungstate and nitrogen source on the dry weight and nitrogen yields, and molybdenum and tungsten content, of white clover (*Trifolium repens*). Plant and Soil, *45*: 201–212.

Tyagi V.V.S. (1974): Stimulatory effects of sodium tungstate on the growth of the blue-green alga, *Anabaena doliolum*. Annals of Botany, *38*: 485–491.

Van Assche F., Clijsters H. (1990): Effects of metals on enzyme activity in plants. Plant, Cell and Environment, *13*: 195–206.

Yemm E.W., Willis A.J. (1954): The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal, *57*: 508–514.

Received on June 23, 2011

Corresponding author:

Prof. Naresh Chander Aery, Mohanlal Sukhadia University, Department of Botany, Laboratory of Geobotany and Biogeochemistry, Udaipur-313039, Rajasthan, India
phone: + 91 294 2413 955 217, e-mail: ncaery@yahoo.com