ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Resource allocation in dormant corms of crocus *sativus L.* and their heavy metal analysis

Syed Sana Mehraj*[#], *Azra N. Kamili, G.A Bhat and Ruqeya Nazir

Centre of Research for Development and Department of Environmental science, University of Kashmir

Manuscript Info

Manuscript History:

Received: 15 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

Crocus *sativus*, Metals, Dormant, Pollution.

Corresponding Author

Syed Sana Mehraj

Abstract

Objective; This study was conducted to investigate the concentrations of heavy metals in soil and saffron corms which have further impacts on various growth parameters. Soil and plant samples were collected during dormant phase from different locations in Kashmir India, and analyzed for Hg, Pb, Mn, Cr, Zn, Cd, Co, Ni, Cu and Fe. **Methods;** All the heavy metals were determined by using Atomic Absorption Spectrophotometer (AAS). For resource allocation fresh and dry biomass was estimated. **Results;** The concentrations of Hg, Pb, Mn, Cr, Zn, Cd, Co, Ni, Cu and Fe exceeded their respective permissible limits in soil and plant samples. Significant variation was found in fresh and dry biomass of samples. **Conclusion;** The metal contents in the samples were found comparatively higher at pollution site thus has significant variation in resource allocation.

INTRODUCTION

Saffron is one among the most important cash crops of Kashmir Himalayas. It is the most precious and most expensive spice (Mehraj *et al.,* 2013) in the world derived from the stigma of the flower of Saffron (Crocus sativus L.). Saffron contains many plant derived chemical compounds that are known to have several biological activities including antioxidant, anti-inflammatory, and antiproliferative (Chang *et al.,* 1964; Abdullaev, 2002;Assimopoulou *et al.,*2005).Kashmir valley was once known to produce one of the finest qualities of saffron as the environment was most suitable for growth and propagation of plant. But now the greatest threat to the quality and quantity of this very plant is pollution. Among the main sources of pollution are brick kilns, stone crushers, automobile exhaust and cement factories and among these cement factories are the most important sources of pollution responsible for destroying saffron (Jan and Bhat, 2006; Rafiq *et al.,* 2008; Jan, 2009; Mehraj *et al.,* 2013) as most of them are located around the only area where saffron is grown in valley. This cement dust contains a number of harmful chemicals which have adversely affected the plant. Besides gaseous and particulate pollutants there are also enhanced levels of toxic heavy metals in the environment of cement factory likely cobalt, lead, chromium, nickel, mercury etc (Baby *et al*.,2008;Omran *et al.,*1986;Ogbonna and Nwosu.,2011; Mandal and Voutchkov.,2011; Addo et al.,2012; Ibrahim *et al.*,2012;Gupta and Sharma.,2013) posing very potential hazard for all living organisms. Increased concentrations of the above pollutants cause progressive reduction in the photosynthetic ability of leaves, mainly a reduction in growth and productivity of plants. In this study we examined the corm health, as corm directly affects the quality and quantity of saffron, as it is the main reservoir of nutrients for growth and development of plant.

Metal toxicity in plants has been reported by various authors (Bollard and Butler, 1966; Brown and Jones., 1975; Foy et al., 1978; Chidambaram et al., 2009). Heavy metal pollutants are stable in the environment but highly toxic to biological organisms (Zou *et al*., 2006; Levent *et al.,* 2009)

1.1 Sites Selected

Sites Selected	Characteristic Features
Site I	Site was adjacent to cement factory in its south-western direction at an altitude of 1650masl (5413.3 feet) within the geographical co-ordinates of 34°02′ 08.61″N latitude and 075°01′02.2″E longitude. The factory is at a distance of about 2.5 km from the main town and was in receipt of heaviest dust pollution. The site was 0 km away from the factory.
Site II	This site was located 3 km away from the cement factory in the same direction at an altitude of 1648 masl (5406.8 feet) within the geographical co-ordinates of 34°02′37.7″N latitude and 074°57′31.0″E longitude at Wayun. This site was also in receipt of heavy cement dust.
Site III	This was located at 5 km away from the cement factory in the same direction at an altitude of 1645 masl (5396.9feet) within the geographical co-ordinates of 34°01′53.8″N latitude and 074°57′32.5″E longitude at Balhama.
Site IV	This was located at 7 km away from the cement factory in the same direction at an altitude of 1645 masl (5396.6feet) within the geographical co-ordinates of 34°02′15.0″N latitude and 074°55′30.0″E longitude at Sempora.
Site V	This was situated very close to Lethpora and represented the control/reference site. This site was about 12 kms (Ariel distance of 5-6 kms) away from the cement factory towards the south at an altitude of 1642 masl (5387.1feet) within the geographical co-ordinates of 34° 01’81.61’’N 074° 57’ 33.2’’E. The site was under saffron cultivation and was apparently not in receipt of any cement dust from the factory.

2. Materials and Methods

2.1 Sample collection

During the dormant phase of saffron plant, soil and corm samples were collected from five sites while moving away from the cement factories.

2.2 Reagents and standards

Heavy metal analysis was done (AOAC, 1995) using atomic absorption spectroscopy in plant samples using wet digestion method (Meena *et al.*, 2010). Standards of Hg, Cd, Pb, Ni, Cr, Zn, Mn, Cu, Co and Fe procured from Merck, Germany, were used as reference analytes for quantitative estimation of heavy metals as well as accurate calibration and quality assurance of each analyte. The standard stock solutions (1000 ppm) were diluted to obtain working standard solutions ranging from 1 ppm to 10 ppm and stored at 4°C. A calibration curve was plotted between measured absorbance and concentration (ppm). All the samples were analyzed in triplicate using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Germany).

2.3 Sample preparation

The soil samples were mainly collected to a depth of 15 cm. The soil samples were dried thoroughly and then sieved through a <2 mm sieve. Then, the sieved soil samples were digested. While as plant samples were collected separately from different sites. The collected material was washed thoroughly with running tap water followed by washing with DDW (double distilled water) to remove the dust particles and possible parasites. They were shade dried, powdered and stored in closed air tight bottles for further experimentation.

2.4 Analytical procedure

A 1g of the powdered sample was weighed into a Teflon conical flask. 20 ml of the digestion mixture (a mixture of sulphuric acid, perchloric acid and nitric acid in ratio 1: 4: 40 by volume) was added and left to stand overnight. Thereafter, the flask was heated at 70^{o}C for about 40 minutes and then, the heat was increased to 120°C. The mixture turned black after a while and the digestion was complete when the solution became clear with appearance of white fumes. The digest was diluted with deionized 20ml of water and

for 15minutes. This was then allowed to cool, transferred into 100ml volumetric flasks and diluted to the mark with deionized water. The sample solution was then filtered using whatmann filter paper (pore size 0.45μ, Axiva) into a screw capped polyethylene bottle and stored for heavy metal determination using Atomic Absorption Spectrophotometer (AAS, Perkin Elmer Germany) with a digital read out system. All experiments were done in triplicate for precision and accuracy of the results. .

2.5 Fresh and Dry Weight of Corms

Biomass of corms was calculated by weighing the fresh corms collected in a quadrat of 25 cm^2 area. At each site five quadrats were randomly sampled and the results were calculated and expressed as gm/cm^2.
Dry weight of corms were then taken by drying of the corms in an oven at 60°C of temperature and then weighed again.

2.6 Statistical analysis

Results of the research were analyzed for mean and standard deviation. All experiments were done in triplicate for precision and accuracy of the results.

3. RESULTS

Graphical abstract

Saffron corms **Saffron soil**

Metal analysis

Table 1: Estimation of heavy metals (µg/g) in soil and corms during dormant phase

Sites	I		II		III		IV		V	
Metals µg/g	**Soil**	**Corm**	**Soil**	**Corm**	**Soil**	**Corm**	**Soil**	**Corm**	**Soil**	**Corm**
Mercury	1.7± 0.004	0.770± 0.06	1.37± 0.006	0.510± 0.06	1.2± 0.002	0.399± 0.04	1.11± 0.001	0.321± 0.02	0.47± 0.001	0.07± 0.001
Cadmium	0.32± 0.004	1.09± 0.005	0.32± 0.007	0.1± 0.008	0.25± 0.007	0.15 ±0.002	0.24± 0.003	0.17± 0.007	0.27± 0.003	0.11± 0.005
Lead	3.78± 0.002	6.8± 0.003	3.8± 0.001	2.6± 0.001	1.81± 0.003	0.8 ±0.002	0.6± 0.003	0.61± 0.002	0.4± 0.001	0.35± 0.001
Nickel	3.21± 0.006	4.84± 0.004	2.75± 0.011	3.16± 0.002	2.66± 0.003	0.82 ±0.004	2.5± 0.006	0.78± 0.003	0.03± 0.003	0.8± 0.005
Chromium	10.12± 0.008	0.33± 0.006	7± 0.006	0.8± 0.007	6.21± 0.007	0.88 ±0.005	5.5± 0.004	0.01± 0.005	4.67± 0.009	0± 0.001
Zinc	2.45± 0.011	0± 0.002	3.02± 0.003	0± 0.003	3.59± 0.001	0 ±0.001	3.24± 0.001	0± 0.003	3.99± 0.003	0.12± 0.001
Magnese	79.1 ±0.027	4.28 ±0.001	75 ±0.016	3.66 ±0.004	68.3 ±0.06	2.18 ±0.002	64.7 ±0.03	2.04 ±0.003	61.2 ±0.028	2.31± 0.003

Copper	195.2 ±2	47.86 ±3	172.7 ±1	32.08 ±2	145.8 ±4	23.85 ±5	131.5 ±3	23.94 ±4	111 ±5	15.5 ±1
Cobalt	1.22± 0.015	0.17± 0.011	0.9± 0.014	0.15± 0.016	0.22± 0.005	0.14 ±0.007	0.28± 0.003	0.07 ±0.009	0.3± 0.006	0.03± 0.002
Iron	22.9 ±0.031	12.1 ±0.022	18.7 ±0.044	10.2 ±0.019	9.3 ±0.007	10.1 ±0.006	2.12± 0.005	5.72± 0.005	1.39± 0.003	3.11± 0.011

Site I = JK Cements (0Km away from the source); Site II = Wayun (3Km away from the source); Site III = Balhama (5Km away from the source); Site I V= Sempora (7Km away from the source); Site V= Lethpora; Reference site

Table 2: Estimation of fresh and dry weight (g/quadrat) of corms during dormant phase of saffron

Site	Dormant phase 2013		Dormant phase 2014	
	Fresh wt. of corms	Dry wt. of corms	Fresh wt. of corms	Dry Wt. of corms
I	208±0.7	201±3	207±0.2	201±1
II	255±5	244±5	260±5	250±5
III	268±7	259±6	270±3	261±4
IV	280±9	269±6	280±0.4	271±4
V	286±3	275±3	293±2.3	286±2

Site I = JK Cements (0Km away from the source); Site II = Wayun (3Km away from the source); Site III = Balhama (5Km away from the source); Site I V= Sempora (7Km away from the source); Site V= Lethpora; Reference site

3.1 Heavy metal contents and resource allocation

The mean concentration levels of heavy metals found in saffron corm during dormant phase is summarized in Table 1. A highest level was found at site I while as least concentration was found at site V as described below:

3.1.1 Mercury

During dormant phase of the saffron plant mercury estimation in soil and corms was found 1.7µg/g at Site I; 1.37µg/g at Site II; 1.2µg/g at Site III; 1.11µg/g at Site IV and 0.47µg/g at Site V was noticed in the soil while as in case of corms 0.770µg/g at Site I; 0.510µg/g at Site II; 0.399µg/g at Site III; 0.321µg/g at Site IV and 0.07µg/g at Site V was recorded.

3.1.2 Cadmium

During dormant phase of the saffron plant cadmium estimation in soil and corms was found 0.32µg/g at Site I; 0.32µg/g at Site II; 0.25µg/g at Site III; 0.24µg/g at Site IV and 0.11µg/g at Site V was noticed in the soil while as in case of corms 1.09µg/g at Site I; 0.1µg/g at Site II; 0.15µg/g at Site III; 0.17µg/g at Site IV and 0.11µg/g at Site V was recorded.

3.1.3 Lead

During dormant phase of the saffron plant lead estimation in soil and corms was found 3.78µg/g at Site I; 3.8µg/g at Site II; 1.81µg/g at Site III; 0.6µg/g at Site IV and 0.4µg/g at Site V was noticed in the soil while as in case of corms 6.8µg/g at Site I; 2.6µg/g at Site II; 0.8µg/g at Site III; 0.61µg/g at Site IV and 0.35µg/g at Site V was recorded.

3.1.4 Nickel

During dormant phase of the saffron plant nickel estimation in soil and corms was found 3.21µg/g at Site I; 2.75µg/g at Site II; 2.66µg/g at Site III; 2.5µg/g at Site IV and 0.03µg/g at Site V was noticed in the soil while as in case of corms 4.84µg/g at Site I; 3.16µg/g at Site II; 0.82µg/g at Site III; 0.78µg/g at Site IV and 0.8µg/g at Site V was recorded.

3.1.5 Chromium

During dormant phase of the saffron plant cadmium estimation in soil and corms was found 10.12µg/g at Site I; 7µg/g at Site II; 6.21µg/g at Site III; 5.5µg/g at Site IV and 4.67µg/g at Site V was noticed in the soil while as in case of corms 0.33µg/g at Site I; 0.8µg/g at Site II; 0.88µg/g at Site III; 0.01µg/g at Site IV and 0µg/g at Site V was recorded.

3.1.6 Zinc

During dormant phase of the saffron plant zinc estimation in soil and corms was found 2.45µg/g at Site I; 3.02µg/g at Site II; 3.59µg/g at Site III; 3.24µg/g at Site IV and 3.99µg/g at Site V was noticed in the soil while as in case of corms 0µg/g at Site I; 0µg/g at Site II; 0µg/g at Site III; 0µg/g at Site IV and 0.12µg/g at Site V was recorded.

3.1.7 Magnese

During dormant phase of the saffron plant magnese estimation in soil and corms was found 79.1µg/g at Site I; 75µg/g at Site II; 68.3µg/g at Site III; 64.7µg/g at Site IV and 61.2µg/g at Site V was noticed in the soil while as in case of corms 4.28µg/g at Site I; 3.66µg/g at Site II; 2.18µg/g at Site III; 2.04µg/g at Site IV and 2.31µg/g at Site V was recorded.

3.1.8 Copper

During dormant phase of the saffron plant copper estimation in soil and corms was found 195.2µg/g at Site

I; 172.7µg/g at Site II; 145.8µg/g at Site III; 131.5µg/g at Site IV and 111µg/g at Site V was noticed in the soil while as in case of corms 47.86µg/g at Site I; 32.08µg/g at Site II; 23.85µg/g at Site III; 23.94µg/g at Site IV and 15.5µg/g at Site V was recorded.

3.1.9 Cobalt

During dormant phase of the saffron plant cobalt estimation in soil and corms was found 1.22µg/g at Site I; 0.9µg/g at Site II; 0.22µg/g at Site III; 0.28µg/g at Site IV and 0.3µg/g at Site V was noticed in the soil while as in case of corms 0.17µg/g at Site I; 0.15µg/g at Site II; 0.14µg/g at Site III; 0.07µg/g at Site IV and 0.03µg/g at Site V was recorded.

3.1.10 Iron

During dormant phase of the saffron plant iron estimation in soil and corms was found 22.9µg/g at Site I; 18.7µg/g at Site II; 9.3µg/g at Site III; 2.12µg/g at Site IV and 1.39µg/g at Site V was noticed in the soil while as in case of corms 12.1µg/g at Site I; 10.2µg/g at Site II; 10.1µg/g at Site III; 5.72µg/g at Site IV and 3.11µg/g at Site V was recorded.

3.1.11 Resource allocation

The observations with regard to average fresh and dry biomass ($g/25cm^2$) of the live corms of Crocus sativus at the five comparative sites was evaluated

In dormant phases it varies from $208\pm1g/25cm^2$ in 2013 and $207\pm0.2\ g/25cm^2$ in 2014 at site I while as maximum values was found at site V with average of $286\pm3g/25cm^2$ and $293\pm2.3g/25cm^2$ during two consecutive years.

4. Discussion

Soil is a valuable and non-renewable resource essential for survival and growth of plants thus supporting every life on earth. However in the modern world, numerous soil pollutants restrict the growth of plants. With the ongoing technological advancements in industrialization and urbanization process, release of toxic contaminants like heavy metals in the natural resources has become a serious problem worldwide. Metal toxicity affects crop yields, soil biomass and fertility. Presence of heavy metals, like mercury, arsenic, nickel, cobalt, cadmium, copper, lead and chromium in soil can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences in all life forms (Sethy and Ghosh, 2013). Metals and chemicals in higher concentration hamper the plant growth and production mainly associated with the physiological, biochemical and genetic elements of the plant system. Accumulation of metals in saffron soil will affect the mineral uptake and thus growth of corm. Impact of Cadmium on corm of saffron was studied and it was concluded that higher concentration may affect catalase activity in corms (Hadizadeh and Keyhani)

Saffron is a growing perennial plant, regenerating from the vegetative multiplication of its underground corms. Numerous scar like buds covered with scaly leaves, which vary in number (2-20) depending on the corm size (1.0 – 6.0 cm). A corm produces upto five flowers.

Metal accumulation in corms was significantly higher at site I which is effected by cement pollution, thus affect the various biochemical, genomic and metabolic processes in the plant, the fact is supported by a huge literature (Pandey and Sharma., 2002;Rahman *et al.*,2005; Singh *et al*., 2011; Mohamed., 2011; Fukao and Feriana., 2011)˙ Both fresh and dry biomass ($g/25cm^2$) of Saffron corms from the various locations suffering from the cement dust pollution of varied intensity indicated that it was accumulated in higher amounts at sites suffering from higher intensities of cement dust and was thus directly related to the degree of intensity of cement dust deposition, the mean/average values are quite apparently indicative of this phenomenon. Cooley, 1987 have indicated that reduction in total biomass due to air pollutants is often accompanied by a change in partitioning of photosynthate in different plant components.

The plant analyzed here is of huge biological and economic significance and is periodically used in cuisine and other side dishes in India and all over world. Overall it is a part of every food. In this work, results presented here clearly show that the examined plant is suffering from heavy metal stress which may affect the growth of this novel spice which is a good source of minerals like copper, potassium, calcium, manganese, iron, selenium, zinc and magnesium.

Conflict of interest statement

We declare that we have no conflict of interest

Acknowledgements

Authors are thankful to DST, Government of India for funding of work under WOS-A Scheme, vide order no: 400/2013.

References

Abdullaev, F. I., 2002. Cancer Chemopreventive and Tumoricidal Properties of Saffron (Crocus sativus L.), Journal of Experim. Biol. and Medic., 1.

Addo, M.A., Darko, E.O., Gordon, C., Nyarko, B.J.B., Gbadago, J.K., Nyarko, E., Affum, H.A., and Botwe, B.O.,2012. Evaluation of heavy metals contamination of soil and vegetation in the vicinity of a cement factory in Volta region, Ghana.Inter. J. of Sci. and tech,2:1.

AOAC., 1995.Official methods of analysis of AOAC International, 16th edn. AOAC International, Arlington, USA.

Assimopoulou, A. N., Papageorgiou, V. P., and Sinakos, Z.,2005. Radical Scavenging Activity of Crocus sativus L. Extract and Its Bioactive Constituents, Phyto.Res.,11.

Baby, S., Singh, N. A., Shrivastava, P., Nath, S. R., Kumar, S. S., Singh, D., and Vivek, K.,2008. Impact of dust emission on plant vegetation of vicinity of cement plant.J.Environ. Engin.AndManag.,1: 31-35.

Bollard, E.G., and Butler, G.W., 1966. Mineralnutrition of plants.Annu. Rev. Plant Physiol., 17:77-112.

Bradford, M. M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72: 248–254.

Brown, J. C., and Jones, W. E., 1975. Heavy metal toxicity in plants.I.A. crisis in embryo.Commun. Soil Sci. Plant Anal.,6: 421-438.

Chang, P. Y., Kuo, W., Liang, C. T., and Wang, C. K.,1964. The pharmacological action of Crocus sativus L., Yao.HsuehHs. Pao.,11.

Chidambaram, A., Sundaramoorthy, P., Murugan, A., Sankar, K., and Ganesh, B.L. 2009. Chromium induced cytotoxicity in blackgram (Vignamungo L.) Iran. J. Environ. Health Sci. Eng., 6:17-22.

Cooley, D.R., Manning, W.J., 1987. The impact of ozone on Assimilate partitioning in plants; a review, Enivor. Pollution.,47: 95-113.

Foy, C.D., Chaney, R.L., White, M.C., 1978. The physiology of metal toxicity in plants.Annu.Rev.Plant Physiol., 29: 511-566

.

Fukao, Y., and Feriana, A. 2011. V-ATPase dysfunction under excess zinc inhibits Arabidopsis cell expansion, Plant signal Behav., 6:1253-5.

Gupta, S., and Sharma, S.,2013. Effect of heavy metal present in cement dust on soil and plants of Nokha (Bikaner), Curt.world Environ., 8 :299-303.

Hadizadeh, M., Keyhani, E. Toxic effects of cadmium and catalase activity in the corms pf crocus sativus L. University of Tehran.

Ibrahim, H.H., Yauri, B.U.A., Muhammad, C., and Umar, A.,2012. Assessment of pollution potentialities of some Portland cement, Nigerian J. of Basic and App.Scien.,20:182-184.

Jan, A., and Bhat, G. A., 2006. Impact of cement dust on the morphology of Saffron (Crocus sativus) and three species of horticultural trees. J. Res. and Dev., 6: 129-133.

Jan, A., 2009. Impact of cement dust pollution on agricultural crops with special reference to Saffron cultivation in Pampore area. Ph.D Thesis submitted to University of Kashmir.

Lee, Y.P., Takahashi, T., 1966. An improved colorimetric determination of amino acid with the use of ninhydrin. Anal.Biochem. 14, 71-77.

Levent, K.I., Okan, A., and Cuneyt, A. K.,2009. Genotoxic effects of industrial wastewater on Allium cepa L. Afr. J. Biotech., 9: 1919-1923.

Mandal, A., and Voutchkov, M., 2011. Heavy metals in soils around the cement factory in Rockfort, Kingston, Jamaica, Inter. J. of Geoscien.,2:48-58.

Mehraj, S.S., Bhat, G.A., Balkhi, H.M., Gul, T.,2013. Health risks for population living in the neighborhood of a cement factory. Afri. J. of Environ. Scie. and Techn., 7:1044-1052.

Mehraj, S. S., Balkhi, H. M.,2013. Importance and Impact of Cement Industry on Saffron (Crocus Sativus). Interna. J. of Curr. Res.,03: 602-604.

Mohamed, H.I., 2011. Molecular and biochemical studies on the effect of gamma rays on lead toxicity in cowpea (Vigna sinensis) plants. Biol Trace Elem Res.,144:1205–18.

Meena, A.K., Bansal, P., Kumar, S., Rao, M.M., Garg, V.K.,2010. Estimation of heavy metals in commonly used medicinal plants: a market basket survey. Environ Monit Assess.,170:657–660.

Ogbonna, P.C., and Nwosu, A.N.,2011. Metal concentration in soil and plants in abandoned cement factory, Int. Conference on biotechnology and environment management, IPCBEE.,18.

Omran, A,l,M,A,R., Magrabym,S.E.E.l., Nadeem, M.E.A., Eter, A.E.L.E., and Page, A.L., Miller, R.H., and Keeney, D.R.,1986. Methods of Soil Analysis, Part 2--Chemical and Microbiological Properties. 2nd edn, Agronomy., 9: ASA,SSSA Publ., Madison, Wisconsin, USA.

Pandey, N., Sharma, C.P.,2002. Effect of heavy metals Co2+, Ni2+, and Cd2+ on growth and metabolism of cabbage. Plant Scie.,163: 753–758.

Rafiq, S. K., Ganai, B. A., and Bhat, G. A., 2008. Impact of automobile emissions on the productivity of Crocus sativus L. Inter. J. Environ. Res.,4: 1735-6865.

Rahman, H., Sabreen, S., Alam, S., Kawai, S.,2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. Jour. of Plant Nutrit.2005; 28: 393–404.

Sethy, S.K., Ghosh, S.2013. Effect of heavy metals on germination of seeds. J Nat Sc Biol Med.,4: 272 -275.

Singh, H.P., Kaur, G., Batish, D.R., Kohli, R.K., 2011. Lead (Pb)-inhibited radicle emergence in Brassica campestris involves alterations in starch-metabolizing enzymes. Biol Trace Elem Res., 144:1295–301.

Zou, J.H., Wang, M., Jiang, W.S., and Liu, D.H.,2006. Effects of hexavalent chromium (vi) on root growth and cell division in root tip cells of Amaranthusviridis, L. Pak. J. Bot., 38:673-681.