American-Eurasian J. Agric. & Environ. Sci., 16 (1): 104-109, 2016
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2016.16.1.12758

Analysis of Heavy Metals and Resource Allocation During Dormant Phase in *Crocus sativus* L. (Saffron) near Cement Factories

Syed Sana Mehraj, Azra N. Kamili, G.A. Bhat and Requeya Nazir

Centre of Research for Development University of Kashmir, Srinagar-J&K India-190006

Abstract: 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. All the heavy metals were determined by using Atomic Absorption Spectrophotometer (AAS). For resource allocation fresh and dry biomass was estimated, total free amino acids was estimated by Lee and Takahashi while as total soluble protein was estimated by Bradford method. The concentrations of Hg, Pb, Mn, Cr, Zn, Cd, Co, Ni, Cu and Fe exceeded their respective permissible limits in the soil and plant samples. Significant variation was found in fresh and dry biomass, free amino acid content and in total soluble protein of samples. *It can be concluded that* the metal contents in the samples were found comparatively higher at pollution site thus has significant variation in resource allocation.

Key words: *Crocus sativus* · Metals · Dormant · Pollution · AAS

INTRODUCTION

Saffron (*Crocus sativus*) is one among the most important cash crops of Kashmir Himalayas. It is the most precious and most expensive spice [1] 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[2,3,4].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 [5-8] 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 [9-15] 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 Bollard, and Butler [16], Jan [7], Foy *et al.* [18] and Chidambaram [19]. Heavy metal pollutants are stable in the environment but highly toxic to biological organisms [20, 21].

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

Corresponding Author: Syed Sana Mehraj, Centre of Research for Development, University of Kashmir, Srinagar-J&K India-190006.

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.

MATERIALS AND METHODS

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.

Reagents and Standards: Heavy metal analysis was done [22] using atomic absorption spectroscopy in plant samples using wet digestion method [23] 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).

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.

Analytical Procedure: One gram 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°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 boiled 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.

Fresh and Dry Weight of Corms: Biomass of corms was calculated by weighing the fresh corms collected in a quadrate of 25 cm^2 area. At each site five quadrates were randomly sampled and the results were calculated and expressed as g/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.

Total Free Amino Acids: Total free amino acids was extracted and determined following the method of Lee and Takahashi [24].

Total Protein: Total proteins were estimated by Bradford's method [25].

Statistical Analysis: Results of this study were analyzed for mean and standard deviation. All experiments were done in triplicate for precision and accuracy of the results.

RESULTS

Heavy Metal Contents and Resource Allocation: Dormant phase in saffron is comprised of only corms, so metal analysis in this phase includes soil and corm only. The soil and corm samples were analyzed for ten metals. Mercury 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 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 estimated. Similarly 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 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. 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 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 analyzed. The mean concentration levels of heavy metals found in saffron corm during dormant phase is summarized in Table 1.

Similarly 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 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 estimated. 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.

During the phase, 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 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 estimated. Similarly manganese 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 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. Similarly 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 estimated in the soil while as in case of corms 47.86µg/g at Site I; 32.08µg/g at

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
Manganese	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/quadrate) of corms during dormant phase of saffron

	Dormant phase 2013		Dormant phase 2014	
Site	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

Table 3: Estimation of soluble proteins (µg/g) in saffron plant during vegetative and dormant phases

Concentration (µg/g)	
Dormant phase 2013	Dormant phase 2014
172±2	98± 2.5
297±5.5	279±20
1283±1.1	1031.7±27
1294±1.5	1056.7±40
1322±2.6	1120±26.4

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 (12Km away from the source)

Table 4: Estimation of free amino acids (mg/g) in saffron plant during vegetative and dormant phases

Concentration (µg/g)	
Dormant phase 2013	Dormant phase 2014
13± 0.05	13.3±0.4
9.9±0.05	9. 3±0.1
8. 7±0.5	8. 7±0.5
2.9±0.11	3±0.2
2.6±0.1	2.76±0.3

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 (12Km away from the source)

Site II; 23.85µg/g at Site III; 23.94µg/g at Site IV and 15.5µg/g at Site V was recorded. 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.

During the dormant phase 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.

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 phase during 2013 it varies from 208±1g/25cm^2 in 2013 and 207±0.2 g/25cm^2 in 2014 at site I, while as maximum values was found at site V with average of 286±3g/25cm^2 and 293±2.3g/25cm^2 during two consecutive years.

Fresh and Dry Weight of Corms: 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 Average fresh biomass of corms growing at the factory site was minimum, while as maximum at site V during both years of study.

It varies from 208±1g/25cm^2 in 2013 and 207±0.2 g/25cm^2 in 2014 at site I, while as maximum values was found at site V with average of 286±3g/25cm^2 and 293±2.3g/25cm^2 during two consecutive years.

Total Soluble Proteins and Free Amino Acids: Total soluble proteins shows the decreasing trend, while moving from pollution site towards reference site. During dormant phase of the saffron plant total free amino acids were estimated in corms which was 13mg/g at Site I; 9.9mg/g at Site II; 8.7mg/g at Site III; 2.9mg/g at Site IV and 2.6mg/g at Site V was noticed in the first year of study, while as during second year the concentration varies from 13.3mg/g at Site I; 9.3mg/g at Site II; 8.7mg/g at Site III; 3mg/g at Site IV and 2.76mg/g at Site V was recorded which also showed decreasing trend while moving from pollution site towards reference site.

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 [26]. 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 [27].

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 [28-32]. 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 [33] 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. Similarly decrease in free amino acids and increase in total proteins at site I indicates that there is huge abiotic stress at site I. 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.

ACKNOWLEDGEMENTS

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

REFERENCES

1. Mehraj, S.S. and H.M. Balkhi, 2013. Importance and Impact of Cement Industry on Saffron (*Crocus sativus*). Interna. J. Curr. Res., 03: 602-604.
2. Chang, P.Y., W. Kuo, C.T. Liang and C.K. Wang, 1964. The pharmacological action of *Crocus sativus* L., Yao.HsuehHs. Pao., pp: 11.
3. Abdullaev, F.I., 2002. Cancer Chemopreventive and Tumoricidal Properties of Saffron (*Crocus sativus* L.), Journal of Exp. Biol. and Medic., pp: 1.
4. Assimopoulou, A.N., V.P. Papageorgiou and Z. Sinakos, 2005. Radical Scavenging Activity of *Crocus sativus* L. Extract and Its Bioactive Constituents, Phyto. Res., pp: 11.
5. Jan, A. and G.A. Bhat, 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.
6. Rafiq, S.K., B.A. Ganai and G.A. Bhat, 2008. Impact of automobile emissions on the productivity of *Crocus sativus* L. Inter. J. Environ. Res., 4: 1735-6865.
7. 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.
8. Mehraj, S.S., G.A. Bhat, H.M. Balkhi and T. Gul, 2013. Health risks for population living in the neighborhood of a cement factory. Afri. J. of Environ. Sci. and Techn., 7: 1044-1052.
9. Baby, S., N.A. Singh, P. Shrivastava, S.R. Nath, S.S. Kumar, D. Singh and K. Vivek, 2008. Impact of dust emission on plant vegetation of vicinity of cement plant. J. Environ. Engin. and Manag., 1: 31-35.
10. Omran AlMAR, Magraby SEEl, Nadeem MEA, Eter AELE and A.L. Page, 1986. Miller RH and Keeney DR. Methods of Soil Analysis, *Part 2*--Chemical and Microbiological Properties. 2nd Ed., Agronomy. 1986; 9: ASA,SSSA Publ., Madison, Wisconsin, USA.
11. Ogbonna, P.C. and A.N. Nwosu, 2011. Metal concentration in soil and plants in abandoned cement factory, Int. Conference on biotechnology and environment management, IPCBEE, pp: 18.
12. Mandal, A. and M. Voutchkov, 2011. Heavy metals in soils around the cement factory in Rockfort, Kingston, Jamaica, Inter. J. Geoscien, 2: 48-58.
13. Addo, M.A., E.O. Darko, C. Gordon, B.J.B. Nyarko, J.K. Gbadago, E. Nyarko, H.A. Affum and B.O. Botwe, 2012. Evaluation of heavy metals contamination of soil and vegetation in the vicinity of a cement factory in Volta region, Ghana. Inter. J. Sci. and Tech., 2: 1.
14. Ibrahim, H.H., B.U.A. Yauri, C. Muhammad and A. Umar, 2012. Assessment of pollution potentialities of some Portland cement, Nigerian J. of Basic and App.Scien., 20: 182-184.
15. Gupta, S. and S. Sharma, 2103. Effect of heavy metal present in cement dust on soil and plants of Nokha (Bikaner), Curt.World Environ., 8: 299-303.
16. Bollard, E.G. and G.W. Butler, 1966. Mineralnutrition of plants. Annu. Rev. Plant Physiol., 17: 77-112.

17. Brown, J.C. and W.E. Jones, 1975. Heavy metal toxicity in plants. I.A. crisis in embryo. Commun. Soil Sci. Plant Anal., 6: 421-438.
18. Foy, C.D., R.L. Chaney and M.C. White, 1978. The physiology of metal toxicity in plants. Annu. Rev. Plant Physiol., 29: 511-566.
19. Chidambaram, A., P. Sundaramoorthy, A. Murugan, K Sankar and B.L. Ganesh, 2009. Chromium induced cytotoxicity in blackgram (*Vigna mungo* L.) Iran. J. Environ. Health Sci. Eng., 6: 17-22.
20. Zou, J.H., M. Wang, W.S. Jiang and D.H. Liu, 2006. Effects of hexavalent chromium (vi) on root growth and cell division in root tip cells of Amaranthus viridis, L. Pak. J. Bot., 38: 673-681.
21. Levent, K.I., A. Okan and A.K. Cuneyt, 2009. Genotoxic effects of industrial wastewater on *Allium cepa* L. Afr. J. Biotech., 9: 1919-1923.
22. AOAC. Official Methods of Analysis of AOAC International, 16th Ed. *AOAC International*, Arlington, USA, 1995.
23. Meena, A.K., P. Bansal, S. Kumar, M.M. Rao and V.K. Garg, 2010. Estimation of heavy metals in commonly used medicinal plants: a market basket survey. Environ Monit Assess., 170: 657-660.
24. Lee, Y.P. and T. Takahashi, 1966. An improved colorimetric determination of amino acid with the use of ninhydrin. Anal. Biochem., 14: 71-77.
25. 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.
26. Sethy, S.K. and S. Ghosh, 2013. Effect of heavy metals on germination of seeds. J. Nat Sc. Biol. Med., 4: 272-275.
27. Hadizadeh, M. and E. Keyhani, XXXX. Toxic effects of cadmium and catalase activity in the corms pf *Crocus sativus* L. University of Tehran
28. Pandey, N. and C.P. Sharma, 2002. Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. Plant Sci., 163: 753-758.
29. Rahman, H., S. Sabreen, S. Alam and S. Kawai, 2005. Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. J. Plant Nutr., 28: 393-404.
30. Singh, H.P., G. Kaur, D.R. Batish and R.K. Kohli, 2011. Lead (Pb)-inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. Biol Trace Elem Res., 144: 1295-301.
31. 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.
32. Fukao, Y. and A. Feriana, 2011. V-ATPase dysfunction under excess zinc inhibits Arabidopsis cell expansion, Plant signal Behav., 6: 1253-5.
33. Cooley, D.R. and W.J. Manning, 1987. The impact of ozone on Assimilate partitioning in plants; a review, Enivor. Pollution, 47: 95-113.