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Impact of bio-fertilizers and different levels of lead on pigment content of wheat (*Triticum aestivum* L.)

Alireza Pazoki[1*], Mohammad Nabi Ilkaee[2], Mahdi Davoodi Far[3], Farid Golzardi[4]

[1]*Department of Agronomy and Plant Breeding, Yadegar-e-Imam Khomeini (RAH) Shahre-rey Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran*

[3]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, Roudehen Branch, Islamic Azad University, Roudehen, Iran*

[4]*Young Researchers and Elite Club, Karaj Branch, Islamic Azad University, Karaj, Iran*

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Abstract

Among heavy metals, lead is a potential pollutant that readily accumulates in soils and sediments. Although lead is not an essential element for plants, it gets easily uptake, accumulated in different plants sections and had destructive effects on leaf pigments in photosynthetic reaction. For the purpose of evaluating pigment content of wheat under different lead, PGPR and mycorrhiza levels, a Pot culture experiments was done during 2012-2013 in Islamic Azad University, Karaj and Yadegar-e-Imam Khomeini (RAH) Shahre-rey Branches, as factorial based on completely randomized design with 4 replications. The lead amounts in 4 levels (0, 300, 600 and 900 mg/kg of soil), PGPR (Azosperillium, Azotobacter and Pseudomonas) in 2 levels (Application and non-application) and mycorrhiza in 2 levels (Application and non application) were considered. The lead concentration decrease was noticed in all the pigments amounts. The PGPR application increased Chl a (56.23 µg/ml), Chl b (34.63 µg/ml) and Chl a+b (90.38 µg/ml) and decreased carotene (0.46 ppm) and xantophyll (45.16 ppm). Mycorrhiza consumption could add Chl a, Chl b, Chl a+b and reduce carotenoids, but its effect was less than PGPR. The maximum reduction of chlorophylls (% of control) was noticed under the influence of higher dose of lead (900 mg /kg) and non application of PGPR and mycorrhiza and for preventing photosynthetic deficiency, carotene and xantophyll content increased.

***Corresponding Author:** Alireza Pazoki ✉ pazoki@iausr.ac.ir

Introduction

Phytoremediation mechanisms mostly contain phytoextraction and phytostailization. Phytoextraction indicates to the uptake of metals from soils using collecting them into the harvestable shoot parts of plants, although phytostabilization contains to the introduction to the soil of metal tolerant plants to decrease the movement of metals leaching into groundwater. The amounts of translocation from roots to shoot parts mainly depend to the species of plant, kind of metal, or soil metal bioavailability. Phytormediation can be used in areas with medium to low soil contaminate amounts, where physical and chemical soil remediation methods are very expensive (Moreno *et al.*, 2005). Heavy metal toxicity and the risk of their residual in the soil and as a result in food chain is one of the main environmental and health difficulties of our modern population. Introductory sources of pollution is from the burning of fossil fuels, mining and melting of metallic ferrous ores, urban wastes, fertilizers, weedicides, and wastewater (Peng *et al.*, 2006; Xiong, 1998). Lead toxicity is a recognized difficulty. Many researches indicate that the toxicity depends on chemical structure, the rout of its administration, and concentration, time and intensity of exposure. (Kulikowska *et al.*, 1994).

Lead at 500 ppm in soil (or) solid waste considered the substance as "hazardous waste". Maximum permissible amount of lead in drinking water is 0.05 ppm. Sunflower maybe has high resistance and should be able of up taking high amounts of lead (Usha *et al.*, 2011). Accordingly to Gruca-Królikowska and Wacławski (2006), the main reason for the decline in productivity of plants growing on heavy metal contaminated areas is a significant decrease in the photosynthesis efficiency due to interference of chlorophyll biosynthesis. In photosynthesis, the harvesting of solar photons by the accessory pigments have very important roles in photosynthetic pathway (Van Grondelle *et al.*, 1994). Light-harvesting complex II (LHCII) is the most abundant light-harvesting accessory pigment-protein complex in plants. It binds about half of the chlorophylls (Chl's) present in the chloroplast. The complex is generally isolated as a trimmer, which is also believed to be the natural form (Jansson, 1994).

Carotenoids of the higher plants are consisting of two groups of pigments, the carotenes and their oxygen-containing derivatives, xanthophylls. In green plant parts as leaves carotene and xanthophylls (lutein, neoxanthin, violaxanthin, and zeaxanthin) constitute the principal part of pigments? In photosynthesizing tissues, carotenoids are the inseparable elements of chlorophyll-containing pigment-protein complexes of thylakoid membranes. The principal functions of carotenoids: photo protection, light harvesting, structural, and participation in photochemical processes in photosystem I and photosystem II are presented (Ladygin and Shirshikova, 2006; Weedon, 1979).

As the serious deterrence of the Calvin cycle activity in those transformed plants is anticipate causing a steady-state reduction of photosynthetic electron carriers, this finding can be seen as another illustration of xanthophylls biosynthesis regulation dependent on the reduction state of the electron transport chain. The apparent collecting of the accumulations of ELIPs and xanthophylls-cycle carotenoids recorded, with both phenomena responding evident to similar light switches, is stable with the recommended that ELIP or its algal homolog and the xanthophylls cycle might be interrelated in their biological duties (Krol *et al.*, 1995; Adamska *et al.*, 1993; Levy *et al.*, 1993). However, the xanthophylls cycle has key roles in protecting plants against the ability damaging effects of intensity light, the mechanism for the damage of excess energy using xanthophylls (measured as NPQ) is controversial. Smith and Read (1997), stated that Mycorrhiza fungus are one of the most important soil microorganisms which has very effective role in heavy metals absorption during phytoremediation process.

In the present research, an attempt is made to elucidate the effect of different lead, PGPR and

mycorrhiza levels on chlorophylls, and carotene and xanthophylls contents in wheat. The knowledge of physiological and biochemical basis of lead phytotoxicity effects on pigment content can help us to evaluate the roles of this light harvesting substances in photosynthesis process and introducing the suitable biological methods for increasing tolerance against lead stress.

Materials and methods

Experimental design

Due to study the effect of PGPR and mycorrhiza on pigment content of wheat (Bahar variety) under different lead levels, a green house pot culture experiments were conducted during 2012- 2013 in Islamic Azad University, Karaj and Yadegar-e-Imam Khomeini (RAH). The experimental design was as factorial on the basis of completely randomized design (CRD) with 4 replications. The lead concentration in 4 levels (0, 300, 600 and 900 mg/kg of soil), PGPR (Azosperillium, Azotobacter, Pseudomonas) in 2 levels (Application and non-application) and mycorrhiza (Mix variety) in 2 levels (Application and non application) were considered. The ratio 3:1:1 of sand, clay and manure fertilizer were used as media. Wheat seeds were obtained from Seed and Plant Improvement Institute, Karaj, Iran. Seeds were poured in 3% (v/v) of formaldehyde for 3 minutes and washed with distilled water for 4 times to avoid fungal infection. The seeds were sown directly in 7 kg soil capacity pots. To avoid loss of nutrients and trace elements out of the pots, plastic plate were placed under each pot and the collected leaches were put back to experimental pots.

Chlorophyll content

Total chlorophyll concentration is a unifying paramentter for indicating the effect of specific interventions. Concentrations of Chl_a and Chl_b and total chlorophyll (Total Chl= Chl_a + Chl_b) were measured by the Lichtenthaler and Welburn (1983). They introduced the following equations to determine chlorophyll a and chlorophyll b content in 80% acetone extracts:

Chlorophyll a (µg/ml) = 12.21 (A_{663}) - 2.81 (A_{646})

Chlorophyll b (µg/ml) = 20.13 (A_{646}) - 5.03 (A_{663})

Carotene and xanthophylls content

Carotene and xanthophylls contents were determined using absorbance of xanthophylls fraction at 445 mµ and carotenes fraction at 445 and 451 mµ. For convenience, normal hexane can be used as a blank for all absorbance measurements without introducing significant error. Evaluation amounts of pigments in total extract, xanthophylls extraction and carotenes extraction as follows (Blessin, 1962):

$$Pigment, ppm = \frac{A \times V \times 1000}{a \times L \times W}$$

A: Absorbance, V: Volume, L: Cell pass in cm, W: Sample weight (g), a: Absorptivity in carotenoid. Carotenes are recalculated as equivalent xanthophylls to adjust all values to the same wave length and reference standards (Blessin, 1962).

Table 00. Calculations are based on the fallowing wave lengths and Absorptivity.

Pigment	Wavelength (*mµ*)	Absorptivity (*L/g- cm*)
Total pigments	445	231
Xanthophylls	445	231
Carotenes	451	251
Carotenes	445	231

Data analysis

All data were analyzed by the SAS software for Windows Standard Version, and the differences between individual means were evaluated using the Duncan Multiple range test at 5% levels of probability.

Results

The results indicated that more factors simple effect were significant on experimental traits, the results are presented in Table 1. Doses of lead were found to have inhibitory effects on chlorophyll elements and additive influence on carotenoids, so exposure in lead concentration to 900 mg Pb/kg caused a significant decrease in chlorophyll a (28.63 µg/ml), chlorophyll b (16.2 µg/ml), chlorophyll a+b (44.41 µg/ml) and

decrease in carotene (0.69 ppm) and xantophyll (79.24 ppm) (Table 2). The simple effect of different lead concentration showed that they were placed in different statistical groups. The simple effects of PGPR on experimented traits were noticed that after its consumption during wheat growth stages, all studied characters significantly changed, therefore the chlorophyll a (56.23 µg/ml), chlorophyll b (34.62 µg/ml), chlorophyll a+b (90.38 µg/ml) increased and carotene (0.46 ppm) and xanthophylls (45.16 ppm) decreased (Table 2). The findings illustrated that PGPR application was more effective on pigment contents than mycorrhiza (Table 2). Simple effect of mycorrhiza on pigment contents showed that instead of chlorophyll b and a+b, other traits had significant changes (Table 1). In this case, the highest chlorophyll a (54.75 µg/ml), chlorophyll b (33.21 µg/ml), chlorophyll a+b (87.98 µg/ml) and lowest carotene (0.48 ppm) and xanthophylls (47.09 ppm) were obtained. The findings showed that all double and triple interaction effects were not significant on pigment content of wheat, although in this conditions, the maximum reduction for chlorophyll a+b was noticed in Pb 900 mg Pb/kg and non application of PGPR (36.45 µg/ml) and lack of mycorrhiza (39.84 µg/ml) (Table 3 and 4). The total carotenoid content which more evaluates via carotene and xanthophylls was noticed that in sever lead stress (900 mg Pb/kg), application of PGPR can 13.5 % and 17.8 % significantly reduction in carotene and xanthophylls alternatively (Table 3).

Table 1. Analysis of variance for experimental characters.

S.O.V.	d.f.	Mean of Square (M.S.)				
		Chl a	Chl b	Chl a+b	Carotene	Xanthophylls
Lead (L)	1	33.86**	21.67**	55.47**	0.20**	1.76**
PGPR (P)	1	4.94*	7.04**	10.88**	0.4*	0.19**
Mycorrhiza (M)	1	1.97$^{n.s}$	2.79**	4.98*	0.01$^{n.s}$	0.10$^{n.s}$
P × L	3	0.31$^{n.s}$	0.03$^{n.s}$	0.32$^{n.s}$	0.0001$^{n.s}$	0.01$^{n.s}$
M × L	3	0.12$^{n.s}$	0.003$^{n.s}$	0.10$^{n.s}$	0.0001$^{n.s}$	0.009$^{n.s}$
M × P	1	0.27$^{n.s}$	0.36$^{n.s}$	0.34$^{n.s}$	0.002$^{n.s}$	0.00002$^{n.s}$
M× P × L	3	0.01$^{n.s}$	0.01$^{n.s}$	0.01$^{n.s}$	0.00002$^{n.s}$	0.006$^{n.s}$
Error	48	0.78	0.36$^{n.s}$	1.06	0.007	0.04
CV (%)	30	12.50	11.03	11.55	12.29	12.59

ns, * and **: No significant and significant at %5 and %1 level of probability respectively.

Table 2. Simple effect of Lead, PGPR and Mycorrhiza on pigment contents of wheat.

Treatments		Chl a (µg/ml)	Chl b (µg/ml)	Chl a+b (µg/ml)	Carotene (µg/ml)	Xanthophylls (µg/ml)
Lead (mg/kg)	0	75.05 a	44.98 a	119.27 a	0.32 d	16.60 c
	300	60.20 b	35.80 b	96.10 b	0.44 c	42.63 b
	600	46.47 c	27.52 c	74.04 c	0.56 b	63.30 a
	900	28.63 d	16.02 d	44.41 d	0.69 a	79.24 a
PGPR	Non application	48.94 b	27.54 b	76.49 b	0.54 a	55.73 a
	Application	56.23 a	34.62 a	90.38 a	0.46 b	45.16 b
Mycorrhiza	Non application	50.42 a	28.95 b	78.89 b	0.52 a	53.80 a
	Application	54.75 a	33.21 a	87.98 a	0.48 a	47.09 a

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%.

Table 3. Mean comparison of Lead and PGPR on pigment contents of wheat.

PGPR	Lead (mg/kg)	Chl a (µg/ml)	Chl b (µg/ml)	Chl a+b (µg/ml)	Carotene (µg/ml)	Xanthophylls (µg/ml)
Non application	0	71.72 a	40.41 a	112.52 a	0.35 a	20.23 a
	300	57.09 a	32.39 a	89.42 a	0.48 a	47.28 a
	600	43.24 a	24.30 a	67.55 a	0.60 a	68.42 a
	900	23.72 a	13.06 a	36.45 a	0.74 a	87.00 a
Application	0	78.38 a	49.54 a	125.81 a	0.29 a	12.98 a
	300	63.31 a	39.21 a	102.79 a	0.41 a	37.98 a
	600	49.71 a	30.75 a	80.53 a	0.51 a	58.17 a
	900	33.54 a	18.97 a	52.38 a	0.64 a	71 49 a

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%.

Table 4. Mean comparison of Lead and Mycorhiza on pigment contents of wheat.

Mycorrhiza	Lead (mg/kg)	Chl a (µg/ml)	Chl b (µg/ml)	Chl a+b (µg/ml)	Carotene (µg/ml)	Xanthophylls (µg/ml)
Non application	0	72.84 a	42.24 a	113.12 a	0.34 a	19.26 a
	300	58.01 a	33.65 a	95.05 a	0.46 a	45.22 a
	600	45.06 a	25.49 a	70.53 a	0.57 a	66.55 a
	900	25.77 a	14.42 a	39.84 a	0.72 a	84.17 a
Application	0	77.26 a	47.72 a	125.21 a	0.30 a	13.95 a
	300	62.38 a	37.95 a	100.16 a	0.43 a	40.04 a
	600	47.88 a	29.55 a	77.55 a	0.54 a	60.04 a
	900	31. 48 a	17.61 a	48.99 a	0.66 a	74.31 a

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%.

Table 5. Mean comparison of PGPR and Mycorhiza on pigment contents of wheat.

Mycorrhiza	PGPR	Chl a (µg/ml)	Chl b (µg/ml)	Chl a+b (µg/ml)	Carotene (µg/ml)	Xanthophylls (µg/ml)
Non application	Non application	45.98 a	24.72 a	70.89 a	0.57 a	60.31 a
	Application	54.86 a	33.18 a	86.88 a	0.47 a	47.29 a
Application	Non application	51.90 a	30.36 a	82.08 a	0.51 a	51.15 a
	Application	57.61 a	36.06 a	93.87 a	0.45 a	43.02 a

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%.

Discussion

However, it is important to evaluate changes in the 4 important components of pigments, chlorophyll a (chl a), chlorophyll b (chl b), carotene and xanthophylls. This is due to this important issue that heavy metals could affect each pigment at different amounts creating changes in some section of plants physiology and not in others to access a sufficiently high chlorophyll concentration of wheat used in the process is the basis of induced phytoremediation efficiency. These Investigation findings showed considerable and significant decrease in total chlorophyll content (30.5%) in 900 mg Pb/g without PGPR application, as compared to the using (Table 3), These results were consistent with Cenkci *et al* (2010) findings, so Lead transformation and accumulation in shoot seedlings sections and especially in leaves of fodder turnip caused a significant decrease in chlorophylls biosynthesis and concentration and genomic template stability.

According to Gruca-Krolikowska and Wacławski (2006), the most important factor for the decrease in productivity and growth of plants on heavy metal contaminated regions is a considerable decrease in the photosynthesis efficiency due to disorder of chlorophyll biosynthesis. In some evaluations can be some verification of this idea, because there was a considerable inhibitory effect of moderate concentration (600 mg Pb/kg) in the chlorophyll a + b ranging from 80.53 µg/ml to 67.55 µg/ml in PGPR application and non application conditions and 77.55 µg/ml to 70.53 µg/ml in mycorrhiza consumption and normal treatment. Sharma and Dubey (2005) stated that surplus lead concentrations gained a number of toxicity signs in plants such as increase in growth, chlorosis and blackening of the root parts, this comply with the observations of this study. Consequently, lead in the soil has been shown to be able to complex other plant elements such as phosphorus, therefore explanation them both access for uptake (Xie *et al*., 2006). Fresh and dry weight of plant was reduced significantly at every concentration of Pb (19, 20 & 21 mg lit-1), whereas the maximum decrease with the higher concentration of Pb (21 mg lit-1) was noticed in variety UPAS – 120 as compared to variety ICPL-151. Reduce in plants biomass was might be due high loss of moisture and Relative Water Content (RWC). The decrease in dry weight might be due to decline in photosynthesis and chlorophyll 'a' synthesis as suggested by Sinhal (2005); Joshi *et al.* (1999) and Okhi (1978). A concentration dependent decrease was also found in chlorophyll amounts. Prasad & Prasad (1987) proposed that heavy metals stress deterrent chlorophyll biosynthesis via interacting with sulphydryl (-SH) group of the two enzymes viz., δ-Amino laevulinic acid (ALA) dehydratase and Protochlorophyllide reductase, involved in chlorophylls biosynthesis. Our findings revealed that composition of Azosperillium, Azotobacter and Pseudomonas could stimulate chlorophylls biosynthesis and its survival and decrease accessory pigments carotene and xanthophylls. The similar expression was shown in Glick *et al* (1999) results that PGPRs can stimulate plant growth via supporting bio-available phosphorus absorption, biological nitrogen fixation for plant consumption, sequestering micro nutrient as iron for, making phytohormones such as auxins, cytokinins and gibberellins, and reducing plant ethylene amounts.

Mychorhiza application showed conflicting effect on chlorophylls and antenna pigments (Carotene and Xanthophylls). In similar result it was determined that fungies as *Fusarium oxysporum* increase metal bioavailability, absorption and translocation, adding root and shoot biomass, chlorophyll biosynthesis and doubled phytoextraction potential of HE *S. alfredii* (Li and Wong, 2011; Xinxian *et al*., 2010; Xiong *et al*., 2008). 5-aminolevulinic acid (5-ALA) is a no protein amino acid which exists in every living Organism and playing the role of a chlorophyll precursor at plants (Jarosz, 2012; Tanaka *et al.,* 2005).

Conclusion

Our finding indicated that PGPR and mycorrhiza consumption by stimulating IAA could be considered

as a practical methods for stimulating chlorophylls biosynthesis against lead polluted soils. So application of this heavy metals anti stress microorganisms at none, moderate and high concentrations can effectively increase wheat chlorophylls and prevent increasing yield and yield components of wheat. In lead stress conditions, wheat added accessory pigments "carotene and Xanthophylls" to compensate all chlorophyll forms decrease to prevent photosynthesis deficiency.

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References

Adamska I, Kloppstech K, Ohad I. 1993. The effect of free radical enhancers and scavengers on accumulation of early light-inducible protein during light stress. Naturforsch **48**, 391- 396.

Blessin CW. 1962. Carotenoids of corn and sorghum (Analytical procedures). 46th annual meeting , Dallas, Texas, April **39**, 236- 242.

Cenkci S, Hakkı Ci˘gerci I, Yıldız M, Özay C, Bozda˘g A, Terzi H. 2010. Lead contamination reduces chlorophyll biosynthesis and genomic templatestability in Brassica rapa L. Environmental and Experimental Botany **67**, 467-473. doi:10.1016/j.envexpbot.2009.10.001

Glick BR, Patten CL, Holguin G, Penrose DM. 1999. Biochemical and genetic mechanisms used by plant growth-promoting bacteria. Imperial College Press, London.

Gruca-Krolikowska S, Wacławek W. 2006. Metale w środowisku. Cz. II. Wpływ metali ciężkich na rośliny. Chem. Dydakt Ekological Metrology **11 (1-2)**, 41- 54.

Jarosz Z. 2012. Wpływ nawozu Pentakeep V naplonowanie oraz zawartość wybranych makro-imikroelementów w sałacie. Annales UMCE **11 (1)**, 1- 8.

Jensen A. 1978. Chlorophylls and carotenoids. In: Handbook of phycological methods, physiological and biochemical methods. J.A. Hellebust and J.S. Craige (edt.) pp. 59-70 Cambridge University Press, Cambridge.

Joshi VN, Rathore SS, Arora SK. 1999. Effect of Chromium on growth and development of cowpea (*Vigna unguicalata* L.). India Journal of Environmental Protection **19**, 745- 749.

Krol M, Spangfort MD, Huner NPA, Oquist G, Gustafsson P, Jansson S. 1995. Chlorophyll a/b-binding proteins, pigment conversion, and early light-induced proteins in chlorophyll b-less barley mutant. Plant Physiology **107**, 873- 883.

Kulikowska E, Moniuszko-Jakoniuk J, Miniuk K, Kaluzynski A. 1994. Wplyw cynku na redystrybucje otowiu w ustroju szczura narazonego na 500 ppm otowiu. Acta PolonicaToxicology **2**, 148- 152.

Ladygin VG, Shirshikova GN. 2006. Current Image of Carotenoid Functions in Chloroplasts of Eukaryotes, Zhurnal Obshchei Biologii **67**, 163- 189.

Levy H, Tal T, Shaish A, Zamir A. 1993. Cbr, an algal homolog of plant early light-induced proteins, is a putative zeaxanthin binding protein. Journal of Biological Chemistry **268**, 892- 896.

Li WC, Wong MH. 2011. Interaction of Cd/Zn hyper accumulating plant (*Sedum alfredii*) and rhizosphere bacteria on metal uptake and removal of phenanthrene. Journal of Hazardous Materials **209-210**, 421- 433. doi: 10.1016/j.jhazmat.2012.01.055. Epub 2012 Jan 23.

Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. Biochemical Society Transactions **11**, 591- 592.

Moreno DA, Villora G, Soriano MT, Castilla N, Romero L. 2005. Sulfur, chromium, and selenium accumulated in Chinese cabbage under direct covers. Journal of Environmental Management **74**, 89- 96. doi: 10.1080/15226510903534554.

Okhi K. 1978. Pb Concentration in soybean as related to growth, photosynthesis and carbonic anhydrase activity. Physiology Plant **18**, 79- 82.

Peng K, Li X, Luo C, Shen Z. 2006. Vegetation composition and heavy metal uptake by wild plants at three contaminated sites in Xiangxi area, China, Journal of Environmental Science and Health Part A **40**, 65- 76. DOI:10.1080/10934520500298838

Prasad DDK, Prasad ARK. 1987. Effect of Lead and Mercury on chlorophyll synthesis in mungbean plants. Phytochem **26**, 881- 883.
DOI: 10.1016/S0031-9422(00)82310-9

Sharma P, Dubey RS. 2005. Lead toxicity in plants. Brazilian Journal of Plant Physiology **17(1)**, 35- 52. http://dx.doi.org/10.1590/S1677-04202005000100004

Sinhal VK. 2005. Phytotoxic, Cytogenetic and Biochemical effects of Pb2+ & Pb2+ in *Vigna mungo* (L). Hepper. Ph.D. Thesis, M.J.P. Rohilkhand University Bareilly, India.

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. USA. San Diego, Calif. 2nd Ed, Academic press. p: 605.

Su-Qin Z, Ming-Wei C, Ben-Hua J, De-Mao J, Jian-Sheng L. 2011. Roles of xanthophylls and exogenous ABA in protection against NaCl-induced photo damage in rice (*Oryza sativa* L) and cabbage (*Brassica campestris*). Journal of Experimental Botany **14**, 1- 9. doi: 10.1093/jxb/err170. Epub 2011 Jun 3.

Tanaka T, Iwai K, Watanabe K, Hotta Z. 2005. Development of 5-aminolevulinic acid for agriculture uses. Regul. Plant Growth Development **40 (1)**, 22- 29.

Usha R, Vasavi A, Thishya K, Jhansi Rani S, and Supraja P. 2011. Phytoextration of lead from effluents by sunflower (*Heliantus annus* L.). Rasayan Journal of Chemistry **4(1)**, 8- 12.

Weedon BCL. 1979. Carotenoid Research-Past, Present and Future, Pure Appllied Chemistry **51**, 435- 445.

Xie ZM, Wang BL, Sun YF, Li J. 2006. Field demonstration of reduction of lead availability in soil and cabbage (*Brassica chinensis* L.) contaminated by mining tailings using phosphorus fertilizers. Journal of Zhejiang University Science **7(1)**, 43- 50. doi=10.1631/jzus.2006.B0043

Xinxian L, Xuemei C, Yagang C, Woon-Chung WJ, Zebin W, Qitang W. 2011. Isolation and characterization endophytic bacteria from hyper accumulator *Sedum alfredii* hence and their potential to promote phytoextraction of zinc polluted soil. World Journal of Microbiology and Biotechnology **27**, 1197- 1207.

Xiong ZT. 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyper accumulator *Brassica pekinensis* Rupr, The Bulletin of Environmental Contamination and Toxicology **6**, 258- 291.

Xiong J, He Z, Liu D, Mahmood Q, Yang X. 2008. The role of bacteria in the heavy metals removal and growth of *Sedum alfredii* Hance in an aqueous medium. Chemosphere **70**, 489- 494.