ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 4, Issue, 3, pp. 683-687, March, 2014

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

ANTAGONISTIC EFFECT OF CALCIUM (Ca^{2+}) ON CADMIUM (Cd) VIZ. CHLOROPHYLL, PROTEIN AND OIL YIELD OF MUSTARD PLANT *(BRASSICA JUNCEA* L.) VAR. PUSA BOLD

[1*]Nazir Ahmad Bhat, [2]Aabid Hussain Mir, [1]Lal, E. P. and [1]Manzoor Ahmad Rather

[1]Department of Biological Sciences, SHIATS Allahabad -211007 (U.P) India
[2]Department of Environmental Studies, North-Eastern Hill University, Shillong-793022

ARTICLE INFO

Article History:
Received 14th December, 2013
Received in revised form
04th January, 2014
Accepted 15th February, 2014
Published online 25th March, 2014

Key words:
Growth regulator; Stress; Alleviation; Toxicity; Chlorsis

ABSTRACT

The Current study was conducted to study the antagonistic effect of Calcium (Ca^{2+}) on Cadmium (Cd) in the soil in which mustard (*Brassica juncea* L.) var. Pusa Bold was cultivated. The treatment of Ca-Cd was given to the soil after 15 days of seed sowing. The Cd-toxic treatment showed retarded growth, photosynthesis, protein content, yield and oil content as well as chlorsis of young leaves, such symptoms were not observed in Ca- alleviated plants. Ca^{2+} alleviated plants showed higher growth, photosynthesis, protein content, yield and oil content. The lowest growth was found in the higher concentration of Cd (1200ppm/plot). However when Ca was applied, it was found that it compensate the effect of Cadmium in low concentration but when higher Cadmium concentration was present, Ca was ineffective to compensative the effect of Cd.

INTRODUCTION

Heavy metal pollution of soils has become buzzword these days and is considered a major environmental problem the modern world is facing. Such metals tend to accumulate in the soil, translocate into plant tissues, enter food web, and then threaten the human health. Some heavy metals are linked to produce various diseases in humans (Adriano, 1992, Zaman and Zereen 1998, ATSDR 2004). Cadmium is considered to be one of the most toxic heavy metals that can be absorbed by plants and translocated readily to shoots. Crops that are harvested for their leaves often have the weakest barrier to metal uptake, whereas the barrier for root crops is usually greater. Metal concentrations are typically lower in the edible seed or fruit than in the leaves, stems or roots. However, even this barrier cannot be assumed to be effective for all metals or crops. It is non-essential to biota, toxic to humans and has cumulative effect (Singh and Melaughlin 1994). Opposite to this Ca^{2+} is a crucial regulator of growth and development in plants, the ion participates in nearly all aspects of plant development (Harper *et al.,* 2004). Studies have shown a compelling interaction between Ca^{2+}, the cell wall, and the cell growth (Brewbaker and Kwach, 1963). It has also been known for many years that Calcium plays and important role in

Corresponding author: Nazir Ahmad Bhat,
Department of Biological Sciences, SHIATS Allahabad -211007 (U.P) India

It has also been known for many years that Ca^{2+} plays an important role in controlling membrane structure and function (Burstorm, 1968). A general idea is that Ca^{2+} by binding to phospholipids stabilizes lipid bilayers and thus provides structural integrity to cellular membranes. From a physiological point of view, a frequent observation is that Ca^{2+} controls membrane permeability (Epstein, 1972; Hanson, 1984). In order to maintain integrity there occurs a strong interaction between Calcium to the plasma membrane (Wang *et al*., 1992). An increase of Calcium concentration under Cadmium stress would be a possible mechanism for reducing the toxic effects of Cadmium, and a decreased Calcium concentration under Cadmium toxicity may be a symptom of a damaged intercellular defense system (Jiang *et al.,* 2003). Cadmium has numerous negative effects on plant cells, such as membrane distortion, production of toxic metabolites and reactive oxygen species (ROS) and inhibition of photosynthesis (Hassan *et al.,* 2005). Thus, it is imperative to develop approaches to decrease Cd uptake in plants to a permissible level. Mustard (Rapeseed) group of crops is among the oldest cultivated plants in human civilization. It is a group of oilseed crops which assumes the significance in Indian National Economy by occupying the second position next to groundnut and is considered as a 'cash crop'. India is the largest rapeseed- mustard growing country in the world, occupying the first position in area and second position in production after China. Globally, India accounts for 21.7%

and 10.7% of the total acreage and production (USDA, 2010). During the last seven years, there has been a considerable increase in productivity from 1540 kg/ha in 2003-04 to 1950 kg/ha in 2009-2010 and production has also increased from 39.42 mt in 2003-04 to 59.93 mt in 2009-2010. Indian mustard (*Brassica juncea* L.*)* being one of the most economically important oilseed crop and green vegetables in most countries of the world. It is a fast growing plant and it is easy to detect toxic effects of surplus amount of metals when grown in the growth medium. Therefore, the current study was conducted to investigative the interactive effect of Calcium and Cadmium with respect to growth, photosynthesis and yield of Indian mustard (*Brassica juncea L.)*.

MATERIALS AND METHODS

The present study was conducted at the Crop Research Farm, Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences Allahabad (U.P.), during rabi season 2010 – 2011. The experimental area lies 25.88 ^{0}N and 81.15 ^{0}E. The experiments were laid out in randomized block design (RBD, 3×3 factorial). The experiment had three levels of each Cadmium (Cd) and Calcium (Ca) i,e. Cd = 0 ppm, Cd = 600 ppm and Cd = 1200 ppm / plot and Ca = 0 ppm, Ca = 600 ppm and Ca = 1200 ppm/ plot replicates thrice. The area of each plot was 1square meter with plant to plant distance 10 cm. A uniform dose of fertilizers was applied (90 kg Nitrogen, 60 kg Phosphorus and 40 kg Potassium per hectare) in the form of Urea, DAP and MoP and mixed with soil during land preparation. For the germination tests, the bold and healthy seeds of mustard (*Brassica juncea* L.*)* var. Pusa Bold were selected. Seeds were surface sterilized by $HgCl_2$ (Mercuric chloride) and were sown at an identical depth of 2 cm. After germination thinning was done to maintain fifteen healthy uniform plants in each plot. The treatments (Calcium and Cadmium) were given to the soil after fifteen (15) days of sowing. Cadmium Chloride ($CdCl_2$) and Calcium Sulphate ($CaSO_4$) was used as source of Cadmium and Calcium.

Growth parameters

Several growth parameters were used as plant height, number of leaves, leaf area and plant dry weight was recorded in 30, 60 and 90 days after sowing (DAS) stages of mustard plant. Number of flowers per plant was recorded at peak flowering stage i.e. 60 days after sowing.

Estimation of biochemical parameters

The chlorophylls and carotenoids were estimated by the method of Arnon (1949). 1 gm of fresh leaves was suspended in10 ml of 80% ethanol and homogenized thoroughly with the help of motor and pestle. The whole contents were transferred into a centrifuge tubes. The contents were allowed to stand over-night and centrifuged at 6000 RPM. The supernatant was collected and the absorbance was read at 480, 510, 645 and 663nm on the spectrophotometer. The chlorophyll and carotenoid content (mg/g fresh weight) were calculated by following equations:

- Chlorophyll-a = 12.7 (A_{663}) –2.60(A_{645}) x V/1000 x w
- Chlorophyll-b = 22.9 (A_{645}) – 4.68(A_{663}) x V/1000 x w
- Carotenoid = 7.6 (A_{480}) – 1.49 (A_{510}) x V/1000 x w
- Total chlorophyll = 20.2 (A_{645}) + 8.02 (A_{663}) x V/1000 x w

(Where, V is the volume of acetone and w is the weight of sample used). Protein content was estimated by the method of Lowery *et al*., 1951. Oil content was determined by following the solvent extraction technique in which 3 gm of mustard seeds were crushed in Na_2SO_4 and the resultant powder containing oil was taken in test tubes. 20 ml of hexane was poured in the test tubes as mobile phase. Elute containing oil was stored in a voile and hexane was evaporated in hot water bath. The remaining oil was weighed and its %age in seeds was calculated as

Oil %age = oil content/seed weight x 100.

The data recorded during the course of investigation was subjected to statistical analysis as per method of analysis of variance (Fisher, 1950).The significance and non-significance of treatment value (variance ratio) was compared with table value of 'F' at 5% level of significance. If calculated value exceeded the table value, the effect was considered to be significant.

RESULTS AND DISCUSSION

Plant Growth Parameters

All the levels of cadmium (Cd) (0 ppm, 600ppm and 1200 ppm) affected all the growth parameters (plant height, number of leaves, leaf area, plant dry weight and Number of flowers). Plant height, number of leaves, leaf area, number of flowers and biomass per plant (g/dry weight) was significantly reduced. The maximum reduction of plant height was found with Cadmium treatment of 1200 ppm/plot at 5% level of significance depicted the plant height 7.73 cm, 19.17 cm and 38.03 cm and the maximum increase of plant height was found with Calcium at 600 ppm and 1200 ppm which was found to be 9.57cm and 10.00 cm for 30 days after sowing (DAS), 36.47cm and 36.90cm for 60 days after sowing (DAS) and 104.43cm and 104.27cm after the assessment period of 90 days after sowing (DAS) respectively.

The number of leaves was reduced by 3.63%, 9.10% and 10.63% during 30, 60 and 90 DAS respectively at 1200 ppm of Cadmium. While the number of leaves were observed to be increased by 5.77%, 10.67% and 12.57% during 30, 60 and 90 DAS respectively at 600 ppm Calcium treatment in comparison to control. The maximum leaf area of 26.33 cm^2 and 126.44 cm^2 during 60 and 90 days after sowing (DAS) was observed with 1200 ppm Calcium treatment. Flowering number was increased by using the 1200 ppm Calcium dose and it touched the mean number of 66.23 flowers at peak flowering stage. The maximum flowering inhibition of 35.37 was recorded by 1200 ppm Cd dose. In case of biomass the maximum increase was found by 600 ppm Ca treatment having 2.00, 8.83 and 31.33 g during 30, 60 and 90 DAS respectively, while as the maximum reduction of dry weight 0.77, 7.07 and 26.07 was observed by 1200 ppm Cd treatments after 30, 60 and 90 DAS respectively. The interactive effects of Ca and Cd remained statistically non-significant (Table 1). Calcium application (1200 ppm of Ca/plot) alleviated the Cd toxicity and suppressed the processes caused by Cadmium. The effect of Calcium was more pronounced with the lowest level of Cadmium.

Table 1. Effect of different levels of Cadmium (Cd), Calcium and their interactions on Plant height, No. of leaves, leaf area (cm^2) and Plant Dry weight (g/DW) of mustard (*Brassica Juncea* L.) var. Pusa Bold at different stages

Treatments	Plant height (cm)			No. of leaves			Plant Dry weight (g/Dw)			Leaf Area (cm^2)	
	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	30DAS	60DAS	90DAS	60DAS	90DAS
Control	9.13	35.73	103.90	5.73	10.53	12.47	1.97	8.70	31.27	24.56	122.86
Ca_{600} ppm	9.52	36.47	104.43	5.77	10.67	12.57	2.00	8.83	31.33	25.49	124.91
Ca_{1200} ppm	10.00	36.90	104.27	5.67	10.90	12.47	1.83	8.87	31.23	26.33	126.44
Cd_{600} ppm	9.33	28.83	86.50	4.40	9.60	11.20	1.53	8.00	29.53	15.43	56.29
$Cd_{600+}Ca_{600}$	9.27	31.60	87.10	4.73	10.03	11.67	1.66	8.23	30.33	17.70	72.59
$Cd_{600+}Ca_{1200}$	9.23	32.37	87.70	4.30	9.50	11.83	1.67	8.30	30.47	18.57	76.02
Cd_{1200}	7.73	19.17	38.03	3.63	9.10	10.63	0.77	7.07	26.07	9.36	51.29
$Cd_{1200}+Ca_{600}$	8.00	20.50	38.40	3.77	9.30	10.67	0.83	7.53	26.43	9.60	54.23
$Cd_{1200}+Ca_{1200}$	8.10	21.27	40.57	4.07	9.13	11.10	0.80	7.40	26.17	9.66	54.57
C.D at 5% due to Cd	0.599	1.382	1.043	0.322	0.371	0.375	0.255	0.482	0.648	1.686	2.955
C.D. at 5% due to Ca	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "	" "
C.D. at 5% due to Inter.	1.037	2.394	1.806	0.558	0.642	0.650	0.441	0.835	1.122	2.919	5.118

Table 2. Effect of different levels of Cadmium (Cd), Calcium (Ca) and their Interactions on No. of flowers, chlorophyll content, carotenoids, protein content and %age oil content per plant of mustard (Brassica Juncea L.) var. pusa bold at different stages

Treatments	No. of flowers	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Total Chlorophyll	Carotenoid	Protein	Oil Content
Control	64.10	0.90	0.610	1.530	0.195	6.28	30.57
Ca_{600} ppm	65.46	0.95	0.621	1.526	0.198	6.33	33.53
Ca_{1200} ppm	66.23	0.96	0.616	1.539	0.196	6.27	36.47
Cd_{600} ppm	46.02	0.78	0.362	0.817	0.083	4.57	27.20
$Cd_{600+}Ca_{600}$	48.41	0.78	0.368	0.826	0.85	4.74	28.42
$Cd_{600+}Ca_{1200}$	49.38	0.80	0.419	0.841	0.092	4.83	29.37
Cd_{1200}	35.37	0.58	0.289	0.751	0.56	2.43	25.17
$Cd_{1200}+Ca_{600}$	36.18	0.59	0.286	0.761	0.059	2.65	25.77
$Cd_{1200}+Ca_{1200}$	36.23	0.64	0.288	0.770	0.051	2.69	26.23
C.D at 5% due to Cd	0.726	0.089	0.083	0.354	0.001	0.113	0.599
C.D. at 5% due to Ca	" "	" "	" "	" "	" "	" "	" "
C.D. at 5% due to Inter.	1.258	0.154	0.144	0.614	0.002	0.196	1.038

Biochemical parameters

Relative to control chlorophyll content (chl. a, chl. b and total chl.) and carotenoids was decreased significantly at 5% level with increase in Cadmium level in the soil (Table 2). The Ca-Supplementation to Cadmium exposed plants improved the content of chlorophyll and carotenoids. Chlorophyll content (chl. a, chl. b and total chl.) was reduced by 0.58, 0.286 and 0751 due to 1200 ppm Cadmium per plot of soil respectively as comparison to control. The maximum increase was observed in Calcium (600 ppm) and Calcium (1200 ppm) treatments. The carotenoids showed maximum increase in Calcium (600 ppm) treatment with 0.198 and gradually declined with increasing effect of Cadmium and Calcium. The maximum reduction was observed at Cadmium (1200 ppm) treatment i.e. 0.056, 0.059 and 0.051. The higher dose of Cd (1200 ppm) per plot reduced the protein content of mustard leaves significantly at 5% level of significance. The maximum increase was found in Calcium (600 ppm) treatments with 6.33 while as the maximum decline was observed in Cadmium (1200 ppm) treatments with 2.43 as compared to control. However, when Calcium was applied, it was able to compensate the effect of cadmium in low concentration. The maximum oil content was recorded in the plants treated with highest calcium (Ca^{2+}) concentration (36.47) as compared to control and Cadmium treated plants. However, the least %age of oil was recorded in plants treated with highest Cd concentration (25.17). In interactions there was a sharp increase in Cd (600 ppm) + Calcium (1200 ppm) treatment, which were 76.02 as compared to other interaction. Addition of Cadmium to the soil resulted in a general reduction in the growth of mustard plants in terms of plant height, no. of Leaves, leaf area and plant dry mass per plant.

However, this substantial difference should be found in the growth parameters in their response to Cd toxicity, found more sensitive to 1200 ppm cadmium per plot of soil, followed by 600 ppm cadmium per plot of soil, a result that is consistent with previous results of Dixit *et al.* (2001), Hassan et al. (2005) and Anjum *et al.* (2007). The present investigation revealed that growth (plant height) of mustard showed maximum increase in 1200 ppm of calcium treatment and gradually declined with synergetic effect of cadmium (Cd). However the interactive effects of Calcium (Ca) and Cadmium (Cd) also reduces the plant height that was less than that of Cadmium treated alone. So, calcium inhibits translocation of Cadmium at lower concentration.

Similarly, Cadmium and Aluminum have been found to reduce the plant height of soybean in proportion to their doses (Imram *et al*., 2007). The study also revealed that leaf number, leaf area and seed per plant decreased with increased concentration of cadmium. It was also found that the higher doses of Cd reduced the biomass. Same effect has been observed in case of Ni (Fuenter *et al.,* 2007). The total biomass production (TBP), absolute growth rote (AGR) and net assimilation rate (NAR) were significantly increased by calcium (Ca) and Pyridoxine application (Moinuddin *et al*., 2001). The addition of Cadmium reduced the flower number severely but not the fruit setting (Table 2). The cadmium treatment is also known to delay the flowering (Wani *et al.,* 2007). However, in the present experiment when Ca was applied, it was able to compensate the effect of cadmium (Cd) in low concentration but when high concentration was present, Ca was in effective to compensate the effect of Cadmium. The accumulation of heavy metals by plants from contaminated soils and nutrient solutions results in impaired metabolism and retard growth.

Studies have shown that σ -amino levulanic acid dehydratase (ALAD), a metal sensitive enzyme regulate chlorophyll synthesis. Lead inhibits ALAD activity by 5%. The decrease activity of ALAD may be due to interaction of Pb^{++} and Cd with –SH groups of enzyme at active sites. (Granick *et al.,* 1973). The increase in ALAD activity with age reflects the growth and transformation of plastids into chloroplast which follows chlorophyll synthesis. (Kumar *et al.* (1991) suggested that a strong interaction between Calcium and cell wall constituents may be important in providing sufficient Calcium to the plasma membrane to maintain its integrity. An increase of Calcium concentration under Cadmium stress would be a possible mechanism for reducing the toxic effects of Cadmium, and a decrease of Calcium concentration under Cadmium toxicity may be a symptom of a damaged intercellular defense system. Similar results have been reported by Brune & Dietz (1995) that the Calcium contents first increased and then decreased at very high Cadmium concentrations. The chlorophyll content and total plant dry weight were also recorded to be more sensitive to Cd than to Ni (Ayub *et al.,* 2009). The leave contents of chlorophyll and carotenoids in both species and the negative effect increased with Cd concentration (Simonova *et al* 2007). The level of soluble proteins declined with the increase in concentration of Cadmium and Calcium. Higher concentrations of Cadmium may break-down enzymes and proteins with disulphide bonds into thiosulphonates and thiols. Consequently protein synthesis may be hindered. Though, present oil content showed marked decrease by the treatment of Cadmium and some recovery when Calcium was applied to some extent.

Conclusion

In the current study it was found that the Cadmium retards the plant activities as well its higher concentration causes the chlorosis and necrosis of the leaves in mustard. However when at the same concentration calcium was applied, the plant growth was normal and in addition to this, calcium antagonized the negative effects of the cadmium at lower level. But the antagonizing capacity decreased with increased cadmium concentration. Therefore it is concluded that calcium is very important for the regulation of growth in mustard.

Acknowledgement

The authors (s) are highly thankful to Head Department of Biological Sciences, SHIATS Allahabad for providing necessary facilities during the course of work.

REFERENCES

Adriano, D. C. 1992. In Biogeochemistry of trace metals*: Lewis Publication, BocaRaton, Fla- 109-158.*

Anjum, N.A.; Umar, S.; Ahmad, A.; Iqbal, M. and Khan, N.A. 2008. Sulphur protects (*Brassica compestis* L.) from cadmium toxicity by improving leaf ascorbate and glutathione. *Plant growth Regulation*, 54(3): 271-279.

Arnon, D.C. 1949. Copper enzymes in isolated chloroplast. Poly-phenol/oxidase in *Beta vulgaris*, *PI. Physiol*., 24: 1-15.

ATSDR. Agency for Toxic Substances and Disease Registry. 2004. Toxicological profile for Lead. Atlanta: *U.S. Department of Health and Human Services.*

Ayub S, Khan FA and Varshney D, 2009. Effect of heavy metals on a medicinally important plants. *Proceeding of international conference on Emerging Technologies in Environmental Science and Engineering, October 26-28, 2009, Aligarh Muslim University, Aligarh,pp. 1028-1033.*

Bhewbaker, J.L., and Kwack, B.H. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot* 50: 859-865.

Bora, P.C. 1997. Effect of gypsum and lime on performance of *Brassica* varieties under rainfed condition. *Indian Journal of Agronomy*, 42(1): 155-158.

Burstrom, H.G. 1968. Calcium and plant growth, *Biol. Rev.(Camb.)* 43: 287-316.

Dixit V, Pandey V & Shyam R 2001. Differential oxidative response to cadmium in roots and leaves of pea (*Pisum sativum* L. CV. Azad). *J Exp Bot* 52: 1101-1109

Epstein, E. 1972. Mineral nutrition of plants. *Principles and Perspectives.* (New York: Jahn Wiley and Sons).

Fuenter D, Disante KB, Valdecantos A, Cortina J & vallijo VR, 2007. Sensitivity of mediterranem woody seedlings to coppers, nickel and zinc. *Chemosphere*, 66:412-420.

Granick, J.L., Sassa, S.Granick S., Levere, R.O. and Kappas A. 1973. Biochem. Med. 8,149.

Gulshan Kumar, Rana, P. Singh, and Sushila, 1991. Nitrate assimilation and Biomass production in *Sesamum indicum*, seedlings in lead enriched environment. *Water, air and soil pollution* 66: 163-171.

Haasan M.J, Wang F, Ali S, Zhang G 2005b. Toxi effect of Cadmiun on rice as affected by nitrogen fertilizer form*. Plant soil 277:359-365.*

Hanson, T.B. 1984. The functions of Calcium in plant nutrition. *In advances in plant nutrition, P.B. Tinker and A. Lauchli, eds (New York: Praeger publishers),* 149-208.

Hassan JM, Wang Z & Zhang G 2005. Sulphur alleviates growth inhibition and oxidative stress caused by cadmium toxicity in rice. *J Plant Nuts*. 28: 1785-1800.

Horper, J.F., Breton, G., and Harmon, A 2004. Decoding Ca^{2+} signals through plant protein kinases. *Annu. Rev. Plant Biol.* 55: 263-288.

Imran H S, Weik, Jilani G and Zh G, 2007. Interactions of cadmium and almunium toxicity in their effect on growth and physiological parameters in soybean*. Journal of Zhejiang university of scince, 8 181-188.*

Jiang, X.J.; Luo, Y.M.; Zhao, Q.G.; Baker, A.J.M.; Christie, P. and Wong, M.H. 2003. Soil Cd availability to Indian mustard and environment risk following EDTA addition to Cd contaminated soil. *Chemosphere*, 50(6): 813-818.

Lowery, O.H., Rosebrough, N.J., Farr A.L. and Randall, R.J. 1951. *J. Bio-chemi.,* 193-265.

Moinuddin, Khan, Samiullah, Khan, Khan, N.A. and Khan, M. 2001. Response of mustard and wheat to pre-sowing seed treatment with pyridoxine and basal level of calcium. *Indian Journal of plant physiology*, 6(3): 300-305.

Obata . H and Umebayashi M 1997. Effect of cadmium on mineral nutrient concentration in plants differing in tolerance for cadmium. *J. Plant Nutr.* 20: 97-105.

Simonova, E.; Henselova, M.; Masorovicova, E. and Kohenova, J. 2007. Comparison of tolerance of *Brassica juncea* and *Vigna radiata* to Cadmium. *Biologia Plantaorum*, 51(3): 488-492.

Singh B.R and Mclaughlin M.J 1999. Cadmium in soils and plants. Kluwer Academic Publishers, Boston, USA, 257-267.

Singh, S.N. Yunus, M. Kulsheshtha, K. Srivastava, K. and Ahmed K.J. 1985. Responses of *Calendula officinalis L.* to long term fumigation with SO_2. *Environ. pol. (Ser.A)*; 39: 17-25.

Wang J, Evangelou B P, Nielsen M T.1992. Surface chemical properties of purified root cell walls from two tobacco genotypes exhibiting different tolerance to manganese toxicity. *Plant Physiol. 100:496-501*

Wani P .A, Khan M. S and Zaidi. A; 2007. Impact of heavy metal toxicity on plant growth symbiosis, seed yield and Nitrogen and metal uptake in chickpea. *Australian Journal of Experimental Agriculter, 47(6):712-720.*

Zaman, M. S and F.Zareen 1998. Growth responces of Redish plants to soil Cadmium and Lead Contamination. *Bull. Environ. Contam. Toxicol.61:44-50.*

****