Original Article **Asian J Agri Biol, 2014, 2(2):119-128.**

THE EFFECT OF Cr (VI) ON SILK WORM (BOMBYX MORI) FED ON IN VITRO ACCUMULATED MULBERRY LEAVES

Muhammad Arslan Shoukat[1], Shahzad Ashraf[2*], Muazzam Ali[3], Zafar Iqbal[4], Mirza Imran Shahzad[2], H. R. Chaudary[2], Nuzhat Sial[3], Zahra Batool[3].

1. Department of Agriculture, Yazman, Bahawalpur2. University College of Veterinary and Animal Sciences, TheIslamia University of Bahawalpur, Bahawalpur3. Department of Life Sciences, TheIslamia University of Bahawalpur, Bahawalpur4. Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan.

ABSTRACT

Heavy metals take route to higher trophic levels by first of all accumulating in harvestable parts of plants like leaves. Higher the trophic level the amount of metal increase and affect every higher trophic level. Silkworm (*Bombyx mori)* is used as template to assess the biotransformation of heavy metal in a food chain from soil to plant then insect. The mulberry plants were irrigated with synthetic effluent of Cr (VI) (100mg/L with pH 4.5) and the treated soil, mulberry plant leaves, silkworm body, cuticle, alimentary canal, silk glands, silk cocoons and their excreta were sampled to check Cr (VI) contents accumulated by using atomic absorption spectrometry (AAS). The concentration of Cr (VI) in soil and mulberry leaves tend to increase with increase in irrigation times. On the other hand, the contents of Cr (VI) in *B. mori* larvae and the excreta were in considerable amount but decrease with the increase in larval instars, yet most of Cr (VI) remains in its body. Cr (VI) found in *B. mori* body was liable for toxic effects on its life cycle and the body growth and silk production was also inhibited under the effect of Cr (VI) accumulation. A considerable amount of Cr (VI) was also found deposited in the silk glands, cuticle and alimentary canal, and concentration of Cr (VI) in larval body increased *B. mori* death rate significantly.

Keywords: *Bombyx mori*, sriculture, bio-transformation, heavy metal.

INTRODUCTION

Silk is one of the most expensive fibers used in textile industry, produced naturally by silk worm (B. mori). Pakistan along with China, India, South Korea and India are the major producers of natural silk. The silk worms not only provide us precious fiber but also has great role in ethhnozoology, as a good remedy for blood pressure, diabetes and other heart diseases (Fernemore and Parkash, 1992). Mulbury leaves are one of the key food sources for silk worms but some of the other plant leave also proved experimentally good as peepal (Nasreen et al., 1989). All over sub-tropical, tropical regions and temperate regions Mulbery tree are grown Mulberry (Morus sp.) is a deciduous, deep-rooted fast growing, perennial tree. Currently, heavy metal contamination is effecting the growth as its root system supports the heavy metal uptake from the soil and deposits it in its leave parts, which are the most preferred source of food for silk worm (Rajaram et al., 2013). Pollution in soil caused by heavy metals is a worldwide problem and is an issue of great environmental concerns (Kabata-Pendies and Pendias, 1992, Kashem and Singh, 1999). As the heavy metals are ingredient of many enzymes and proteins so they are considered essential and crucial for normal development of plant. Toxicity, inhibition of growth and reduce in yield of plants is caused by increase in amount of both essential and non-essential heavy metals in the soil (Costa and Morel, 1993). Use of mineral fertilizers, pesticides, sewage sludge application and anthropogenic source such as the smelting industry, mining, are the main source from where much higher amount is arrived (Michael et al., 2007). Chromium is one of the well-known heavy metal, having significant impacts as proved toxic for agronomic crops (Ghosh and Singh, 2005).

The present research is planned to study the phytoremediation of Cr (VI) by mulberry (*Morusalba* L.) plants and its later effects on silkworm who feed on the mulberry leaves also effect on silk quality produce by silk worm. The objective of the study is to check the effect of Cr from synthetic pollutant discharge in

**Corresponding author:* e-mail: drshahzadbalouch@yahoo.com

industrially polluted areas on the growth and productivity of silkworm larvae feeding on mulberry.

MATERIAL AND METHODS

Production of Cr (VI) contaminated biomass:

Under the prevailing environmental conditions at Post Graduate Agriculture Research Station which is situated at Jhang Road, Faisalbad, *Morusalba* L. plants were carefully chosen grown in field and irrigated twice a week with serial dilutions of Cr (VI) containing solution ($K_2Cr_2O_7$ solution with water) with pH maintained at 4.5. Leaves were collected after a pre-determined time of 90 days.

Rearing of silkworm:

The silkworm eggs were obtained by Forest Department from Lahore and these were kept in incubator at 25 ± 1 ^{o} C and 75 ± 5% R.H. the eggs were hatched within 3 days. The caterpillars which were newly emerged were moved to the rearing trays and were split into two divisions; 1) Treatment fed on grown Cr (VI) contaminated mulberry leaves and 2) was control fraction and was fed by non-contaminated fresh mulberry leaves. In the 5th instar of caterpillars some caterpillars were selected. The larvae which left behind were left to pupate. When the larvae are near to pupate the larvae were shifted into pupating chambers made by cardboard. After collection of pupae they are dried in electric oven at 60 ^{o} C for 48 hours, weighted, reeled, and compared with which of control larvae and results were piles up. Later washing with de ionized distilled water (DDW), the treated collected leaves from experimental plants were divided in two fractions, one for feeding of silkworm larvae and second for atomic absorption spectroscopy (AAS) analysis.

Dissection of silkworm larvae:

After the completion of 5th instar the larvae were dissected. First the carefully chosen larvae were place into formalin for almost 2 hours and then dissected. The silk glands, alimentary canal and cuticle are obtained after dissection. The obtained body parts after dissection were dried in electric oven for 48 hours at 60^{o}C. after drying the material was exposed to digestion with nitric acid.

The collection and storage of material for analysis:

Fresh weight of samples was taken and then the samples were dried in an electric oven at 60 ^{o} C for 48 hours. Later the dried material were ground and digested (in Nitric acid and Hydrogen per Oxide), filtered and analyzed. Afterward irrigation of $K_2Cr_2O_7$ solution in water and soil samples were collected fortnightly to detect the deposition of Chromium in the soil. The samples were dried and digested by using method defined by Amacher (1996) as 1.0g of dried soil was taken in Pyrex flask and mixed with 10ml of $HNO_3$and left for whole night. Then samples were heated at hot plate at 200^{o}C, after cooling it added 1 mL of $HNO_3$and $HClO_4$.Heated at 200 ^{o}C unless fumes appeared. Sample was cool down and added 1:10 HCl, again heated to 70 °C for one hour. Allowed to cool and made the volume 50 mL by using 1% HCl and filtered and stored for Atomic Absorption Spectrophotometer.

Analysis of samples and standards:

Javed et al in 2007 used Atomic absorption Spectrophotometer (AAS) Perkin Elmer, Model A Analyst 300 equipped with air acetylene flame, for the analysis of toxic heavy metal i.e. chromium in the samples.

Atomic absorption spectrophotometer analysis:

Hollow cathode lamps of respective metal ions that provide narrow spectral lines of moderate intensity with air acetylene system were used. Solution containing metal ions was introduced into the flame through aspiration. The metal ions through thermal dissociation were converted into atomic vapors. Atomic vapors were in ground state in which electronic arrangement was stable however part of it absorbed thermal energy emitted from the flame, thus being jumped into excited state in which energy level was higher. When light beam irradiated from hollow cathode lamp, atomic vapors in ground state changes to excited state. The amount of intensity from spectrum to be absorbed was proportional to the number of atoms in the ground state or concentration of metal ions. The results were in the form of spectra between concentration and absorbance (Itanna, 2002).

RESULTS AND DISCUSSION

This study was conducted to examine the gradual increase of model heavy metal (Cr) in whole the food chain starting from soil to the mulberry and silk worm fed on. The impacts of this accumulation were also investigated by weighing cocoons and determining the mortality rate. The results shown that the accumulation in the soil was increased with the continuous irrigation with Cr (VI) containing solution. After first irrigation the concentration level was 89.06 ±0.02 and while 60 day regular irrigation the value increased to 221±0.02pm. The heavy metal adhere to the soil particles so consecutive irrigation increased adhered metal ions and remain there due to non-remediation practice. The present continuous irrigation can be parallel to the continuous effluent release from industries containing a bunch of heavy metals. Same was the results were taken by Oklandorf et al.(1986) when studied Zn concentrations in the soil of densely industrial area due to continuous flooding of the area with industrial waste water vs non industrial area. The Cr up taken by mulberry selected to irrigate by Cr (VI) solution. The leaves taken to check the accumulation of up taken metal and analysis revealed same pattern. The number of irrigations and time found directly proportional to the concentration. After 15 days of irrigation the first sample collected the deposition of metal was 262.96 ± 0.01 ppm and the maximum deposition was observed in the last sample of leaves, after 75 days of sampling which was 425.37 ± 0.01 ppm. On the other hand, the metal concentrations in non-treated plants showed minimum accumulation of Cr (VI) in mulberry leaves was studied i.e., 4.9± 0.01, 6.2 ± 0.01, 11.71 ± 0.01, 11.82 ± 0.01, 12.62 ± 0.01 ppm after the 15, 30, 45, 60 and 75 days respectively. To feed silkworms, leaves were taken from both treated and non-treated mulberry plants, and accumulation in various parts of the worm was determined. The accumulation of Cr (VI) in the larvae was found to increase with the feeding duration as 16.3 ±0.02 of the first instar to 69.2 ±0.01 ppm of the fifth while opposite to it; the larvae fed n non-contaminated leaves declined with every instar. Prince et al., (2001) reported that Cd shows almost similar results regarding accumulation in silkworm. After 5th molt, the silk glands developed and the concentration of Cr (VI) was 289 ± 0.01 ppm and 17 ±0.01 ppm in the larvae fed on contaminated leaves and non- contaminated respectively. As from research of Ali (2010), it shows that with the change in the concentration of Cr (VI) in the effluents the deposition of Cr (VI) in the silk glands of silk worm changes. The minimum deposition was 4.56 ± 0.05 mg/kg and maximum deposition was 36.67 ± 0.04 mg/kg at the 25ppm and300 ppm effluent concentration respectively. Similarly the deposition of metal in the glands decreases as pH increases as it was maximum. The accumulation of Cr (VI) in silkworm larvae alimentary canal was 131 ± 0.01. On the other hand, the larvae reared in control conditions the deposition rate found significantly lower as 9 ± 0.01 ppm. The concentration of Cr(IV) in the excreta also increased with the succeeding molts as the 5th instar had 193.31± 0.01 ppm Cr (VI) deposited in faeces while the minimum pass out of metal was found in the 1st instar i.e. 3.91 ± 0.02 mg/ kg of Cr (VI). No significant difference found in the concentration Cr in the all the instars fed on non-contaminated leaves as 10.16 ± 0.01, 10.97 ± 0.01, 11.01 ± 0.01, 11.59 ± 0.01, 11.98 ± 0.01 ppm during 1st, 2nd, and 3rd, 4th and 5th instar respectively. Many of the previous studies revealed that the concentration of heavy metals in silk worm also depend upon the concentration of metal used in irrigation as at 400 ppm concentration the maximum pass out of heavy metal was observed i.e., 22.3 mg/kg in 5th instar silkworm larvae and the minimum concentration of heavy metal i.e. in synthetic effluent was found at 25 ppm is 9.87.While he was checking the effect of different concentration at different pH levels by Khan (2009). Lisa (1986) and Kazou et al., (1984) reported that by excretion most of the heavy metals were exerted out the larval body.

The accumulation of the heavy metal used (Cr) resulted in significant decrease in silk formation evident from the comparative weights of cocoon fed on treated vs control food. Fresh cocoon weights were 7.2 ± 0.006g and 7.004 ± 0.011 g at 100 mg/L concentration of solution and pH value of 4.5. Whereas the controls were having higher weight of cocoons is 11.13 ± 0.004 and 11.75 ± 0.002 which clearly mean that the silk formation rate was higher in worms fed on non-contaminated leaves. Dry cocoon weights were 2.6 ± 0.001 and 2.7 ± 0.010 for Cr (VI) treatment and for control treatment the dry weights were 3.97 ± 0.002 and 4.215 ± 0.006. Working with other heavy metals, many of the previous investigations revealed the parallel results as Khan (2009) found that Pd accumulation also lowers the production of silk by concentrating the silk glands.Silk worm larvae, s mortality were also tested per 100 larvae in each larval instars and the maximum mortality was found in 1st instar i.e., 11 ± 0.04 and minimum mortality was found in 5th larval instar i.e., 4 ± 0.04. The mortality rate decreased with the succession of molts due to development of resistance and making Cr (VI) as body component, while still the mortality rate in case of treatment stood higher than control ones where the mortality rate remain significantly lower at every instar as maximum in first instar (5 ± 0.05) and the successive molts 3 ± 0.06, 3 ± 0.06, 2 ± 0.05, 2 ± 0.02 during 2nd, 3rd, 4th and 5th instar respectively. Arnandova and Grekoy (2003) also recorded the enhanced mortality rate with increase in accumulation of heavy metals in insect bodies. Along with the metal toxicity, Khan (2009) studied the pH variation effect on mortality rate and found maximum rate at pH 5. The results revealed that the leaves taken from plants grown in polluted areas can significantly decrease production rate and can result in significant loss of this small industry. The plants should grow in control conditions and continuous examination of soil and leaves is necessary to avoid such damages. Soil treatment strategies also require removing this heaping phenomenon of heavy metals. The quality study of silk is still requiring to determine the effect of accumulation on silk produced.

The Effect of Cr (VI) On Silk Worm (Bombyx Mori) Fed on In Vitro Accumulated Mulberry Leaves

Accum. Of Cr in Insater no/Category		1	2	3	4	5	Cocoon
Accumulation of Cr (VI) in silkworm larvae(ppm)	Treatment	16.3 ±0.02	34.61 ±0.01	47.10 ±0.01	62.0 ±0.02	69.2 ±0.01	x
	Control	8 ± 0.01	4 ± 0.01	4 ± 0.01	3 ± 0.01	3 ± 0.01	x
Accumulation of Cr (VI) in the feaces of silkworm larvae (ppm)	Treatment	3.91 ± 0.02	3.28 ± 0.01	264.2 ± 0.01	234.07 ± 0.02	193.31 ± 0.01	x
	Control	10.16 ± 0.01	10.97 ± 0.01	11.01 ± 0.01	11.59 ± 0.01	11.98 ± 0.01	x
Accumulation in silk glands(ppm)	Treatment	x	X	x	x	289 ± 0.01	x
	Control	x	X	x	x	17 ±0.01	x
Effect of Cr (VI) accumulation on silk yield(gm) Fresh	Treatment	x	x	x	x	x	7.2 ± 0.006
	Control	x	x	x	x	x	2.6 ± 0.001
Dry	Treatment	x	x	x	x	x	2.6 ± 0.001
	Control	x	x	x	x	x	4.215 ± 0.006
Effect of Cr (VI) accumulation on the body weight of silk worm larvae(gm)	Treatment	0.0159 ± 0.004	0.08 ± 0.005	0.69 ± 0.001,	4.88 ± 0.003	14.3 ± 0.002	
	Control	0.021 ± 0.001	0.017 ± 0.003	0.97 ± 0.006	5.42 ± 0.005	17.4 ± 0.004	
Effect of Cr (VI) accumulation on the body length of silk worm larvae(cm)	Treatment	0.5 ± 0.004	1.09 ± 0.005	2.15 ± 0.001	4.68 ± 0.003	5.37 ± 0.002	
	Control	0.49 ± 0.001	1.46 ± 0.003	2.41 ± 0.006	4.83 ± 0.005	5.71 ± 0.004	
Mortality rate of silk worm larvae(n)	Treatment	11 ± 0.02	9 ± 0.04	8 ± 0.01	6 ± 0.03	4 ± 0.04	
	Control	5 ± 0.05	3 ± 0.06	3 ± 0.06	2 ± 0.05	2 ± 0.02	

REFERENCES

Aery, N. C. and Jagetiya, B. L. 1997.Relative toxicity of Cadmium, Lead and Zinc on Barley. Comm. Soil. Sci. and Plant.Analysis., 28: 949-960

Afzal, W., 2009. Transportation of Zinc to Bombyxmori L. from Mulberry Plant (Morus alba L.) grown at soil irrigated with Zinc containing Effluents. M.Sc. (Hons.) Thesis, Deptt. Agri. Entomol., Univ. Agri., Faisalabad, Pakistan., 55-59.

Ahalya, N., T. V. Ramachandra and R. D. Kanamadi. 2003. Biosorption of heavy metals. Res. J. Chem. Environ., 4: 71-79.

Ahmad, N., 1987. Comparative studies on the effect of food, on larval development and silk yield in an exotic and indigenous strain of silkworm. M. Sc. (Hons.) Thesis, Deptt. Agri. Entomol., Univ. Agri., Faisalabad, Pakistan., 83-86.

Ali, A and M.T. Mehdii, 1972.Rearing of Japanese strain of Bombyxmori L. in Iraq. Bull. Biol. Res. Cent. (Baghdad), 5: 33-48. (Biol. Abst., 55 (7): 3740-1973.

Ali, M. 2010. Transportation of Chromium (VI) to Bombyxmori L. from mulberry plant (Morusalba) grown at soil irrigated with Chromium (VI) containing effluents. M.Sc. (Hons.) Thesis, Deptt.Agri.Entomol., Univ. Agri., Faisalabad, Pakistan.

Ali, R. and M. Younus, 1970.Rearing of silkworm on alternate host plants. Pak. J. Agri.Sci., 7 (3-4): 155-160.

Anonymous, 1993. Rearing of Silkworm (in Urdu), Pak. Agric. Res. Counc., Islamabad. Pp.31.

Arnaudo**,** M., 1964.Effect of Cobalt on the life cycle of the silkworm (Bombyxmori L.).Atti. Soc. Ital. Sci. Vet., 8:279-282.

Arnaudova, k. and Grekov, D. 2003. A study on the development and productivity of mulberry silkworm larvae Bombyxmori L. fed leaves from heavy metal polluted areas. J. Environ. Port. Eco., 4: 619-622.

Asakura, T., 2006. Some observations on the structure and function of the spinning apparatus in the silkworm Bombyxmori L., Biomacromolecules., 8(1): 175-181.

Ashfaq, M. and M. Aslam, 2006.Renewal of Sericulture Industry. Middle-East J. Sci.Res., 1(1):58-59.

Ashfaq, M., M. I. Khan and M. A. Hanif, 2009.Use of Morus alba–Bombyxmorias a Useful Template to Assess Pb Entrance in the Food Chain From Wastewater. J. Environ. Entomol. 38(4): 1276-1282.

Ashfaq, M., Ali, S. and Hanif, M. A. 2010, Bioaccumulation of cobalt in silkworm Bombyxmori L. in relation to mulberry, soil and wastewater metal concentrations. Process. Biochem.44: 1179-1184

Ashfaq, M., and A. A. Sail, 2001 b. The effect of optimum doses of mineral nutrients N. P, K, Ca, Mg and Cu in various combinations through root supplementation on the larval development and Silk yield of Bombyxmori L. Pak. Entomol., 23(1-2): 103-106. Assess, 69(3):231-233.

Ashfaq, M., W. Afzal and M. A. Hanif, 2010. Effect of Zn (II) deposition in soil on mulberry-silk worm food chain.African J. Biotech., 9(11): 1665-1672.

Baker, A. J., Rotkittikhun, P. and Kruatrachue, M. 2010. Tolerance and accumulation of lead in Vetiveriazizanioides and its effect on oil production. J. Environ. Biol. 31:

Barcelo, J., C. Poschenrieder and B. Gunse. 1986. Water relations of chromium (VI) treated bush bean plants (Phaseolus vulgaris L. cv. Contender) under both normal and water stress conditions. J. Exp. Bot., 37:178-187.

Bartlett R. J. and B. James, 1983. Behavior of chromium in soils: VII. adsorption and reduction of hexavalent forms. J. Environ. Qual., 12(2):177-181.

Bartlett, R.J. 1988. Mobility and bioavailability of chromium in soils. Adv. Environ. Sci.

Begonia M.T., G.B. Begonia, M. Ighoavodha and D. Gilliard, 2005. Lead accumulation by tall fescue (Festucaarundinaceaschreb.) grown on a lead-contaminatd soil. Inter.J.Environ Res. Publ.Health, 2(2):228-233.

Benzarti, S., S. Mohri and Y. Ono, 2008. Plant Response to Heavy Metal Toxicity: Comparative Study Between the HyperaccumulatorThlaspicaerulescens (Ecotype Ganges) and Non-accumulator Plants: Lettuce, Radish, and Alfalfa. Environ. Toxicol., 10: 607-616.

Bhoopathy, S., and N. Gunasegar, 1998.Effect of Potassium dicrhromate on the Pre and Post cocoon characters in a hybrid silkworm, Bombyxmori L. J. Eperi. Zool., 1(1):61-67.

Biman, B., Mandal and Kundu, S. C. 2009.Non-mulberry. Silk gland fibrion protein 3- D Scaffold for enhanced differentiation of human mesenchymal stem cells into osteocutes. Acta.Biomaterialia, 5: 2579-2590

Bischoff, C., 1999. Effects of heavy metal stress on carbohydrate and lipid concentration in the haemolymph and total body tissue of parasitized Lymantriadispar L., larvae (Lepidoptera). Comparative Pharmacology and Toxicology., 112: 87-92

Boon, D. Y. and Soltanpur, P. N. 1992. Lead, Cadmium and Zinc contamination of Aspen Garden soils and vegetation. J. Environ.Quality., 21: 82-86

Cary, E. E., W. H. Allaway and O. E. Olsen. 1977. Control of chromium concentrations in food plants. 1 absorption and translocation of chromium by plants. J. Agric. Food Chem., 25:300-304.

Chatterjee, N. and Z. Luo, 2009. Effect of two different Cr-(III)-organic compounds exposure to Saccharomyces cerevisiae. Toxicol. Environ. Chem., 14:36-47.

Chen, H. M., Zheng, C. R. and Shen, Z. G. 2000. Chemical methods and phytoremediation of soil contaminated with heavy metals. Chemosphere, 41: 229-234

Chidambaram, A., P. Sundaramoorthy, A. Murugan K. S. Ganesh, L. Baskaran, 2009. Chromium induced cytotoxicity in blackgram (vignamungo l.). J. Environ. Health. Sci. Eng., 6(1): 17-22

Chojnacka, J, Chiojnacki, A. and Gorecki, H. 2004. Bioavailability of heavy metals from polluted soils to plants. Sci. of The Total Environ.337: 175-182

Clemens, S., 2001.Molecular mechanisms of plant metal tolerance and homeostasis. Planta, 212: 475-486.

Costa, G. and J.L. Morel, 1993. Cadmium-uptake by Lupinusalbus (L.): cadmium excretion, a possible mechanism of cad,ium tolerance. Journal of Plant Nutrition, 16:1921-1929.

Czekala, J., S. Gladysiak, M. Jakubas, 1996.The effects of acidity on the content of soluble lead, cadmium, chromium and iron in humus horizon of Luvisol (in Polish).Zesz. Probl.Post.NaukRol., 434:867.

Dhawan, S. and K P. Gopinathan, 2003. Expression pattern of Cubitus interrupts from the mulberry silkworm (Bombyxmori L.) in late developmental stages. Develop. Genes & Evol., 213 (4):166-177.

Dias, A.C.B., M.A. Pavan, M. Miyazawa and D.C. Zocoler, 2003. Plant residues: Short term effect on sulphate, borate, zinc and copper adsorption by an acid oxisol. Brazilian Archives of Biol. & Tech., 46(2): 199-202.

Erin, M. L., Pierce, L. M., Patricia, L., Gillis, Chris, M. W. and Donnell, O.J. 2009.Cadmium transportby the gut and malpighian tubules of Chironomusriparius. Aquatic Toxicol., 92 (3): 179-186.

Faying, L., L. Nianchum, N. Dekui, G. Xiangxi and Z. Peicong, 2007. Ecological engineering remediation of heavy metal polluted soils. Trans. Chinese soci. Agri. Enginee., 23(3):80-84

Fendorf, S. E., 1995. Surface reactions of chromium in soils and waters.Geoderma., 67:55 - 71.

Fenemore, P. G. and A. Prakash, 1992.Applied Entomology.Willey Eastern Limited, New Dehli, India.Pp. 115.

Fernades, J.C. and F.S. Henriques. 1991. Biochemical, Physiological and structural effects of excess copper in plants. The Botanical Review, 57:246-273.

Fotakis, G. and J. A. Timbrell, 2006.Role of trace elements in cadmium chloride uptake inhepatoma cell lines.Toxicol.Lett., 164: 97–103.

Gal J., Hamilton, A. Hursthouse, P. Tatner, F Stewart, and R.Welton. 2008. Cobalt and secondary poisoning in the terrestrial food chain: data review and research gaps to support risk assessment. Environ Int., 34: 821–38.

Gao, Y., Zhou, P., Mao, L., Zhi, Y. Zhang, C., Shi, W. 2010. Effect of plant species co-existence on soil enzyme activities and soil, microbial, community structure under Cd and Pb combined pollution. J. Environ. Sci. (China)..22: 1040-1048

Ghosh, M. and S. P. Singh, 2005.A review of phytoremediation of heavy metals and utilization of its byproducts. Appl. Ecol. Environ. Res., 3: 1-18.

Gisbert, C. and R.R. De Haro 2003. A plant genetically modifiedthatbaccumulatesPb is especially promising for phytoremediation. Biochem.Biophy. Res. Comm., 303:440-445.

Gongalsky, K. B. 2006. Bioaccumulation of metals by soil-dwelling insects in a uranium production area. European J. Soil.Bio., 42:180–185.

Gorur, G., 2005. Developmental instability in cabbage aphid (Brevicorynebrassicae) populations exposed to heavy metal accumulated host plants. Ecol. Indi., 6:743-748.

Gower, A. M., G. Myers, M. Kent and M. E. Foukes, 1994.Relationships between macro invertebrate communities and environmental variables in metal-contaminated streams in S-W England. Freshwater Biol., 32: 19-22.

Hamilton, E. I., 1994.The geobiochemistry of cobalt.Sci Total Environ.,150(1–3): 7–39.

Hassler, C. S., Behra, R. and Wilkinson, K. J. (2005).Impact of Zn (II) Bioaccumulation and homeostatis in Chlorella kesslerii. Aquatic Toxicol; 74: 139-149.

Hassler, C. S., Behra, R. and Wilkinson, K. J. (2005).Impact of Zn (II) Bioaccumulation and homeostatis in Chlorella kesslerii. Aquatic Toxicol; 74: 139-149.

He, M., Z. Wang and H. Tang, 1998.The chemical, toxicological and ecological studies in assessing the heavy metal pollution in Liver, China. Water Res., 510-518.

Hursthouse, A.S., 2001. The relevance of speciation in the remediation of soils and sediments contaminated by metallic elements-an overview and examples from Central Scotland, UK. J. Environ. Monit., 3(1): 49-60.

Iqbal, M., 2000. Impact of optimum doses of mineral nutrients N, P, K, Ca, Mg, and Cu in various combination on larval development and silk yield by silk Bombyxmori L. through root injection. M.Sc. (Hons.) Thesis, Deptt.Agri. Entomol., Univ. Agri., Faisalabad, Pakistan.

Jamil, S., 1994. Effect of feeding Zinc treated peepal leaves on the development of silkworm Bombyxmori L. larvae and silk yield. M.Sc. (Hons.) Thesis, Deptt.Agri. Entomol., Univ. Agri., Faisalabad, Pakistan.

Jamshaid, K. 1994. Effect of feeding manganese (Mn) treated peepal leaves on larval development and silk yield of Bombyxmori L., M.Sc. (Hons.) Thesis, Deptt.Agri. Entomol., Univ. Agri., Faisalabad, Pakistan.

Jia-Xi, W. and Yong- Mei. 1998. Flouride effects on the mulberry silkworm system. Environ. Poll., 52: 11-18

Kabata-Pendias, A., 2001.Trace Elements in the Soil and Plants. CRC Press, Boca Raton., 45: 23-29.

Kabata-Pendias, A., H. Pendias, 1999.Biogeochemistry of trace elemnts (in Polish). PWN Warszawa., 279-287.

Kadukova J. and Kalogerakis, N. 2007. Lead accumulation from non-saline and saline environment by Tamarixsmyrnensis B. Eur. J.Soil. Biol., 43:216-223

Kamilova, E., and B. Tsarev, 2006. Mortality of silkworms due to air pollution: environmental indicators induced by ecological stress. Environ. Toxicol., 110(2): 241-250.

Kasheem, M. A. and Singh, B. R. 1999.Heavy metal contamination of soil and vegetation in the vicinity of industries in Bangladesh. Water, Air and soil pollution, 115: 347-361

Kazou T. Suzuki, Y. Aoki, M. Nishikawa, Masui. H. and Matsubara, F. 1984. Effect of Cadmium- feeding on tissue concentration of elements in germ-free silkworm (Bombyxmori) larvae and distribution of cadmium in the alimentary canal.Comparative pharmacology and toxicology. 79: 249-253

Keeling, S.M., R.B Stewart, C.W.N. Anderson and B.H. Robison. 2003. Nickle and cobalt phytoextraction of the hyper accumulator BerkhyaCoddii implications for polymetallicphytomining and phytoremediation. International Journal of Phytoremediation, 5:290-294.

Khan, M. A., 1997.The impact of optimum dosagers of N, P, K along with Ca, Mg, and Cu on the larval development and silk yield of silkworm (Bombyxmori L.) M.Sc. (Hons.) Thesis, Deptt.Agri. Entomol., Univ. Agri., Faisalabad, Pakistan.

Khan, M. I., 2009. Effect of heavy metal (Lead) on silkworm (Bombyxmori L.) larvae reared on mulberry (Morusalba L.) grown in Lead impregnated soil. M.Sc. (Hons.) Thesis, Deptt.Agri.Entomol., Univ. Agri., Faisalabad, Pakistan.

Khan, M. N., Wasim, A. A., Sarwar, A. and Rasheed, M. F. 2011. Assessment of heavy metal toxicants in the roadside soil along the N-5, National Highway, Pakistan. Environ. Monit. Assess. 19

Kochi, S. C. and Kaliwal, B. B. 2006.The effects of potassium bromide on biochemical contents of the fat body and heamolymph of crossbred races of silkworm.Mprusalba L. Caspian. J.Environ. Sci., 4: 17-24

Koptsik, J. 2003. Heavy metals in soil near the nickel smelter: chemidtry, spatial variation and impacts on the plant diversity. Journal of Environmental Monitoring 5:441-450.

Kuhad, M. S., Malik, R. S., Singh, R., and Singh. A. 1989. Studies on mobility and accumulation of heavy metals in agricultural soils receiving sewerage water irrigation. J. Ind. Soc. Soil. Sci., 37: 290-294

Liang, Y., Gao, B., Harris, W. and Cao, X. 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ. Sci. Technol., 45: 4884-4889

Lisa, A., Seidman, G., Bergtrom, David, J. and Charles, C., Remsen. 1986. Accumularion of cadmium by the fourth instar larva of the fly Chironomusthummi. Tissue and cell., 18: 395-405

Lokanath, R., K. Shivashankar and kasivisvanthan, 1986.Effect of foliar application mg and micro nutrients to mulbverry on the quality and production of cocoons. Indian J. Seri., 25(1):40-41.

Lokanath, R., Shivashankar, K. and Kasivisvanthan, 1986.Effect of foliar application of Mg and Micro nutrients to Mulberry on the quality and production of cocoons. Ind. J. Seri., 25:40-41.

Mahmood, K., M. Ahmed and A. H. Gilani, 1987. Effect of feeding leaves of Morusalba L. and Moruslaevigata on larval growth and silk yield of Bombyxmori L. Pakistan J. Zool., 19(3):239-243.

Mario, S., Neri, R., Bellanca, A., and Manta, D. S. 2002. Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. The.Sci. of.Total.Environ., 300: 229-243

Mary A., D. Peter, E. William, E. Walton, T. John and T. Trumble, 2006. Acute and chronic activity of perchlorate and hexavalent chromium contamination on the survival and development of Culexquinquefasciatus Say (Diptera: Culicidae). Environ. Pol., 144: 759 - 764.

McGrath, S. P., 1982. The uptake and translocation of trivalent and hexavalent chromium and effects on the growth of oat in flowing nutrient solution and in soil. New Phytol., 92: 381-390.

Mengel, K. and E.A. Kirkby. 1982. Principals of plant nutrition. International Potash Institute,Bern, Switzerland. Pp: 655.

Michael, K., T. Pavel, S. Jirina, C. Vladislav and E. Vojtec¡h, 2007**.**The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere., 67: 640-651.

Mortvedt, J.J. and Giordano, P.M., 1975. Response of corn to zinc and chromium in municipal wastes applied to soil. J. Environ. Qual. 4:170-174.

Muchuweti, M., J. W. Birkett, E. Chinyanga, R. Zvauya, M. D. Scrimshaw and J. N. Lester, 2006. Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: implications for human health. AgricEcosyst Environ., 112: 41–8.

Nagpal, N.K., 2004. Water quality guidelines for cobalt.Ministry of Water, Land and Air Protection, Water Protection Section, Water, Air and Climate Change Branch, Victoria.

Naheed, S., Ariz, N. and Beg, S. 1998. Qualitative assessment of effluents and accumulation of heavy metals in some vegetables. Environ. Technol., 9:251-259

Nakamura, M., Y. Horie and E. Shakamoto, 1982. Absorption and utilization of iron by silkworm Bombyxmori L. App. Entomol Zool., 17(4):543-549.

Nasreen, A., M. Akhtar, M. Ashfaq and S. Qamar, 1989. Alternate host plants of silkworm Bombyxmori L. Pakistan J. Agri. Sci., 26(1):105-106.

Nayna, G. P., V. C. Sumitra, 2005.Effects of Mercury and Chromium on Peroxidase and IAA Oxidase Enzymes in the Seedlings of Phaseolus vulgaris.Turk J. Biol.,29: 15-21.

Nicola, J. 2003. Assessment of nutritional status and trace element contamination of Holm oak woodlands through analysis of leaves and surrounding soils. Sci. of Total Environ., 311: 191-203

Oklandorf, M. H., Hoffman, D. J., Saik, M. K. and Aldrich, T. W. 1986.Embryonic mortality and abnormalities of aquatic insects. Sci. Total. Environ. 52: 49-63.

Ooik T. V., M. J. Rantala and I. Saloniemi, 2005. Diet-mediated effects of heavy metal pollution on growth and immune response in the geometrid moth

Epirritaautumnata.Environ. Pollut., 145:348-354.

Otabbong, E., 1989. Chemistry of Cr in some Swedish soils. 1. Chromium speciation in soil extracts. Acta Agric. Scand., 39:119.

Pane, L., C. Solisio, A. Lodi, G. L. Mariottini and A. Converti, 2008. Effect of extracts from Spirulinaplatensisbioaccumulating cadmium and zinc on L929 cells.Ecotoxicol. Environ. Saf., 70: 121–126.

Peltier, E. F., Webb, S. M., Gaillard, J. (2008).Zinc and lead sequestration in an imparted wetland system. Adv. Environ. Res. 8: 103-112.

Perez-Espinosa A., R. Moral, A. Cortes and M. Perez-Murcia, 2005.Co phytoavailability for tomato in amende3d calcareous soils.Biores. Tech., 96(2):649-655.

Pollution prevention fact, 2002..Chromium and Chromium Compounds.Ohio 43215 (614): 644-3469.

Poulton, B.C., Beitinger,T.L. and Stewart, K.W., 2005.The effect of hexavalent chromium on the critical thermal maximum and body burden of Clioperlaclio (Plecoptera:Perlodidae).Archives of Environ. Conta.&Toxicol., 18(4341):594-600.

Prince, S. P. M. 2002.Cadmium toxicity in mulberry with special reference to the nutritional quality of leaves. J. Plant. Nutri. 25: 689-700

Prince, S. P. M., Senthilkumar, P. and Subburam, V. 2001.Mulberry-silkworm food chain- a template to assess heavy metal mobility in terrestrial ecosystem. Environ. Monitor. Asses., 69: 231-238.

Prince, W. S., P. S. kumar and V. Subburm, 2001.Mulberry-silkworm food chain- a template to assess heavy metal mobility in terrestrial ecosystem. Environ. Monitor. Asses., 69: 231-238.

Prince, W. S., P. S. Kumar, K. D. Doberschutz, and V. Subburam, 2002.Cadmium toxicity in mulberry with special reference to the nutritional quality of leaves. J. Plant. Nutri. 25: 689-700.

Rabitsch , W. B. 1997. Tissue- specific accumulation pattern of Pb, Cd, Cu, Zn, Co, and Mn in Workers of three Ant Species (Formicidae, Hymenoptera) from a metal- polluted site. Arch. Environ. Contam.Toxicol., 32: 172-177.

Rajesh, K., Kumar, A. P.., Sharma, P. N., 2010. Morphology and oxidative physiology of sulphur- deficient Mulberry plants. Environmental and Experimental Botany., 68: 301-308.

Rajesh, K., Kumar, P. and Sharma, P. N., 2006. Magnesium deficiency induced oxidant stress and antioxidant responces in mulberry plants. Sci. Horticulturae., 108:7-14

Rigueiro, P., 1998. Silkworm as an Engineering Material. J. App. Polymer Sci., 70:2439-2447.

Rossato, L. Macfariane, J., Doley, D. and Schmidt, S. 2011., Metal binding particles alleviated lead and zinc toxicity during seed germination of metallophyte grass Astreblalappacea. J. Hazard. Matter, 190: 772-779

Rutkowski, T., L. Raschid-Sally, and S. Buechler, 2007. Wastewater irrigation in the developing world-Two case studies from the Kathmandu valley in Nepal. Agricult. Water Manage., 88: 84-91.

Sabir, S., 1991. Effect of feeding iron treated Peepal (Ficusreligiosa) leaves on the development and silk yield of silkworm Bombyxmori L., M.Sc. (Hons.) Thesis Deptt.Agri. Entomol., Univ. Agri. Faislabad, Pakistan.

Scott, C. D., 1992. Removal of dissolved metals by plant tissue.Biotechnol.Bioeng., 39:1064–68.

Sial, R. A., M. F. Chaudhary, S. T. Abbas, M.I. Latif and A.G. Khan, 2006. Quality of effluents from Hattar Industries Estate, Pakistan. J. Zhejiang Uni. Sci., 7(12):974- 980.

Siddique, K. M., 1990. Sericulture in Pakistan. Pak. J. Forestry., 4(2): 23-27.

Singh, J. and Singh, V. K. 2007. Heavy metal accumulation in crops grown in an industrial region irrigated with wastewater. J. Ecophysiol. Occup.Hlth. 7: 145-150

Skeffington, R.A., Shewry, P.R., and Peterson, P.J., 1976. Chromium uptake and transport In barley seedlings (Hordeumvulgare L.). Planta 132:209-214.

Sorensen, M. A., P. D. Jensen, W. E. Walton and J. T Trumble, 2006. Acute and chronic activity of perchlorate and hexavalent chromium contamination on the survival and development of Culexquinquefasciatus Say (Diptera: Culicidae). Environ. Pollu., 144: 759-64.

Srivastava, S., R. Kapoor, A . Thathola, and R. P. Srivastava, 2006. Nutritional quality of leaves of some genotypes of Mulberry (Morusalba). International Journal of Food Science and Nutrition, 57:305-313.

Stephen, B. and T. Kakouli-Duartea, 2008.The effects of chromium VI on the fitness and on the β-tubulin genes during in vivo development of the nematode Steinernemafeltia. J. Sci. Total Environ., 404: 56-67.

Subhas, C., K. Biraja, C. Dash, D. Rupesh and D. L. Kaplan, 2008. Natural protective glue protein, sericin bioengineered by silkworms: Potential for biomedical and biotechnological applications. J. Polymer Sci., 33: 998–1012.

Suzuki, M., M. Takahashi, T. Tsukamoto, S. Watanabe, S. Matsuhashi, J. Yazaki, N. Kishimoto, S. Kikuchi, H. Nakanishi, S. Mori and N. K. Nishizawa, 2006.Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. Plant J., 48(1): 85-97.

Tanhan, P., Kruatrachue, M., Pokeethitiyook, P., Chaiyarat, R. 2007. Uptake and accumulation of cadmium , Lead and zinc by Siam weed (Chromolaenaodorata L.). Chemosphere., 68: 323-329.
Technol., 20:267-304.

Tucker, F. B., K. X. Wang, S. L. Lu and L. J. Xu, 2004. Influence of form and quantity of chromium on the development and survival of two silkworm (Bombyxmori L.) races. J. Environ. Sci., 15:744-48.

United Nations Industrial development Organization. 2000. Industrial policy and the environment in Pakistan. NC/ Pak/97/18: 14-15

Vishvanath, A. P and K. Krishnamurthy, 1984.Effect of foliar spray of micronutrients on the larval development and cocoon characters of silkworm, (Bombyxmori L.) Indian J. Seric., 21-22(10): 1-6.

Wang, K. R., Gong, H., Wang, Y. and Van Der Zee, S. E. A. T. M. 2004. Toxic effects of cadmium on Morusalba L. and Bombyxmori L. Pl. Soil., 261: 171-180

Wyszkowska, J., J. Kucharski, E. Jastrzębska and A. Hlasko, 2001.The biological properties of soil as influenced by chromium contamination. Polish J. Environ. Stud., 10(1): 37-42.

Wyszkowska, J., 2002. Soil Contamination by Chromium and Its Enzymatic Activity and Yielding.Polish J. Environ. Stud., 11(1): 79-84.

Xu, Y. M., Wang, L., Liang, X. F., Sun, Y., Qin, X. 2011. Effect and mechanism of immobilization of cadmium and lead compound contaminated soil using new hybrid material. Huang.Jing.Ke.Xue., 32: 581-588

Zhou, D. M. and Wang, P. 2011. A novel approach for predicting the uptake and toxicity of metallic and metalloid ions.Plant. Signal. Behav.1-6.

Zhu, X., L Lijin, S. Jirong, Y. Yuanxiang, J. Xiaojun and Z. Qingsong, 2008.Effects of Zinc and Chromium Stresses on Quality of Rice Grain. J. Bioinform. Biomedi. Eng., 2: 4475 – 4479, 191: 305-318.