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Transportation of Chromium (VI) to *Bombyx mori* L. from mulberry Plant (*Morus alba* L.) grown at soil irrigated with Chromium (VI) containing effluents

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ABSTRACT

The research was conducted to determine Chromium (VI) toxicity in population *Bombyx mori*. The synthetic wastewater used to irrigate soil to evaluate the impact of pH (4 to 8) at 100 mg/L and initial Chromium (VI) concentrations (25 mg/L to 300 mg/L) at 5 pH in its bio accumulation in *B. mori* foodchain. By using atomic absorption spectrophotometer (AAS) analysis the amount of Chromium (VI) determined in soil, mulberry plants, *B. mori* larvae, silk glands and silkworm feces. The results showed that local cobalt pollution can be indicated by using *B. mori* as template as its body length, body weight and mortality rate was found to be strongly related to Chromium (VI) concentration. Higher the Chromium (VI) amount in mulberry leaves causes more toxicity to *B. mori* population. At 300 mg/L Cr (VI) concentration and pH 4 there was maximum deposition of Chromium (VI) in soil, mulberry plants, *B. mori* larvae, faeces and silk glands from the synthetic effluent. The maximum deposition was 123.5 ± 0.03 mg/kg, $89.76 \pm .031$ mg/kg, 23.31 ± 0.019 mg/kg, 41.32 ± 0.069 mg/kg and 35.67 ± 0.04 mg/kg observed respectively.

Key words: Silkworm, *Bombyx mori*, *Morus alba*, Cr (VI), Bioaccumulation

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INTRODUCTION

Contamination of soil by heavy metals is a major environmental problem, causes toxic effect, wide distribution, persistence, and transferability to plants of these metals compared with other pollutions and can lead to various diseases (Huang *et al.*, 2015; Huang *et al.*, 2016). Contamination usually results from Industrial activities, such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application and generation of municipal waste (Kabata-Pendias, 2001). Liberation from industries including electroplating, refining of petroleum, tanning, batteries, textile, metallurgy and dyes manufacturing are one of the most important sources of chromium contamination (Yang *et al.*, 2015). Zn (II) is one of most important nutrient that is necessary at low concentration for the activity of several enzymes, when it accumulates in the cells, serious damage to metabolic pathways and also causing toxicity (Albergoni *et al.*, 1980). The living

organisms gradually damage by bioaccumulation of heavy metals (Spiegel, 2002). Zn (II) is the heavy metal majority of industrial wastes contain the greatest concentration of it (Boardman and Mc Guire, 1990).

Essential micronutrients for plants contain heavy metals but they cause damage plant growth when their concentration exceed the threshold of phytotoxicity (Bennett, 1993). Leslie *et al.* (1999) found evidence of bioaccumulation of Cr and physical abnormalities in many surviving caddis fly larvae. Cr (VI) seems to inhibit growth by act principally on plant roots (Mukherji and Roy, 1978). Cr causes oxidative damage by destruction of membrane lipids and DNA damage or may even cause the death of plant species (Singh *et al.*, 2013). Chromium Cr(VI) is one of the most toxic, especially when compared to trivalent chromium (Korshoj *et al.*, 2003). The consumption of mulberry leaves was reduced in both silkworm races while Qiufeng×Baiyu larvae showed the

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higher reduction in leaf consumption (Tucker *et al.*, 2003). The entry of Cr (VI) by all exposure routes cause lethal, allergenic, carcinogenic and irritation effects (Tan *et al.*, 2015). Phytoremediation is a technique to cleanup of polluted soils, which combine the disciplines of soil chemistry, plant physiology and soil microbiology. Industrial effluents and municipal wastewater containing Heavy metals are also being used to irrigate agricultural land. If concentrations of heavy metals in waste water exceeded may be lethal for humans and animals (Noureen *et al.*, 2015). Various natural materials have been examined for the uptake of Cr(III) and Cr(VI), such as cross-linked chitosan (Bhatt *et al.*, 2015; Li *et al.*, 2017), waste-containing chitin (Fabbricino *et al.*, 2013), bio-composite of mango (Akram *et al.*, 2017), live white rot fungi (Hanif and Bhatti, 2015), seaweed (Bertagnolli *et al.*, 2014), raw agricultural waste (Moussavi and Barikbin, 2010), and biochar (Han *et al.*, 2016).

Industrial effluents and municipal wastewaters have Cr (VI). It can enter into the food chain through accumulation in plants and animals. Nowadays environmental pollution and degradation are major problems of the world. Therefore, the present research trial was conducted to determine the phytoremediation of Cr (VI) by *Morus alba* plants and its subsequent transport to *B. mori* larvae. The present study will be helpful in evaluating ecological transportation of Cr (VI).

MATERIALS AND METHODS

Production of “Cr (VI)” Contaminated Mulberry Biomass

The research trial was conducted from September 2008 to 2009 at Post Graduate Agriculture Research Station Faisalabad, *Morus alba* L. plants selected and irrigated with salts containing Cr (VI). Row to row and plant to plant distance is five feet. For each treatment five plants were selected. After seven days of selection, the plants exposed to different treatment of pH (4-8) at 100 mg/L and varying concentration of Cr (25-300mg/L) at pH 5 by irrigating plants with salts containing Cr (VI). To avoid from contamination and ensure the usage of given wastewater only Plastic sheets were used. The control treatments were irrigated with canal water. Collected plant leaves, massively washed with deionized distilled water (DDW) and then silkworm larvae feed on these leaves. After a predetermined period of 15 days leaf and soil sampling were done. Sampling of the silkworm larvae, silk glands and their excreta were done at the end of all the five larval instars and used for AAS analysis. First these were place in an oven for drying at 70°C till constant weight. Later, the dried material changes into powdered form by grinding. 1 g of dried and powdered *M. alba* leaves, silk worm larvae, soil, silk glands and Faeces.

Chemical Reagents

The chemicals $K_2Cr_2O_7$, HNO_3 , H_2O_2 , HCl, NaOH and Cr (VI) (1000mg/L) atomic absorption spectrometry standard solution was used and purchased from Fluka chemical.

Cr (VI) Solutions

The stock solution of Cr (VI) with concentration of 1000 mg/L prepared by dissolving 2.8288 g of $K_2Cr_2O_7$ in distilled water. The Cr (VI) solution of required concentration prepared by diluting stock solution appropriately.

Digestion of Mulberry Leaves and Silkworm Larvae

According to the method described by Zubair *et al.* (2008), 1 gram of dried and powdered leaves of mulberry, *B. mori* larvae, faeces, silk glands and wet soil digested.

Determination of Cr

For the determination of Cr (VI) concentration in mulberry leaves, *B. mori* larvae, silk glands, and faeces and soil samples carried out by flame atomic absorption spectrometry using a Perkin-Elmer Analyst 300 atomic absorption spectrometer equipped with an air acetylene flame.

Rearing of Silkworm Larvae

In sericulture lab *B. mori* larvae were reared under controlled conditions of temperature and R.H. The three replications were used in each treatment. Larvae were feed four times a day with interval of six hours.

Statistical Analysis

The whole experiment was repeated 3 times. Microsoft Excel Version office Xp was used for statistical analysis.

RESULTS AND DISCUSSION

Cr (VI) Contents in Soil

Cr (VI) synthetic effluents with pH ranging from 4 to 8 and with initial Cr (VI) concentrations ranging from 25 to 300 mg/L used to irrigate the soil, in which mulberry plants were planted, was irrigated with cobalt concentration in soil before irrigation using wastewater was 2.5 ± 0.05 mg/kg. When wastewater is used to irrigate soil then, the concentration of Cr (VI) in soil increased significantly (Figs. 1,2). The maximum amount of Cr (VI) reside in soil at pH 4 and 300 mg/L initial concentration in synthetic wastewater. It was found that when concentration of Cr (VI) effluents increase then Cr (VI) contents in soil also increased (Fig. 1). After 75 days of irrigation it reached to its maximum value of 41.32 ± 0.03 mg/kg at the highest concentration of 300 mg/L. After the same time period the lowest Cr (VI) contents were recorded to be 15.65 ± 0.058 mg/kg at 25 mg/L dose rate. When the pH increases then Cr (VI) contents in soil also decreased, after 75 days at pH 4 were maximum (123.54 ± 0.03 mg/kg) and at pH 8 minimum (52 ± 0.01 mg/kg). The contents were decreased after pH 4 as shown in (Fig. 2). Bartlett and Kimble (1976) reported that adsorption of Cr (VI) is maximum at acidic pH and less at neutral to alkaline pH. The intensity of adsorption depends on soil structure and texture,

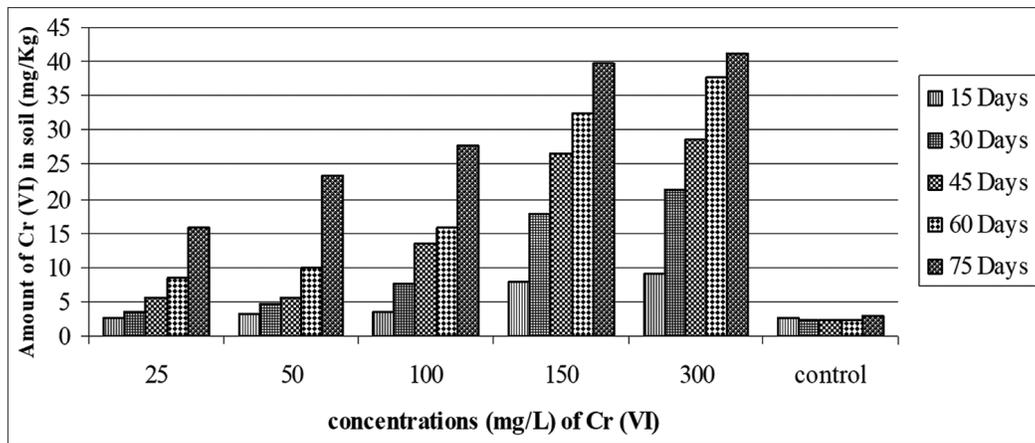


Figure 1: Accumulation of Cr (VI) In Soil (mg/Kg) at different Concentrations

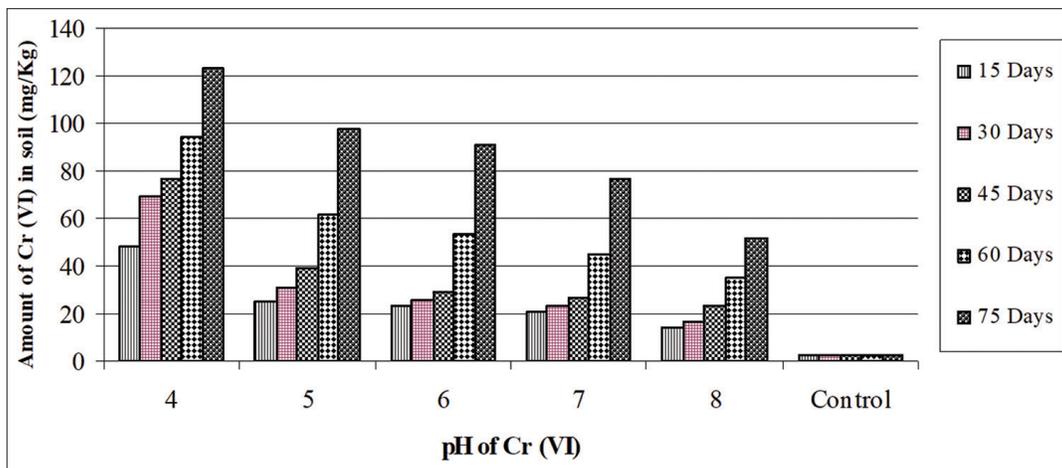


Figure 2: Accumulation of Cr (VI) In soil (mg/Kg) at different pH Levels

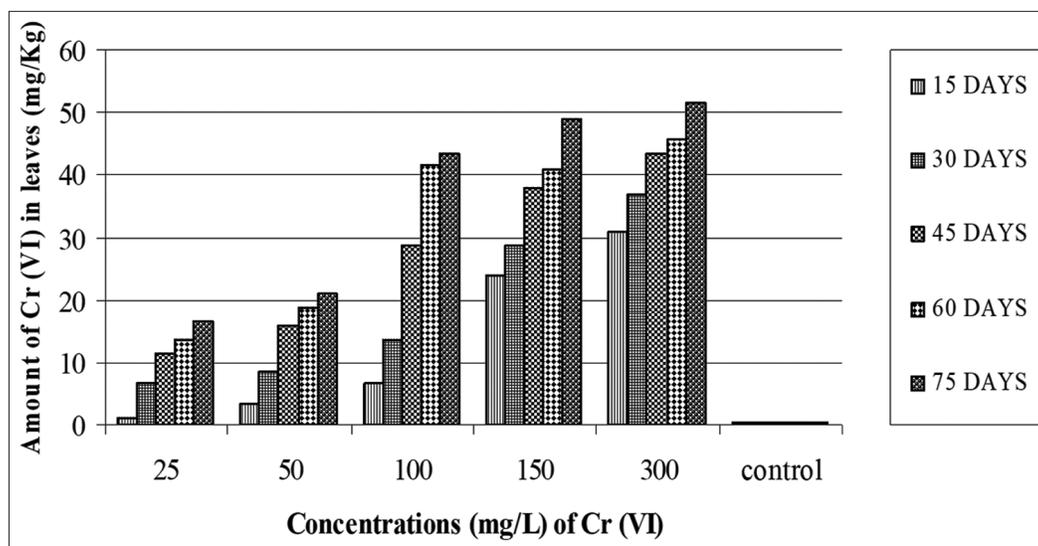


Figure 3: Accumulation of Cr (VI) In Leaves (mg/Kg) at different concentrations

as well as on pH and the presence of competing legends such as phosphate. The wetlands irrigated by industrial wastewater

have higher concentration of Zn as compare to unpolluted soil (Kadlec & Knight, 1996).

Cr (VI) Contents in Mulberry Leaves

When the initial concentration of chromium in wastewater at 300 mg/L then amount of Cr (VI) bio accumulated in mulberry leaves was 51.43 ± 0.001 mg/kg. The Cr (VI) bioaccumulation in mulberry leaves was also reduced when the initial Cr (VI) concentration was low (Figure 3). The lowest Cr (VI) contents in mulberry leaves were 16.56 ± 0.123 mg/kg at 25 mg/L Cr (VI) in wastewater. After 75 days results of micro plot experiment are reported. In Cr (VI) uptake pH of wastewater is one of most important parameter. When soil pH 4 then maximum bioaccumulation of Cr (VI) in mulberry leaves occurred were 89.76 ± 0.031 and at pH 8 minimum was 23.23 ± 0.02 . The bioaccumulation of Cr (VI) in mulberry suggests that it could harmful for animal and human life. To reduce the chance of severe damage to life and ecosystem the plant biomass present in highly polluted areas should be carefully disposed off. James (1996) reported that plants that accumulate Cr might also have the ability to accumulate Pu or U. Chromium (VI) is generally regarded

greatest human health risk as compare to Cr (III) because it is more toxic, more soluble, and more mobile

Cr (VI) Contents in Silkworm Larvae

Cr (VI) bioaccumulation in the larval body was maximum (28.56 ± 0.025 mg/kg and 14.32 ± 0.297 mg/kg) at 400 mg/L Cr (VI) concentration and soil pH 4 respectively. The lowest values were 3.1 ± 0.174 mg/kg and 10.57 ± 0.018 mg/kg at 25 mg/L and soil pH 8 after the completion of 5th larval instar (Figs. 5, 6). The life cycle of *B. mori* consists of five instars. The life span of 1st, 2nd, 3rd, 4th and 5th silkworm instars is of 2–3, 4–5, 6–7, 6–7 and 4–6 days, respectively. Accumulation of Cr (VI) gradually decreased from 1st instar to 5th. A detailed review of literature was made to study bioaccumulation of Cr (VI) in insects. But according to the information of the authors there was not a single study carried out in this regard. Hower the other heavy metals accumulation in invertebrates has been extensively quantified in past by Blackmore and Wang (2004). Shoukat *et al.* (2014) reported that the concentration of Cr

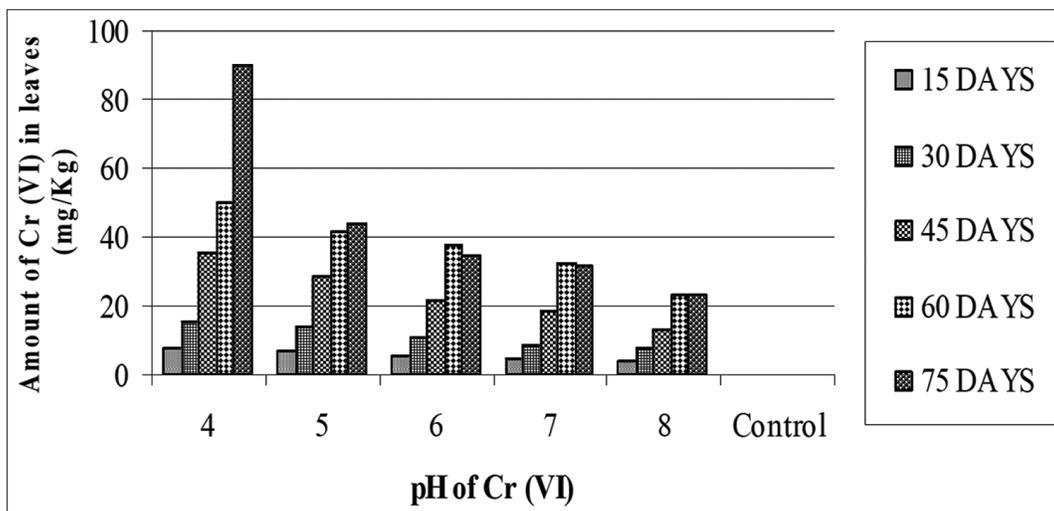


Figure 4: Accumulation of Cr (VI) In Leaves (mg/Kg) at different pH Levels

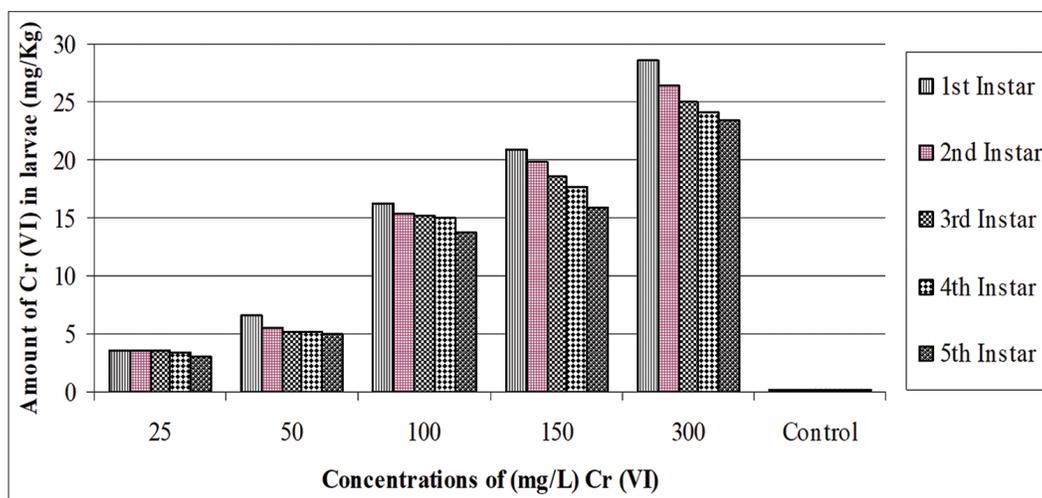


Figure 5: Accumulation of Cr (VI) In Larvae (mg/Kg) at different concentrations

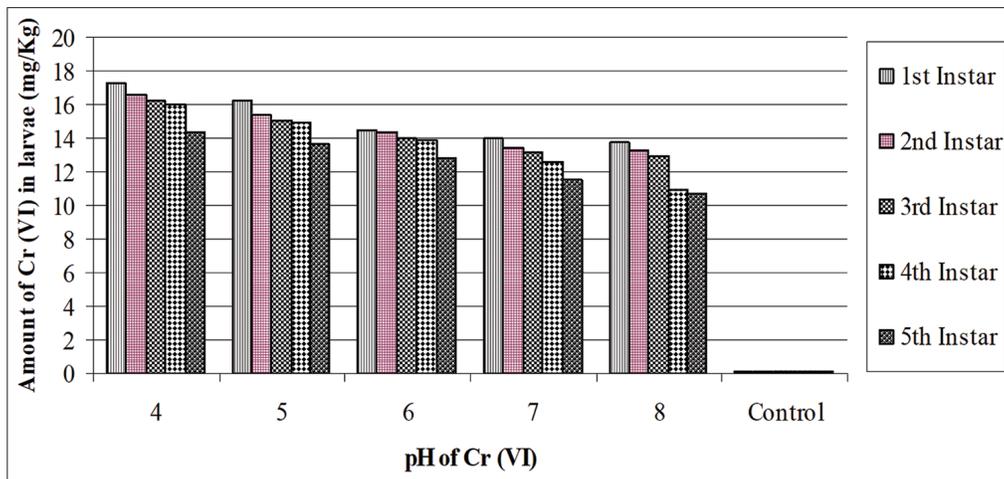


Figure6: Accumulation of Cr (VI) In Larvae (mg/Kg) at different pH Levels

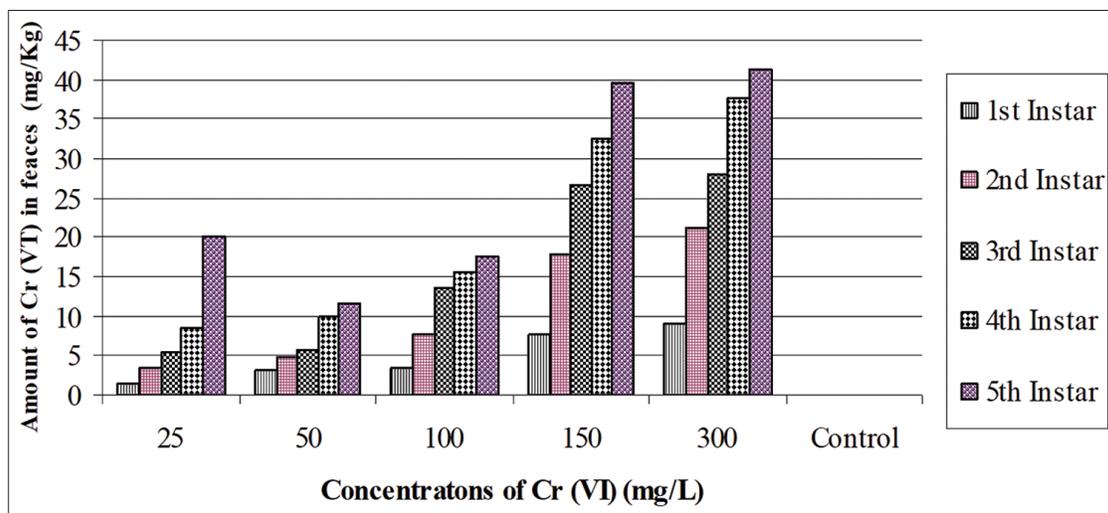


Figure 7: Accumulation of Cr (VI) In Faeces (mg/Kg) at different Concentrations

(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

Cr (VI) Contents in Silkworm Faeces

The silkworms Faeces were analyzed to determine the Cr (VI) contents (Figs. 7, 8). When Cr (VI) concentration is 300 mg/L and pH is 4 then maximum Cr (VI) concentration in Faeces was found to be $(41.32 \pm 0.069 \text{ mg/kg}$ and $19.34 \pm 0.22 \text{ mg/kg}$) respectively. After the completion of 5th larval instar, when concentration of Cr(VI) is 25 mg/L and soil pH is 8 The lowest values were $(20 \pm 0.189 \text{ mg/kg}$ and $12.87 \pm 0.108 \text{ mg/kg}$). In control treatment the accumulation is almost negligible. Metal toxicity in silkworm body occurred due to Cr (VI) that is still present inside the insect body. The same type of conditions

was drawn by Hernandez-Hernandez *et al.* (1990). The result suggested that presence of Cr in mulberry leaves significantly affected digestive rate of silkworm larvae.

Cr (VI) Contents in Silk Glands

The *B. mori* silk glands were examined to determine the Cr (VI) contents (Figs. 9,10). The maximum Cr (VI) concentration in glands was found to be $(35.67 \pm 0.04 \text{ mg/kg}$ and 10.45 ± 0.05) at 300 mg/L of Cr (VI) concentration and pH 4 respectively. After the completion of 5th larval instar, at 25 mg/L and soil pH is 8 then lowest values were $(4.56 \pm 0.05 \text{ mg/kg}$ and $4.08 \pm 0.04 \text{ mg/kg}$). In control treatment the accumulation is almost negligible. According to the information of the authors there was not a single study carried out in this regard.

Bombyx mori body length, body weight and mortality rate

Body length, body weight and mortality rates of *B. mori* were altogether influenced due nearness of Cr (VI) in higher

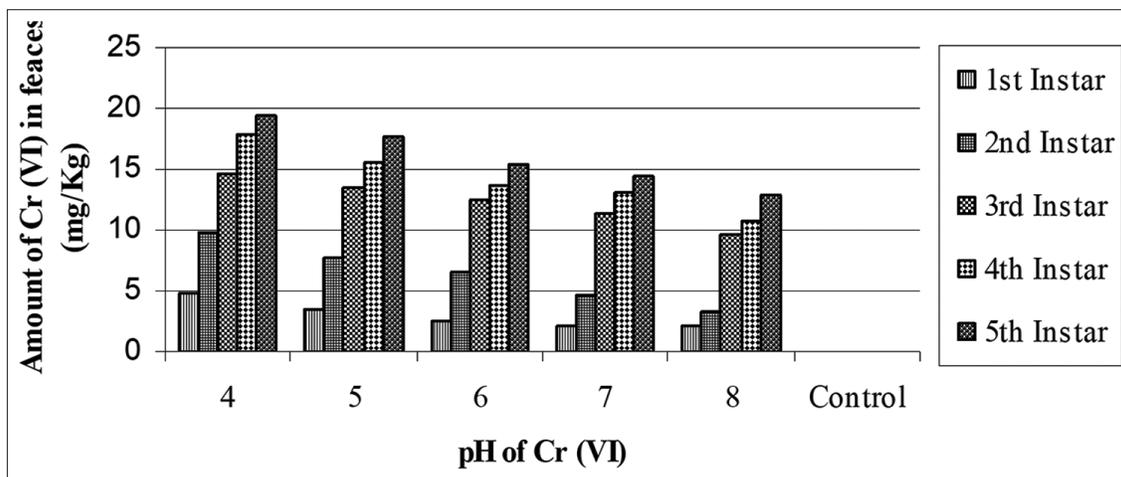


Figure 8: Accumulation of Cr (VI) In Faeces (mg/Kg) at different pH Levels

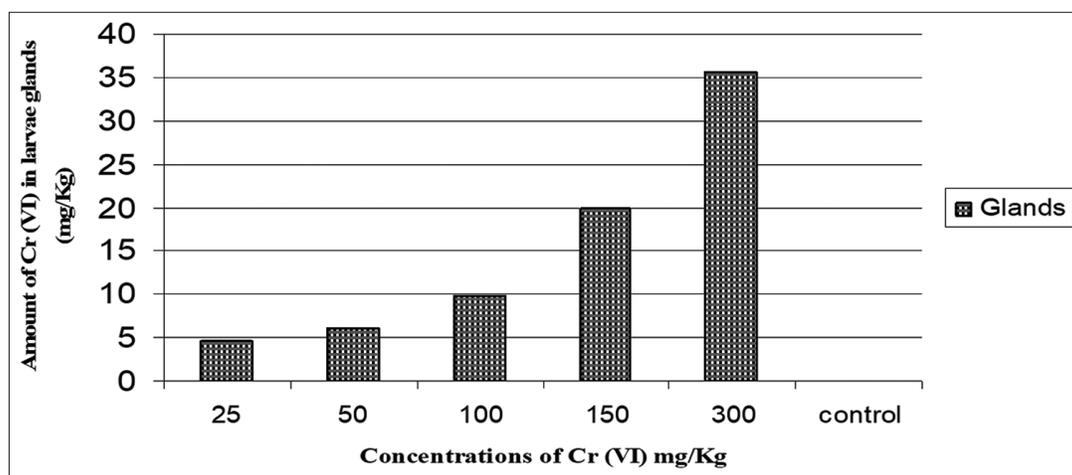


Figure 9: Accumulation of Cr (VI) in silk glands of silk worm larvae (mg/Kg) at different Concentrations

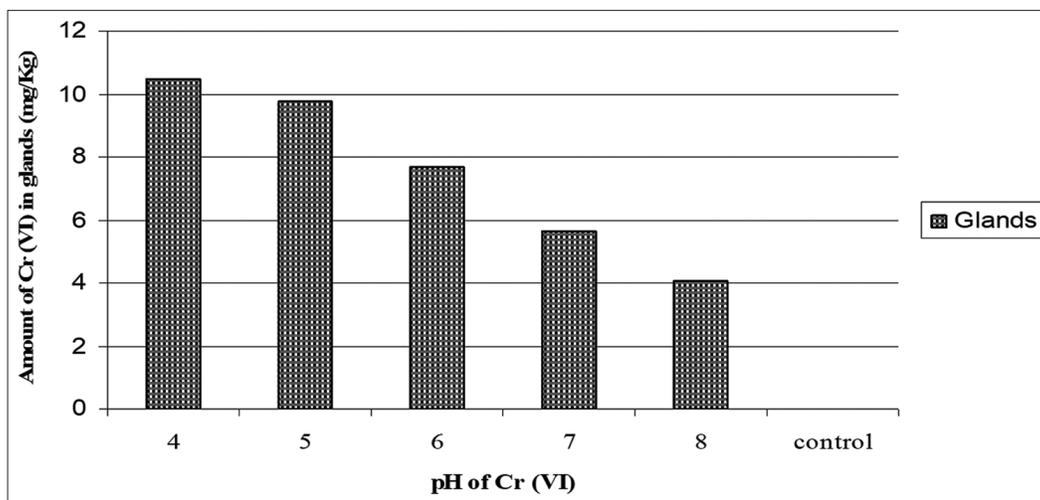


Figure 10: Accumulation of Cr (VI) in silk glands of silk worm larvae (mg/Kg) at different pH

fixations in larval body (Tables 1, 2, 3). The control larvae were found to have more body length and body weight and less mortality rate as contrast with those larvae that stored

Cr (VI) into their bodies. The water defiled with chromium Cr (VI) likewise has harmful impact on mosquito (Sorensen et al., 2006).

Table 1: Body Length (cm) at different concentrations and pH levels of Cr (VI)

Treatment	Test group	1 st Instar	2 nd Instar	3 rd Instar	4 th Instar	5 th Instar
Cr (Vi) Conc. mg/L	Control	.7 ± .05	1.3 ± .05	1.8 ± .05	3.95 ± .05	5.95 ± .05
	25	.56 ± .031	1.2 ± .031	1.6 ± .031	3 ± .031	4.5 ± .031
	50	.51 ± .017	.9 ± .017	1.5 ± .017	2.5 ± .017	4.5 ± .017
	100	.48 ± .03	.7 ± .03	1.4 ± .03	2.25 ± .03	4.2 ± .03
	150	.44 ± .04	.7 ± .04	1.39 ± .04	2.20 ± .04	4.1 ± .04
pH	300	.42 ± .02	.65 ± .02	1.35 ± .02	2.02 ± .04	3.9 ± .02
	4	.51 ± .031	.7 ± .031	1.36 ± .031	3.3 ± .031	4.6 ± .031
	5	.52 ± .017	.8 ± .017	1.31 ± .017	3.1 ± .017	4.7 ± .017
	6	.54 ± .03	.9 ± .03	1.4 ± .03	3.4 ± .03	4.7 ± .03
	7	.55 ± .04	1.1 ± .04	1.44 ± .04	3.5 ± .04	5 ± .04
8	.61 ± .02	1.3 ± .02	1.8 ± .02	3.9 ± .02	5.2 ± .02	

Table 2: Body Weight at different concentration and pH levels of Cr (VI)

Treatment	Test group	1 st Instar	2 nd Instar	3 rd Instar	4 th Instar	5 th Instar
Cr (Vi) Conc. mg/L	Control	.045 ± .05	.241 ± .05	1.152 ± .05	7.808 ± .05	18.751 ± .05
	25	.039 ± .031	.184 ± .031	.854 ± .031	6.70 ± .031	16.61 ± .031
	50	.032 ± .018	.161 ± .017	.749 ± .017	6.450 ± .017	16.09 ± .017
	100	.031 ± .04	.138 ± .03	.692 ± .03	6.280 ± .03	15.611 ± .03
	150	.025 ± .04	.131 ± .04	.65 ± .04	6.201 ± .04	15.035 ± .04
pH	300	.029 ± .03	.12 ± .02	.605 ± .02	5.601 ± .02	14.035 ± .02
	4	.02 ± .02	.126 ± .02	.690 ± .031	6.145 ± .031	15.836 ± .02
	5	.017 ± .04	.108 ± .04	.610 ± .017	6.305 ± .017	14.125 ± .017
	6	.018 ± .03	.115 ± .03	.595 ± .03	6.146 ± .03	15.653 ± .03
	7	.022 ± .017	.134 ± .017	.562 ± .04	6.650 ± .04	16.032 ± .04
8	.025 ± .031	.138 ± .031	.605 ± .02	6.264 ± .02	17.661 ± .02	

CONCLUSION

There was critical increment in Cr (VI) focus in soil subsequent to flooding it with metal defiled water. Mulberry plants seemed to search and gather Cr (VI) into its leaves, taking into account its potential move and bioaccumulation in higher tropical dimension living beings. In spite of the fact that *B. mori* discharged substantial amount of Cr (II) yet at the same time the greater part of Cr (VI) lived inside its body. There is likewise

Table 3: Mortality Rate in Silkworm Larvae at different concentration and pH levels

Treatment	Test group	1 st Instar	2 nd Instar	3 rd Instar	4 th Instar	5 th Instar
Cr (Vi) Conc. mg/L	Control	9 ± .05	7 ± .05	6 ± .05	4 ± .03	2 ± .04
	25	14 ± .031	12 ± .031	10 ± .031	9 ± .031	5 ± .031
	50	16 ± .017	13 ± .017	12 ± .017	10 ± .017	9 ± .017
	100	17 ± .03	16 ± .03	13 ± .03	13 ± .03	10 ± .03
	150	20 ± .04	18 ± .04	15 ± .04	14 ± .04	11 ± .04
pH	300	21 ± .02	19 ± .02	17 ± .02	15 ± .02	10 ± .02
	4	19 ± .031	15 ± .031	13 ± .031	11 ± .031	9 ± .031
	5	18 ± .017	14 ± .017	11 ± .017	10 ± .017	8 ± .017
	6	16 ± .03	12 ± .03	10 ± .03	9 ± .03	7 ± .03
	7	15 ± .04	11 ± .04	9 ± .04	8 ± .04	7 ± .04
8	11 ± .02	10 ± .02	8 ± .02	7 ± .02	6 ± .02	

metal amassing in silk organs of *B. mori*. These discoveries unmistakably recommended that Cr (VI) nearness in watery effluents utilized for plant water system ought to be carefully checked.

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