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BIOACCUMULATION OF CHROMIUM (III) IN SILKWORM (*BOMBYX MORI* L.) IN RELATION TO MULBERRY, SOIL AND WASTEWATER METAL CONCENTRATIONS

M. Ashfaq, S. Ahmad, M. Sagheer, M. A. Hanif*, S. K. Abbas and M. Yasir

Department of Agricultural Entomology, University of Agriculture, Faisalabad-38040, Pakistan
*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan
Corresponding author's address Saqi1472@gmail.com

ABSTRACT

Heavy metals contamination in soil is one of the major environmental concerns due to large quantities of industrial waste in Faisalabad. The research experiments was conducted to determine the entrance of Cr (III) into the food chain of *Bombyx mori* (silk worm) from mulberry plants irrigated with Cr (III) containing synthetic effluents. For this purpose, the samples of soil, plant, silk glands, silkworm larvae and their excreta were used to determine Cr (III) amount by using Atomic Absorption Spectrometry (AAS). The amount of Cr (III) accumulated into soil, in the *Morus alba* leaves and *B. mori* larvae increased with pH of the effluent. *B. mori* excreted considerable amount of Cr (III) but still most of Cr (III) remained inside its body. The maximum Cr (III) amount observed in soil, leaves, larvae and faeces were 99.89± 1.02, 95.67 ± 0.58, 61.32 ± 0.036 and 58.97 ± 0.451 mg/kg, respectively at pH 5 and at 200 concentration (200 mg/L). Cr (III) present in *B. mori* body produced toxic effects on its life cycle. First instar of *B. mori* was most affected by Cr (III) toxicity. Body length, body weight of *B. mori* decreased with increase in bio-accumulation Cr (III) amount in larval body. Higher Cr (III) concentration in larval body increased *B. mori* larval mortality significantly up to 22/100 larvae.

Key words: Waste water**,** mulberry, silkworm, chromium (III).

INTRODUCTION

Heavy metals contamination is due to 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). In the current years, increasing attention has focused on heavy metal concentrations of vegetables all over the world. Heavy metals have positive and negative roles in human life, Ozturk *et al.* (2011).The pollution of aquatic ecosystems caused by heavy metals from industrial and domestic sources lead to the bioaccumulation of these toxicants through the food web, He *et al.* (1998).The bioaccumulation of heavy metals over large areas and long time periods, which may cause in the gradual damage to living organisms, necessitates careful monitoring of the input, mobility, and effects of these pollutants, Spiegel (2002). There is no evidence that Cr can any physiological function in plants, however at low concentrations (1μM), Cr stimulates plant growth, Ghosh and Singh (2005). Higher amount of heavy metals originates from anthropogenic sources such as the mining and smelting industry, use of mineral fertilizers and pesticides, and sewage sludge application, Michael *et al*. (2007). Heavy metals, metalloids, and radionuclides may be immobilized or taken up by plants through phytostabilization, phytoextraction, or phytofltration, Clemens (2001); Ahalya *et al*. (2003). Heavy metals also enters in the environment through erosion and leaching from the soil, domestic and industrial waste discharges, lead dust follow out from the atmosphere and combustion of petroleum products. When fishes are exposed to these heavy metal ions in aquatic environment. Due to this, fishes cause heavy metals toxicity, Ahmad and Bibi (2010). Heavy metals, absorbed through the root systems, induce chlorosis of leaf and inhibition of root penetration and growth, Scott (1992). In fact, heavy metals have a significant toxicity for humans, animals, microorganisms and plants, Fotakis and Timbrell (2006). Excessive accumulation of heavy metals in agricultural soils, through wastewater irrigation, may not only result in soil contamination, but also affects the food quality and safety, Muchuweti *et al.* (2006).

Solubility of Cr (III) in soil was dependent on pH and decreased dramatically at pH > 4.5. At pH values less than 3, the predominant form of Cr in the Cr (III) system was Cr (III). The toxicity symptoms of Cr appeared as severe wilting and chlorosis of plants. Chlorosis appeared in the upper leaves of Cr-affected plants, Turner and Rust (1971). Tucker *et al*. (2004) found that even at low concentrations of 400 mg/L Cr (III) or Cr (VI) ions, growth of Qiufeng×Baiyu and Qingsong×Haoyue was significantly depressed and high death rate in both silkworm races.

Heavy metals containing industrial effluents and municipal wastewater are also being used to irrigate agricultural land. Cr (III) is commonly detected in industrial effluents and municipal wastewaters. It has an ability to be accumulated in plants and animals and thus, can enter into the food chain. Environmental pollution and degradation are the worst problems of the world nowadays.

The present research was planned to study transportation of chromium (III) to *Bombyx mori.* L from mulberry plant grown in soil irrigated with chromium (III) containing effluents and its subsequent effects on silkworm which feed on the leaves of these plants.

MATERIALS AND METHODS

Production of “Cr (III)” Contaminated Mulberry Biomass: Under the environmental conditions prevailing at Post Graduate Agriculture Research Station, Faisalabad, Pakistan *Morus alba L.* plants were selected and irrigated with water containing Cr (III). Each of row to row and plant to plant distance was five feet. Five plants were selected for a single treatment. After seven days of selection, the plants were exposed to different conditions of pH (3-5) and varying concentration of Cr (25-200 mg/L) offered or given (exposed) by irrigating plants with salts containing Cr (III). Subsequently, plant leaves was collected after a predetermined time of fifteen days. After washing extensively with deionized distilled water (DDW), these collected leaves were divided into two fractions. One fraction was used to feed the silkworm larvae and other was used in atomic absorption spectroscopic (AAS) analysis. The silkworm larvae from all the five instars, excreta leaves and silk glands were used for AAS analysis. First these were dried in an oven at 70^0C until constant weight. Later, the dried material was grounded into powdered form.

Chemical Reagents: Chemical reagents of analytical grade were used in these studies, purchased from Fluka chemicals. The chemicals used in this study were Cr $(NO_3)_2$, HNO_3, H_2O_2, HCl, NaOH and Cr atomic absorption spectrometry standard solution (1000mg/L).

Cr (III) Solutions: The stock solution of Cr (III) with concentration of 1000 mg/L was prepared by dissolving 7.695 g of Cr $(NO_3)_2$, in distilled water. The Cr (III) solution of required concentration was prepared by diluting stock solution appropriately.

Digestion of Mulberry Leaves and Silkworm Larvae and Determination of Cr (III): The samples were wet digested by taking 1 g of dried material in 20 ml of conc. $HN0_3$ followed by heating at 60°C for 15 min. Then 5 ml of H_20_2 were added as discoloring agent and then whole mixture was heated at 100°C for 10 min. After cooling, sample is filtered and diluted up to 50 ml using DDW (Shafqat *et al*., 2008). Determination of Cr (III) concentration in mulberry leaves, silkworm larvae and soil samples were carried out by flame atomic absorption spectrometry using a Perkin-Elmer Analyst 300 atomic absorption spectrometer equipped with an air acetylene flame.

Statistical Analysis: All experiments were replicated thrice. Microsoft Excel Version office XP was used for statistical analysis and the obtained results were reported as mean ± SD.

RESULTS AND DISCUSSION

In past, few studies were carried out to observe the entrance of Cr (III) into food chain but with a limited plan of work. In this regard, the present research can play very important role in evaluating the transportation of Cr (III) from inorganic sources to different life forms.

Cr (III) contents in soil: The mulberry plants were grown in different plots. These plots were irrigated with synthetic effluents having pH variation from 3 to 5. Cr (III) initial concentration ranged from 25 to 200 mg/L. The concentration of Cr (III) in soil before synthetic effluent irrigation was 15.56 ± 0.01 mg/kg. The amount of Cr (III) concentration observed in soil samples taken from the plots irrigated with synthetic effluent having different pH and concentrations of Cr-III are given in Figs. 1 and 2. The concentration of Cr (III) in soil irrigated with synthetic effluents of different pH was observed with an interval of 15 days up to a maximum of 75 days. The plants were irrigated with synthetic effluents on weekly basis. The residues of Cr (III) were more in soil samples which irrigated with higher concentration of synthetic effluents. Residues of Cr (III) were also increased with the increase in post application duration. The Cr (III) concentration in soil was increased with the increase in pH and maximum up to 75 days (Fig. 1). The concentration of Cr (III) in soil after 75 days of irrigation with synthetic effluents was noted to be 99.89± 1.02mg/Kg at pH 5. The Cr (III) concentration in soil was increased with increase in concentration of Cr (III) in synthetic effluents (Fig. 2).
Fendorf (1995) stated that soil Cr was mobilized when soils were flooded and then drained or incubated with organic matter.

Accumulation of Cr (III) in mulberry plants: Results given in Fig. 3 and 4 are shown the accumulation of Cr (III) in mulberry plants. The Cr (III) concentration was observed only in the leaves of mulberry plant. The leaves were a source of Cr (III) transfer to larvae of *B. mori*. The results of the present study indicate that the concentration of Cr (III) in leaves of mulberry was increased with increase in concentration and pH of synthetic effluents. From the results of this experiment, it can be suggested that the bioavailability of Cr (III) can be reduced by decreasing the pH of effluents being used for irrigation at certain limit. The accumulation of metals in vegetation was found to be problematic for organisms of higher trophic level (Peltier *et al*., 2008). Ashfaq *et al*. (2009a; 2010) reported the same kind of results.

Bioaccumulation of Cr (III) in *Bombyx mori:* Fig. 5 shows the bio-transportation of Cr (III) to *Bombyx mori* from *Morus alba* leaves at different soil pH values whereas Fig.6 depicts the effect of Cr (III) concentration in soil on its Bio-transportation to *Bombyx mori* from *Morus alba*. The Cr (III) amount in *B. mori* body was higher in treated soils than controlled one. Cr (III) bioaccumulation in *B. mori* body was higher (61.32 ± 0.036 mg/kg) at high pH and high initial metal concentration of soil at which mulberry plants were grown. Some other research workers studied Cd accumulation in silkworm larvae and found similar results like Kazuo *et al*. (1984), Prince *et al*. (2001) and Ashfaq *et al*. (2009b).

Amount of Cr (III) in *Bombyx mori* faeces: The contents of Cr (III) in the *B. mori* faeces are indicated in Figs. 7 and 8. The results showed that *B. mori* have somewhat efficient Cr (III) excretion system although large quantity of Cr (III) resides accumulated in its body. The Cr (III) excretion was highest (58.97 ± 0.451 mg/kg) at pH 5 and concentration of 200 mg/L once inside the organism. Afzal (2009) and Ali (2009) reported that most of the heavy metal was excreted out of the larval bodies through faeces.

Amount of Cr (III) in *Bombyx mori* silk glands: The accumulation of Cr (III) silk glands are shown at different concentration levels and pH in (Fig. 9). Increasing trend in the accumulation of Cr (III) silk glands was found as the concentration and pH of the effluent applied increased. The maximum of accumulation of Cr (III) was found when the concentration applied was 200 mg/kg and its value was noted as 6.5 ± 0.019 mg/kg.

***Bombyx mori* body length, body weight and mortality rate:** *B. mori* body length and body weight were significantly reduced by Cr (III) concentration in larval body (Tables 1 - 2) and mortality rate increased (Table 3). Control larvae showed more body length and body weight and less mortality rate in comparison to those larvae that accumulated Cr (III) into their bodies. Some other research workers also noted negative impact of heavy metal accumulation on larval growth of silkworm larvae like Ali (2009) ; Ashfaq *et al*. (2010). Tucker *et al*. (2004) reported same kind of effects of Cr (III) on silkworm larvae. This metal increased the mortality rate of silkworm larvae.

Table1. Body Length (cm) of *Bombyx mori* larvae affected by pH and Cr (III) concentration in synthetic effluent

Treatment	Test group	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
	Control	**0.78±0.03**	**1.4±0.04**	**3.02±0.032**	**5.46±0.025**	**7.05±0.04**
Cr(III) amount (mg/L)	25	0.72±0.004	1.01±0.017	2.82±0.099	5.07±0.17	6.62±0.13
	50	0.67±0.002	0.98±0.067	2.71±0.034	5.01±0.12	6.51±0.059
	100	0.63±0.005	0.92±0.017	2.69±0.046	4.75±0.069	6.43±0.054
	150	0.58±0.014	0.83±0.094	2.68±0.024	4.74±0.029	5.93±0.32
	200	0.55±0.012	0.82±0.04	2.66±0.07	4.63±0.083	5.84±0.21
pH	3	0.62±0.04	0.88±0.064	3.29±0.085	4.82±0.159	6.61±0.053
	3.5	0.78±0.03	1.4±0.04	3.02±0.032	5.46±0.025	7.05±0.04
	4	0.42±0.01	0.78±0.017	2.57±0.064	4.06±0.035	5.91±0.064
	4.5	0.51±0.014	0.79±0.024	2.67±0.024	4.18±0.087	6.54±0.209
	5	0.55±0.06	0.82±0.06	2.94±0.041	4.78±0.027	6.21±0.172

Table 2 Body weight (g) *Bombyx mori* larvae affected by pH and Cr (III) concentration in synthetic effluent.

Treatment	Test group	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
	Control	**0.043±0.02**	**0.26±0.02**	**1.154±0.01**	**7.853±0.04**	**17.905±0.05**
Cr(III) amount (mg/L)	25	0.017±0.032	0.072±0.35	0.844±0.36	6.471±0.19	16.901±0.51
	50	0.016±0.018	0.07±0.25	0.809±0.71	6.113±0.16	16.787±0.94
	100	0.014±0.002	0.068±0.29	0.767±0.12	5.956±0.31	16.566±1.23
	150	0.014±0.004	0.065±0.78	0.637±0.16	5.857±0.15	16.389±0.99
	200	0.013±0.005	0.062±0.16	0.604±0.44	5.315±0.16	15.971±0.56
pH	3	0.018±0.034	0.067±0.22	0.735±0.19	5.463±0.29	16.319±0.22
	3.5	0.017±0.017	0.065±0.82	0.672±0.25	5.056±0.98	16.218±0.14
	4	0.014±0.003	0.061±0.35	0.652±0.44	4.752±0.12	16.313±0.82
	4.5	0.013±0.004	0.058±0.65	0.641±0.43	4.661±0.91	16.282±0.47
	5	0.011±0.002	0.055±0.18	0.613±0.49	4.543±0.71	15.443±0.53

Table 3. Morality rate *Bombyx mori* larvae affected by pH and Cr (III) concentration in synthetic effluent.

Treatment	Test Group	1st Instar Dead/100 larvae	2nd Instar Dead/100 larvae	3rd Instar Dead/100 larvae	4th Instar Dead/100 larvae	5th Instar Dead/100 larvae
	Control	8±0.05	6±0.05	5±0.01	3±0.01	1±0
Cr(III) amount (mg/L)	25	13±0.04	10±0.07	8±0.04	6±0.05	3±0.05
	50	15±0.06	11±0.05	10±0.05	7±0.05	6±0.04
	100	19±0.03	15±0.03	12±0.05	10±0.02	9±0.05
	150	21±0.01	17±0.04	12±0.02	12±0.02	9±0.01
	200	22±0.07	20±0.05	15±0.02	13±0.01	10±0.01
pH	3	19±0.04	17±0.03	13±0.04	8±0.03	5±0.06
	3.5	20±0.02	18±0.04	15±0.05	10±0.04	6±0.03
	4	22±0.05	20±0.05	15±0.04	13±0.05	9±0.04
	4.5	24±0.01	21±0.03	17±0.03	16±0.06	10±0.07
	5	20±0.01	19±0.02	13±0.01	13±0.03	9±0.04

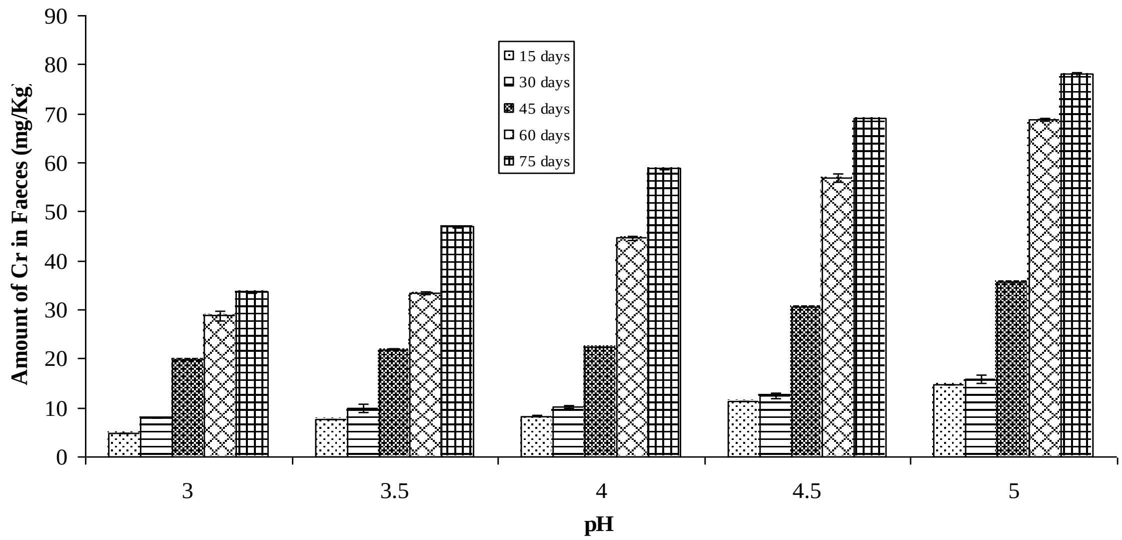

Fig. 1: Effect of synthetic effluent pH on Cr (III) deposition in soil.

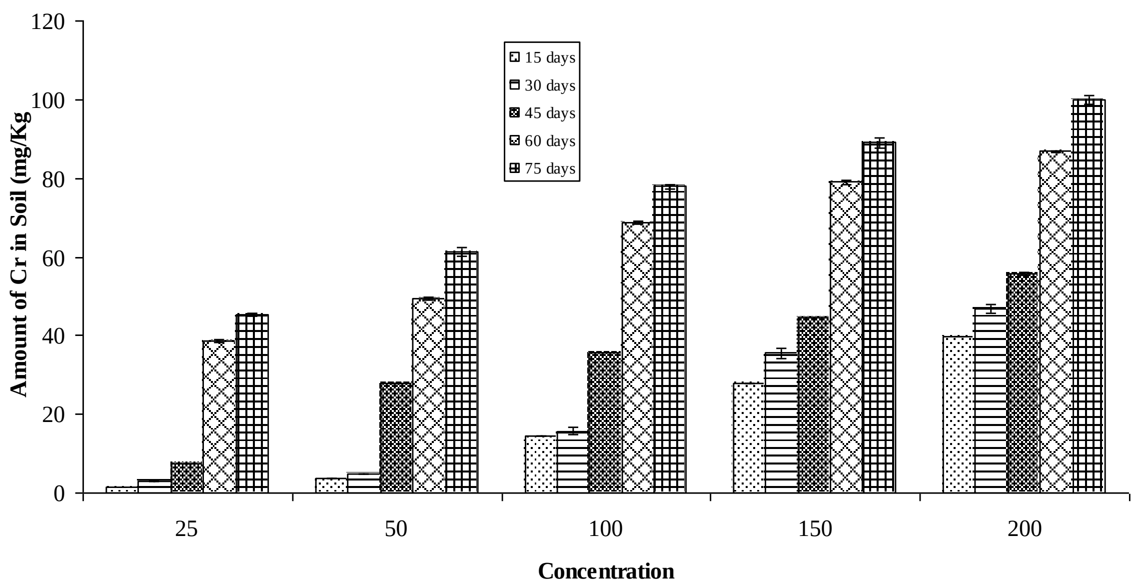

Fig. 2: Deposition of Cr (III) in soil when synthetic effluent was applied with different concentrations of Cr (III).

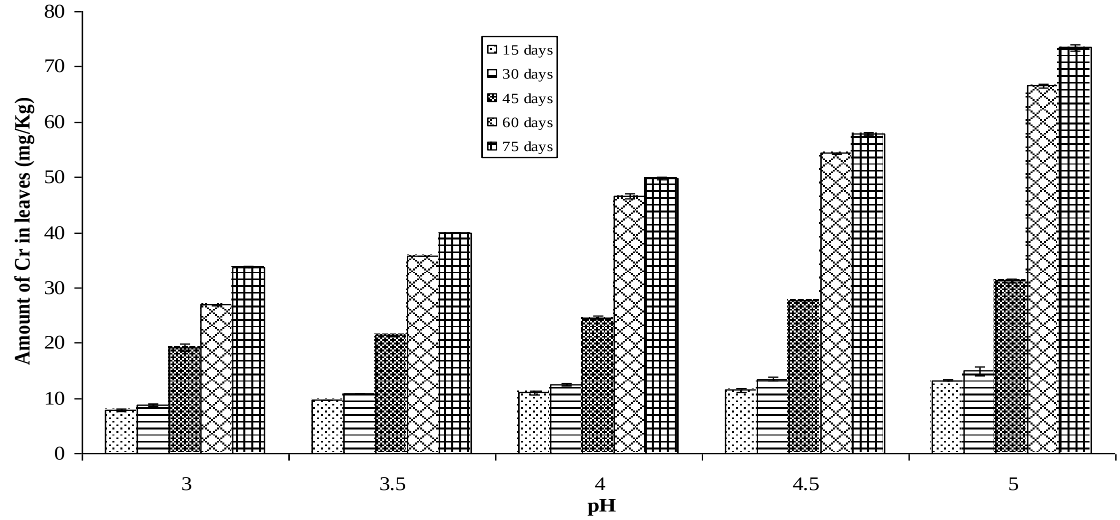

Fig. 3: Bioaccumulation of Cr (III) by *Morus alba* at various soil pH values.

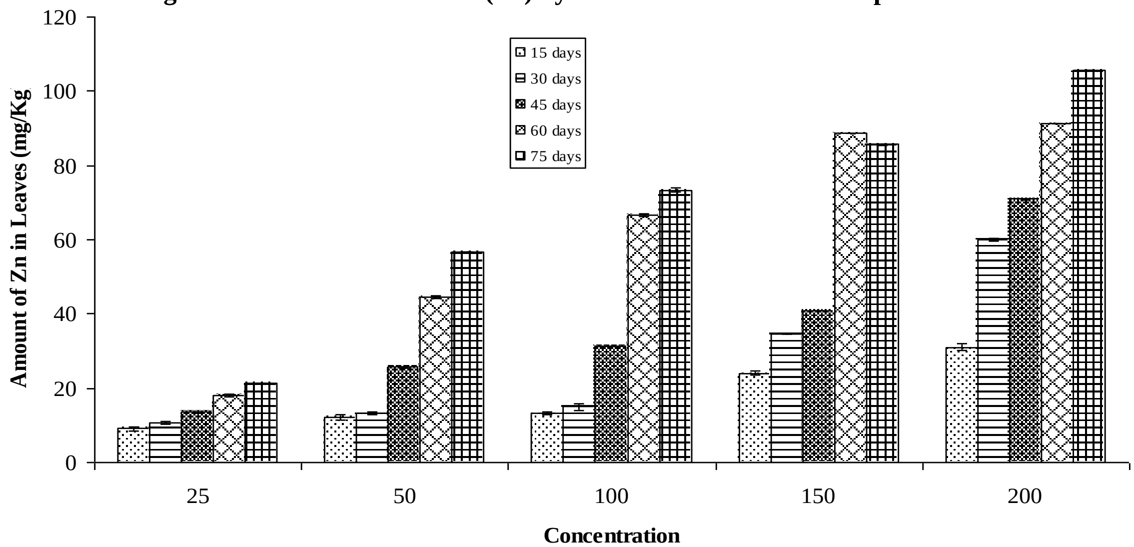

Fig. 4: Effect of Cr (III) concentration in soil on its bioaccumulation by *Morus alba*.

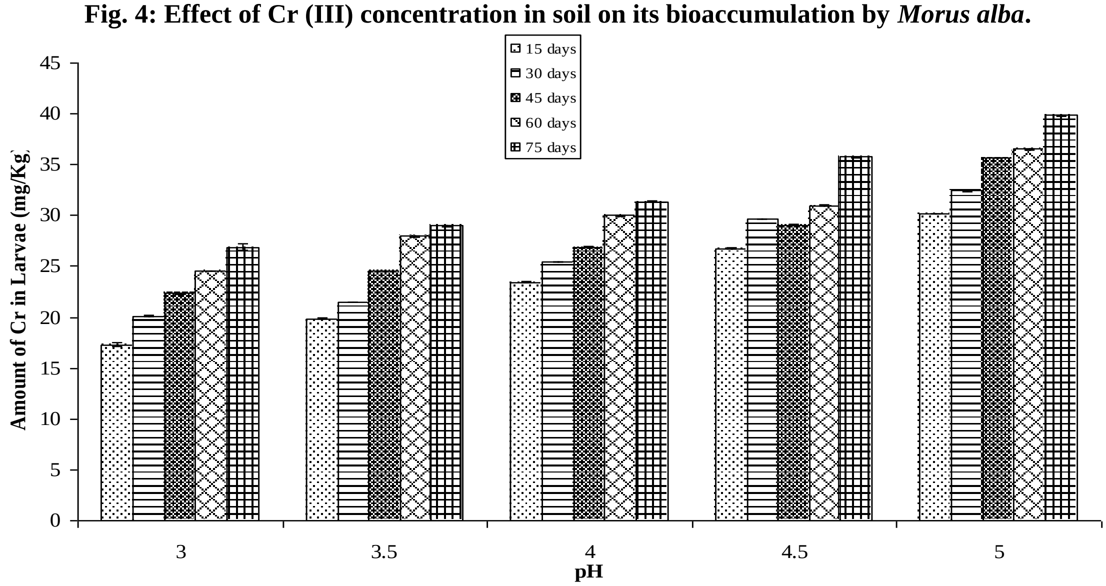

Fig. 5: Bio-transportation of Cr (III) to *Bombyx mori* from *Morus alba* leaves at various soil pH values.

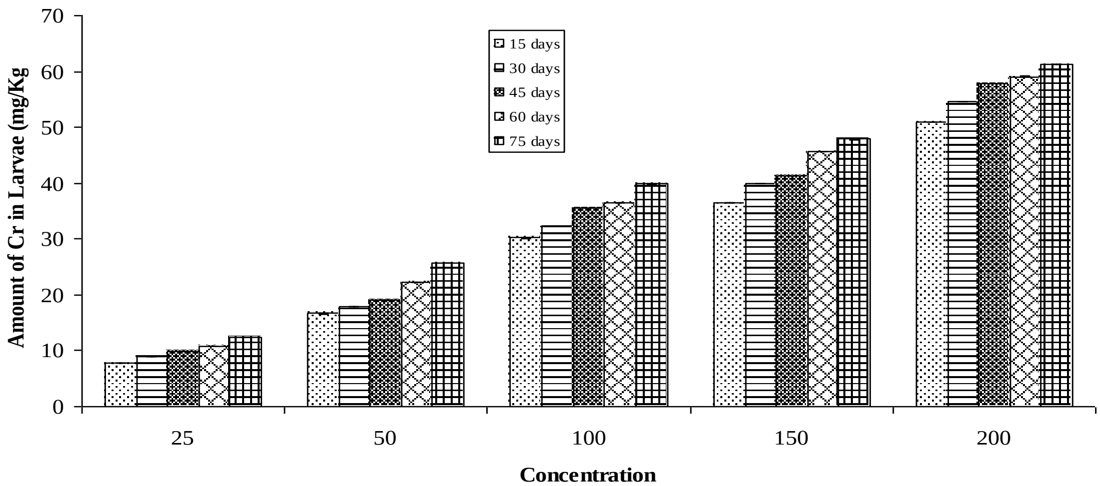

Fig. 6: Effect of Cr (III) concentration in soil on its Bio-transportation to *Bombyx mori* from *Morus alba*.

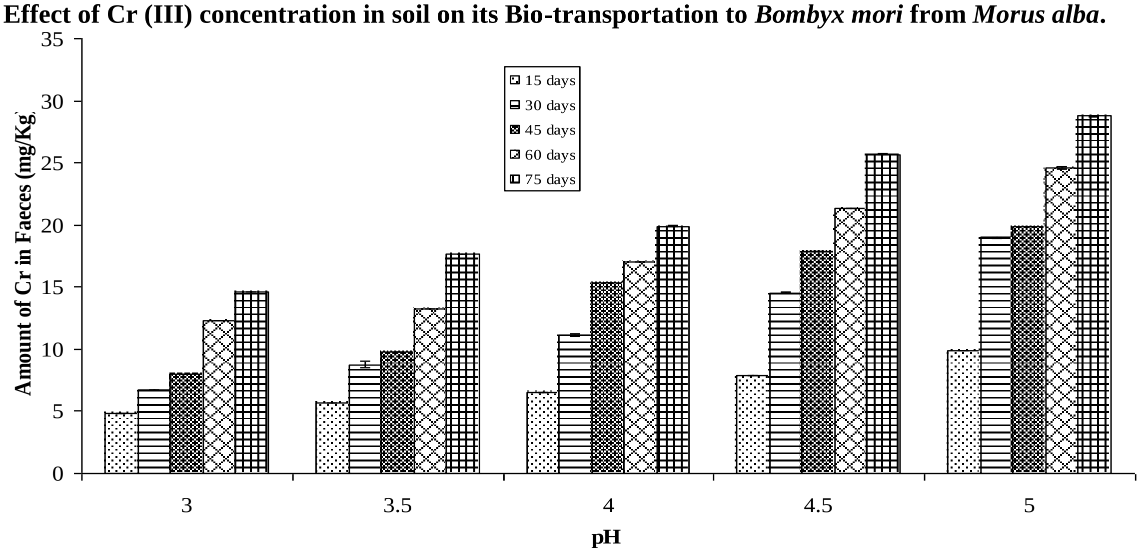

Fig. 7: Cr (III) concentrations in *Bombyx mori* excreta at various soil pH values.

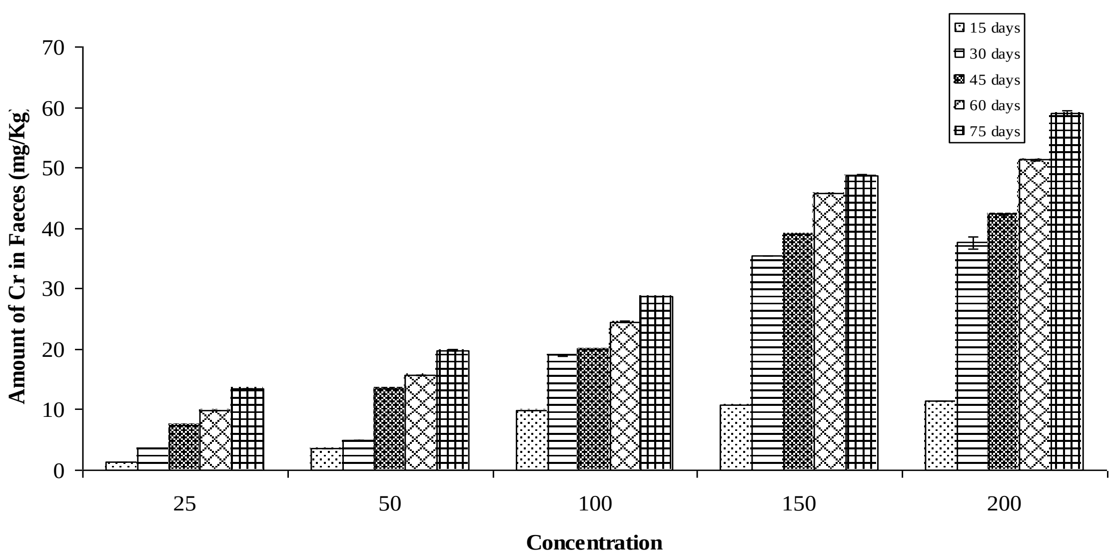

Fig. 8: Effect of Cr (III) concentration in soil on its amount in *Bombyx mori* excreta.

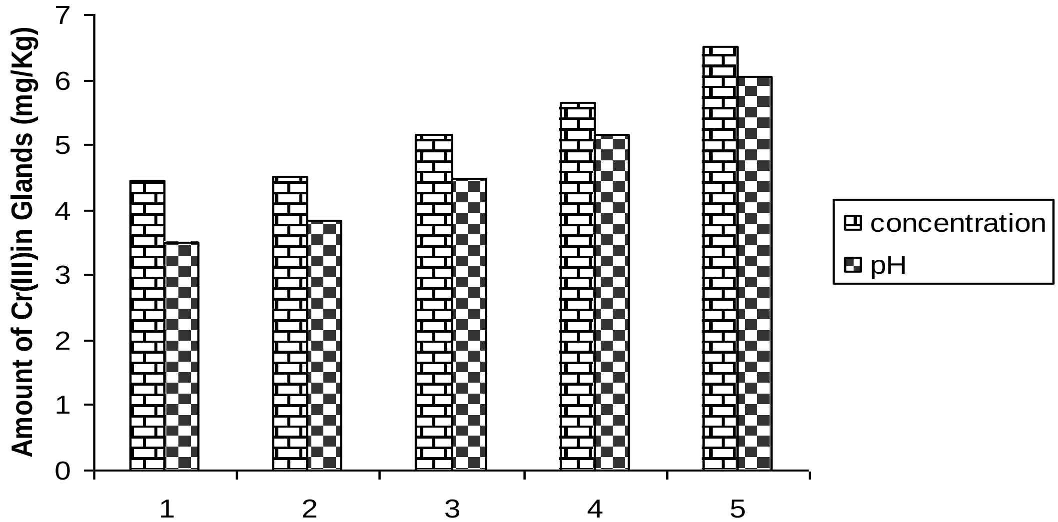

Fig. 9: Cr (III) concentrations in *Bombyx mori* silk Glands at various concentrations and soil pH values.

Conclusions: The following conclusions were drawn from the above results: It has been concluded that *B. mori* excreted large quantity of Cr (III) but still most of Cr (III) remained inside its body. The symptoms of Cr toxicity appeared as severe wilting and chlorosis of plants. The synthetic effluents irrigation was managed on weekly basis. The present study suggested that heavy metals accumulation imposed negative impacts on larval growth of silkworm larvae. These conclusions clearly suggested that Cr (III) presence in aqueous effluents used for plant irrigation should be strictly monitored.

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