African Journal of Biotechnology Vol. 9(11), pp. 1665-1672, 15 March, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1441
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Effect of Zn(II) deposition in soil on mulberry-silk worm food chain

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Accepted 30 December, 2009

The present study was conducted to evaluate the entrance of Zn(II) into the food chain of *Bombyx mori* (silk worm) from mulberry plants irrigated using Zn(II) containing synthetic effluents. The soil, plant, silkworm and their excreta were sampled to determine Zn(II) amount by using atomic absorption spectrometry (AAS). The amount of Zn(II) deposited by synthetic effluent to soil was increased with pH of the effluent. However, the bioaccumaltion of Zn(II) in *Morus alba* leaves and *B. mori* larvae was high when the effluent pH was in the acidic range. *B. mori* excreted considerable amount of Zn(II) but still most of Zn(II) resided inside its body. The maximum Zn(II) amount detected in soil, leaves, larvae and faeces were 386.51 ± 0.03, 142.85 ± 0.001, 91.375 ± 0.019 and 42.13 ± 0.69 mg/kg, respectively. Zn(II) present in *B. mori* body was responsible for toxic effects on its life cycle. First instar of *B. mori* was most affected by Zn(II) toxicity. Body length, body weight of *B. mori* decreased with increase in bioaccumlated Zn(II) amount in larval body. Higher Zn(II) concentration in larval body increased *B. mori* death rate significantly.

Key words: *Bombyx mori*, *Morus alba*, Zn(II), food chain, bioaccumulation.

INTRODUCTION

Heavy metals contamination in soil is a major environmental problem. Contamination usually results from indus-trial 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). 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). Zn(II) is essential at low concentration for the activity of several enzymes, when present in excess it accumulates in the cells, causing toxicity and serious damage in metabolic pathways (Albergoni et al., 1980). The bioaccumulation of heavy metals results in gradual damage to living organisms (Spiegel, 2002). Zn(II) is the heavy metal present in the greatest concentration in the majority of industrial wastes (Boardman and Guire, 1990). Several heavy metals are essential micronutrients for plants but they can damage the plant growth if they exceed the threshold of phytotoxicity (Bennett, 1993). For example, Zn(II) becomes toxic if it exceeds a maximum soil concentration of 400 mg/kg (Kabata-Pendias and Pendias, 1984). Zn(II) is primarily an ecological risk, because it is known to adversely affect aquatic receptors and can be phytotoxic at high concentrations (United States Environmental Protection Agency, 2003). Unlike organic compounds, metals cannot be degraded or destroyed under biotic conditions (Ghosh and Singh, 2005). Trace metals, even essential ones, are toxic when present above threshold levels (Rehfeldt and Sochting, 1991; Gower et al., 1994).

Trace elements and heavy metals enter into an agro-ecosystem through both natural and anthropogenic processes. Soil inherits trace elements from its parent material. Heavy metals containing industrial effluents and municipal wastewater are also being used to irrigate agricultural land. Zn(II) 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.

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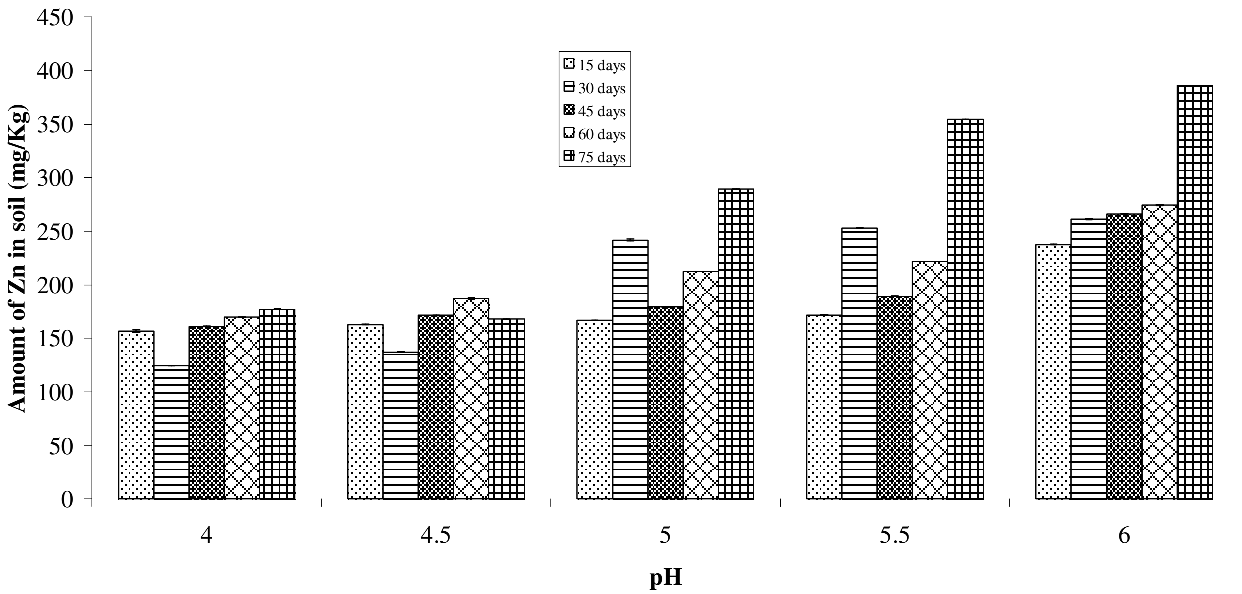

Figure 1. Effect of synthetic effluent pH on Zn(II) deposition in soil.

In this regard the present research was planned to study the phytoremediation of Zn(II) by *Morus alba* plants and its subsequent transport to *Bombyx mori* larvae. The present study will be helpful in evaluating ecological transportation of Zn(II).

MATERIALS AND METHODS

Production of Zn(II) contaminated mulberry biomass

Under the environmental conditions prevailing at the University of Agriculture, Faisalabad, Pakistan, *M. alba* plants were grown in soil irrigated using Zn(II) containing synthetic effluents. Each row to row and plant to plant distance was kept constant at five feet. Three plants were selected for a single treatment. After seven days of cultivation, the plants were exposed to different conditions of pH (4 - 6) and varying concentration of Zn(II) (25 - 800 mg/l) by irrigating plants with synthetic effluents. *M. alba* plants leaves were collected after a predetermined time of fifteen days. After washing extensively with deionized distilled water (DDW), these collected leaves were used to feed the silkworm larvae. The soil, silkworm larvae (from all the five instars) and leave samples were dried in an oven at 70°C till constant weight was obtained. Dried samples were subsequently grounded into the powdered form. 1 g of dried and powdered *M. alba* leaves, silkworm and soil were wet digested according to the method described by Zubair et al. (2008).

Chemical reagents

The following analytical grade chemicals used in the present study were purchased from Fluka Chemicals: Zn(II) $(NO_3)_2$, HNO_3, H_2O_2, HCl and Zn(II) (1000 mg/l) atomic absorption spectrometry standard solution.

Zn(II) solutions

Stock solution of Zn(II) with a final concentration of 1000 mg/l was prepared by dissolving 11.38 g of Zn(II) $(NO_3)_2$ in distilled water. The Zn(II) solution of required concentration was prepared by diluting stock solution appropriately.

Digestion of mulberry leaves and silkworm larvae: Determination of Zn(II)

The determination of Zn(II) concentration in mulberry leaves, silkworm larvae and soil samples was carried out by flame atomic absorption spectrometry using a Perkin Elmer Analyst 300 atomic absorption spectrometer equipped with an air acetylene flame. The analytical wavelength for Zn(II) determination was set at 213.856 nm (Javed et al., 2008). The transportation of Zn(II) through various inorganic and organic sources is schematically presented in Figure 1.

Statistical analysis

All experiments were triplicated. The obtained results were plotted using mean ± SD values.

RESULTS AND DISCUSSION

The study of entrance of heavy metals into food chain is although very important but with a limited properly planned work. In this regard, the present study can play very important role in evaluating the transportation of heavy metals from inorganic sources to different life forms. The present study evaluated Zn(II) transformation from inorganic sources to living organisms.

Zn(II) contents in soil

The mulberry plants were grown in soil irrigated with

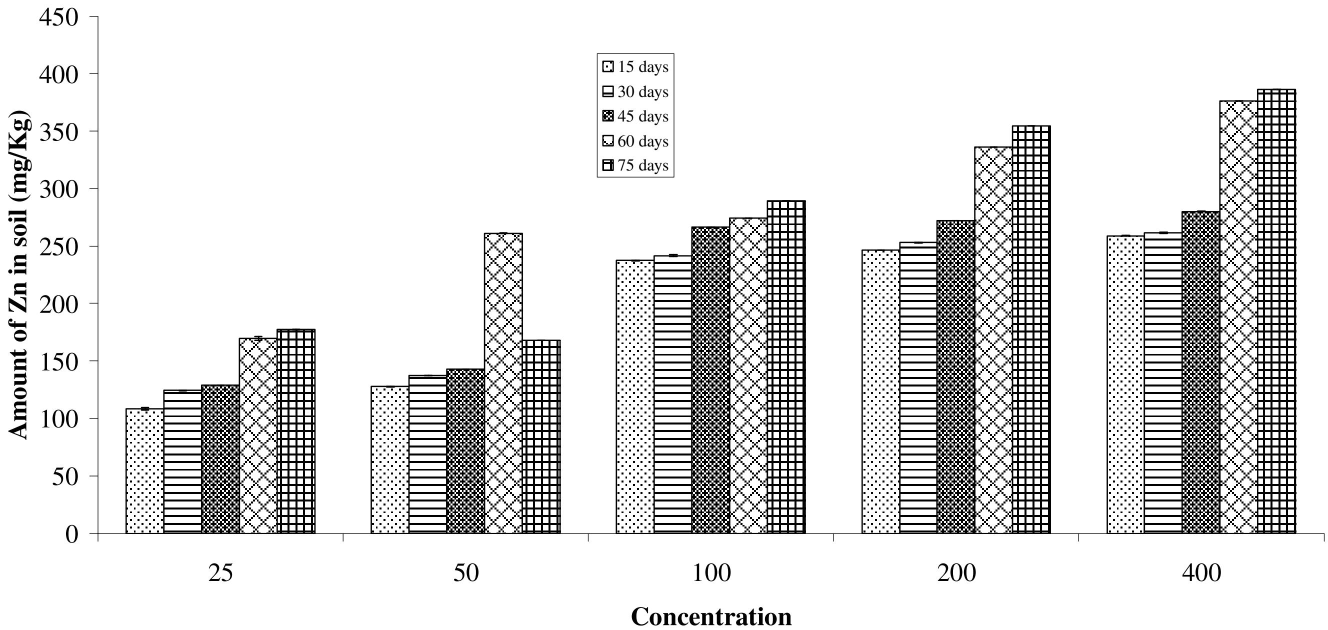

Figure 2. Effect of Zn(II) concentration in synthetic effluent on its deposition in soil.

synthetic effluents whose pH varied from 4 to 6 and Zn(II) initial concentration ranged from 25 to 400 mg/l. The concentration of Zn(II) in soil before irrigation using synthetic effluent was 15.56 ± 0.01 mg/kg. Results of total Zn(II) concentration in soil after irrigation using synthetic effluent at different pH and concentration values are shown in Figures 1 and 2. pH and initial Zn(II) concentration were already found as important parameter in metal entrance to food chain by Peltier et al. (2008). The same authors also concluded that aqueous Zn(II) is the most bioavailable form of this metal even at low concentrations. The concentration of Zn(II) in soil irrigated with synthetic effluents of different pH was determined with an interval of 15 days up to a maximum of 75 days. The synthetic effluents irrigation was managed on daily basis. The Zn(II) concentration in soil increased with the increase in pH and maximum upto 75 days (Figure 1). The concentration of Zn(II) in soil after 75 days of irrigation with synthetic effluents was found to be 386.51 ± 0.03 mg/Kg at pH 6. Peltier et al. (2008) observed that approximately, one quarter to one third of zinc in the surface sediment was present either as dissolved species or loosely bound surface complexes, primarily to clay mineral. This fraction could be expected to be easily available to plants and biota present in the area. The Zn(II) concentration in soil was also increased with initial concentration of Zn(II) in synthetic effluents (Figure 2). Wetlands impacted by industrial activities were found to contain much higher concentration of Zn(II) in comparison to unpolluted soils (Kadlec and Knight, 1996; Oklandorf et al., 1986). In this regard, it was suggested that concentration of Zn(II) in industrial effluents should be strictly controlled.

Zn(II) accumulation in mulberry plants

Zn(II) accumulation by mulberry plants is shown in Figures 3 and 4. The Zn(II) concentration was determined only in mulberry leaves as it is the source of Zn(II) transfer to *B. mori* population. In general, the Zn(II) concentration in mulberry leaves increased with the increase in Zn(II) concentration in synthetic effluents. The maximum Zn(II) concentration accumulated in leaves was 142.85 ± 0.001 mg/kg. The same type of results, in case of Zn(II) accumulation by *Chromolaena odorata* was reported by Tanhan et al. (2007). The results of the present study indicated that the concentration of Zn(II) mulberry leaves decreased with increase in pH of synthetic effluents. From the results of this experiment, it can be suggested that the bioavailability of Zn(II) can be reduced by increasing the pH of effluents being used for irrigation. The accumulation of metals in vegetation is found to be problematic from a bioaccumulation stand point by Peltier et al. (2008). They also found that plant species serve as food sources for a number of higher trophic level organisms.

Zn(II) contents of *Bombyx mori* body

The contents of Zn(II) in the *B. mori* body are illustrated in Figures 5 and 6, respectively. *B. mori* accumulated different amounts of Zn(II) in body at different treatment levels given to soil at which mulberry plants were grown. The Zn(II) burdens in *B. mori* body were higher in treated soils than controlled ones. Zn(II) bioaccumulation in *B. mori* body was higher (91.375 ± 0.019 mg/kg) at low pH and

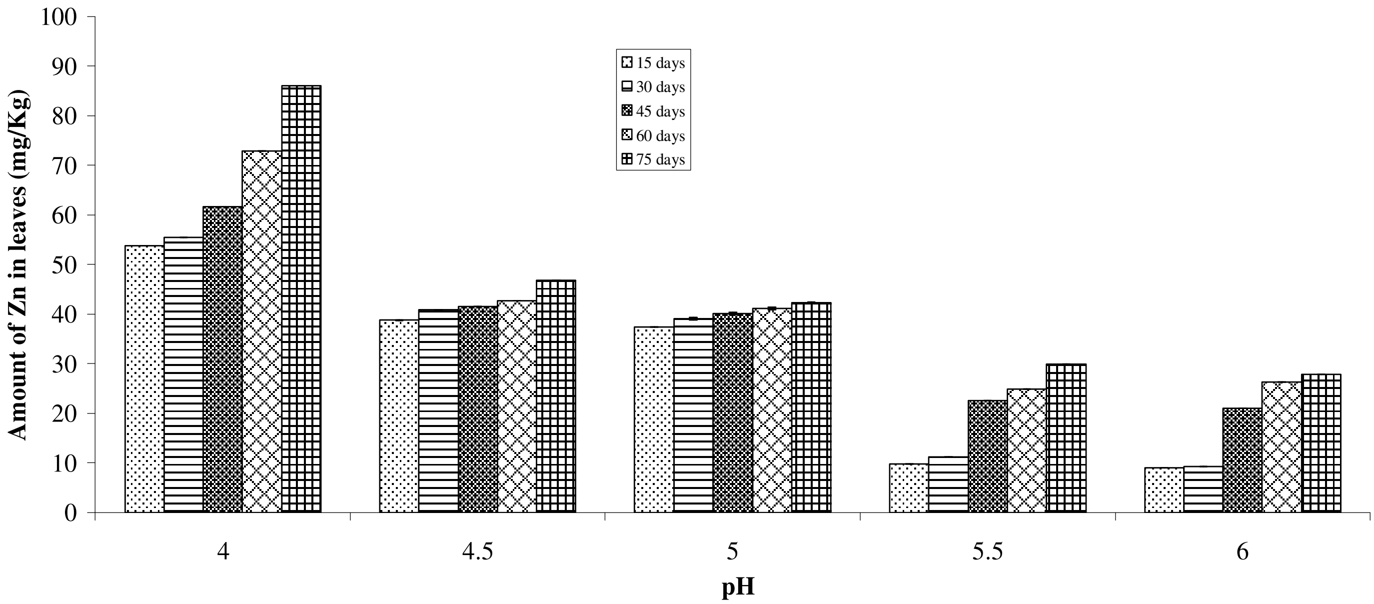

Figure 3. Bioaccumulation of Zn(II) by *Morus alba* at various soil pH values.

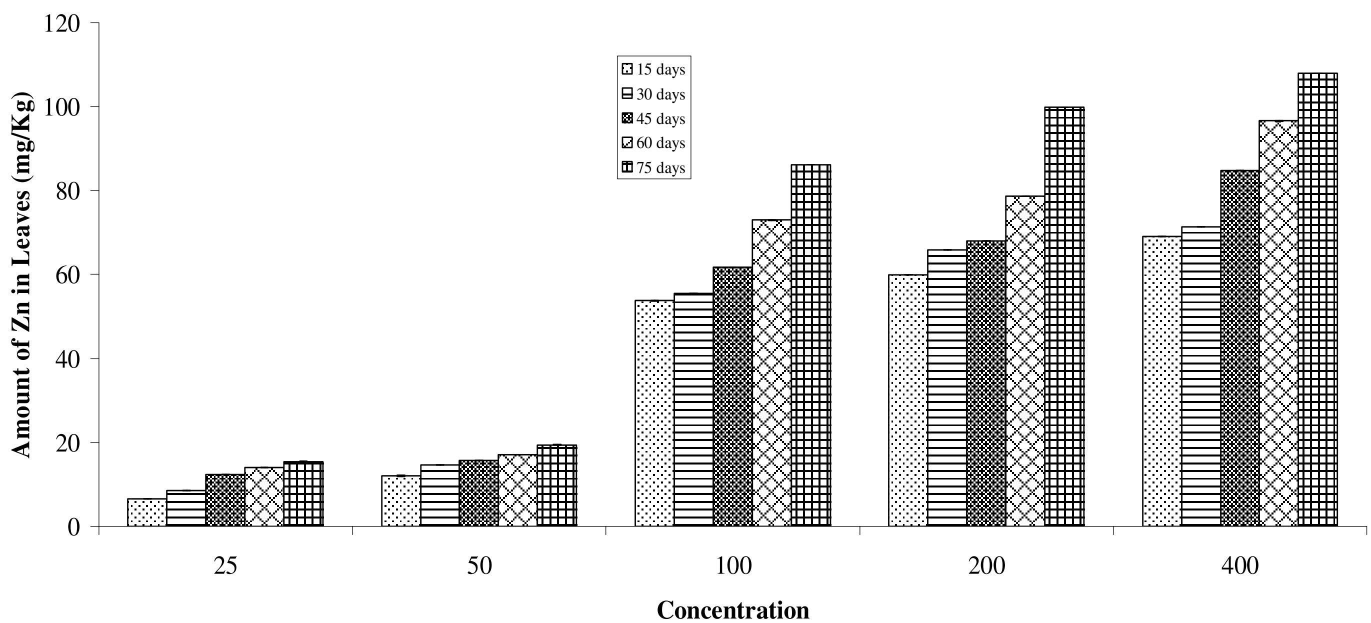

Figure 4. Effect of Zn(II) concentration in soil on its bioaccumulation by *Morus alba*.

high initial metal concentration of soil at which mulberry plants were grown. The zinc accumulation in other invertebrates has been extensively quantified in the past [Blackmore and Wang, 2004; Kizilkaa, 2005; Spurgeon et al., 2000).

Amount of Zn(II) in *Bombyx mori* faeces

B. mori faeces were analyzed for Zn(II) contents and results are shown in Figures 7 and 8. The results indicated that *B. mori* have somewhat efficient Zn(II) excre-tion system although large quantity of Zn(II) remained accu-mulated in its body. The Zn(II) excretion was highest (42.13 ± 0.69 mg/kg) at pH 4 and concentration of 400 mg/l once inside the organism, zinc may react with several biological ligands that are responsible for zinc storage or detoxification (Hassler et al., 2005; Garnham et al., 1992).

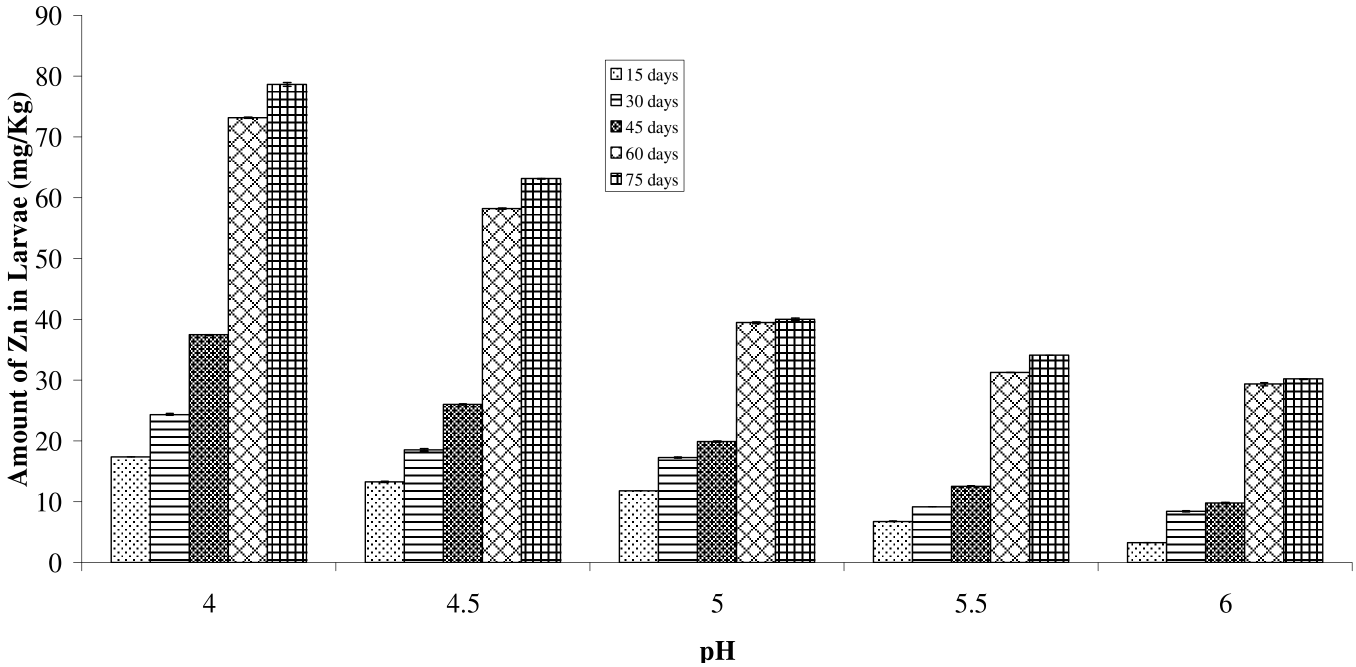

Figure 5. Biotransporation of Zn(II) to *Bombyx mori* from *Morus alba* leaves at various soil pH values.

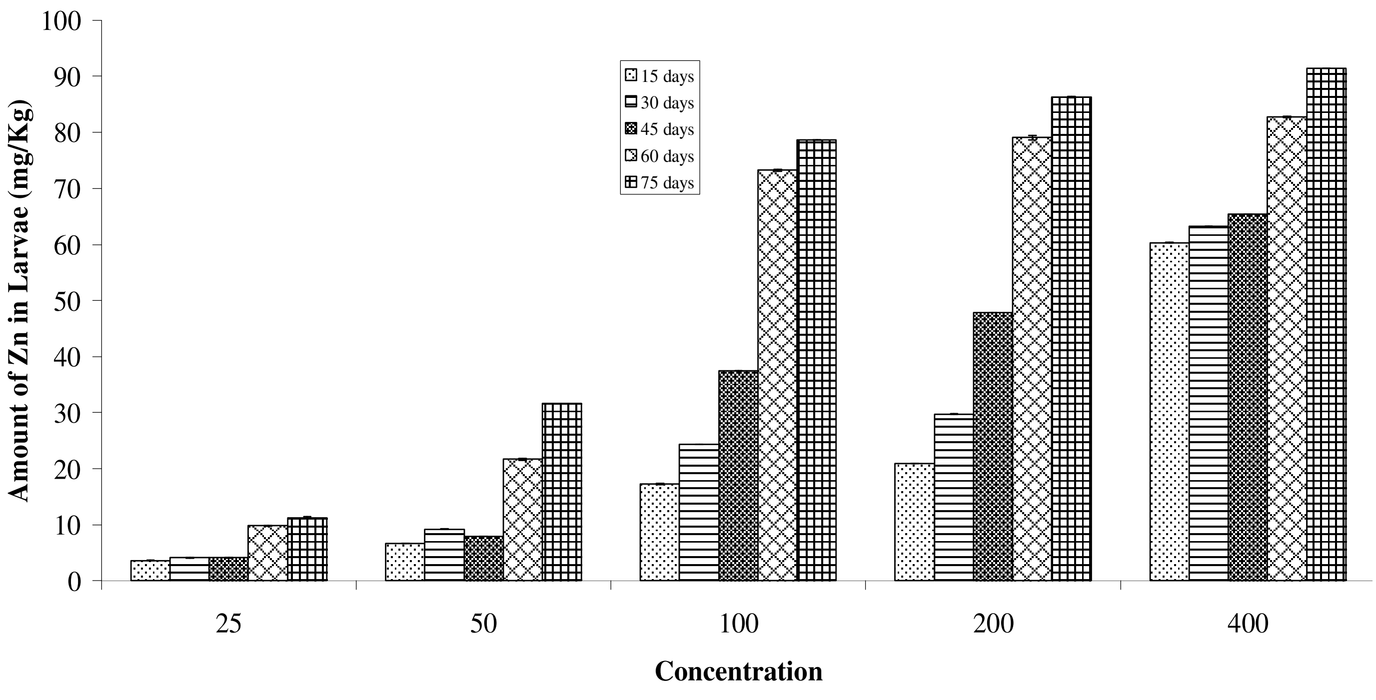

Figure 6. Effect of Zn(II) concentration in soil on its Biotransporation to *Bombyx mori* from *Morus alba*.

Bombyx mori body length, body weight and mortality rate

B. mori body length, body weight and mortality rates were significantly affected by Zn(II) concentration in larval body (Tables 1 - 3). Control larvae were found to have more body length and body weight and less mortality rate in comparison to those larvae that accumulated Zn(II) into their bodies. Spurgeon et al. (2000) carried out similar type of study and evaluated Zn(II) effect on earthworm

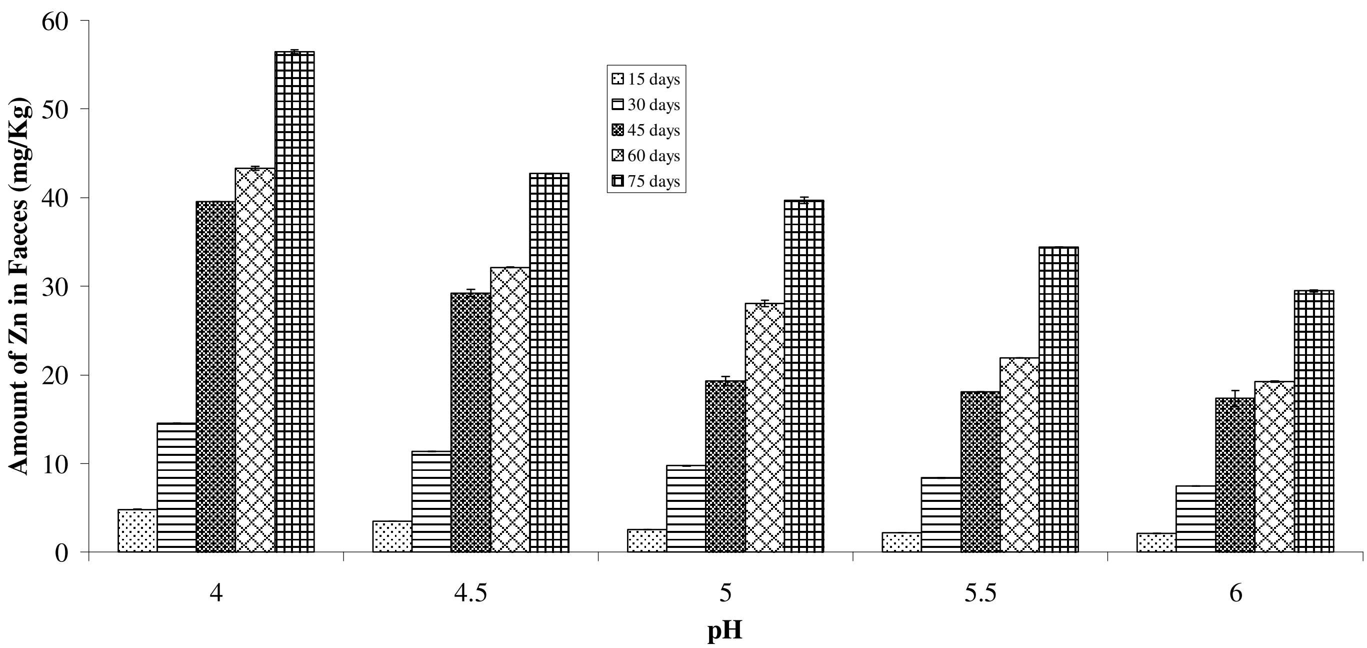

Figure 7. Zn(II) concentration in *Bombyx mori* excreta at various soil pH values.

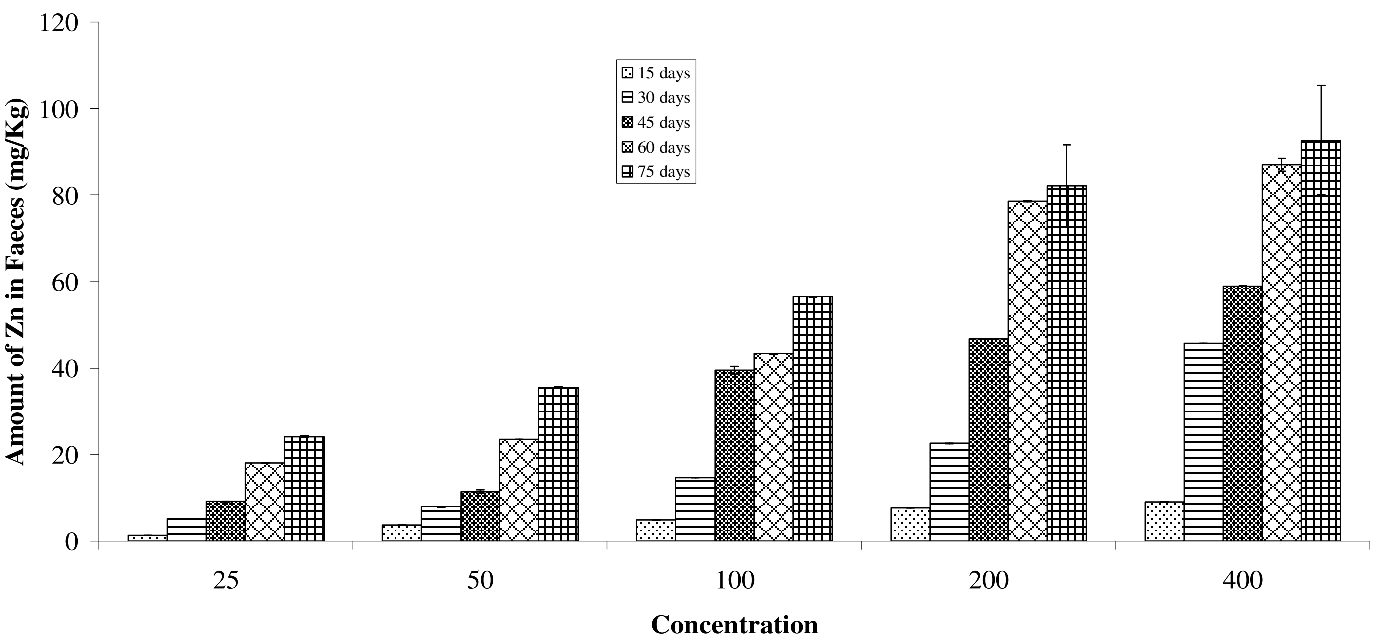

Figure 8. Effect of Zn(II) concentration in soil on its amount in *Bombyx mori* excreta.

population.

Conclusion

The following conclusions were drawn from the obtained results:

1. There was significant increase in Zn(II) concentration in soil after irrigating it with metal contaminated water.
2. Mulberry plants appeared to scavenge and accumulate Zn(II) into its leaves, allowing for its potential transfer and bioaccumulation in higher tropical level organisms.
3. Although *B. mori* excreted large quantity of Zn(II) but

Table 1. Body Length (cm) of *Bombyx mori* larvae affected by pH and Zn(II) concentration in synthetic effluent.

Treatment	Test group	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
	Control	0.70 ± 0.10	1.35 ± 0.11	1.75 ± 0.10	4.00 ± 0.09	6.20 ±
Zn(II) amount (mg/l)	25	0.55 ± 0.11	1.10 ± 0.09	1.70 ± 0.08	3.00 ± 0.08	4.90 ± 0.09
	50	0.50 ± 0.10	0.80 ± 0.08	1.50 ± 0.07	2.50 ± 0.10	4.20 ± 0.08
	100	0.49 ± 0.09	0.70 ± 0.05	1.40 ± 0.09	2.25 ± 0.07	4.10 ± 0.07
	200	0.45 ± 0.08	0.70 ± 0.05	1.40 ± 0.05	2.20 ± 0.08	4.10 ± 0.04
	400	0.40 ± 0.02	0.65 ± 0.07	1.35 ± 0.04	2.00 ± 0.06	4.00 ± 0.06
pH	4	0.50 ± 0.05	0.80 ± 0.06	1.35 ± 0.05	3.20 ± 0.07	4.50 ± 0.07
	4.5	0.52 ± 0.06	0.80 ± 0.05	1.30 ± 0.07	3.10 ± 0.04	4.70 ± 0.04
	5	0.52 ± 0.05	0.90 ± 0.07	1.40 ± 0.05	3.20 ± 0.03	4.70 ± 0.05
	5.5	0.55 ± 0.04	1.10 ± 0.04	1.45 ± 0.08	3.50 ± 0.02	5.00 ± 0.03
	6	0.60 ± 0.03	1.20 ± 0.02	1.55 ± 0.07	3.80 ± 0.05	5.20 ± 0.02

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

Treatment	Test group	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
	Control	0.019 ± 0.005	0.090 ± 0.002	0.501 ± 0.001	3.962 ± 0.004	8.891 ± 0.005
Zn(II) amount (mg/l)	25	0.014 ± 0.004	0.075 ± 0.004	0.405 ± 0.006	3.500 ± 0.008	8.700 ± 0.002
	50	0.012 ± 0.008	0.070 ± 0.007	0.375 ± 0.005	3.250 ± 0.005	8.650 ± 0.006
	100	0.010 ± 0.007	0.068 ± 0.004	0.350 ± 0.007	3.200 ± 0.004	8.610 ± 0.007
	200	0.010 ± 0.009	0.067 ± 0.001	0.325 ± 0.008	3.115 ± 0.006	8.600 ± 0.004
	400	0.090 ± 0.005	0.060 ± 0.002	0.305 ± 0.005	3.000 ± 0.006	8.509 ± 0.005
pH	4	0.011 ± 0.004	0.079 ± 0.003	0.280 ± 0.003	3.155 ± 0.005	8.600 ± 0.003
	4.5	0.013 ± 0.006	0.080 ± 0.005	0.295 ± 0.001	3.160 ± 0.001	8.650 ± 0.005
	5	0.015 ± 0.003	0.082 ± 0.002	0.310 ± 0.009	3.175 ± 0.006	8.790 ± 0.009
	5.5	0.014 ± 0.002	0.083 ± 0.009	0.350 ± 0.008	3.205 ± 0.009	8.795 ± 0.008
	6	0.016 ± 0.001	0.085 ± 0.007	0.490 ± 0.007	3.335 ± 0.001	8.800 ± 0.007

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

Treatment	Test group	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar
	Control	10 ± 0.65	8 ± 0.61	7 ± 0.62	5 ± 0.63	3 ± 0.64
Zn(II) amount (mg/l)	25	13 ± 0.60	11 ± 0.50	10 ± 0.40	8 ± 0.55	5 ± 0.45
	50	15 ± 0.50	13 ± 0.45	12 ± 0.55	10 ± 0.50	9 ± 0.50
	100	18 ± 0.45	17 ± 0.40	12 ± 0.45	13 ± 0.40	10 ± 0.40
	200	19 ± 0.50	18 ± 0.35	15 ± 0.50	13 ± 0.45	11 ± 0.45
	400	21 ± 0.55	18 ± 0.45	17 ± 0.60	14 ± 0.50	10 ± 0.50
pH	4	20 ± 0.35	15 ± 0.50	12 ± 0.45	10 ± 0.45	7 ± 0.45
	4.5	18 ± 0.45	13 ± 0.55	11 ± 0.50	10 ± 0.50	8 ± 0.30
	5	17 ± 0.50	12 ± 0.40	10 ± 0.35	8 ± 0.40	7 ± 0.45
	5.5	15 ± 0.65	12 ± 0.45	10 ± 0.45	8 ± 0.35	7 ± 0.50
	6	12 ± 0.50	10 ± 0.50	8 ± 0.30	7 ± 0.45	6 ± 0.55

still most of Zn(II) resided inside its body.

These findings clearly suggested that Zn(II) presence in aqueous effluents used for plant irrigation should be strictly monitored.

ACKNOWLEDGMENT

The authors are thankful to Rose Laboratory Staff, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan, for their help in carrying out the present study.

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