Current World Environment Vol. **11**(1), 218-227 (2016)

Toxicity of Zinc on Growth of an Aquatic Macrophyte, *Ipomoea aquatica* Forsk

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http://dx.doi.org/10.12944/CWE.11.1.27

(Received: March 28, 2016; Accepted: April 15, 2016)

ABSTRACT

The effects of different concentrations of Zn on growth of an aquatic macrophyte, *Ipomoea aquatica* Forsk. were studied. Fresh weight, dry weight, shoot length, root length, number of nodes, development of leaves, and chlorophyll and carotene contents were the different growth parameters considered. Toxicity symptoms like browning and decaying of roots could be observed in plants treated at 22.7 mg L^{-1} Zn as early as 3rd day of experiment while yellowing of older leaves appeared during the later period of exposure. High concentration of Zn (12.71 – 22.7 mg L^{-1}) significantly inhibited the growth of plant while lower Zn concentrations up to 4.09 mg L^{-1} enhanced its growth. However, at 7.26 mg L^{-1} Zn the chlorophyll as well as total carotene content in leaf of *I. aquatica* were significantly reduced from that in control on 5th day of exposure and subsequently the reduction was observed in lower concentrations. Thus, *I. aquatica* can be employed in biomonitoring of Zn polluted aquatic ecosystems using root browning, root and shoot growth inhibition, and chlorophyll and total carotene contents as sensitive biomarkers.

Keywords: Aquatic ecosystems, Biomonitoring, Carotene, Chlorophyll, Root browning.

INTRODUCTION

Natural environment, particularly the aquatic ecosystem is being disturbed by anthropogenic activities with rise in industrialization and urbanization. The decline in water quality of fresh water systems threatens its sustainability and has become one of the major environmental issues. The current scenario of Indian freshwater resources has been studied by many workers and appealed for their management as environmental problems. The increased concentrations of heavy metals in receiving water, especially lakes and rivers worsened the situation and threatened the biological components through bioaccumulation along the food chain and ultimately affect human being as well[1-5].

Zn which is an essential micronutrient associated with metabolic activities in organisms can turn toxic at higher concentrations. Its concentration in various Indian aquatic ecosystems has reached alarming levels as reported by a number of scientific studies[4,6-9]. The main sources of Zn in fresh water ecosystems includes effluents from electroplating industries, smelting and refining, mining, paper industries, domestic sewage and agricultural run-off[10]. The immersion of idols and tazias is also one of the major reasons for increase level of Zn in lakes and rivers all over India[11-13]. Zn is one of the major constituents of paints for decorating both idols and tazias, and it has been reported that the number of idol immersion is being increased each year, which could increase load of heavy metals[14].

Although Zn is a micronutrient, it becomes hazardous at high levels[15]. The growing concern in environmental aspects as well as the narrow window between its essentiality and toxicity[16] has generated interest in studying its effects on aquatic plants which play an important role in ecosystem functioning. In addition, a regular testing of Zn pollution in fresh water is required in the Indian context. Thus,

the present study was carried out with the aim of exploring the potential of an aquatic macrophyte, *Ipomoea aquatica* Forsk. to be used in biomonitoring and phytotoxicity studies. This plant was selected on the basis of its geographical distribution, availability and adaptability. It is widely distributed not only in India, but in the entire South, South East, and East Asia[17] and well adapted to wide range of habitats. It is also reported to be grown in other parts of the world like Africa, Australia and United States of America (Austin, 2007)[18]. Further, it is perennial and mostly grows in moist soil, inundated floodplains, ditches, ponds, canals and sluggish rivers[19] and easy to cultivate due to its ability to proliferate by fragmentation, and produce adventitious roots and lateral branches that bear flowers and leaves, from its nodes[20].

A number of studies have been conducted on Zn toxicity using aquatic plants. Growth inhibition was observed in *Eichhornia crassipes*[21], *Lemna gibba*[22], *Phragmites australis*[23], *Iris psuedacarus*[24], *Spirodela polyrhiza*[25] and *Salvinia natans*[26]. Another study reported the inhibition of growth induced by Zn stress in three aquatic macrophytes viz. *Lemna minor*, *Elodea canadensis* and *Leptodictyum riparium*, where *L. riparium* turned out to be the most resistant species with 50% growth inhibition at 100 µM Zn[27]. In contrast to this, studies on Zn toxicity to *A. aquatica* is scarce. This study was, therefore, taken up to understand and assess the toxic effects of Zn on this aquatic macrophyte.

MATERIALS AND METHODS

Ipomoea aquatica Forsk. was collected from uncontaminated ponds of Irongmara area in Cachar district, Assam, India, and washed with tap water. Stock cultures were grown following standard method[28]. The plants were grown in hydroponic tubs till new branches developed. These new branches were cut and planted in pots containing soil flooded with 50 % Hoagland nutrient media. The pH of nutrient media was adjusted at the range of 5.8-

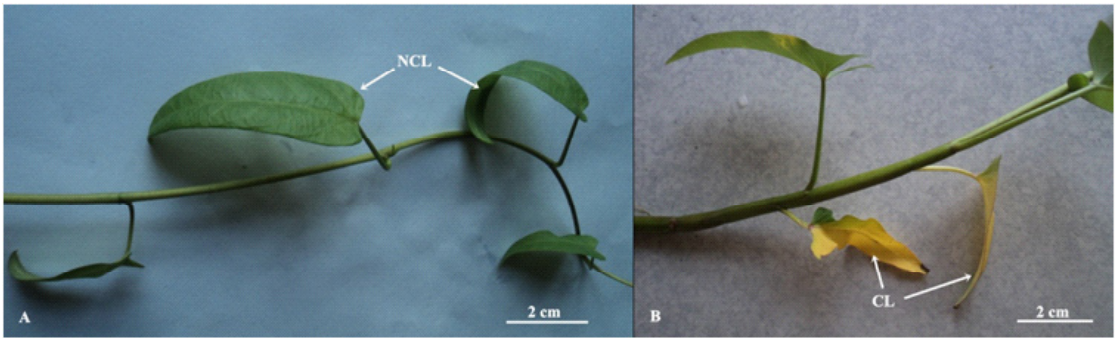

Fig.1: Zn induced change in appearance of leaf in *I. aquatica*; A- Non chlorotic leaf (NCL) of control; B- Chlorotic leaf (CL) of plant treated at 22.7 mg L^{-1} Zn

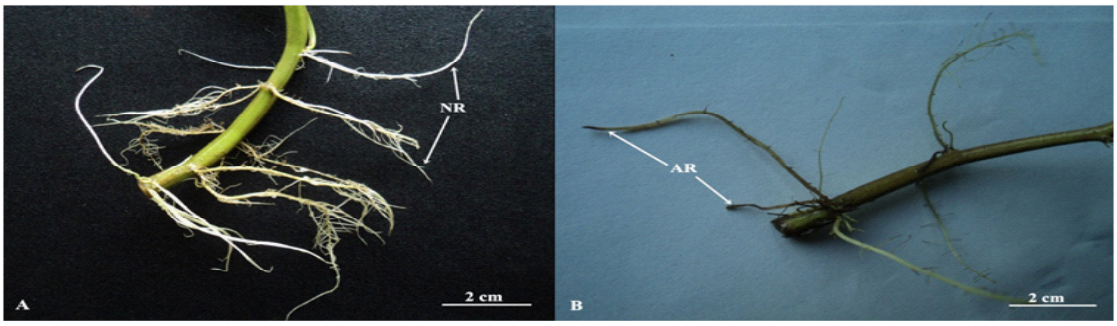

Fig. 2: Zn induced darkening of root in *I. aquatica*; A- Normal root (NR) of control; B- Affected root (AR) of plant treated at 22.7 mg L^{-1} Zn

6.2. Healthy and fully grown shoots of similar shoot height were cut from the same mother plant, washed with tap water and acclimatized in 50 % Hoagland nutrient media for one week at 25-27°C, 12 h light with an intensity of 100-120 $\mu mol^{-2} s^{-1}$ and 12 h dark periods. These acclimatized plants were exposed to half strength Hoagland nutrient media added with graded concentrations of Zn as $ZnSO_4.7H_2O$ (actual Zn concentrations: 0.23 mg L^{-1}, 2.27 mg L^{-1}, 4.09 mg L^{-1}, 7.26 mg L^{-1}, 12.71 mg L^{-1} and 22.7 mg L^{-1}) for 15 days. Plants grown in 50 % Hoagland media without added Zn served as control. Water loss due to evaporation or transpiration was compensated by renewal of solutions every week. At the termination of the experiment on 15th day all control and Zn-treated plants were removed, washed with distilled water, and softly blotted to remove excess water before fresh weight of the plants, their root length and lateral roots, and leaf area were measured. This was followed by drying in hot air oven at 60 ^{0}C till constant weight. Other growth parameters such as shoot length, new leaves, and number of nodes were measured at 3 day intervals. The percentage of primary roots bearing lateral roots (PRL %), and the ratio of lateral roots (LR) to the number of primary roots bearing lateral roots (PRL) was calculated. All these data were also obtained before the plants were exposed to Zn. Leaf area was measured by using ImageJ (http://imagej.nih.gov/ij/) software. All leaves were neatly clipped at their petioles and properly spread to take the image of the entire leaf area. Toxicity symptoms on leaf and root, such as darkening of roots and appearance of chlorosis were noted during the experiment. For chlorophyll estimation, fresh leaf was homogenized with 80 % acetone, centrifuged, and the absorbance of the supernatant was taken at 662, 645, and 470 nm for chlorophyll a (chl a), chlorophyll b (chl b) and total carotene, respectively, in a spectrophotometer. The concentrations of these pigments were calculated following standard formula[29] with the extraction solution used as blank.

Statistical significance of differences among the data sets was determined with One-way ANOVA, with multiple comparisons made by Tukey test. All tests were done using SPSS 20 software for Windows.

Table 1: Appearance of darkening of roots in *I. aquatica* on exposure to graded concentrations of Zn (n=5)

Zn Conc. (mg L^{-1})	Number of plants with darkened roots				
	Day 3	Day 6	Day 9	Day 12	Day 15
0.23	0	0	0	1	1
2.27	0	0	0	1	1
4.09	0	1	2	2	2
7.26	0	1	2	3	3
12.71	1	2	2	4	5
22.7	3	5	5	5	5

RESULTS AND DISCUSSION

Plants at all concentrations of Zn could survive the total exposure period of 15 days, although some toxicity symptoms were encountered in plants exposed to higher concentrations of Zn. Thus the survival pattern of the plant reflected its tolerance capacity up to Zn concentrations of 22.7 mg L^{-1}. Among aquatic macrophytes, *Typha agustifolia* and *Colocasia esculenta* were found to be the most tolerant plants to heavy metals[30]. These two plants did not show any significant toxicity symptoms when they were grown in sediment contaminated with Zn concentration of 363 mg kg^{-1}. *Eichhornia crassipes* when exposed to 20 mg L^{-1} Zn did not show any morphological symptom of toxicity[31]. The present study revealed that plants treated at 22.7 mg L^{-1} showed yellowing of older leaves (**Fig. 1**), while significant blackening of root tips occurred at 22.7 mg L^{-1} Zn on the 3rd day of exposure (**Fig. 2**).

Blackening of root tips was also observed in plants treated at other concentrations with longer exposure time (**Table 1**). One way ANOVA showed significant reduction in length of PR at 7.26 - 22.7 mg L^{-1} Zn (**Table 1**) which might be correlated to gradual decaying of roots along the length. Zn at 200 µg ml^{-1} reduced root biomass in *Iris psuedacorus*[24]. However, symptoms like blackening and decaying of roots were not reported, although plants exposed to other heavy metals like Cd showed browning of root tips[32]. Dark root tips in *Vicia faba*[33] and cell death in root tissues of *Talinum triangulare*[34] were also reported due to oxidative stress induced by Pb accumulation. Thus, these symptoms of toxicity in

roots can be used as a tool in biomonitoring of Zn pollution in water. The suppression of development of new PR led to a significant reduction in the PRL % as well as lateral roots in plants treated at 12.71 – 22.7 mg L^{-1} Zn (**Table 2**). A significant reduction at $p < 0.05$ in LR: PRL was also observed in plants treated with 7.26 – 22.7 mg L^{-1} Zn at the end of 15 day exposure as revealed by one way ANOVA and multiple comparisons by Tukey test (**Table** 2). Zn at concentrations of 0.23 and 2.27 mg L^{-1}, on the other hand, enhanced growth of roots in *I. aquatica*.

Despite the toxic symptoms exhibited by *I. aquatica*, growth in terms of fresh weight and

Table 2: Effects of Zn on root length, number of PR, PRL % and LR:PRL of *I. aquatica* at the end of 15 days

Zn Conc. (mg L^{-1})	Increase in length of PR (cm)	No. of new PR	PRL %	LR:PRL
Control	23.77±2.43^{ab}	15.67±2.67^{a}	53.26±14.77^{abc}	5.81±1.33^{a}
0.23	39.23±9.68^{a}	16.33±1.33^{a}	68.88±6.20^{ab}	5.00±0.25^{a}
2.27	32±5.04^{ab}	19.33±2.40^{ab}	72.14±10.90^{a}	3.49±0.60^{ab}
4.09	20.7±2.92^{b}	24±1.15^{b}	40.09±11.25^{abc}	2.92±0.79^{ab}
7.26	3.33±4.08^{c}	9.67±1.45^{c}	38.15±5.08^{bc}	0.15±0.88^{bc}
12.71	-1.97±4.22^{c}	5±1.73^{cd}	32.81±8.88^{c}	-1.20±1.69^{c}
22.7	-4.73±1.17^{c}	1.67±1.20^{d}	-20.21±11.40^{d}	-1.92±1.05^{c}

LR – Lateral roots; PR – Primary roots; PRL % - percentage of primary roots bearing lateral roots; Values are given as mean±SE; Values with different superscript letters in the column indicate significant differences at $p < 0.05$; '-'Decrease in each parameter with respect to the corresponding initial value

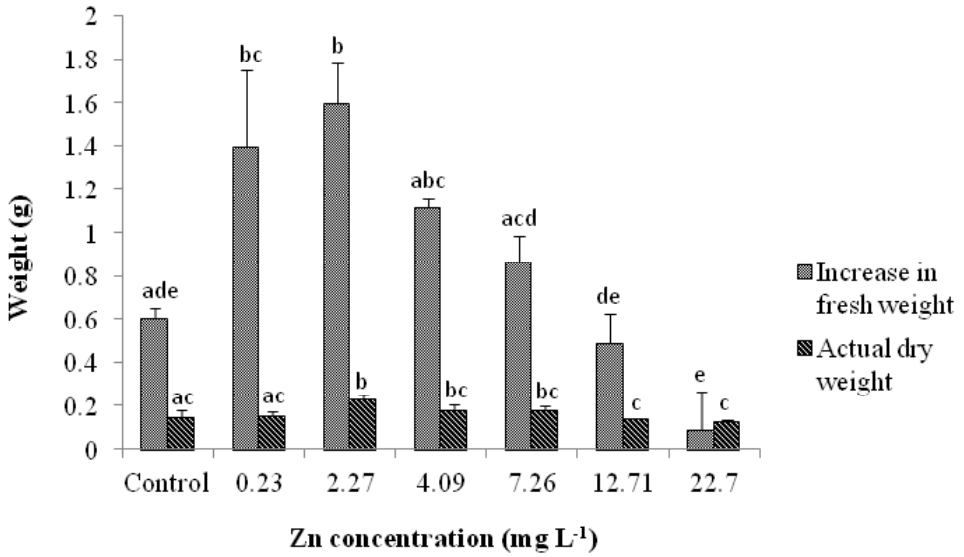

Fig. 3: Effects of Zn on fresh and dry weight of *I. aquatica* at the end of 15 days exposure; Values are given as mean±SE; Values with different superscript letters indicate significant differences at $p < 0.05$

dry weight was not affected at Zn concentrations of 12.71-22.7 mg L^{-1}, while 0.23-7.264.09 mg L^{-1} Zn enhanced growth (**Fig. 3** & **4**). On the other hand, one way ANOVA with multiple comparisons by Tukey tests showed that Zn concentrations of 7.26 – 22.7 mg L^{-1} had significant effect on number of new nodes (**Fig. 4**). There was also decline in increase in shoot length with significant effect at 22.7 mg L^{-1} Zn as revealed by one way ANOVA at $p < 0.05$. Zn being a micronutrient probably enhanced photosynthesis and protein metabolism, thus increasing the growth of plants at low level of Zn[35]. Growth of *Sesuvium portulacastrum* was enhanced by Zn concentrations up to 300 mg kg^{-1}[36]. On the other hand, Zn at 2mM concentration could decrease shoot height substantially in *Phragmites*

Table 3: Effects of Zn on leaf fall, new leaf and leaf area of *I. aquatica* at the end of 15 days

Zn Conc. (mg L^{-1})	No. of leaf fallen	No. of new leaf	Total leaf area (cm^2)
Control	1.33 ± 0.33^{ab}	3 ± 0^{abd}	29.30 ± 4.3^{a}
0.23	0.33 ± 0.33^{a}	2 ± 0^{ad}	33.46 ± 3.65^{a}
2.27	1.67 ± 0.33^{ab}	2.67 ± 0.33^{abc}	35.47 ± 1.36^{a}
4.09	1.67 ± 0.67^{ab}	3.67 ± 0.33^{bc}	32.37 ± 7.03^{a}
7.26	3 ± 0.58^{bc}	2.67 ± 0.88^{cd}	25.14 ± 4.15^{a}
12.71	3.67 ± 0.33^{c}	2.33 ± 0.33^{d}	17.72 ± 2.99^{a}
22.7	4.33 ± 0.88^{c}	0 ± 0^{e}	15.65 ± 1.51^{b}

Values are given as mean±SE; Values with different superscript letters in the column indicate significant differences at $p < 0.05$

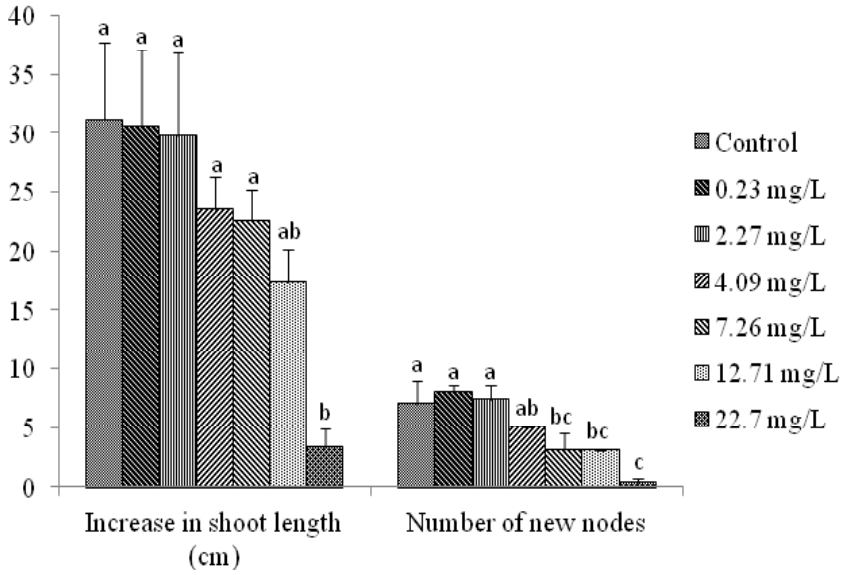

Fig. 4: Effects of Zn on increase in shoot length and number of nodes in *I. aquatica* at the end of 15 days exposure; Values are given as mean±SE; Values with different superscript letters indicate significant differences at $p < 0.05$

australis[23]. In another study, growth of *Hydrilla verticillata* was slightly affected by 0.1 and 1 mg L^{-1} ZnO nanoparticle treatment for 3 weeks while 1000 mg L^{-1} significantly reduced growth of the plant[37]. Thus, there is a wide variation in response to heavy metals among plants[38,39].

The results of the present study revealed that Zn at low concentrations induced new leaf

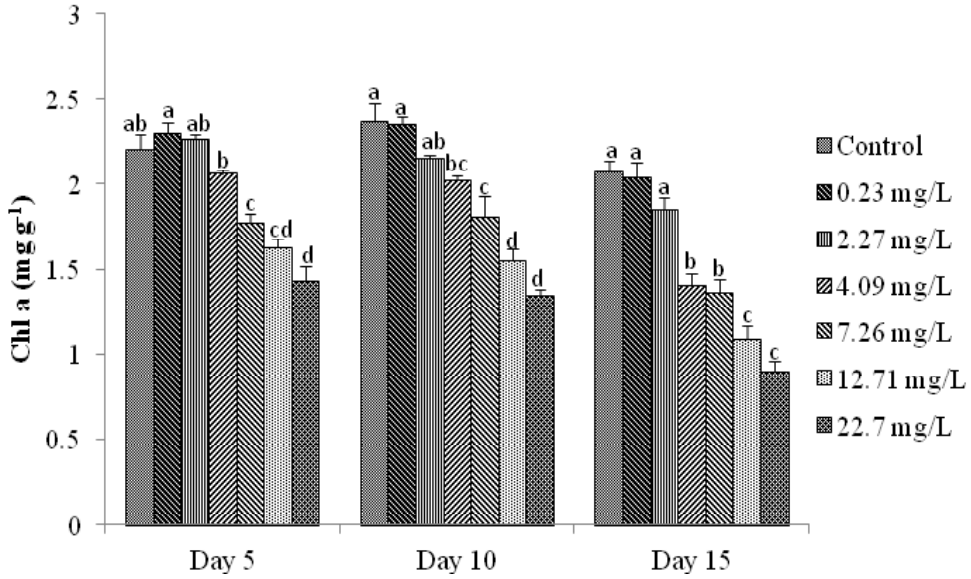

Fig. 5: Effect of Zn on content of chl a in leaf of *I. aquatica* on day 5, 10 and 15 of exposure; Values are given as mean±SE; Values with different superscript letters indicate significant differences at $p < 0.05$

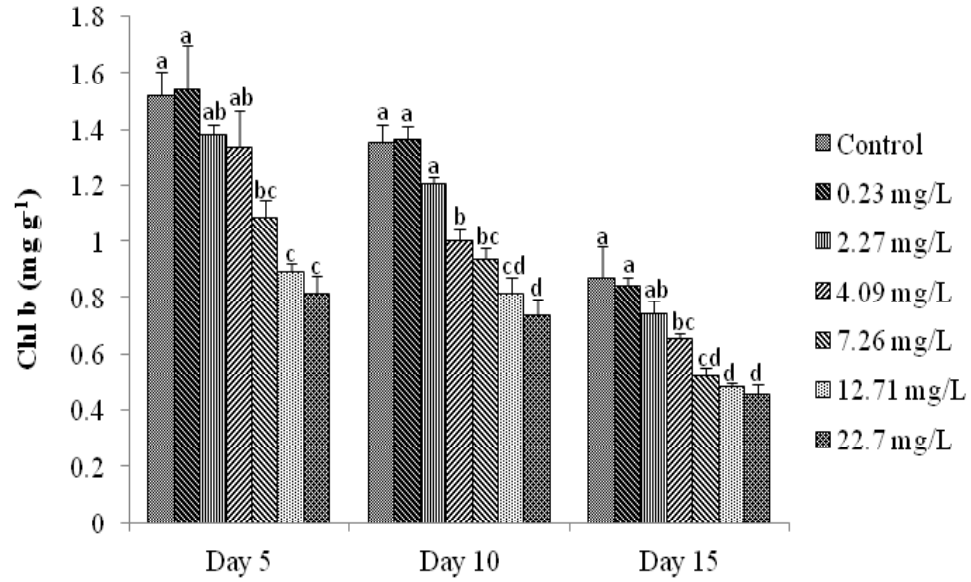

Fig. 6: Effect of Zn on content of chl b in leaf of *I. aquatica* on day 5, 10 and 15 of exposure; Values are given as mean±SE; Values with different superscript letters indicate significant differences at $p < 0.05$

development as well as increased leaf area in *I. aquatica*, while at higher concentrations it affected leaf growth (**Table 3**). One way ANOVA showed significant differences in number of new leaf as well as leaf area among control and 22.7 mg L^{-1} Zn treated plants at $p < 0.05$. Similar results were also reported in *Sesuvium portulacastrum* grown in soil amended with 100 – 600 mg kg^{-1} Zn[36]. High concentrations of Zn also enhanced shedding of older leaf with significantly higher number of leaf fall at 12.71 and 22.7 mg L^{-1} Zn from that in control. High Zn concentration resulted growth retardation and induces leaf senescence by disturbing key metabolic processes such as photosynthetic activity, pigment content and antioxidant systems[40, 41].

The present study revealed that low Zn concentrations (0.23 and 2.27 mg L^{-1}) enhanced the content of chl a in leaf of *I. aquatica* while it was significantly affected at 7.26 – 22.7 mg L^{-1} Zn concentrations on 5th day of exposure as shown by one way ANOVA at $p < 0.05$. A dose and time – dependant reduction of chl a content was observed in subsequent days of exposure (**Fig. 5**). Similar results were also observed in case of chl b and total carotene content (**Fig. 6** & **7**). Chlorophyll and total carotene content can be considered as effective biomarkers since significant decline was obtained at 7.26 mg L^{-1} Zn after 5th day of exposure. In addition, symptoms like leaf yellowing was not observed at this concentration till the end of the experiment. Significant reduction in chlorophyll content with leaf yellowing was also observed in *Phragmites australis* treated with 1000 mg L^{-1} ZnO nanoparticles[37], although this dose is much higher than the dose of Zn which showed significant effect in the present study. Chl a, Chl b and carotenoid content in *Salvinia natans* was also significantly reduced by Zn treatment at the level of 10 mg L^{-1}[26].

Thus, the results of this study revealed that *I. aquatica* can successfully be employed in toxicity studies in aquatic ecosystems polluted with Zn at a threshold limit of about 12 mg L^{-1} by using root browning, reduction of growth in root and shoot and pigment content as tools for biomonitoring. This plant being resistant to Zn in terms of survival capacity coupled with its adaptive nature to wide range of habitats, has the prospect of being used in further studies like phytoremediation of Zn polluted areas.

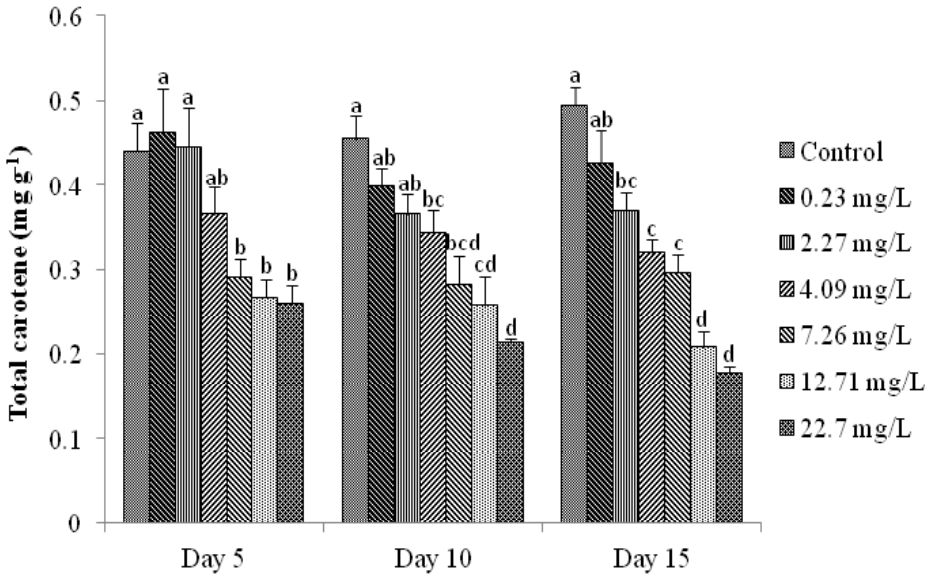

Fig. 7: Effect of Zn on content of total carotene in leaf of *I. aquatica* on day 5, 10 and 15 of exposure; Values are given as mean±SE; Values with different superscript letters indicate significant differences at $p < 0.05$

ACKNOWLEDGEMENT

LBC would like to thank the University Grant Commission, New Delhi, India for financial assistance in the form of UGC-BSR Fellowship during the course of the study.

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