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Zinc (Zn) accumulation and its Toxicity effect on *Salvinia molesta* Mitchell and *Spirodela polyrhiza* (L.) Schleid

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Abstract

Due to increasing awareness of toxic heavy metals contamination to the environment, studies of metal accumulation from the view point of metal removal from the contaminated water have been performed. The use of biological systems for removing metals from metal solution has the potential to achieve greater performance at lower cost. This is an emerging biological application based on 'Green Liver Concept' and operates on the principles of biogeochemical cycling.

The present study focuses on zinc toxicity on morphology and some biochemical parameters of *Salvinia molesta* Mitchell and *Spirodela polyrhiza* (L.) Schleid. The laboratory experiments were conducted for the assessment of morphological index parameters (MIP), biochemical parameters and metal (Zn) accumulation profile in test plants at various concentrations (viz. 5,10, 20, 30 and 40 ppm *Salvinia* and *Spirodela* at regular interval for 12 days. *Salvinia* and *Spirodela* shows visible symptoms like withering of roots, chlorosis and necrosis at higher concentration. With respect to *Spirodela* at higher concentration the lower surface of leaf turns pink to whitish. However, the test plants showed normal growth at lower concentration (5.0 ppm). The estimation of biochemical parameters *i.e* total chlorophyll, protein and carbohydrate of test plants showed a significant increase at lower concentration (5

ppm) of zinc and showed significant decrease with increase in exposure concentration and duration. Metal accumulation by test plants was maximum at 4 days exposure duration and marginal at subsequent concentrations and exposure duration. With respect to biochemical parameters the concentrations are significant. However, metal accumulation is significant at different concentrations and exposure duration.

Heavy metal pollution is a major environmental problem facing the modern world[8,36]. The global heavy metal pollution is increasing in the environment due to increase of human activities. However, it is gaining importance day by day due to its obvious effect on human health through the food chain[18]. Due to the increasing awareness of toxic heavy metals contamination to the environment, studies of metal accumulation from the view point of metal removal from the contaminated water have been performed[4,6]. Conventional methods including precipitation, oxidation, reduction, ion exchange, filtration, electrochemical treatment, membrane technologies and evaporation recovery are expensive or ineffective[33]. The use of biological systems for removing metals from low metal solution (1 to 100 mg/g) has the potential to achieve greater performance at lower cost.

Aquatic plants and / or algae are known to accumulate metals and other toxic elements from contaminated water[34]. The bio-removal process using aquatic plants often exhibits two staged uptake process: on initial fast, reversible, metal binding process (biosorption) followed by a slow, irreversible, ion sequestration step (bioaccumulation). The initial metal biosorption by different parts of cells can occur via complexation coordination, chelation of metals, ion exchange, adsorption and micro precipitation. The bioaccumulation process is an active mode of metal accumulation by living cells[4,22]. This process is dependent on the metabolic activities of the cell, which inturn can be affected by the presence of metallic ions[33]. The accumulation of metals at higher concentration causes retardation of growth, biochemical activities and also generation of –SH groups containing enzymes.

The main goal of this study was to determine the performance of the test plants i.e *Salvinia molesta* and *Spirodela polyrhiza* to the different concentrations of Zinc (Zn) on morphology, biochemical constituents and accumulation of metal profile from the experimental pond under laboratory conditions.

Salvinia and *Spirodela*, free floating aquatic plants for unpolluted water bodies is maintained in a cement pots (1 meter diameter) under conditions at a temperature 28-30°C. About 20 g of young healthy *Salvinia* and *Spirodela* were acclimatized for two weeks in Arnon and Hoagland nutrient solution maintaining pH between 7.1-7.4. The concentrations of Zinc in the polluted water are in the range of 5, 10, 20, 30 and 40.0 mg/l and tap water as a control. Morphological Index Parameters (MIP) viz, root length, leaf length and breadth were observed for 12 days at interval of 4 days. Photographs of *Salvinia* and *Spirodela* which were taken by using

Canon's Power Shot G_2 digital camera were treated with different concentrations of zinc. For the further study the plants were harvested at the end of 4, 8 and 12 days exposure and are thoroughly washed with distilled water and used for the estimation of total chlorophyll, protein and carbohydrate and also for morphological observations. Plants harvested after 48 hrs were dried at 80^0C for 2 days for metal extraction.

The fresh test plant samples of 1 g is macerated in 100 ml of 80% (v/v) chilled acetone by using pestle and mortar. The centrifuged and supernatant was used for the estimation of total chlorophyll by standard Arnon method using 652 nm against the solvent (80% acetone as a blank)[12]. The protein was estimated by Lowry's method[7] using Bovine Serum Albumin (BSA) as a standard, using 660 nm and carbohydrates by phenol sulphuric acid method (Dubois) using glucose as standard at 490 nm[1]. Morphological characters were identified with the help of photographs, using Canon's Power Shot G_2 digital camera.

The estimation of metal Zn in the test plant was carried out by using standard method[23]. The dried and powdered 1 g plant material was digested by using mixed acid digestion method in Gerhardt digestion unit. The digested samples were diluted with double distilled water and filtered through Whatman filter paper No. 44. The estimation of Zn was done by atomic absorption spectrophotometer (AAS) (GBC 932 Plus Austrelia) with air acetylene oxidizing flame and metal hollow cathode lamp at 217.00 nm wavelength. Working standards (SISCOP-CHEM-Bombay Lab) were used for the calibration of instrument.

Statistical Analysis :

Data are presented as mean values ± SE from two independent experiments with three replicates each. Data were subjected to Two – way ANOVA to know significance between concentrations and between exposure duration for the accumulation of heavy metal, (Zn). Further, Dunet's test is also applied for multiple comparisons between control and other concentrations. Two – way ANOVA test also extended to know the significance between concentration and duration for biochemical parameters.

Toxic effect of Zinc on morphology :

The test plant showed luxuriant growth, shows increase in the lamina and breadth at low concentration (5.0ppm) in both test plants. In *Salvinia* at (5.0 ppm) of zinc was found to promote the laminal length by 1.48 ± 0.020 cm, 1.5 ± 0.04 cm, 1.666 ± 0.14cm and breadth by 1.6 ± 0.169cm, 1.666 ± 0.14cm and 1.7 ± 0.027cm at 4, 8 and 12 days exposure duration. Similarly root length by 2.61 ± 0.216 cm, 2.2 ± 0.205 cm and 2.4 ± 0.216 cm. In *Spirodela* at the lower concentration (5.0 ppm) showed increase in laminal length by 0.866 ± 0.027 cm, 1.0 ± 0.01cm and 1.1 ± 0.072cm and breadth by 0.633 ± 0.072 cm, 0.666 ± 0.054 cm and 0.766 ± 0.054 cm at 4, 8 and 12 days exposure duration respectively. Similarly the root length by 2.766 ± 0.144cm, 2.866 ± 0.165cm and 3.0 ± 0.216cm at 4, 8 and 12 days exposure duration respectively.

Morphometric assay is one of the quantitative tool for the assessment of toxicants measured by using Morphological Index Parameter (MIP). The rate of inhibition in the root and leaf (Fronds) is directly proportional to the concentration of zinc and in both test plants. Two way ANOVA test states that the concentrations are significantly toxic at 5% level but duration is not significant. MCA test also represents maximum deviation is at higher concentration compared to control. Both the test plants showed normal growth at their respective lower concentration (5.0 ppm).

However, in *Salvinia* at 40 ppm concentration shows severe inhibition of laminal length by 1.1 ± 0.081 cm, 1.033 ± 0.072cm and 0.933 ± 0.081cm and laminal breadth by 1.0 ± 0.08 cm, 0.966 ± 0.072 cm and 0.911 ± 0.081 cm at 4, 8 and 12 days exposure duration. Similarly the root length inhibition was noticed to 0.933 ± 0.222 cm, 0.9 ± 0.047 cm and 0.8 ± 0.216 cm at 4, 8 and 12 days exposure duration respectively. Similarly, in *Spirodela* also at 40 ppm concentration shows severe inhibition of laminal length by 0.600 ± 0.047 cm, 0.533 ± 0.027 cm and 0.366 ± 0.027 cm and laminal breadth by 0.60 ± 0.047 cm, 0.466 ± 0.027 cm and 0.311 ± 0.027 cm at 4, 8 and 12 days exposure duration. Similarly the test plant (*Spirodela*) also exhibited root inhibition by 1.566 ± 0.098 cm, 1.10 ± 0.047 cm and 0.80 ± 0.216 cm at 4, 8 and 12 days exposure duration (Tables-1 and 2).

The higher concentration of Zn (10 ppm to 40 ppm) exhibited toxicity symptoms like chlorosis, fall of leaves were observed about particularly after 8 days to 12 days exposure brownish marks were observed in *Salvinia*, however, in *Spirodela* the plants have lost their roots, leaves turned green to yellow on the upper surface, lower pink colour tends to creamy white in colour. Our results of toxicity symptoms of Zn at higher concentrations observed and were similar to Saygideger[20]. Sobero confirmed elongation of root in some members of Lemnaceae at different concentration of Zn[14].

Toxicity effect of Zn on Biochemical parameters :

The chlorophyll content was very sensitive to heavy metal toxicity. The results found that at 5 ppm of Zn is found to augment chlorophyll synthesis and was directly proportional to the concentration and exposure duration in both test plants. The chlorophyll of *Salvinia* increased by 3.08% (0.701 mg/g), 4.62% (0.724 mg/g) and 5.22% (0.725 mg/g) respectively at 4, 8 and 12 days exposure duration to 5 ppm Zn in comparison to control. The chlorophyll content in *Spirodela* increased with increase in the exposure duration at an exposure concentration of 5 ppm Zn to 0.721 mg/g, 0.752 mg/g and 0.806 mg/g respectively at 4, 8 and 12 days exposure duration (Tables 3 and 4).

A number of heavy metals are required by the plants as micronutrients[20] and they act as a cofactors of enzymes as part of prosthetic groups and involved in a wide variety of metabolic pathways, but higher concentration of heavy metals are toxic to plants[2]. Heavy metals in ecosystem induces physiological and genetical changes in plants[5]. The zinc is one of the important nutrient for various metabolic processes and required in minute quantities[24].

It is evident from the data that the lower concentration of 5 ppm stimulates chlorophyll synthesis. Pandey reported the stimulation of chlorophyll synthesis at lower concentration of Nickel in *Spirodela*[26]. The 5.0 ppm of Zn promotes the chlorophyll synthesis from 0.701 mg/g to 0.745 mg/g in *Salvinia* and 0.721 mg/g to 0.806 mg/g in *Spirodela* during 4 and 12 days. The enhancement percentage of chlorophyll at 12 days exposure is 5.22% in *Salvinia*, 15.14% in *Spirodela* when compared to its respective controls. Choudhary and Ramachandra observed stimulatory effect of 1.5 mg/lt Zn on *Nostoc muscorum* including chlorophyll, carbohydrate and protein content[15]. Plant possess unique ability to evolve tolerance by the induction of phytochelatin (PC)[5,16].

However, the higher concentration of Zn found to inhibit the chlorophyll synthesis in both test plants. The present inhibition is 12.5% (0.595 mg/g), 23.26% (0.529 mg/g) and 42.1% (0.410 mg/g) at 40 ppm concentration respectively at 4, 8 and 12 days exposure duration respectively in comparison to control in *Salvinia*. Similarly *Spirodela* also shows inhibitory effect at 40 ppm of zinc towards chlorophyll synthesis by 1.57% (0.628mg/g), 19.18% (0.590 mg/g) and 41.88% (0.465 mg/g) respectively at 4, 8 and 12 days exposure duration. The severity of chlorophyll inhibition increases with increase in the duration of exposure. The percent inhibition of chlorophyll at 12 days exposure is 12.1% in *Salvinia* and 41.88% in *Spirodela* when compared to their respective control. Van Asche and Clijsters reported the chlorophyll biosynthesis is attributed to the interaction of metals with –SH group involved in catalytic activity[2]. Algal cultures exposure to higher concentrations of Zn exhibited chlorosis and cell lysis. The degradation of light harvesting pigments might be attributed to the disruption of thylakoid membranes within the cell[3]. The inhibition of photosynthesis is mainly because of proteolytic PC halide reductase complex and the synthesis of δ amino levulinic acid (ALA)[30].

Two way ANOVA represents biochemical toxicity to the test plants, concentrations were significant at $P>0.01$ level but duration is not significant. (Tables 3 and 4).

The increase in carbohydrate content at 5 ppm, Zinc promotes the carbohydrate content by 52mg/m (18.3%), 66.0mg/m (24.52%) and 76.0mg/m (28.8%) respectively at 4,8 and 12 days exposure. In *Spirodela*, the carbohydrate synthesis is increased at 5 ppm exposure to 32.0 mg/m (6.66%), 38.0mg/m (11.76%) and 44.0mg/m (18.91%) respectively at 4,8 and 12 days in comparison.

The carbohydrates form an important organic constituent of the plant tissues. Our investigation showed that at lower concentrations of Zn (5ppm) enhances the carbohydrate synthesis from 52mg/m to 76mg/m in *Salvinia* and 32mg/m to 44mg/g in *Spirodela* from 4 to 12 days exposure respectively. The enhancements of carbohydrates at 12 days exposure is 28.8% in *Salvinia* and 18.91% in *Spirodela* when compared to their respective controls. Increased carbohydrate content in *Nostoc muscorum* at lower concentrations of Nickel and Magnesium observed[23]. Lower concentration of Zn activates the enzyme or is incorporated into metalloenzymes in electron transport system (ETS)[31].

Table-1. Effect of Zinc on morphology of *Salvinia molesta*

Conc-entr-ation (ppm)	Exposure Duration (in days)								
	4	8	12	4		8		12	
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control 0	1.966±0.19	1.9±0.216	1.91±0.216	1.4±0.080	1.366±0.788	1.4±0.080	1.433±0.027	1.511±0.027	1.501 ± 0.027
5	2.61±0.216	2.2±0.205	2.4±0.216	1.43±0.027	1.6±0.169	1.5±0.047	1.666±0.144	1.666±0.144	1.7±0.027
10	1.866±0.144	1.73±0.205	1.712±0.216	1.4±0.080	1.466±0.144	1.366±0.027	1.433±0.027	3.966±0.196	1.366±0.788
20	1.366±0.284	1.33±0.284	1.116±0.216	1.3±0.788	1.4±0.080	1.233±0.072	1.333±0.151	1.111±0.196	1.3±0.708
30	1.066±0.222	1.03±0.098	1.0±0.284	1.16±0.072	1.066±0.072	1.033±0.072	1.033±0.072	1.0±0.047	0.966±0.557
40	0.933±0.222	0.9±0.047	0.8±0.216	1.1±0.081	1.0±0.08	1.033±0.072	0.966±0.072	0.933±0.081	0.911±0.081

Values are expressed in cms

Mean values ± Standard Error

Table-2 . Effect of Zinc on morphology of *Spirodela polyrhiza*

Conc-entr-ation (ppm)	Exposure Duration (in days)								
	4	8	12	4		8		12	
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control 0	2.000±0.047	2.0±0.047	2.2±0.216	0.666±0.054	0.566 ± 0.054	0.733 ± 0.072	0.600 ± 0.0	0.733 ± 0.072	0.610 ± 0.272
5	2.766±0.144	2.866±0.165	3.0±0.216	0.866±0.027	0.633±0.072	1.0±0.0	0.666±0.054	±0.072	0.766±0.054
10	2.333±0.054	2.3±0.816	2.1±0.04	0.766±0.047	0.666±0.072	0.733±0.072	0.633±0.054	0.666±0.054	0.533±0.027
20	1.900±0.216	1.633±0.047	1.333±0.816	0.7±0.047	0.566±0.027	0.7±0.047	0.566±0.027	0.4±0.027	0.300±0.027
30	1.666±0.216	1.566±0.098	1.116±0.216	0.666±0.054	0.533±0.027	0.6±0.047	0.511±0.027	0.5±0.0	0.400±0.47
40	1.566±0.098	1.100±0.047	0.800±0.216	0.600±0.047	0.600±0.047	0.533±0.027	0.466±0.027	0.366±0.027	0.311±0.027

Values are expressed in cms

Mean values ± Standard Error

Table-3. Two way ANOVA for biochemical effects of Zn on *Salvinia molesta*

	Total chlorophyll	Protein	Carbohydrate
F-Value (between concentration)	10.027**	10.56**	6.787**
F-Value (between duration)	2.66	1.24	0.013

**Significant at P < 0.01 level

Table-4. Two way ANOVA for biochemical effects of Zn on *Spirodela polyrhiza*

	Total chlorophyll	Protein	Carbohydrate
F-Value (between concentration)	5.808**	23.84**	15.468**
F-Value (between duration)	0.488	0.249	0.045

**Significant at P < 0.01 level

Table-5 . Two way ANOVA with Dunet's test for multiple comparison for accumulation of Zn by aquatic macrophytes

	Salvinia	*Spirodela*
F-Value (between concentration)	5621.50**	2247.42
F-Value (between duration)	13.90**	6.723
Dunet's value	265.164	386.60
Control V/s 5.0 ppm	2690.41	2880.33
Control V/s 10.0 ppm	5202.50	5446.00
Control V/s 20.0 ppm	10768.08	11573.66
Control V/s 30.0 ppm	15645.00	13784.33
Control V/s 40.0 ppm	17804.50	16866.00

** Significant at P < 0.01 level

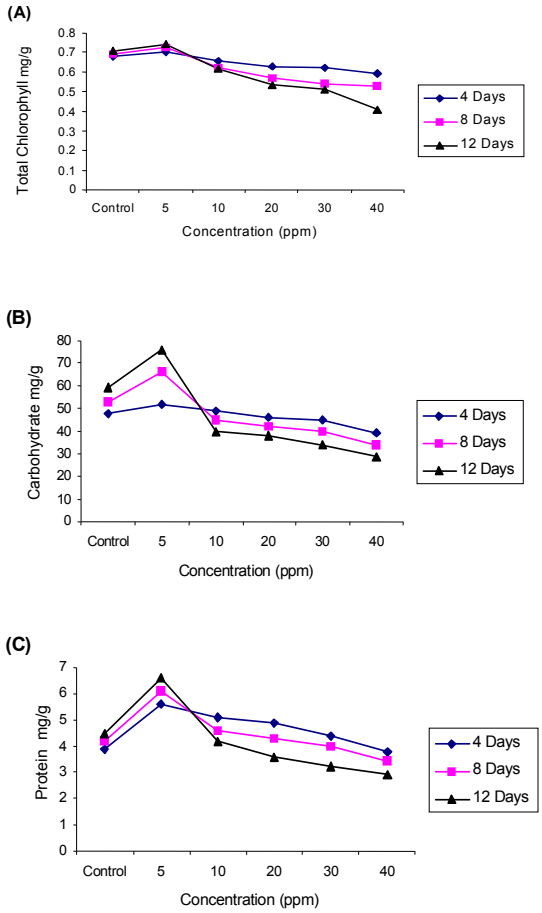

Fig: 1. Biochemical effects of Zinc on *Salvinia molesta*
(A) Total Chlorophyll (B) Carbohydrate
(C) Protein

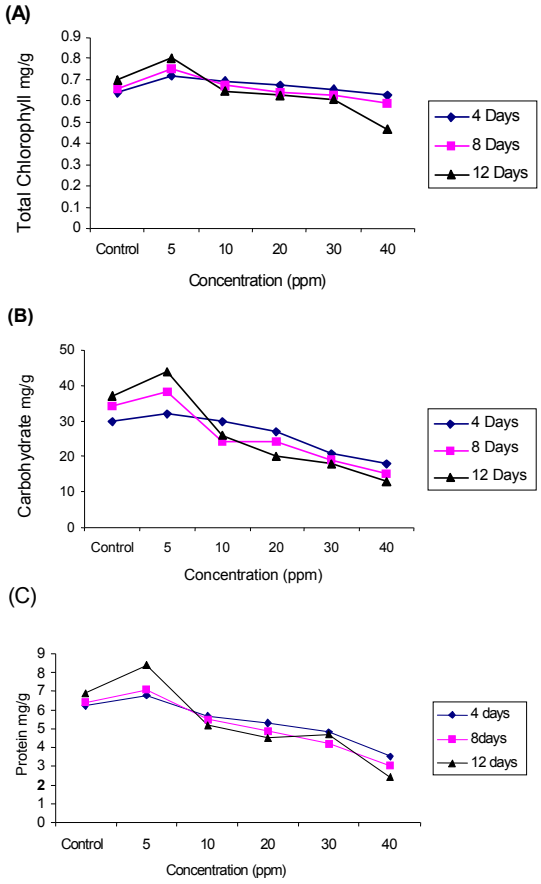

Fig: 2 . Biochemical effects of Zinc on *Spirodela polyrhiza*
(A) Total Chlorophyll (B) Carbohydrate
(C) Protein

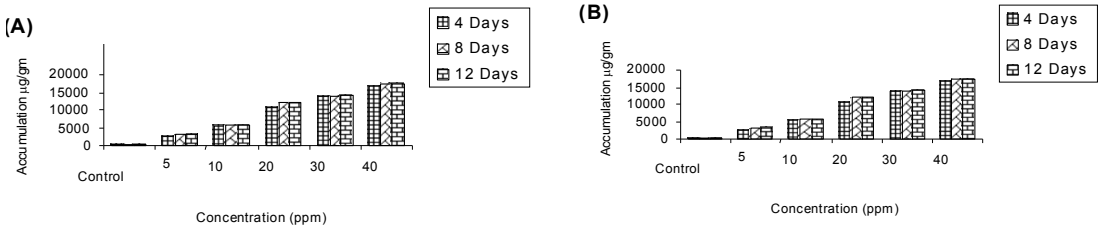

Fig: 3. Accumulation profile of Zinc by aquatic macrophytes
(A) *Salvinia* (B) *Spirodela*

However, the higher concentration of Zn 10, 20, 30 and 40 ppm of Zn, *Salvinia* was found to exhibit inhibition of carbohydrate synthesis. The 40 ppm Zn inhibited the synthesis of carbohydrate to 39.0mg/g, 34.0 mg/g and 29.0 mg/g respectively at 4, 8 and 12 days of exposure duration in comparison to control. In *Spirodela* at 40 ppm of Zn the inhibition of carbohydrate synthesis is 16.0 mg/g (46 %), 15 mg/g (55.88%) and 13.0 mg/g (64.87%) respectively at 4, 8 and 12 days exposure duration, the reduction in carbohydrate content can be attributed to the reduced rates of photochemical activities[17] and also Succinic Dehydrogenase (SDH) of all in cells indicate oxygen stress and energy crisis and mitochondria disturbances[9]. Our experimental results shows that 40 ppm Zn inhibit the carbohydrate synthesis from 39 mg/g to 29 mg/g in *Salvinia* and 18 mg/g to 13 mg/g in *Spirodela* from 4 to 12 days exposure duration respectively. The percent inhibition of carbohydrate during 12 days exposure is 50.85% in *Salvinia* and 64.87% in *Spirodela* in comparison to its respective control.

The 5 ppm of Zn in *Spirodela* promotes the protein synthesis and is directly proportional to exposure duration. The rate of increase is 6.8mg/g (9.67%), 7.1 mg/g (10.93%) and 48.4mg/g (21.73%) respectively at 4, 8 and 12 days exposure duration. In *Salvinia* also at 5 ppm of Zn promotes the protein synthesis by 47.8% (5.6mg/g), 45.58% (6.1mg/g) and 46.6% (6.6 mg/g) respectively at 4, 8 and 12 days exposure in comparison to control. The proteins are one of the most important group of biomolecules, includes maintenance of osmotic balance, storage of some particular elements, enzymes to catalyse biochemical reactions. Our investigation revealed the stimulation percentage of protein during 12 day exposure is 46.66 % in *Salvinia*, 21.73% in *Spirodela* compare to control. Similar observations were in *Potamogeton pectinatus* at lower concentration of Zn[27]. Phytochelatin (PC) produces enzymes like Glutathione reductase and PC synthetase bind and sequesters metal toxicity in the plant cell and increases the protein content at lower concentration[21,35].

However, the higher concentration of 40ppm in *Salvinia* was decreased by 2.57% (3.8mg/g), 19.05% (3.4mg/g) and 40.82% (2.9mg/g). Similarly in *Spirodela* at 40ppm of Zn, the percent inhibition was 43.54% (3.5mg/g), 53.12% (3.0mg/g) and 65.2% (2.4mg/g) at 4,8 and 12 days exposure in comparison to respective control (Fig. 1 and 2). Further 40ppm of Zn found to inhibit the protein synthesis from 3.8mg/g to 2.9mg/g in *Salvinia* and 3.5mg/g to 2.4mg/g in *Spirodela* from 4 and 12 days exposure respectively. The percent inhibition of protein is 40.82% in *Salvinia* and 65.82% in *Spirodela* during 12 days exposure duration, compared to control. The decline in protein content at high metal concentration may be due to the oxidation of protein[33] and also due to increased activate of protease or other catabolic enzymes which are activated and destroy the proteins. The high concentration of Zn metal ions binds with –SH functional groups resulting in the disruption of protein synthesis pathway.

Profile of Metal accumulation :

Fig. (3) shows the concentration of Zn accumulation in *Salvinia* and *Spirodela* was directly proportional to its concentration and exposure duration. The accumulation of

metal in test plants at 4 days duration is more pronounced irrespective of exposure duration. However, at remaining duration of exposure it remains marginal.

The *Salvinia* exposed to 5ppm found to accumulate 2675.25 µg/g, 2998.25 µg/g and 3121.75 µg/g during 4, 8 and 12 days exposure duration respectively. However, at subsequent exposure duration, it shows marginal increase in their accumulation. *Salvinia* grown in experimental ponds containing 40ppm of Zn found to accumulate 17525.00 µg/g, 18130.75 µg/g and 18481.75 µg/g respectively at 4, 8 and 12 days exposure (Fig. 3). The *Spirodela,* test plant exposed to 5ppm of concentration accumulated 2802.0 µg/g, 3088.0 µg/g and 3403.0 µg/g respectively at 4, 8 and 12 days exposure. Similar to *Salvinia*, *Spirodela* at 40ppm concentration, Zn accumulation was 16925.0 µg/g. 17125.0 µg/g and 17200.0 µg/g respectively at 4, 8 and 12 days exposure. (Fig. 3).

Application of Two way ANOVA, it is found that both concentration and exposure duration are significant at $P < 0.01$ level in *Salvinia*. Further Dunet's test is applied for multiple comparison between control and different concentration, it is clear that all treatment means significantly differ with control. However, in case of *Spirodela*, by applying Two way ANOVA it is found that both concentration and exposure duration are not statistically significant (Table-5).

The Zinc is an essential micronutrient required for several metabolic activities of the plants and has a long biological half life[19]. Heavy metal pollution of water is a major environmental concern, is increasing at alarming rate due to anthropogenic activities. In the present investigation aquatic macrophytes viz, *Salvinia* and *Spirodela* are used to measure accumulation status.

It was found that *Spirodela* to accumulated maximum content of Zn (3403.0 µg/g) from the experimental pond containing 5.0 ppm zinc followed by *Salvinia* (3121.75 µg/g) during an exposure duration of 12 days. The accumulation of Zn in the plants exposed to 40ppm are as follows: *Salvinia* (1848.75 µg/g) following *Spirodela* (17200 µg/g) during 12 days exposure. The accumulation of Zn in plants exposed to 40 ppm of Zn are as : *Salvinia* (18481.75 µg/g) followed by *Spirodela* (17200 µg/g) during 12 days exposure. Our findings confirms observation of Jain and they found that Pb anf Zn content in *Azolla pinnata* and *Lemna minor* increased at initial concentration[25]. The rate of accumulation of Zn in *Potamogeton pectinatus* increases with increase in the exposure duration[37]. There are two phases in metal absorption by the plants. The first phase is rapid while 2nd phase is slow and extended. Our results are in total agreement with the above statement. The increase in the accumulation might be due to increased number of binding sites for the complexation of heavy metal ions, leading to the increased absorption, however, slow accumulation may be attributed to binding ions to the plants and establishment of equilibrium status between adsorbant and adsorbate.

It is concluded from the findings that the morphological, biochemical responses and metal accumulation profile by *Salvinia* and *Spirodela* were directly proportional to

concentration of metal and maximum metal uptake was recorded at 4 days exposure and later it was marginal at subsequent concentrations and exposure duration, *Spirodela polyrhiza* is found to be suitable candidate for toxicity evaluation. However, the *Salvinia molesta* is the tolerant species and can be used for remediation of heavy metals from the aquatic ecosystem and environmental monitoring.

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References :

1. Allen, S.E., H.M. Grimshaw, J.A. Parkinson and C. Quarmby (1974) Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford.
2. Arnon, D.I. (1949) *Plant Physiol, 24: 1-15*.
3. Azharbaig, M.B., K. Joseph, K. Vijay Rao and Jayantha (1999) *J. Ecotoxicol. Environ.*, *1:* 156-161.
4. Brix, H. and H.H. Schierup (1989) Ambio, *18:* 100-107.
5. Choudhary, M.P. and (2005) *Journal of Environmental Biology.*, *26*(1): 129-134.
6. Dammal, D., W.B. Green, M.T. Henzl, J.M. Hosea, R.H. Mc Pherson, J. Sneddon and M.D. Alexander, (1986) Recovery of heavy metals by immobilized algae. In: Thompson R (ed.) Trace metal removal from Aqueous solution, Whitstable Litho Ltd. . 1-24.
7. Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, (1956) *Annul. Chem.*, *28:* 350-356.
8. Dushenkov, V., P.B.A.N. Kumar, H. Motto and I. Raskin, (1995) *Environ. Sci. Tech.*, *29:* 1239-1245.
9. Halliwell, B. and J.M.S. Gutteridge, (1993) Free radicals in biology and medicine. Clarendan Press Oxford.
10. Jain, S.K., P. Vasudevan, and N.K. Jha, (1990) *Water Res., 24*(2): 177-183. 1990.
11. Kuyack, N. and B. Voleksy, (1988) *Biotech Letto. 10:* 137-142.
12. Lowry, O. H., N. J. Rosebrough, R. J. Randall and A. Farr (1951) *J. Biol. Chem.*, *193:* 265-275.
13. Mathad, P., S.B. Angadi and R.D. Mathad (2004) *Asain Jr. of Microbiol. Biotech. Env. Sci.*, *6*(1): 99-106.
14. Mudhakavi, J.P. and B.V. Narayana (1997) *IJEP.*, *18*(2): 108. 1997.
15. Pahlasson, A.M.B. (1989) *Water, Air and Soil Pollut.*, *47:* 278-319.
16. Pandey, S., R.K. Asthana, A.M. Kayastha, N. Singh, and S. P. Singh, (1991) *Plant Physiol.*, *154:* 634-640.
17. Prasad, K.V.S.K., P. Pradha Sardhi, and P. Sharmila, (1999) *Environ. Expt. Bot.*, *42 :* 1-10. 1999.
18. Prasad, M.N.V. Trace metal In: *Plant ecophyscology* (Ed. Prasad, M.N.V.) John Wiley and Son. New York. 1997. 207-249.
19. Rai, A.K. and S. Kumar, (1999) *Applied Microbiol. Biotechno.*, *39:* 661-667.
20. Rajendra, J., Muthukrishnan and P. Gunashekaran, (2003) *Indian Journal of Experimental Biology.*, *41:* 935-944.
21. Romero- Puertas, M.C., F.J. Corpus, M. Rodriguez-Seranno, M. Gornez, and L.A. Dei Rio, (2007) *J. Plant Physiol.*, *164:* 1346-1357.

22. Satyakala, G. and K. Jamil, (1992) *Bull. Environ. Contam. Toxicol.*, *48:* 921-928.
23. Saygideger, S. and M. Dogan, (2009) *Bull. Environ. Contam. Toxicol.*, *73:* 182-189.
24. Sharma, R. K. and M. Agrawal (2005) *J. Environ. Biol.*, (**2** supl) : 301-313.
25. Sibihi, K., O. Cherifi, A. Agarwal, B. Oudra, and F. Aziz, (2012) *J. Mater Environ. Sci*., *3:* 497-506.
26. Singh, N.K., G.C. Pandey, U.N. Rai, R.D. Tripathi, H.B. Singh, and D. K. Gupta, (2005) *Bull. Environ. Contam. Toxicol.*, *74:* 857-863. 2005.
27. Sinha, S., M. Gupta, and P. Chandra, (1994) *Indian J. Envi. Hlth.*, *38:* 51-53.
28. Sinha, S. and K. Pandey, (2003) *Bull. Environ. Contam. Toxicol*., *71:* 1175-1183. 2003.
29. Sobero, M.C., J. Beltarano, and A. E. Ronco, (2004) *Bull. Environ. Contam. Toxicol*., *73:* 416-423.
30. Tripathi, R.D., U.N. Rai, P. Vajpayee, M.B. Ali, E. Khan, D.K. Gupta, M.K. Shukla, S. Mishra, and S. N. Singh, (2003) *Bull. Environ. Contam. Toxicol.*, *71:* 255-262.
31. Vajapayee, P., U.N. Rai, M.B. Ali, R. D. Triphati, V. Yadav, S. Sinha and S.N. Singh (2001) *Bull. Environ. Contam. Toxicol*., *67:* 246-254.
32. Van Assche, F. and H. Clijsters (1990) *Plant. Cell Environ.*, *13:* 195-206.
33. Wang. T.C., J.C. Weissman, G. Ramesh, R. Varadarajan and J.R. Bennemann (1996) *Bull. Environ.Contam. Toxicol. 57:* 779-786.
34. Wilde, E. W. and J. R. Benemann (1993) *Biotech. Adv.*, *11:* 781-812.
35. Wintz, H., T Fox, and C. Vulpe, (2002) *Biochem Soc. Transactions, 30:* 766-768.
36. Yongpisanphop, J., M. K. Chue, and P. Porethitiyook, (2005) *Journal of Environmental Biology., 26*(1): 79-89.