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RESEARCH ARTICLE

PHYTOASSAY OF SEWAGE USING AQUATIC MACROPHYTE *SALVINIA MOLESTA* (MITCHELL)

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ABSTRACT

Anthropogenic activities have contributed huge quantities of industrial and domestic waste water which contains large quantities of xenobiotics that leads to undesirable changes in the physico-chemical environment. The aquatic plants have the potential to accumulate heavy metals, were tolerant and able to withstand on the pollution stress. They serve as a tool for the study phytoremediation of xenobiotics from the aquatic ecosystem. Phytoremediation is an emerging “Green Bioengineering Technology”. Bioengineering is a green, cheaper alternative that hardly cost civil engineering works for environmental reconstructions. The present investigation focuses on morphological and biochemical toxicity and accumulation profile of heavy metals in *Salvinia molesta* Mitchell to the various concentrations of sewage (25, 50, 75 and 100 %) at regular interval of 4 days. *Salvinia* showed visible symptoms like chlorosis, stunted growth and withering of roots at higher concentrations, however, the test plant shows luxurious growth at lower concentration (25%) of sewage. The estimation of biochemical parameters viz, total chlorophyll, protein and carbohydrate of test plants shows significant increase at lower concentrations but decreased with increased concentrations of sewage and exposure durations. The plant accumulation profile of heavy metals is directly proportional to sewage concentration and exposure duration. The accumulation potentiality in *Salvinia* was maximum at 4 days exposure irrespective of concentration. However, at remaining duration of exposure it remains marginal.

INTRODUCTION

The urbanization and industrialization coupled with unprecedented population growth are the main causes for undesirable changes in the physicochemical environment. The anthropogenic activities have contributed huge quantities of industrial and domestic waste water which contains large amount of inorganic and organic materials that form a source of plant nutrients in addition to the presence of various amounts of heavy metals (Brar *et al*., 2000; Bajguz, 2011). Change in environment by pollution leads to undesirable changes in the quality of life and its status. The response of organisms depends on their inherent sensitivity of xenobiotics, sensitive species fails to withstand the stress. The tolerant plant species survive, thrive-well and multiply in polluted environment and serve as an indicator species for environmental quality. Aquatic macrophytes possess tremendous accumulating potential to reduce the level of toxic metals from the sewage (Salt *et al*., 1998). The aquatic plants have the ability to accumulate the heavy metals and have been employed for the treatment of waste water (Rai *et al*., 2003).

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Hence, the present investigation evaluates the morphological, biochemical responses and accumulation of heavy metals in *Salvinia molesta* from the sewage.

MATERIALS AND METHODS

The sewage collected from Srinagar pond was slightly brown in colour having foul smell. The analysis of physicochemical parameters of sewage were carried out by using the standard methods of APHA (1998). The test plant *Salvinia molesta*. Mitchell were sampled from Srinagar pond near Karnatak University, Dharwad (India). The stock plants were maintained under the laboratory conditions and experiments were carried out in triplicates. The young healthy *Salvinia molesta* were selected and acclimatized for two weeks in 4% Hoagland solution in the experimental ponds of 10 L capacity. 50 gms of fresh plants were introduced simultaneously into each of the experimental ponds containing 25, 50, 75 and 100% of sewage and tap water (control) respectively. The experiments were conducted under standard physical conditions in green house of the Department of Botany, BLDEA’s College, Jamkhandi. The plants were harvested at the end of 4, 8 and 12 days exposure and thoroughly washed with distilled water. The 25 gm of plant material was harvested and used for the morphological observation and toxicity evaluation, for the estimation of total chlorophyll, carbohydrate, protein content and accumulation of heavy metals. The fresh plant samples were macerated in 100 ml of 80% (v/v) chilled acetone by using pestle and mortar. The extract was centrifuged and supernatant was used for the estimation of total chlorophyll by Arnon method (1949), carbohydrate estimation by phenol sulphuric acid method (Dubois method, 1956) and protein estimation by Lowry's method (1951). The heavy metal estimation of sewage was carried out by using standard method (Allen *et al*., 1974). The dry powder of one gm plant material was digested using Gerhardt digestion unit. The digested sample was used for the estimation of heavy metals present in the sewage and the plant samples using Atomic Absorption Spectrophotometer (AAS) (GBC 932 plus, Austrelia).

Statistical analysis

Data are presented as mean values and ±SE from experimental data with three replicates. Data were subjected to three way ANOVA with two factor interaction to find out the significance between concentration and exposure duration, concentration and parameters and exposure duration and parameter. Further, Dunet's test is also applied for multiple comparisons between control and other concentrations.

RESULTS AND DISCUSSION

The results of physico-chemical analysis of sewage are presented in Table 1. The sewage is slightly brown in colour with foul smell.

Table 1. The physicochemical analysis of sewage

No.	Parameter	Concentration
1	pH	8.09±0.01
2	Electric conductivity	1.29±0.01
3	Total Dissolved Oxygen (TDS)	685.6±5.50
4	Chloride	455.6±4.50
5	Hardness ($CaCO_3$)	345.4±3.90
6	Calcium (Ca)	98.0±2.64
7	Magnessium (Mg)	74.0±1.0
8	Dissolved Oxygen(DO)	3.83±0.25
9	Sodium (Na)	144.0±2.64
10	Potassium (K)	73.60±2.51
11	Chemical Oxygen Demand (COD)	302.3±3.78
12	Phosphates (PO_4)	2.17±0.06
13	Sulphates (SO_4)	107.66±4.93
14	Bicarbonates (HCO_3)	556.6±5.20
15	Zinc (Zn)	502.3±4.04
16	Copper (Cu)	215.33±5.03
17	Manganese(Mn)	445.0±2.00
18	Iron (Fe)	1613±5.03
19	Nickel (Ni)	373.6±2.50
20	Lead (Pb)	2108.6±3.20
21	Cadmium (Cd)	374.3±3.04

No. 3 to 14 are expressed in mg/l and 15 to 21 are expressed in µg/lt.

Morphological Assay

Morphometric assay of toxicity is one of the quantitative tools for the assessment of toxicants was measured by using Morphological Index Parameters (MIP) (Table 2). The rate of inhibition of growth in root and leaf is directly proportional to the increasing concentrations of sewage. The plants shows better growth other than the normal growth at lower concentration in comparison to control, due to utilization and absorption of the nutrients and decomposition by microorganisms. Similar observation was made by Singh *et al*., 2005 in the germination of *Sorgham* in dairy effluent of 25%. The higher concentration of sewage exhibited toxicity symptoms like chlorosis, curled lamina, leaf margins became dark brown in color. The three way ANOVA with two factor interaction for the effect of sewage on leaf length and breadth, between exposure and concentration is significant at 1% ($p > 0.01$). The excess accumulation of toxic metals in different tissues of the plant is responsible for morphological and biochemical changes due to interference of metal ions in the metabolism Satyakala and Jamil, (1997). Similar observations were made by Kamble and Patil, (2001) in *Eichornea*, Marawari and Sharma, 2005 in *Raphanus sativus* treated with textile waste water.

Biochemical Toxicity Assay

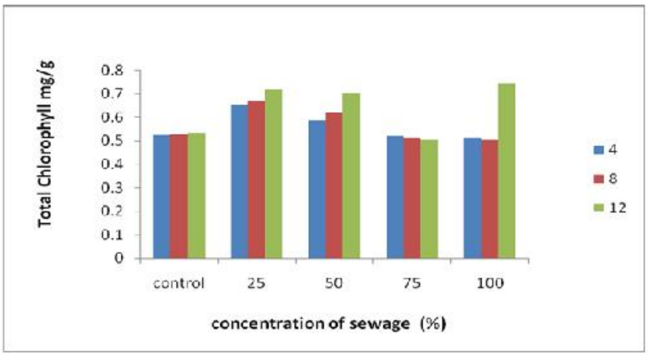
The biochemical data and Fig. 1A shows that 25% and 50% of sewage found to augment the chlorophyll synthesis and is directly proportional to the exposure duration. The chlorophyll content of the experimental plant was increased 0.656±0.03 mg/gm (24.71%), 0.670±0.04 mg/gm (26.65%) and 0.720±0.04 mg/gm (34.57%) at 4, 8 and 12 days exposure at lower concentration of sewage (25%) in comparison to control ponds. The results indicate that 25% sewage was found to be favorable for the synthesis of chlorophyll. The chlorophyll stimulation is associated with the formation of phytochelatins (PCs) which plays a role in detoxification (Prasad, 2004, Sibihi *et al*., 2012; Brahmbhatt *et al*., 2013). The similar observations have been reported in the rice seedlings grown in industrial effluents (Bendra and Mishra, 1983). However, the higher concentration of the sewage found to inhibit chlorophyll synthesis and is directly proportional to the exposure duration. The percent inhibition of chlorophyll at 100% sewage was 0.516±0.03 mg/gm (21.49%), 0.506±0.04 mg/gm (24.47%) and 0.499±0.06 mg/gm (30.69%) (at 4, 8 and 12 days exposure respectively, compared to control.

The above results may be attributed to the presence of heavy metals in the sewage which inhibited the chlorophyll synthesis by interacting with the functional –SH group of enzyme synthesizing chlorophyll (Prasad, 1995).

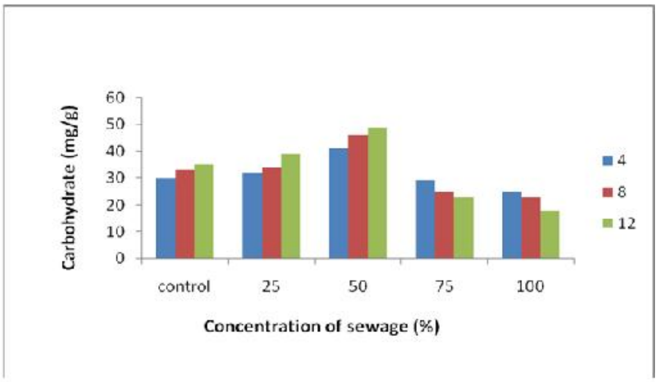
Table 2. Effect of sewage on Morphology of *Salvinia molesta*

Sewage Concentration (%)	Exposure Duration (in days)								
	4	8	12	4		8		12	
	Root length			Leaf size					
				Length	Breadth	Length	Breadth	Length	Breadth
Control	2.43± 0.02	2.66± 0.21	3.03± 0.47	1.36± 0.02	1.2± 0.05	1.4± 0.00	1.26± 0.07	1.46± 0.02	1.3± 0.08
25	2.7± 0.04	3.03± 0.07	3.33± 0.02	1.66 0.02	1.25± 0.02	1.76± 0.02	1.36± 0.02	1.8 ± 0.04	1.36± 0.02
50	2.53± 0.07	2.76± .027	3.06± 0.02	1.53± 0.02	1.22± 0.02	1.56± 0.02	1.33± 0.02	1.6 ± 0.02	1.3 ± 0.47
75	2.5 ± 0.04	2.26± 0.05	1.7 ± 0.12	1.5 ± 0.04	1.2 ± zero	1.36± 0.02	1.26± 0.02	1.3 ± 0.08	1.06± 0.54
100	2.33± 0.09	2.2 ± 0.12	1.33± 0.05	1.5 ± 0.04	1.16± 0.02	1.36± 0.02	1.10± 0.04	1.3 ± 0.47	1.03± 0.02

Values are expressed in cms
Mean-values ± Standard Error



A)



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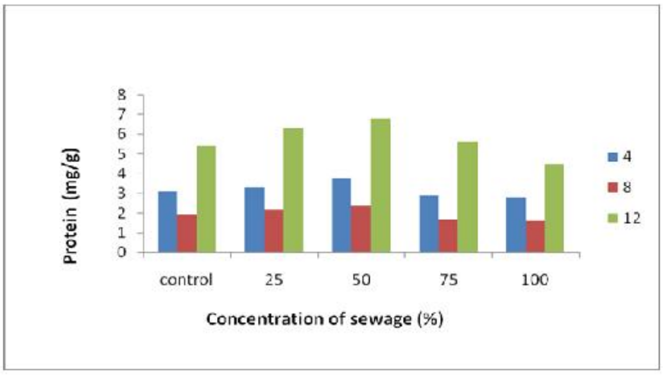
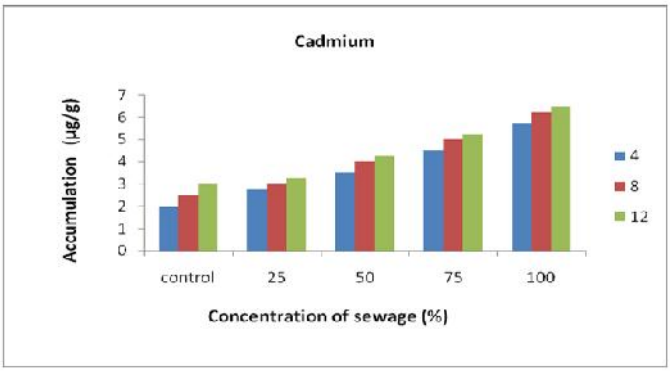


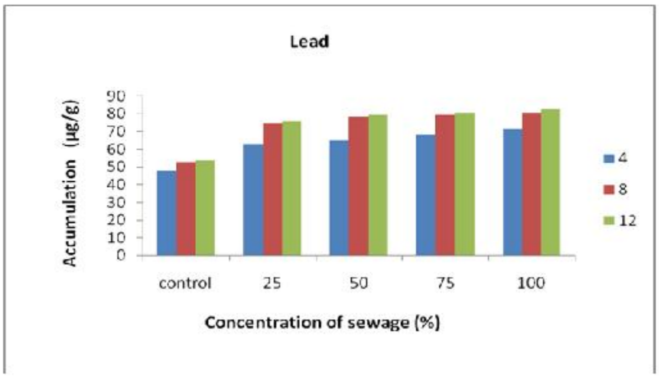
C)

Fig. 1. Biochemical effect of sewage on *Salvinia molesta* (A) Total Chlorophyll (B) Carbohydrate (C) Protein

The heavy metals present in the sewage are the potent inhibitors of biosynthesis of chlorophyll at higher concentration of sewage. The major sites of inhibition are in the formation of proteolytic phytochelatin (PC) halide reductase complex and synthesis of δ aminolevulinic acid dehydrogenase (δALAD), chloroplast enzyme, which catalyzes the pathway of porphobilionogen (PBG) synthesis for δ-aminolevulinic acid (ALA) (Prasad and Prasad, 1987; Singh *et al*., 2005; Sibihi *et al*., 2012). The data reveals that effect of different concentrations of sewage on carbohydrate content (Fig. 1B). The 50% sewage is more promotive for the synthesis of carbohydrate than 25% sewage by 41.6±1.52 mg/gm (36.66%), 46.3±2.51 mg/gm (39.39%) and 49.6±1.52 mg/gm (40.1%) at 4, 8 and 12 days exposure in comparison to control. It was reported that the accumulation of carbohydrate in algal cells, viz, *Anabaena sps*, *Fischerella sps* and *Westiellopsis sps* grown in highly diluted effluent and the carbohydrate content is directly proportional to the chlorophyll content (Renuga, 2005).



A)



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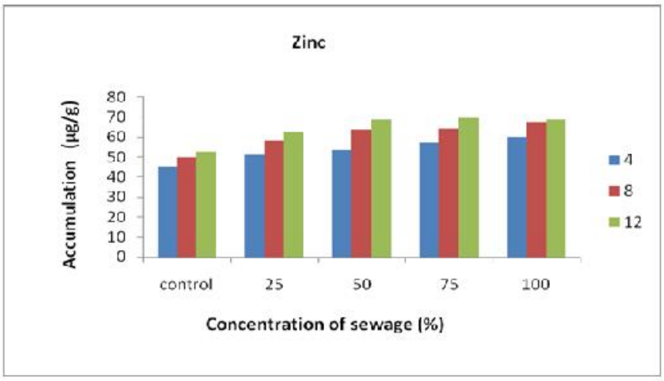

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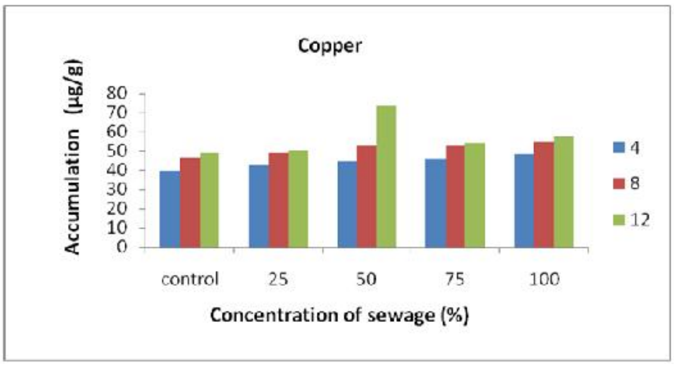

D)

Fig. 2. Accumulation profile of heavy metals of Cd, Pb, Zn and Cu from sewage by *Salvinia molesta*

However, higher concentration of sewage (100%) was found to inhibit the synthesis of carbohydrate by 25.66±2.51 mg/gm (16.66%), 23.06±2.08 mg/gm (30.33%) and 18.12±2.51 mg/gm (48.57%) at 4, 8 and 12 days exposure in comparison to control. The increased concentration of sewage has impact on photosynthetic activity as the heavy metals like Pb, Ni, Cd and Cu damages the chloroplast structure, hinders the photosynthetic assimilation (Hasan *et al*., 2009). The biochemical data and Fig. 1 C. shows that 50% sewage found to augment the protein content. The protein content of the test plant at 50% sewage was 3.8±0.025 mg/gm (22.56%), 2.4±0.15 mg/gm (26.35%) and 6.8±0.173 mg/gm (27.77%) at 4, 8 and 12 days exposure respectively. Similar observation was made in seedlings of *Oryza sativa* at 5% Chloralkali factory effluent (Radha and Panigrahi, 1998).

The 75% and 100 % sewage exhibits inhibitory effect on protein synthesis in test plants upto 8 days, however, later there is an augmentation of protein synthesis. The protein at 100% sewage is 2.8±0.15 mg/gm (9.67%), 1.6±0.15 mg/gm (5.78%) and 4.5±0.10 mg/gm (16.66%) at 4, 8 and 12 days exposure. After 8 days exposure, 100% sewage was found to augment the protein synthesis from 1.6±0.15 mg/gm to 4.5±0.10 mg/gm was noticed due to synthesis of the stress proteins or metallothionines but, later declines due to toxic effect of heavy metals on protein synthesis due to increased activity of protease, blockage of -SH group of protein. Similar observations were made by Ali *et al*., 2003 in *Salix acmophylla* using heavy metals like Cu, Ni, and Pb. It is demonstrated that on the metal accumulation depends on cellular metabolism, where metals binds to the cellular surface Sibihi *et al*., 2012 and Moreno-Caselles *et al*., 2000.

The three way ANOVA with two factor interaction between concentration exposure duration and parameters represents clear significant at $P < 0.05$ level between concentrations and parameters.

Metal Accumulation

Salvinia showed high amelioration potential of heavy metals (viz, Pb, Cd, Zn and Cu) by accumulation in its tissues (Fig. 2). It is apparent from the present study that the accumulation of metals was maximum during 4 days exposure and thereafter marginal increase in accumulation of metals. The order of accumulation in the test plant species is as: Zn > Pb > Cu > Cd from the sewage. The similar observations were made in *Lemna minor* and *Phragmites karka*, was high at lower concentration of waste water and absorption capacity of the plant is marginal at subsequent concentrations and exposure duration (Pandey and Pandey, 2006, Phetsombat *et al*., 2006).

Three way ANOVA represents significant association at $P < 0.01$ between concentration and parameters and exposure and parameters. This representing the high significant in metal accumulation but other factors are also related but at significant level ($p > 0.05$). Two way ANOVA represents concentrations and exposure duration are significant at $P < 0.01$ and Dunet's test also reveals the concentrations are significantly differ with control.

Conclusion

Salvinia serves as a phytoremediation tool for the accumulation extraction of heavy metals from the sewage. Regular harvest of the plant at the interval of 4 days helps to reduce toxicity in polluted aquatic environment. The *Salvinia* is a sewage tolerant species and is a suitable candidate for toxicity evaluation.

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