Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 01, pp.11235-11239, January, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PERFORMANCE ANALYSIS OF THE DUCKWEED (*LEMNA MINOR*) IN THE BIOREMEDIATION OF SEWAGE WATER

***Ajay Kumar Patra**

Department of Zoology, Utkal University, Bhubaneswar-751004, India

ARTICLE INFO

Article History:
Received 04th October, 2014
Received in revised form
17th November, 2014
Accepted 07th December, 2014
Published online 23rd January, 2015

Key words:
Water Quality Renovation,
Sewage Treatment,
Duckweed

ABSTRACT

Duckweeds are monocotyledonous aquatic plants having ability to rapid vegetative reproduction to form genetically uniform clones. In this study, duckweeds (*Lemna minor*) are used for the study of laboratory scale in the reduction of parameters such as BOD, COD, nitrate, nitrite, ammonia, TSS, sulphate, phosphate and chloride under different detention time (10, 12, 15, 20 and 30 days). Reduction values of these parameters were increased with corresponding increase in detention time. Hence duckweed can be used as model system for the study of water pollutants and an alternative choice for the study of some toxic chemicals present in the pollutants.

INTRODUCTION

Wastewater treatment has become a vital part of our daily life and there is a worldwide growing demand for less expensive and eco-friendly wastewater treatment system. However, the conventional systems that are using recently are expensive and incomplete due to high capital costs to remove or reduce various pollutants (Culley *et al.,* 1978). Recently, many complex arrays of techniques are involving in wastewater treatment, but no efficient and practical technologies are bump into clean water discharge requirements. For example, aeration could significantly reduce BOD but cannot control growth of algae. This results in installing and operating a combination of numerous mechanical, biological and chemical treatment components (Harremoes, 1999). Moreover, the wastewater treatment techniques do not completely eliminate all problems such as sludge, odour and sometimes even hazardous byproducts (Polprasert, 1996; Zimmo *et al*., 2000). Several references reported that the algal stabilization ponds and lagoons are considered to be effective low cost method of sewage treatment worldwide, which are inadequate to meet the secondary effluent disposal standards. Duckweeds are the world's smallest angiosperms, fastest growing and simplest of flowering plants, usually reproduce by budding and multiply very quickly. Recent publications demonstrated that duckweeds act as model system for different experiments due to their minute sizes, ability to rapid growth to form genetically uniform clones, easy handling and high sensitivity to organic and inorganic substances (Zhang *et al*., 2010). *Lemnea* sp. are widely used for studies in plant physiology, genetics, ecology and environmental monitoring (Brain and Solomon, 2007; Scherr *et al*., 2008). In present study, duckweeds are used as a model plant for treatment of wastewater, an effective and reliable remover of sewage toxicity with respect to growth inhibition and biochemical responses.

****Corresponding author: Ajay Kumar Patra***
Department of Zoology, Utkal University, Bhubaneswar-751004, India.

MATERIALS AND METHODS

Duckweeds belong to the family "*Lemnaceae*" and consist of four genera: *Lemna, Spirodelia, Wolfia* and *Wolfiela*. Here, used *L. minor* collected from nearby freshwater ponds, cultured in small tanks and was regularly fed for 10 days with diluted raw domestic sewage for necessary acclimatization. The sewage water collected at 10 consecutive days from the drain of thickly populated city of Bhubaneswar (19º 40" N to 20º 25" N latitude and 24º 55" E to 36º 05" E longitude), Odisha during summer period(June,2014), stored and analyzed by the standard methods (APHA, 2005). The water sample analysis and extensive monitoring of the treatment efficiency was performed by collecting weekly samples from the different treatment units of small tanks. All physico-chemical analyses for pH, COD, BOD, ammonia, nitrite, nitrate, total phosphorus, TSS, sulphate, chloride were performed according to standard methods (APHA, 1998).

Prototype ponds were installed at the open laboratory so as to acclimatize, grow and test the performance of the duckweeds in a laboratory-scale (Verma, 2007). Experiments were conducted in open laboratory at temperature 33° ± 1° C. Different chemical parameters of duckweeds were estimated for control and test plants essentially followed the standard methods (EPA, 1975; Zimmo *et al*., 2000). Fronds were carefully taken out by forceps, blotted dry on paper towels and the fresh weight was measured after cultivation of duckweeds at different times (10, 12, 15, 20 and 30 days). Duckweeds growth rate and yield were monitored every 24 h in each tank. After harvesting, thickness of the residual cover was maintained at one layer at the prototype ponds. The harvested biomass was drained, weighed, dried in an oven at 70° C and dry matter content was calculated. The dry matter was powdered in a tissue grinder and 75-100 mg was used for organic N analysis. Protein content was measured by the method of Lowry *et al*., (1951). From rest of the powder (200-250 mg) was taken, burned and ash was analysed for phosphate using the per-sulphate digestion method (APHA, 1998) followed by the vanadomolybdate calorimetric method (APHA, 1998).

Chlorophyll concentration was determined by the method of Lichtenthaler and Wellbum (1983), and Zhang *et al*., (2010) with little modification. Briefly, after fresh weight measurement the duckweed plants were placed into individual eppendorf tubes containing 1 ml ethanol and placed in dark at 4º C for 24 h. A clear solvent solution served as the reference blank. The concentration of chlorophyll a (Ca) and chlorophyll b (Cb) for extraction in ethanol, and the total chlorophyll concentration were calculated as Ca+Cb. Above data was multiplied by the extraction volume (1 ml) and divided by the fresh weight of the duckweed samples (mg chlorophyll/g fresh weight) for the mass standardize of total concentrations. All the experiments were replicated at least three times with at least five replications in each experiment. The data presented in the table were mean of five replicates. The student't' test was employed to calculate the statistical significance value (p-value of <0.05 and 0.01).

RESULTS AND DISCSSION

Results concerning the performance of laboratory scale duckweed tanks in reduction of pH, BOD, COD, TSS, total nitrogen, sulphate, phosphate and chloride under varying detention times (10, 12, 15, 20 and 30 days) have been scientifically displayed (Table 1). The overall reduction of BOD, COD, TSS, total nitrogen and phosphates were quite promising the percentage of which further increased with a corresponding increase in detention time (Table 1). However, the reduction in sulphates and chlorides were not so pronounced. The nitrogen transformation in a floating aquatic plant system was presented in Fig. 1. As shown in Figure 2, selected biochemical parameters showed inhibitory effect in *L. minor* at high concentration of wastewater (50 and 100%), however, increased at low level (25%) after 30 days detention time (Table 2). As shown in Table 3, the wastewater inhibited the chlorophyll and protein content. Few biochemical properties such as chlorophylls increased at low concentrations then gradually decreased with increase in concentration of wastewater (Table 3).

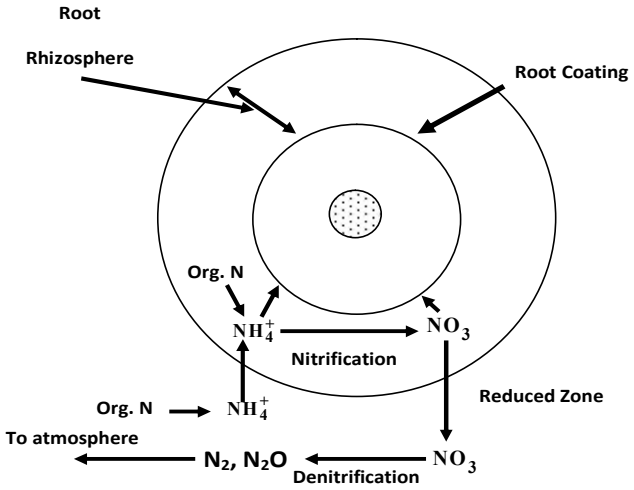

Fig. 1. Nitrogen transformation in a floating aquatic plant system

Table 1. Monitoring of laboratory model duckweed tanks working in series (mean data)

Parameters	Influent	10 days	12 days	15 days	20 days	30 days	% reduction		
							15 day s	20 days	30 days
pH	6.89	7.86	7.28	7.1	7.0	7.0	–	–	–
BOD at 20°C (mg/l)	136	63	27	14	13	12	89.68	90.4	91.1
COD (mg/l)	190	118	60	35	33	32	81.51	82.6	83.1
TSS (mg/l)	321	165	65	55	54.3	54.3	82.8	83.08	83.08
Total solids (mg/l)	1056	983	501	358	351	351	66.09	66.76	66.28
TDS (mg/l)	741	723	442	361	358	357	51.28	51.68	51.82
Nitrate as N (mg/l)	0.86	0.49	0.43	0.39	0.36	0.35	54.65	58.13	59.31
Ammonical Nitrogen (mg/l)	23	18.0	16	9.5	9.0	9.0	58.7	60.8	60.8
Phosphate as P (mg/l)	6.78	6.0	5.0	4.0	3.1	2.5	41.1	54.4	63.2
Sulphate (mg/l)	55	47	45	45	45	45	18.2	18.2	18.2
Sulphide (mg/l)	5.12	1.7	1.7	1.7	1.7	1.7	–	–	–
Chloride	264	258	250	240	250	254	9.09	5.30	5.78

Table 2. Chemical composition of *Lemna minor* from laboratory culture tank

Chemical composition	Control	Tests (30 days at 50% treatment
Protein (µ g– 1 fresh wt.)	67.31±2.5	45.41±4.3
Chlorophyll – a (mg g– 1 fresh wt.)	1.18±0.01	1.05±0.01
Chlorophyll – b (mg g– 1 fresh wt.)	0.46±0.02	0.29±0.02
Chlorophyll – a+b (mg g– 1 fresh wt.)	1.44±0.35	0.71±0.04
Dry matter (g)	0.007±0.001	0.005±0.001
Ca	1.0±0.01	1.5±0.05
K	3.1±0.02	3.9±0.04
P	0.7±0.01	0.9±0.02
Mg	0.3±0.01	0.1±0.01

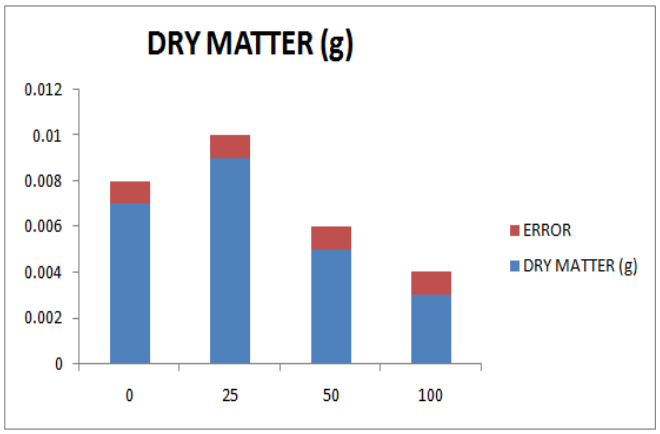

Fig. 2a. Inhibiting effect in *L.minor* of certain biochemical parameter (dry matter)

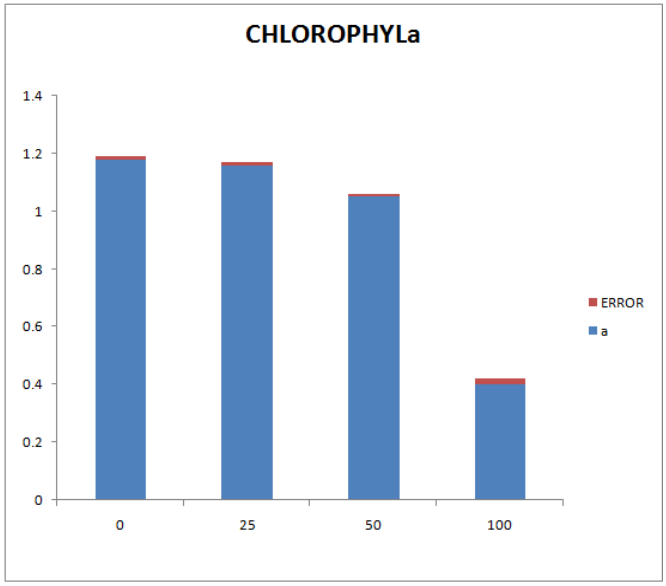

Fig. 2b. Inhibiting effect in *L.minor* of certain biochemical parameter (chlorophylla)

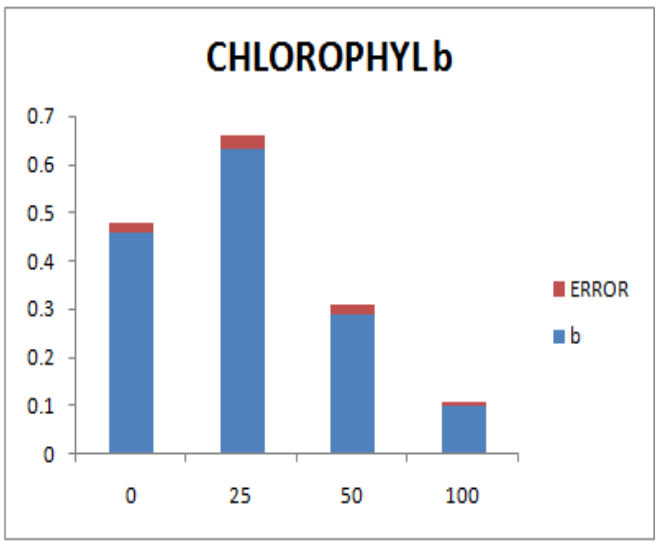

Fig. 2c. Inhibiting effect in *L.minor* of certain biochemical parameter (chlorophyll)

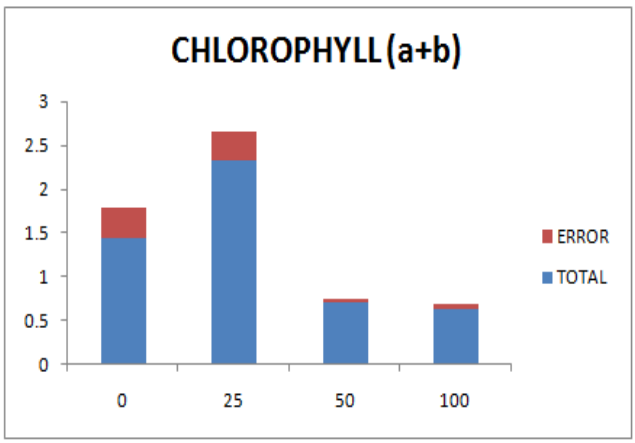

Fig. 2d. Inhibiting effect in *L.minor* of certain biochemical parameters (chlorophyll a+b)

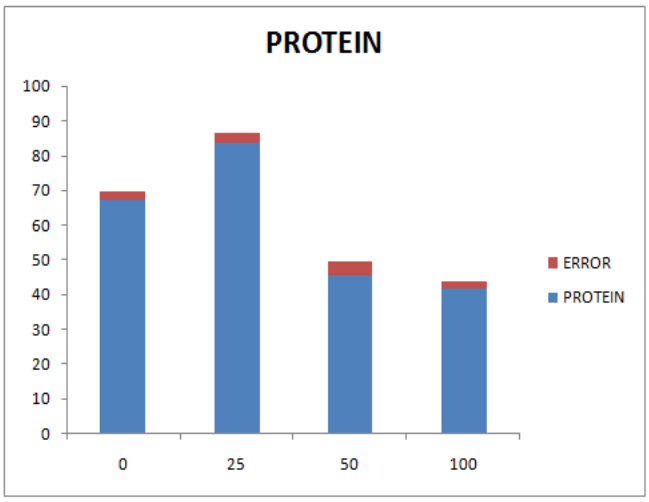

Fig.2e. Inhibiting effect in *L.minor* of certain biochemical parameters (protein

Aquatic ecosystem is the final descend for discharge of many contaminated chemicals used in agriculture, industry and livestock operations without proper treatment. Frequently releases of these pollutants to water bodies spoil the water quality and become inapt for aquatic organisms (Trivedy *et al*., 1990; Forkas *et al*., 2000; Fjalborg and Deve, 2003) due to their persistence, bioaccumulation, toxicity and bio-magnification in the food chain (Lavanya *et al*., 2011). Reduction in dry matter of test plants by polluted water was possibly due to high metal ions content in growth medium (Garg and Chandra, 1994; Pandey, 2006). Moreover, presence of toxic chemicals in the aquatic bodies accumulates in the aquatic plants, animals and leading to toxic effects (Huebert and Shay, 1993; Sen *et al*., 1994) that ultimately transfer to food web when consumed by various living organisms (Sharma *et al*., 2001; Singh and Singh, 2006; Sahu *et al*., 2007). Several publications demonstrated the removal of COD, BOD and TSS from the anaerobic effluent fed to the duckweed ponds varied in different seasons at the HRT of 15 days. Oron *et al*. (1987) demonstrated that COD and BOD removal values for the duckweed plant (*L. gibba*) covered mini-ponds of about 63% and 92% respectively at 20 days using settled sewage.

Table 3. Effect of domestic sewage water on biomass, chlorophyll *a (Ca)*, chlorophyll *b* (Cb) and total (Ca+Cb) (mg g^{-1} fresh wt.) and protein (μ g^{-1} fresh wt.) content in *Lemna minor* after 30 days retention

Sewage Conc.(%)	Dry matter (g)	Chlorophyll			Protein
		a	*b*	Total	
0 (control)	0.007±0.001	1.18±0.01	0.46±0.02	1.44±0.35	67.31±2.5
25	0.009±0.001	1.16±0.01	0.63±0.03	2.32±0.33	83.67±2.7**
50	0.005±0.001	1.05±0.01	0.29±0.02	0.71±0.04	45.41±4.3*
100	0.003±0.001	0.40±0.02	0.10±0.01	0.64±0.05	41.65±2.3**

Values are mean of three replicates; ± S.E. value significant at* = <0.05 level; ** = < 0.01 level.

This is probable that COD, BOD and TSS removal in duckweed ponds is mainly HRT dependent. In present study, it was also recorded the same in experimental prototype ponds efficiencies in the removal of large reduction of pH, BOD, COD, TSS, total nitrogen and phosphate values. Reduction values of the above parameters further increased with corresponding increase in detention time. Many references reported that due to consumption of ammonia, duckweed plants tends to use nitrite and nitrate as nitrogen source only after the ammonia has reached low concentrations (Porath and Pollock, 1982; Nelson *et al*., 1981; El-Shafai *et al*., 2007).

In our experiment, performance of laboratory scale study was found to be comparable with the result obtained in the above field scale duckweed pond eco-system, which could affect a similar high reduction in all the above mentioned parameters.
Chlorophyll is a vital pigment for the photosynthetic activity in plant. Toxic chemicals present in wastewater inhibit the uptake and transportation of useful elements (Fe, Zn and Mn) that lose the capacity of synthesis of pigments (Prasad *et al*., 2001; Liu *et al*., 2004; Xiong *et al*., 2006). The present findings supported the sensitivity of *L. minor* to toxic metals and other pollutants as reported by other researchers (Mohan and Hosettii, 2006; Singh and Singh, 2006; Cayuela *et al*., 2007). *L. minor* accumulates heavy metals in tissues (Wang, 1990; Maine *et al*., 2001; Phetsombat *et al*., 2006) and used for phytoextraction of these toxic metals (Zayed *et al*., 1998). Further, certain macrophytes also used as test plants for indicator of aquatic pollutants (Tremp and Kohler, 1995; Verma *et al*., 1999).

Conclusion

In conclusion, present findings suggest that use of duckweed aquatic plants as a model system for assessing the sensitive and receptive towards water pollutants. Such a liquid-based system is especially well suited for high throughput screening experiments to identify the toxic chemicals present in the pollutants suppressed biomass production, metabolic activities and appeared visible symptoms of toxicity, as well as for the future identification of potential marker agents for the control of pathogenic bacteria present in sewage. Further, the performance of lab-scale study was found to be comparable with the results reported by others in field-scale duckweed pond which could affect a similar high reduction in all the above mentioned parameters. This model needs more detail research towards high-throughput qualitative as well as quantitative analysis due to the system is simple in terms of operation, easy handling,less maintenance and suitable for rural communities.

Acknowledgement

The author gratefully acknowledge to the head of the Zoology Department, Utkal University for necessary laboratory facilities. The author is also grateful to UGC, New Delhi for granting Emeritus Fellowship (20014-2016) to conduct this experiment.

REFERENCES

A.P.H.A. 1998. American Public Health Association Standard Methods for the Examination of Water and Waste Water, 20th Edition, Washington D.C.

A.P.H.A. 2005. American Public Health Association Standard methods for the examination of water & wastewater. 21st edition, Wasinton D.C.

Brain, R.A. and Solomon, K.R. 2007. A protocol for conducting 7-day daily renewal tests with Lemna gibba. *Nat Protoc* 2: 979–987.

Cayuela, M.L., Miliner, P., Slovin, J. and Roig, A. 2007. Duckweed (*Lemna gibba*) growth inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composing. Chemosphere 68: 1985-1991.

Culley, D.D., Gholson, J.H., Chisholm, T.S., Standifer, L.C. and Epps, E.A. 1978. Water quality renovation of animal waste lagoons utilizing aquatic plants. U.S. Env. Prot. Agency, Ada, Oklahoma, pp. 166.

El-Shafai**,** S. A., El-Gohary F. A., Nasr F. A., Peter van der Steen, N. and Gijzen H. J. 2007. Nutrient recovery from domestic wastewater using a UASB-duckweed ponds system. *Bio resource Technology* 98: 798–807.

EPA, 1975. Test methods for assessing the effect of chemicals on plants. In: R. Rubinstein and J. Smith (Eds.), Final Report U.S. Environmental Protection Agency, EPA-560, Washington D.C.

Fjalbong, B. and Dave, G. 2003. Toxicity of copper in sewage sludge. *Environ. Int.* 28, 761-769.

Forkas, A., Salanki, J. and Varanka, I. 2000. Heavy metals concentrations in fish of lake Balaton Lakes and Reservoirs. *Ros. Mange*. 5: 271-279.

Garg, P. and Chandra, P. 1994. The duckweed *Wolfia glubosa* as indicator of heavy metal pollution, Sensitivity to Cr and Cd. *Environ. Monil. Assess*. 29:89-95.

Harremoës, P. 1999. Water as a transport medium for waste out of towns. *Water Science and Technology*. 39: 1-8.

Huebert, D.B. and Shay, M. 1993. The response of *Lemna trisulea* L. to cadmium. *Environ. Pollut.* 80:247-353.

Lavnya, S., Ramesh, M., Kavitha, C. and Malarvizhi, A. 2011. Hematological, biochemical and ionoregulatory responses of Indian major carp *Catla catla* during chronic sublethal exposure to inorganic arsenic. *Chemosphere* 82(7): 977-85.

Lichtenthaler, H.K and Wellburn, A.R. 1983. Determination of chlorophyll-a and b of leave extracts in different solvents. *Biochem. Soc. Trans.* 11: 591-598.

Liu, J., Xiong, Z., Li, T. and Huang, H. 2004. Bioaccumulation and ecophysiological responses to copper stress in two populations of *Rumex dentatus* L. from Cu contaminated and non-contaminated sites. *Envon. Exp. Bot.* 52: 43-51.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein determination with Folin reagent. *J. Biol. Chem.* 193: 256-276.

Maine, M., Duarte, M. and Sune, N. 2001. Cadamium uptake by floating macrophytes. *Water Res*. 35: 2629-2634.

Mohan, B.S. and Hosetti, B.B. 2006. Phytotoxicity of cadmium on the physiological dynamics of *Salvinia natans* L. grown in macrophyte ponds. *J. Envon. Biol*. 27: 701-704.

Nelson, S.G., Smith, B.D. and Best, B.R. 1981. Kinetics of nitrate and ammonia uptake by the tropical fresh water macrophyte Pista stratiotes L. Aquaculture 24: 11–19.

Orun, G., Porath, D. And Jansen, H. 1987. Performance of duckweed species *Lemna gibba* on municipal wastewater effluent renovation and protein production. Biotech. *Bioeng.* XXIX, 258–268.

Pandey, S.N. 2006. Accumulation of heavy metals (Cd, Cr, Cu, Ni and Zn) in *Raphanus sativus*. *J. Environ. Biol*. 27: 381-384.

Phetsombat, S., Kruatrachue, M., Pokethitiyook, R. and Upatham, S. 2006. Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*. *J. Environ. Biol*. 27: 645-652.

Polprasert, C. 1996. Organic waste recycling – Technology and Management, 2nd edn., John Wiley, Chishecter.

Porath, D. and Pollock, J. 1982. Ammonia stripping by duckweed and its feasibility in circulating aquaculture. *Aquat. Bot*. 13: 125–131.

Prasad, M.N.V., Malec, P., Waloszek, A., Bajko, M. and Strzlka, K. 2001. Physiological responses of *Lemna trisulea* (duckweed) to cadmium and copper bioaccumulation. *Plant Sci*. 161: 881-889.

Sahu, R.K., Katiyar, S., Tiwari, J. and Kisku, G.C.2007. Assessment of drain water receiving effluent from tanneries and its impact on soil and plant with particular emphasis on bioaccumulation on heavy metals. *J. Enviorn. Biol*. 28: 685-690.

Scherr, C., Simon, M., Spranger, J. and Baumgartner, S. 2008. Test system stability and natural variability of a Lemna gibba L. bioassay. PLoS One 3: e3133.

Sen, A.K., Mondal, N.G.and Mondal, S. 1994. Toxic effects of chromium (VI) on the plants *Salvinia natans*. *Environ. Ecol*. 12:379-283.

Sharma, K., Chaturvedi, R.K., Bhardwaj, S.M. and Sharma, K.P. 2001. Heavy metals in vegetables and cereals growing around Sanganer town Jaipur, Rajasthan (India). *J. Ind. Bot. Soc*. 80: 103-108.

Singh, V.K.and Singh, J., 2006. Toxicity of industrial waste water to the aquatic plant *Lemna minor* L. *J. Environ. Biol*. 27:385-390.

Tremp, H. and Kohler, A. 1995. The usefulness of macrophyte-monitoring systems, exemplified on eutrophication and acidification of running waters. *Acta Bot. Gall*. 142: 541-550.

Trivedy, R.K., Khatavkar, S.D., Kulkarni, A.Y. and Shrotri, A.C. 1990. Ecology and pollution of the river Krishna in Maharashtra. *In*: River pollution in India, Ashish Publishing House, New Delhi, India, pp. 71-79.

Verma, Y., Hargan, M.C., Ruperelia, S.G. and Kulkarni, P.K. 1999. Toxicity testing of tannery effluents using duckweed bioassay. *Pollut. Res*. 18:497-500.

Verma, Y. 2007. Toxicity assessment of industrial effluents using duckweed bioassay. *J. Environ. Protect*. 27: 260-263.

Wang, W. 1990. Literature review on duckweed toxicity testing. *Environ. Res*. 52: 7-22.

Xiong, Z.T., Liu, C. and Zeng, B. 2006. Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. Ecotoxicol. *Environ. Saf*. 64: 273-260.

Zayed, A., Gowthaman, S. and Terry, N. 1998. Phytoaccumulation of trace elements by Wetland plants: I. Duckweed, *J. Environ. Qual*. 27:715-721.

Zhang, Y., Hu, Y., Yang, B., Ma, F. and Lu, P. 2010. Duckweed (Lemna minor) as a Model Plant System for the Study of Human Microbial Pathogenesis. PLoS ONE 5(10): e13527.

Zimmo, O.R., Al-Sa'ed, R.M., Van der Steen, N.P. and Gijzen, H.J. 2000. Process performance assessment of algae-based and duckweed-based wastewater treatment systems. Proceedings of the First Latin American Conference on Waste Stablisation Ponds. Cali, Colombia, 24-27.

****