ISSN: 0974-4908
http:/rels.seplko.co.in

Res. Environ. Life Sci.
1(1) 5-8 (2008)
rel_sci@yahoo.com

Responses of *Lemna minor* L. (duckweed) plants to the pollutants in industrial waste water

Kavita Singh[1], Ajai Misra[2] and S.N. Pandey[1*]
*Department of Botany, University of Lucknow, Lucknow-226007,India
**Department of Geology, University of Lucknow, Lucknow-226007, India
***e-mail:** snpandey511@gmail.com

Abstract: The duckweed (*L. minor)* plants were treated with industrial waste water. The waste water was collected from common effluent treatment plant, Unnao, UP state, India). The pollutants analyzed in waste water, showed high values of BOD, COD, total solids and total dissolved solids. It also contained various heavy metals *i.e.,* Cr (1.64), Cu (0.16), Fe (0.27), Ni (1.33) and Zn (0.35) mgL^{-1}. The industrial waste water reduced biomass of plants by 50%. The chlorophyll (a, b and total) and biochemical activities (protein, amylase and catalase) were also suppressed by industrial waste water, observed at 7^{th} day of exposure. Chlorophyll b, protein and catalase activity were found stimulatory at 25% diluted level of waste water. The activity of amylase decreased with increase in concentration of waste water. Visible symptoms of toxicity appeared in *L .minor* plants, as upper marginal yellowing of leaf converted into necrotic area and wilting and drying of plants. The mortality of 50% plants observed in duckweed plants at 100% supply of waste water at 7^{th} day. Any visible symptoms were not appeared in duckweed plants at control and 25% waste water supply.

Key words: Pollutants, Industrial waste water, *Lemna minor* L.,Heavy metals

Introduction

The discharge of industrial effluents into the aquatic bodies causes heavy metals toxicity in living organisms. The biomagnification of toxic chemicals / metals in food web are of the great environmental problems in the world today. A huge amount of industrial effluents and domestic sewage are discharged into surface water bodies, with or without proper treatment which contains heavy metals and toxic chemicals (Trivedy *et al.*, 1990; Kaushik *et al.*, 2001). These discharged pollutants become dangerous to aquatic life (Forkas *et al.*, 2000; Fjalborg and Dave, 2003). Occurrence of toxic metals in fresh water bodies affect the people that depend upon these water sources for their daily requirements (Rai *et al.*, 2002; Ayas *et al.*, 2007). For the regulation of quality of industrial effluents, various technologies are available to remove toxic metals from water: ion exchange, reverse osmosis, electrolysis and bioremediation through hyper accumulator plants *etc*.

The toxicity test through some macrophytes, as bioindicator of toxic pollutants may be helpful in monitoring the pollution level in aquatic system. Recently, duckweed of family Lemnaceae, a floating fast-growing plant, small and easy to cultivate, used for toxicity testing of pollutants in waste waters (Wang, 1992; Barber *et al.*, 1995; Verma, 2007). The aquatic plant, *Lemna minor* present a high growth rate, have been used for removal of heavy metals from polluted water bodies (Maine *et al.*, 2001; Cardwell *et al.*, 2002). It has been demonstrated that, aquatic plants accumulate heavy metals (Out ridge and Noller, 1991; Tremp and Kohler, 1995), and produces phytotoxic effects on plants resulting in inhibition of biomass production (Pandey, 2004; Drost *et al.*, 2007). More than 25 numbers of tanneries and other industries discharging their effluents into a common drain after treatment in common effluent treatment plant (CETP), Unnao, UP, India. This waste water used for irrigational purposes on nearby areas of drain, about 2 Km of its length and finally discharged into Sai river, a significant tributary of river Gomti.

Present study describes the toxicity responses of *Lemna minor* (duckweed) to pollutants present in industrial waste water with respect to growth inhibition, biochemical responses and visible symptoms.

Materials and Methods

The waste waters collected at 15 consecutive days from drain of the common effluent treatment plant (CETP), Unnao, UP state, India during summer period (April-June) in the year 2007, stored and analyzed by the standard methods (APHA, 2005). These waste waters pool out to make a composite sample, used as a growth medium for *L. minor* plants. The duckweed *(L. minor)* was collected from fresh water ponds and cultured in Hoagland's nutrient medium. The healthy plants were used in the test, after one week of culturing (EPA, 1975). Experiment was performed on known weight basis in 250 ml glass beaker with 150 ml of waste water and control (distilled water). The *Lemna minor* L. plants were exposed to distilled water (control) and graded industrial waste water (25, 50 and 100%). Experiments were performed in glass house at temperature $28^0C \pm 1^0C$ in bright sunlight (day time). Plants were harvested at seven days of exposure for dry matter yield; chlorophyll and protein contents and enzymes activities (amylase and catalase). Visible symptoms appeared on plants observed regularly. Chlorophyll content was determined by the method of Lichtenthaler and Wellburn (1983). Protein was estimated by the method of Lowry *et al.* (1951). The enzymatic activity *i.e.,* amylase by the method of Katsuni and Fekuhara (1969) and catalase activity by Euller and Josephson (1959) were estimated. All the data presented in the table are mean of three replicates ± SE value. The student 't' test described by

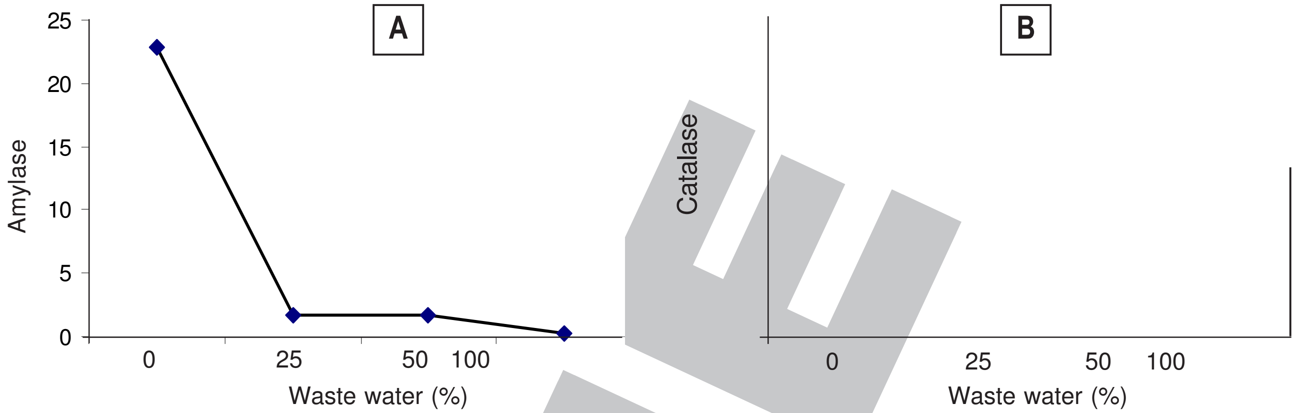

Fig. 1: Effect of industrial waste water on (A) amylase (mg starch hydrolysed g^{-1} fresh weight) (B) catalase activity (ml H_2O_2 Hydrolysed g^{-1} fresh weight) in *L. minor* plants

Fisher (1950) was employed to calculate the statistical significance values.

Results and Discussion

The composite sample of industrial waste waters collected from drain of the common effluent treatment plant (CETP), Unnao, quantified for their some significant pollution parameters and potentially toxic heavy metals (Table 1). The pollutants level in industrial waste water was indicated its higher pollution values. Also high concentrations of heavy metals were measured (Zn, 0.35; Cu, 0.16; Cr, 1.64; Ni, 1.33 and Fe, 0.27). These values indicated high level of Cr and Ni in waste water. The duckweed (*Lemna minor* L.) plants showed inhibitory effect on dry matter yield when treated with high concentrations of waste water (50 and 100%). While, increased 33.3% at diluted concentration (25%) of waste water over control. The reduction in biomass of test plants by polluted water was possibly due to, high Ni and Cr content in growth medium (Garg and Chandra, 1994; Pandey *et al.*, 2002; Rahaman *et al.*, 2005; Pandey, 2006). The presence of toxic chemicals and heavy metals in aquatic bodies due to discharge of industrial effluents, accumulate in the aquatic plants and cause toxicity effects (Rai and Raizada, 1989; Huebert and Shay, 1993; Sen *et al.*, 1994). Ultimately, transfer into food web, when consumed by various living organisms (Sharma *et al.*, 2001; Sahu *et al.*, 2007).

Some biochemical parameters showed inhibitory effect in *L. minor* at high concentration of waste water (50 and 100%), however, increased at low level (25%). Industrial waste water inhibited the chlorophyll and protein content (Table 2). The chlorophyll a and total contents was decreased by 67.2 and 48.6% in *L. minor* when treated with waste water (100%) as compared to control. Some biochemical parameters (chlorophyll b and protein) increased at low concentrations then gradually decreased with increase in concentration of waste water. Chlorophyll is an important pigment of the photosynthetic activity for primary productivity. Many studies have demonstrated influences of heavy metals on chlorophyll and protein contents in higher plants (Prasad *et al.*, 2001; Xiong *et al.*, 2006; Zengin and Kirbag, 2007). Heavy metals inhibit uptake and transportation of other metal elements such as Fe, Zn and Mn by antagonistic effects and therefore, plants lose the capacity of synthesis of pigments (Lin and Wu, 1994; Liu *et al.*, 2004). The activities of catalase and amylase enzymes significantly reduced by exposure of waste water over control in *L. minor*. The catalase activity was significantly increased when treated with waste waters at diluted concentrations (25 and 50%) and decreased 40.3%) at 100% concentration in test plants when compared at 50% dilution. The results supported sensitivity of *L. minor* to toxic heavy metals and pollutants, when present in aquatic bodies as reported by other workers (Mohan and Hosetti (1997), Cayuela *et al.* (2007), Singh and Singh (2006).The tissue of aquatic plants accumulate heavy metals (Wang, 1990; Maine *et al.*, 2001; Phetsombat *et al.*, 2006), by which, used for phytoextraction of these toxic metals (Zayed *et al.*, 1998; Miretzky *et al.*, 2004). Some macrophytes also used as test plants for indicator of aquatic pollutants (Wang, 1986; Verma

Table - 1: Evaluation of pollutants in Industrial waste water from drain of the common effluent treatment plant (CETP), Unnao

Parameters	Average value
pH	07.24 ± 0.01
Electrical conductivity (mmhos/cm)	12.10 ± 0.71
Total solids (mgL^{-1})	7970.00 ±15.50
Total dissolved solid (mgL^{-1})	7840.00 ± 20.11
Phosphate (mgL^{-1})	12.6 ± 1.2
Ca (mgL^{-1})	156.0 ± 8.3
BOD (mgL^{-1})	134.0 ± 1.0
COD (mgL^{-1})	288.0 ± 5.8
Cu (mgL^{-1})	0.16 ± 0.05
Cr (mgL^{-1})	1.64 ± 0.01
Ni (mgL^{-1})	1.33 ± 0.02
Zn (mgL^{-1})	0.35 ± 0.10
Fe (mgL^{-1})	0.27 ± 0.05

Values are mean of three replicates, ± SE (n=3), BOD- Biological oxygen demand,COD- Chemical oxygen demand

Table - 2: Effect of industrial waste water on biomass, chlorophyll a, b and total (mg g^{-1} fresh weight) and protein (µg g^{-1} fresh weight) content in *Lemna minor* L. plants

Waste water concentration (%)	Dry matter yield (g)	Chlorophyll			Protein
		a	b	Total	
0 (Control)	0.006 ± 0.001	1.22 ± 0.01	0.43 ± 0.05	1.46 ± 0.36	65.22 ± 2.1
25	0.008 ± 0.001	1.20 ± 0.01	0.64 ± 0.02	2.33 ± 0.35	82.91 ± 2.9**
50	0.007 ± 0.001	1.02 ± 0.01	0.49 ± 0.01	1.72 ± 0.01	97.43 ± 2.1*
100	0.004 ± 0.001	0.40 ± 0.12	0.18 ± 0.01	0.75 ± 0.05	42.78 ± 2.5**

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

et al., 1999). Visible symptoms of toxicity appeared in *L. minor* as: necrosis followed by chlorosis; wilting and drying of some plants; about 50% mortality of test plants treated with undiluted waste water at day 7. Our findings suggest that, *L. minor* (duckweed) plants are very sensitive towards aquatic pollutants, especially those, having elevated level of Cr and Ni. The pollutants including heavy metals in waters, suppressed biomass production, metabolic activities and appeared visible symptoms of toxicity as mentioned above. More studies are required to develop *L. minor* as test plants for polluted aquatic bodies.

References

APHA: Standard methods for examination of water and waste water, 21st Edition, Washington, D.C. (2005).

Ayas, Z., G. Ekmekci, S.V. Yerli and M. Ozmen: Heavy metal accumulation in water, sediments and fishes of Nallihan Bird Paradise,Turkey. *J. Environ. Biol.,* **28**: 545 – 549 (2007)

Barber, J. T., H.A. Sharma, H.E.Ensley, M.A. Polito and D.A. Thomas : Detoxification of phenol by the aquatic angiosperm *Lemna gibba. Chemosphere,* **31**: 3567-3574 (1995).

Cardwell, A.,D. Hawker and M.Greenway: Metal accumulation in aquatic macrophytes from southeast Qeensland, Australia. *Chemosphere,* **48**: 653-663 (2002).

Cayuela, M. L., P. Millner, J. Slovin and A. Roig: Duck weed (*Lemna gibba)* growth Inhibition bioassay for evaluating the toxicity of olive mill wastes before and during composting. *Chemosphere*, **68:**1985-1991 (2007).

Drost, W., M. Matzke and T. Backhans : Heavy metal toxicity to *Lemna minor*: Studies On the time dependence of growth inhibition and the recovery after exposure. *Chemosphere*, **67:** 36 – 43 (2007).

EPA: Test methods for assessing the effect of chemicals on plants. *In*: R. Rubinstein and J. Smith (Eds.) Final Report U.S. Environmental Protetection Agency. EPA- 560/ 5-75-008. Washington D.C. (1975).

Eulller, H. Von and K.Son Josephson: Method Uber Katalani I Liebigs Anon catalase activity. *Annals of Botany*, **452**: 158-184 (1959).

Fisher, R.A.: Statistical methods for research works. 11th Eds., Oliver and Boyd, Edinburgh (1950).

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

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

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

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

Katsuni, M. and M. Fekuhara: The activity of amylase in shoot and its relation to induced elongation. *Physiol. Plan.*, **22**: 68-75 (1969).

Kaushik, A.,S. Jain,J.Dawra R. Sahu and C. P. Kaushik: Heavy metal pollution of river Yamuna in the industrially developing state of Haryana (India). *Indian J. Environ. Hlth.*, **43**: 164-168 (2001).

Lichtenthaler, H. K. and A.R. Well Burn: Determination of chlorophyll a and b of leaf extracts in different solvents. *Biochem. Soc., Trans*. **11**: 591-598 (1983).

Lin, S. and L. Wu: Effects of copper concentration on mineral nutrient uptake and copper accumulation in protein of copper tolerant and non-tolerant *Lotus purshianus*. *Ecotoxicol. Environ. Saf.*, **29**: 214 - 228 (1994).

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

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein determination with Folin reagent. *J. Biol. Chem*., **193**: 265-276 (1951).

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

Miretzky, P., A. Saralegui and A. Cerelli: Aquatic macrophytes potential for the simultaneous removal of heavy metals. *Chemosphere*, **57**: 997 -1005 (2004).

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

Outridge, P. and B. Noller: Accumulation of toxic trace elements by freshwater vascular plants. *Rev. Environ. Contam. Toxicol*., **121:** 2-63 (1991).

Pandey, A. K., S. Dutta and K.C. Sharma: Impact of marble slurry on subsurface water – A case study on Kishangarh, disst. Ajmer. *Nature Environ. Pollut. Technol*., **1**: 5-11 (2002).

Pandey, S. N.: Accumulation of heavy metals (Cd, Cr, Cu, Ni and Zn) in *Raphanus sativus* L. and *Spinacia oleracea* L. plants irrigatiated with industrial effluent. *J. Environ. Biol*., **27**: 381-384 (2006).

Pandey, S. N.: Industrial effluent and its effect on seed germination and seedling growth of *Zea mays* Linn. and *Oryza sativa* Linn. *Biol. Memoirs*, **30**: 104 -107 (2004).

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

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

Rahaman, H., S. Sarbreen and S. Kawri: Effect of nickel on growth and composition of metal micronutrient in barley plants grown in nutrient solution. *J. Plant Nutrition,* **28**: 393-404 (2005).

Rai, L.C. and M. Raizada: Effect of bimetallic combination of Ni, Cr and Pb on growth, uptake of nitrate and ammonia, C-4 fixation and nitrogenase activity of *Nostock muscorum*. *Ecotoxicol. Environ. Saf.*, **17**: 75-85 (1989).

Rai, U.N., R.D.Tripathi, P. Vajpayee, Jha Vidyanath and M.B Ali: Bioaccumulation of toxic metals (Cr, Cd, Pb and Cu) by seeds of *Euryale ferox* Salisb (Makhana). *Chemosphere*, **46**: 267-272 (2002).

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

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

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

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

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

Trivedy, R. K., S.D. Khatavkar, A.Y. Kulkarni and A.C. Shrotri: Ecology and pollution of the river Krishna in Maharashtra. *In*: River pollution in India. Ashish Publishing House, New Delhi, India. pp. 71-97 (1990).

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

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

Wang, W.: Toxicity tests of aquatic pollutant by using common duck weed. *Envrion. Pollut*., **11**: 1-14 (1986).

Wang, W.: Use of plants for the assessment of environmental contaminants. *Rev. Environ. Contam. Toxicol*., **126**: 87-127 (1992).

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

Xiong, Z. T., C. Liu and B. Zeng: Phytotoxic effects of copper on nitrogen metabolism and plant growth in *Brassica pekinensis* Rupr. *Ecotoxicol. Environ. Saf*., **64**: 273 -280 (2006).

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

Zengin, F. K. and S. Kirbag: Effects of copper on proline, protein and abscisic acid level of sunflower (*Helianthus annus* L.) seedlings. *J. Environ. Biol*., **28**: 561–566 (2006).