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

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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 7th 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 7th 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 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°C ± 1°C 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 Fekuhra (1969) and catalase activity by Euler 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
The level of Cr and Ni in waste water. The duckweed (Lemna minor) showed inhibitory effect on dry matter yield when treated with waste water (100%) as compared to control. Some biochemical parameters (chlorophyll b and protein) and pigment of the photosynthetic activity for primary productivity. Many workers (Mohan and Hosetti, 1997), Cayuela et al., 2006), Phetsombat et al., 2001; Xiong et al., 2007), Singh et al., 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 et al., 2008).

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., 1993; Sen et al., 1994). Ultimately, transfer into food web, when consumed by various living organisms (Sharma et al., 2001; Sahu et al., 2007).

Fig. 1: Effect of industrial waste water on (A) amylase (mg starch hydrolysed g⁻¹ fresh weight) (B) catalase activity (ml H₂O₂ Hydrolysed g⁻¹ fresh weight) in L. minor plants

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average value</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>07.24 ± 0.01</td>
</tr>
<tr>
<td>Electrical conductivity (mmhos/cm)</td>
<td>12.10 ± 0.71</td>
</tr>
<tr>
<td>Total solids (mgL⁻¹)</td>
<td>7970.00 ± 15.50</td>
</tr>
<tr>
<td>Total dissolved solid (mgL⁻¹)</td>
<td>7840.00 ± 20.11</td>
</tr>
<tr>
<td>Phosphate (mgL⁻¹)</td>
<td>12.6 ± 1.2</td>
</tr>
<tr>
<td>Ca (mgL⁻¹)</td>
<td>156.0 ± 8.3</td>
</tr>
<tr>
<td>BOD (mgL⁻¹)</td>
<td>134.0 ± 10.0</td>
</tr>
<tr>
<td>COD (mgL⁻¹)</td>
<td>288.0 ± 5.8</td>
</tr>
<tr>
<td>Cu (mgL⁻¹)</td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td>Cr (mgL⁻¹)</td>
<td>1.64 ± 0.01</td>
</tr>
<tr>
<td>Ni (mgL⁻¹)</td>
<td>1.33 ± 0.02</td>
</tr>
<tr>
<td>Zn (mgL⁻¹)</td>
<td>0.35 ± 0.10</td>
</tr>
<tr>
<td>Fe (mgL⁻¹)</td>
<td>0.27 ± 0.05</td>
</tr>
</tbody>
</table>

Values are mean of three replicates, ± SE (n=3), BOD- Biological oxygen demand, COD- Chemical oxygen demand 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 et al., 2008).
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


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**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 Lemma minor L. plants

<table>
<thead>
<tr>
<th>Waste water concentration (%)</th>
<th>Dry matter yield (g)</th>
<th>Chlorophyll</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>0 (Control)</td>
<td>0.006 ± 0.001</td>
<td>1.22 ± 0.01</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>25</td>
<td>0.008 ± 0.001</td>
<td>1.20 ± 0.01</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td>50</td>
<td>0.007 ± 0.001</td>
<td>1.02 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>100</td>
<td>0.004 ± 0.001</td>
<td>0.40 ± 0.12</td>
<td>0.18 ± 0.01</td>
</tr>
</tbody>
</table>

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


