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REGULAR ARTICLE

BIOCHEMICAL ALTERATIONS DUE TO ACUTE TANNERY EFFLUENT TOXICITY IN *LEMNA MINOR* L.

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SUMMARY

The effect of acute tannery effluent (TE) toxicity on some biochemical parameters in *Lemna minor* L. was studied using different TE concentrations i.e. 0 to 50.0% (v/V) in modified Hoagland's solution and exposure durations of 48 and 96 hours. The *L. minor* plants failed to survive at TE levels beyond 25.0%. The photosynthate (starch) level increased above the control up to the 10.0% TE level, at higher concentrations it decreased to a level below the control and was comparatively higher at 96-hour exposure. Reducing sugar content at 48 hours did not show a clear trend but at 96 hours it followed a clear increasing trend up to 5.0% effluent level and subsequent concentrations showed decrease in reducing sugars. In the case of total soluble sugars at 48 hours, there was an increase up to 10.0% effluent after which it started decreasing till 25.0% level. However, at 96-hour exposure, total soluble sugars were maximum in control and showed a steady decreasing trend with increasing TE concentrations. The soluble proteins increased and were higher than the control at 48 hour exposure. However, 96 hour exposure to 20.0 and 25.0% effluent concentration revealed a marked decrease in soluble protein content. Total free amino acid content followed the trend observed with soluble protein up to 20.0% effluent level, after which their content decreased markedly. The acid phosphatase activity was higher at 48-hour exposure in comparison to 96 hour and showed an increasing trend with increasing effluent concentration at both the exposures. These biochemical constituents can be used as indices for measuring the phytotoxicity and understanding the mechanism and the level of tolerance to tannery effluent in *L. minor*.

Keywords: Acute toxicity, Biochemical Parameters, *Lemna minor*, Tannery effluent.

Nand Lal. Biochemical Alterations Due to Acute Tannery Effluent Toxicity in *Lemna minor* L.. J Phytol 1 (2009) 361-368
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1. Introduction

Tannery effluent (TE) is one of the most complex effluents containing a number of pollutants (Sastry and Madhavakrishna, 1984; Sujatha et al. 1996) and poses great threat to ecosystems by exerting detrimental (direct toxicity, genotoxicity, mutagenicity) effects and disrupts the food chain by reduction in the structure and productivity of aquatic flora (Koteshwari and Ramnibai, 2004). TE contains vegetable tannins in addition to soluble organic matter, suspended solids, chromium, sodium, high chloride and sulphate concentration with a very high pH, high BOD, COD and conductivity (Rao and Kumar, 1981). Some of the salts in the

effluent are nutritious and some are toxic to plant growth. Tannery effluent is highly toxic to animals (Rao and Marriyappan, 1972; Saktivel, 1989), plants (Muthukumar and Mahadevan, 1981) and a variety of microorganisms (Mahadevan et al. 1984). Studies on bioremediation of TE by aquatic macrophytes were carried out by Vajpaye et al. (1995) and Sinha et al. (2002) in which these plants were proved to have the ability to reduce the level of toxic metals from the polluted water.

Decrease in chlorophyll content in *Cicer arietinum* L. seedlings has been observed at higher concentrations (25-100%) of tannery effluent but 5-10% effluent concentration enhanced the total chlorophyll (Rao and Kumar, 1983). Higher effluent levels delayed germination and decreased root and shoot length.

Kumar and Chachan (1993) studied reduction in root and shoot number and their growth in *Allium cepa* due to effect of TE. Madhappan (l993) observed that undiluted TE (beyond 50%) had a toxic effect on germination and growth of *Phaseolus mungo* L. and *P. aureus* L. whereas, 25% effluent concentration had a growth promotion effect. There existed a significant positive relationship between total chlorophyll content and lower effluent concentration. Considerable information is available on the effects of TE pollution on terrestrial flora (crop plants) and water animals, but information on the effect of TE on aquatic flora is scanty. Little quantum work has been done on the effect of TE in relation to biochemical constituents of plants.

In the present work, the toxicity of TE on *Lemna minor* L. was studied using different TE concentrations i.e. 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0 and 50.0% (v/V) for the estimation of biochemical parameters namely, total soluble protein, total free amino acid, starch, total soluble sugar, reducing sugar and acid phosphatase activity.

2. Materials and Methods

The TE used during the experiments was collected in a plastic container from the open-outfall drain of the leather industry, at the mixing point with the Ganges River, at Jajmau, Kanpur (U.P.). TE was analyzed for various Physico-chemical properties (Table 1) as per the method described by APHA, AWWA and WPCF (1975) in water analysis lab at Industrial Toxicology Research Centre, Lucknow. *L. minor* plants were collected from Aquatic Botany Division, N.B.R.I., Lucknow and their cultures were maintained at 20±2°C under 16 hour photoperiod provided by cool, white fluorescent light (2000 lux) using modified Hoagland's nutrient solution (EPA, 1975).

Toxicity tests were conducted in a series of 250 ml beakers, each containing 100 ml of test solution (nutrient solution + TE + distilled water). Each treatment was replicated to eight, 4 replicates for 48 hour and 4 replicates for 96-hour exposure duration. Hundred plants of *L. minor* of the same size from laboratory stock cultures were transferred into each beaker containing different TE concentrations. Plants harvested from different treatments were used for various biochemical estimations.

Harvested plants (separately from each treatment) were divided into two groups and weighed. Plants of first group were homogenized with dH_2O in potter Elvehjem homogenizer in chilled cold condition at the medium speed for 2-3 minutes. This homogenate was used for estimation of total soluble protein,

total free amino acid and acid phosphatase activity. The remaining weighed plants of the second group (separately from each treatment) were homogenized in 80% ethanol in potter Elvehjem homogenizer at full speed for 2-3 minutes. This homogenate was used for estimation of total soluble sugar, reducing sugar and starch content.

Protein was precipitated by adding equal volume of 10% TCA to water homogenate and the solution was centrifuged at 2000 rpm for 10 minutes. The residue was dissolved in 0.1 N NaOH and the protein content were estimated by the method of Lowry et al. (1951). The content of free amino acid was estimated according to the method of Lee and Takahashi (1966). Homogenate in 80% ethanol was centrifuged at 3000 rpm for 15 minutes and the residue obtained from this homogenate + 4 ml of dH2O was heated for 15 minutes in a water bath and macerated with a glass rod. Then 3 ml of 52% perchloric acid (PCA) was added to each sample. The mixture was centrifuged at 2000 rpm for 15 minutes. The content of starch and total soluble sugar was estimated by the method of Montgomery (1957). From 80% ethanol homogenate of plants, the content of reducing sugar was estimated according to the method of Nelson (1944). The Acid phosphatase activity was estimated in μmole/mg of protein/min according to the method of Wooton (1964) and one unit of enzyme activity refers to liberation of 1.0 μM phosphate/mg of protein/min.

Each treatment was carried out in four replicates for each parameter and the recorded results are the arithmetic mean. Data were statistically analyzed using one way analysis of variance on the basis of which LSD values (P=0.05) for any two compared means were calculated.

Table 1: Physico-chemical characteristics of tannery effluent used for toxicity effects in *L. minor*

Parameter	Values in mg/l (Except pH)
Colour	Blackish and highly turbid
Odour	Pungent smell
pH	8.59
Suspended solids	2967.6
Dissolved solids	4683.6
Total solids	7651.2
Volatile solids	1440.0
Fixed solids	6211.0
Oil and grease	3604.0
BOD	330.0
COD	928.0
Total nitrogen	593.6
Chloride	2500.0
Sulphate	2025.0
Nitrate	N.D.
Fluoride	0.93
Phosphate	0.038
Chromium	N.D.
Phenol	784.3*

N.D.- Not detected, *- Phenol expressed in μg/l

3. Results

The *L. minor* plants could grow in the nutrient medium containing TE concentration up to 25.0% (v/V) beyond which TE levels resulted in mortality of plants. TE at 50.0% (v/V) caused 100% mortality of plants within 48 hours whereas low levels i.e. 30.0 and 40.0% resulted in 100% mortality after 72 hours. The results indicate that TE treatment shows a concentration dependent time requirement to attain mortality in *L. minor*. The soluble protein content was 15.87 mg/g in control as well as in 0.5% TE level. The soluble protein level gradually increased with increase in TE concentration in the nutrient medium at the end of 48 hours (Figure 1). Up to the 20.0% TE level, there was a slow increase in TSP whereas at 25.0% TE level a rapid increase in

TSP was observed and attained the maximum value (25.75 mg/g). After a 96 hours exposure period, the TSP level of control was 15.30 mg/g, which was approximately the same as in control at the 48 hours exposure. The value of TSP content gradually increased up to the 10.0% TE level. Further enhancement of TE concentration showed gradual decrease in TSP content to 20.81, 14.47 and 14.29 mg/g in media with 15.0, 20.0 and 25.0% TE, respectively. The minimum protein content was at 25.0% TE concentration (14.29 mg/g), which was less than the control of 48 and 96-hour exposure duration.

The free amino acid content of control was 0.90 mg/g. At 0.5% TE concentration, free amino acid content decreased to 0.55 mg/g. However, the free amino acid content increased gradually to 0.87, 0.98, 0.98, 1.23, 1.35 and 2.13 mg/g in media with 2.5, 5.0, 10.0, 15.0 and 20.0% TE concentration, respectively (Figure 2). At 25.0% TE concentration, free amino acid content showed a rapid and drastic decrease and reached a minimum value of 0.18 mg/g. The free amino acid content of the control at the 96 hours exposure duration was 2.75 mg/g, which was more than thrice the value observed with the control of 48 hours exposure. The free amino acid content decreased to 1.38 mg/g at 0.5% TE. Further increase in TE concentration in the growth medium resulted in gradual increase of free amino acid content and reached a maximum of 5.42 mg/g at 20.0% TE concentration.

At 48 hour TE exposure, the starch content at control and 0.5% TE concentration were almost the same (Table 2). Above 0.5% TE, the starch content gradually increased and reached a maximum (5.80 mg/g) at 10.0% TE as is evident from the data. Subsequent enhancement of TE concentration in culture medium showed gradual and significant decrease in starch content and gone to minimum (2.49 mg/g) at 25.0% TE level.

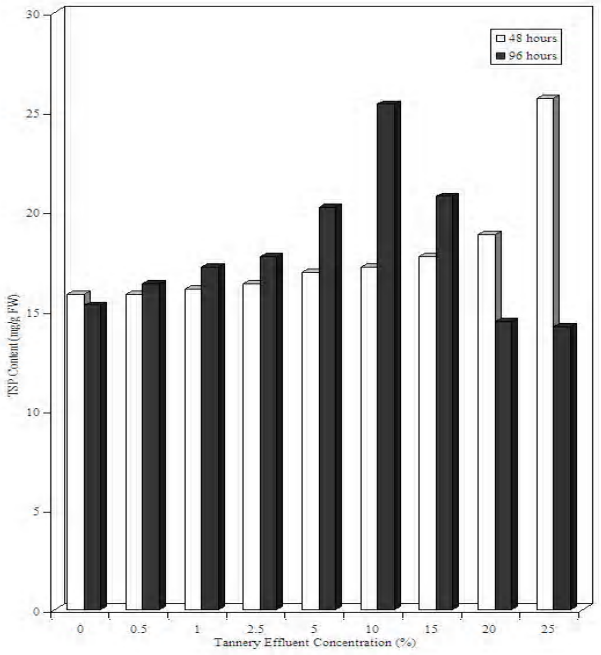

Figure 1. Total soluble protein (TSP) content of *L. minor* plants treated with different concentration of tannery effluents for 48 and 96 hours (LSD at P=0.05 = 1.42 at 48 hours and 1.61 at 96 hours)

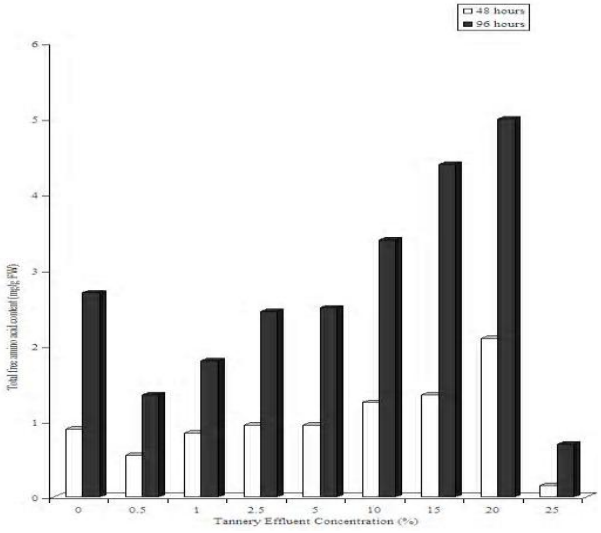

Figure 2. Total free amino acid content of *L. minor* plants treated with different concentration of tannery effluents for 48 and 96 hours (LSD at P=0.05 = 0.08 at 48 hours and 0.18 at 86 hours)

At 96-hour exposure, the control had the starch content of 5.83 mg/g. The starch content

of plants up to 5.0% TE remained unchanged and was approximately same as in control (Table 2). At 10.0% TE, starch content was found to be maximum (6.48 mg/g), however, the starch content gradually decreased at 20.0 and 25.0% TE level in comparison to control and 10.0% TE and reached to minimum (4.54 mg/g). Comparison of starch content at 48 and 96 hours exposure in different treatments revealed increase in its amount with increase in exposure time but this increase was to different degrees on media with 2.5% to 25.0% TE (lowest in media with 10.0% TE).

Table 2: Contents of Starch, soluble sugars and reducing sugars (as mg/g FW) in *L. minor* plants treated with different concentrations of tannery effluent for 48 and 96 hours

TE level (% v/V)	48 hour exposure			96 hour exposure		
	Starch	Soluble sugars	Reducing sugars	Starch	Soluble sugars	Reducing sugars
0 (control)	3.49	1.51	1.80	5.83	1.67	2.32
0.5	3.48	1.66	1.97	5.83	1.43	3.03
1.0	3.55	1.69	2.23	5.85	1.43	3.79
2.5	3.61	1.70	2.81	5.90	1.19	3.79
5.0	4.35	1.88	3.48	5.90	1.19	4.04
10.0	5.80	1.86	3.78	6.48	1.15	3.43
15.0	3.36	1.82	3.32	5.19	1.09	3.32
20.0	2.90	1.54	2.55	5.19	0.95	3.23
25.0	2.49	1.51	1.60	4.54	0.95	2.83
LSD at P=0.05	0.21	0.09	0.11	0.40	0.08	0.14

In case of 48-hour exposure, the free sugar content at control was 1.51 mg/g. As the TE concentration in medium increased, the free sugar content also increased gradually and reached to 1.66, 1.69, 1.70 and 1.88 mg/g at 0.5, 1.0, 2.5 and 5.0% concentration of TE, respectively. Subsequent increase (above 5.0%) in TE concentration caused decrease in free sugars and reached to minimum (1.51 mg/g) at 25.0% TE concentration. This soluble sugar amount was equal to amount observed at control (Table 2). At 96 hour, the free sugar content in control was 1.67 mg/g which was slightly more than free sugar content observed at control of 48 hours exposure, The inclusion of TE at different concentration in culture medium showed gradual decrease in the free sugar content of plant tissues and reached to the minimum (0.95 mg/g) at 20.0% TE concentration (Table 2).

During 48 hours exposure time, the reducing sugar content at control was 1.80 mg/g. As TE concentration increased, the reducing sugar content also increased gradually and reached to maximum (3.78 mg/g) at 10.0% TE concentration (Table 2). However, on further enhancement (above 10.0%) of TE concentration, reducing sugar content decreased and reached to minimum (l.60 mg/g) at 25.0%. TE. In 96 hours, the reducing sugar content at control was 2.32 mg/g, which was least among all the treatments. As TE % increased, the reducing sugar content also increased gradually and was found to be maximum (4.04 mg/g) at 5.0% TE concentration. Further increase in TE concentration showed

gradual decrease in the reducing sugar content and went down to 3.43, 3.23, 3.32 and 2.83 mg/g when treated with 10.0, 15.0, 20.0 and 25.0% concentration of TE, respectively.

Cultivation of *L. minor* plants on different TE level for 48 and 96 hours revealed higher acid phosphatase activity after 48 hours exposure and there was a general inhibition of acid phosphatase activity with increase in exposure time (Figure 3). In case of 48-hour exposure, the control showed 0.0925 units acid phosphatase activity. Acid phosphatase activity was differentially stimulated by different TE concentrations except 1.0% TE concentration, which resulted in marginal inhibition of enzyme activity (0.0895 units). Maximum stimulation of acid phosphatase activity was observed at 0.5% TE (13.33% over control) whereas minimum stimulation was observed on 10.0% TE containing media (3.25% over control).

In case of 96 hours exposure to different TE treatments, *L. minor* plants showed 0.0333 units acid phosphatase activity on TE-free medium. Inclusion of 25.0% TE concentration in medium caused maximum stimulation of enzyme activity (101.2% over control) whereas minimum stimulation was observed with 20.0% TE concentration (2.40% over control). None of the treatments in case of 96 hours exposure time had acid phosphatase activity less than control but TE-stimulated acid phosphatase activity followed a fluctuating trend (Figure 3).

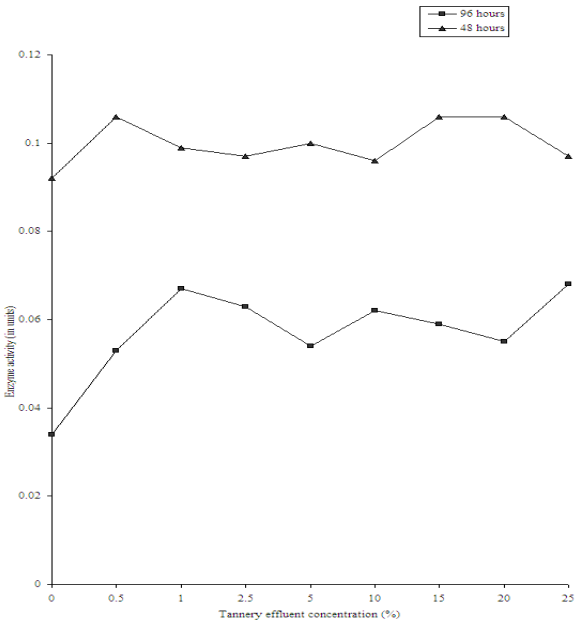

Figure 3. Acid Phosphatase activity in *L. minor* plants treated with different concentrations of Tannery Effluent for 48 and 96 hours (1 unit = 1µm phosphate liberated /mg of protein/min, LSD at P=0.05 = 0.008 at 48 hours and 0.011 at 96 hours)

4. Discussion

The TE contains a number of toxic constituents, but is rich source of nitrogen which aids to availability and assimilation of nitrogen by plant cells. The survival of *L. minor* plants was affected by higher TE concentrations.

The presence of TE induced changes in protein contents and these changes were highly concentration- and time-dependant in nature. Low TE and 48 hour exposure stimulated protein level whereas its opposite was true for high TE and 96 hour exposure. Similar effects of industrial effluent from a fertilizer plant on TSP content have been reported in Guar by Taghvi and Vora (1994) and tannery effluent in several aquatic plants (Sinha et al. 2002).

In case of free amino acids, there was a positive change till 20% TE, after which amino acids content decreased drastically. Positive change seems to be due to nitrogen component of TE whereas at 25% TE level, the toxic constituents, particularly chromium ions negatively affected amino acid level. Similar effect of Te and chromium ions on amino acid biosynthesis via inhibition of nitrate reductase and limiting reduced nitrogen availability is

reported in aquatic macrophytes (Sinha et al. 2002).

Lower concentrations of TE are known to enhance photosynthesis whereas high TE turns to be inhibitory (Borah and Yadav, 1996). In the present study, low TE favoured starch, free sugar and reducing sugar biosynthesis. Comparison of free soluble sugar content in different treatments of 48 and 96 hour exposures revealed decrease in soluble sugar content with increase in exposure time at all the levels of TE, however, in case of control the soluble sugar content showed a marginal increase with increase in exposure time. Since reducing sugars act as substrate for oxidative pathway, the extra energy requirement of plants under stress is fulfilled by a rapid increase in their level. This might be achieved either by not permitting the conversion of total sugars of dark reaction into starch or by enhancing the hydrolysis of starch into reducing sugars with increase in stress quantum.

Increased acid phosphatase activity is reported to help in availability of inorganic phosphate needed for new membrane biosynthesis, repairing of membrane damage and biosynthesis of nucleic acids/nucleoproteins as reported by Borah and Yadav (1996). When *L. minor* plants are treated with TE for long durations, the acid phosphatase activity goes down, which is an indication of decreased anabolic activity in the tissues. Thus, acid phosphatase activity in *L. minor* can be used as indices of phytotoxic effect of TE on cellular anabolic pathways.

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