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THE TOXIC EFFECT OF AN ANIONIC DETERGENT ON A ROOTED FLOATING MACROPHYTE

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ABSTRACT

In this study the toxic effect of a commonly used anionic synthetic detergent on the growth and biochemical characteristics of *Nymphoides indica*, a rooted floating macrophyte was evaluated for different concentrations. The effect was studied for a period of one month. The growth characteristics of the plant were assessed in terms of specific leaf area (SLA). Biochemical analysis was done for total chlorophyll content, total protein, membrane lipid peroxidation and electrolyte leakage from tissues. Chlorophyll Stability Index (CSI) and percent Membrane Injury (MI %) were also assessed. The results revealed that the anionic detergent inflicted adverse effects on all the parameters considered. Lipid peroxidation and electrolyte leakage serve as more relevant parameters for detergent induced stress.

KEY WORD: *Nymphoides indica*; LAS (linear alkylbenzene sulfonate); lipid peroxidation; electrolyte leakage; membrane injury.

INTRODUCTION

Anionic surfactants account for approximately 25-30% of the world's total production of synthetic surfactants and is used in domestic and industrial formulations (Temmink and Klapwijk, 2004). These anionic synthetic detergents containing linear alkyl benzene sulfonate (LAS) are disposed of primarily "down-the-drain" and its distribution is wide spread in fresh water ecosystems; and it can be present in sufficient concentrations in untreated waste water causing long term toxic effects in aquatic organisms (Ankley and Burkhard, 1992). The flora in aquatic systems represented by the algal and macrophytic species are the primary energy sources for aquatic ecosystems and play a significant role in recycling of nutrients and other chemical constituents of the system and hence detergent contamination can influence the balance of an aquatic system.

Surfactant toxicity is primarily a function of the ability of surfactants to adsorb and penetrate the cell membrane of aquatic inhabitants (Gullermina, 2000) and adversely affect metabolic pathways in aquatic flora (Chawla, 1987). Involvement of free radicals that disrupt metabolic pathways during stress is a well-documented phenomenon (Heath and Packer 1968; Kastori *et al.*, 1992). Free radicals and hydrogen peroxide stimulated production of reactive active oxygen species (ROS) such as super oxides, hydroxyl radicals and singlet oxygen stimulated lipid peroxidation damaging the phospholipid membrane of mitochondria and other organelles (Snell and Mullock, 1987) and if prolonged thereafter even denatured the proteins as studied by Stegeman *et al.*, 1991a.

Leaf survival is related to environmental stress (Chabot and Hicks, 1982) and specific leaf area (cm^2 *leaf area per gram dry weight*) as a measure of growth can be used in characterizing leaf morphology of a plant subjected to a particular change (Evans and Hughes, 1961). Chlorophyll, in photosynthetic plants is used as a sensitive parameter to study stress conditions due to a single environmental factor or to a combination of different environmental factors as stress induced disruption of the electron transport chain is considered as one of the most usual ways by which photosynthesis is affected

(Gonzalez *et al.,* 1998). Chlorophyll stability index (CSI), hence served as a reliable indicator of stress resistance since it exhibited an increase in tolerant plants and an index value of above 1.0 was indicative of higher tolerance of the species (Praderm *et al.,* 2003).

The relationship between pollutant sensitivity and lipid peroxidation clearly indicated that pollutants were highly responsible in stimulating lipid peroxidation (Dat *et al.,* 2000; Demiral and Turkan, 2005). Damage to living cells under stress conditions largely depend on the formation of ROS that interact with different macromolecules and the destruction of lipid membranes could amplify the cellular damage by formation of lipid hydro-peroxides and their toxic aldehyde degradation products (Reichheld, *et al.,* 1999). Several workers (De long and Steffen, 1997; Sinha *et al.,* 2005) by their studies confirmed that stress induced lipid peroxidation disrupted membrane functions and inflicted harmful effects on plant cells. Surfactants are known to alter the permeability of biological membranes to water and ions (Part *et al.,* 1985) and cellular damage due to surfactants occurred at membrane levels and promoted the inhibition of intercellular processes (Morreno-Garrido *et al.,* 2001.

In India, it is a common practice to discharge domestic sewage to natural waters without any treatment. The surfactants present in the sewage may get distributed between the water and sediment phases in the water body (Morreno-Garrido *et al.,* 2001). Studies indicate that LAS detergents possess the potential to impose an oxidative stress which leads to various physiological and biochemical responses including membrane damage, pigment bleaching (Fridovich, 1986), lipid peroxidation (Chen *et al.,* 2000) and consequently protein degradation (Jiang and Zhang, 2001). Hence it is important to develop stress assessment techniques for early warning of detergent induced stress and degradation for the effective management of aquatic systems.

The present study has been conceived with an aim to assess the toxic effects of a commonly used anionic synthetic detergent on *Nymphoides indica,* a rooted floating macrophyte found in wetland ecosystems of India.

MATERIALS AND METHODS

Plant material

Nymphoides indica (L) O.Ktze. [Family: Menyanthaceae]

The 'Water Snow Flake' (*Nymphoides indica*), an aquatic rhizomatous dicotyledonous herb was selected as the experimental plant. This perennial floating-leaved aquatic plant is seen to thrive well in shallow ponds, pools, ditches and flooded lowlands of India.

Healthy young plants were collected from a natural site and raised in 35 litre polypropylene tubs containing 5kg of topsoil collected from the site where the experimental plants were collected. The soil used was found to have a pH of 3.83, alkalinity 0.2 $mg\ l^{-1}$, acidity 4.0 $mg\ l^{-1}$, chloride 59.99 $mg\ l^{-1}$ and electrical conductivity 0.483 $mS\ cm^{-1}$. The procedures given by APHA (1998) were followed for analysis of soil parameters.

Experimental Method

Surf Excel Blue; a commonly used anionic synthetic detergent was selected for the study and concentrations of 0.1, 0.25. 0.5 and 1.0 $g\ l^{-1}$ were prepared. This was then added to the tubs containing the acclimatized ***Nymphoides indica*** plants. A control (0.0 $g\ l^{-1}$) was also maintained for comparison and the study was done for a period of 30 days.

The concentrations of the detergent to be administered was determined by a pilot study done considering the general survival capacity of the plants under different concentrations of the detergent. The LAS concentration in the water samples collected from the natural site was also determined to assess the levels of detergent contamination in the natural system from where the experimental plant was collected. Five sets of experiments were carried out for each concentration of the detergent and the mean values were taken.

Analysis of plant characteristics

For analysis the weighed plant parts (leaf and root) were processed. Analysis was carried out on day 1, 3, 5, 10, 15, 20, 25 and 30. The data obtained was tabulated, statistically analyzed and represented.

Growth was assessed in terms of specific leaf area. Biochemical analysis was done for total chlorophyll content, total protein, membrane lipid peroxidation and electrolyte leakage from tissues. The chlorophyll stability index and percent membrane injury were also determined.

Analysis of variance was applied for growth and biochemical changes to test the level of significance between the concentration of the stress applied and

duration of exposure.

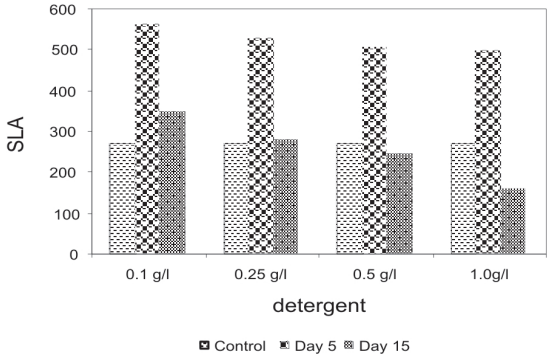
The specific leaf area was determined as per thew method suggested by Zhou and Qiu (2005). The leaf dry weight was determined as per the method of Weatherly (1970). Specific leaf area (SLA) was calculated and expressed in *cm^2 gm $^{-1}$ (dry wt.).* The chlorophyll content in the leaves was analyzed following the Arnon (1949) method and it was expressed in *mg g $^{-1}$ (fresh weight).* Chlorophyll stability index (CSI) was calculated from the total chlorophyll content as per the method suggested by Praderm *et al.* (2003). The method developed by Lowry *et al.* (1951) was followed for the estimation of total protein content in the leaf samples. Membrane lipid peroxidation was estimated in terms of malonedialdehyde (MDA) accumulation employing thiobarbeteuric acid (TBA) test of Heath and Packer (1968). The concentration of MDA was calculated using an extinction coefficient of 155 *mM^{-1} cm^{-1}*. For the study of electrolyte leakage (*mS g^{-1} fresh weight)* from tissues, the method outlined by Bhattacharjee *et al.* (1996), was adopted. The percentage of membrane injury was calculated using the formula of Sullivan (1972).

Statistical analysis

Data (mean values) of the analysis were tabulated and statistically analyzed as per the method followed by Gomez and Gomez (1984). SPSS (Statistical Package for Social Sciences) was employed for the analysis. ANOVA (analysis of variance), with 1-factor CRD (completely randomized design) and 2-factor CRD was applied for growth and biochemical changes to test the level of significance between the control and experimental samples.

RESULTS AND DISCUSSION

Different concentrations of the detergent showed a marked effect on growth when assessed in terms of SLA. The changes were found to be significant. Detergents showed to stimulate the expansion of leaf area initially for all treatments considered and a concentration influenced decrease was noticed after day 5. The reduction in SLA was more pronounced at 1.0 *g l^{-1}* of the detergent and the plant did not survive beyond 15 days (Fig. 1). Macrophytes, the important first order components in aquatic systems respond to exposure to contaminants from molecular level to organism level and exhibit manifestations as impairment in growth, reproduction and developmental abnormalities or decreased survival (Shugart *et al.,* 1992).



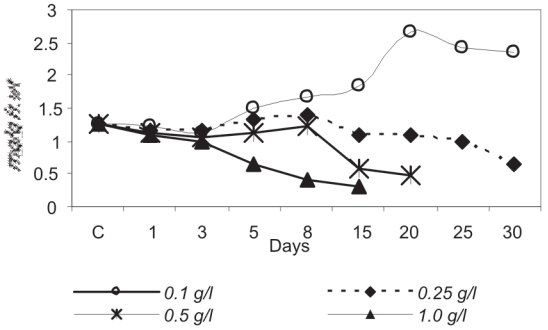
Mean of control =	270.400
VR (conc.) **	CD (conc.) 38.676
VR (day) **	CD (day) 27.348
VR (interaction) **	CD (interaction) 54.696
[** P< 0.01	CD at 5% level]

Fig. 1. Variations in Specific leaf area (cm^2 gm $^{-1}$ dry wt.*)* in *N. indica.*

Increase in SLA noticed at lower detergent levels may be due to the enhanced growth in presence of available phosphates of detergent origin. The LAS detergents contain sodium tripolyphosphate as builder components. The concentration dependent decrease in SLA seen in the experimental plants can be related to the stress induced reduction in both number and size of cells as reported Zhou and Qui (2005). The present study reveals that SLA can be employed as a useful tool to explain the ability of freshwater macrophytes in utilizing resources present in the contaminants at lower concentrations. Detergent influenced changes in biochemical constituents in *N. indica* were evaluated by analysis of total chlorophyll *(mg g^{-1} fr.wt.)*, CSI, total protein *(mg g^{-1} fr.wt.)*, lipid peroxidation *(nmoles MDA g^{-1} fr.wt.)* electrolyte leakage *(mS g^{-1} fr.wt.)* and extent of membrane damage.

It was noticed that both concentration and exposure time influenced the photosynthetic pigment. When compared to control, a significant initial reduction for total chlorophyll was observed as an immediate response and further, an enhancement in concentration of this pigment occurred at lower concentrations of 0.1 *g l^{-1}* At 1.00 *g l^{-1}*.a pronounced decrease in chlorophyll content was observed (Fig. 2). Photosynthetic pigments in aquatic plants serve as potential biomarkers of pollution stress (Masodijek *et al.,* 2000). The variation in pigment concentrations revealed that

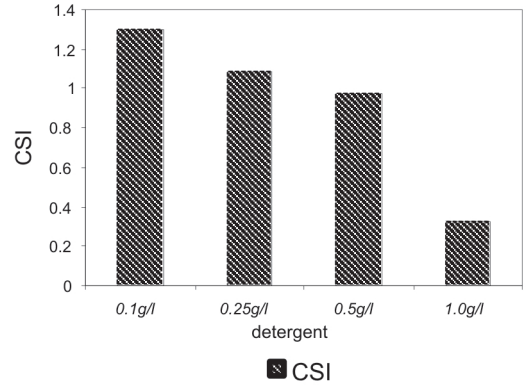
even a small level of the detergent manifested perturbations of the medium and of the photosynthetic capacity of the plants as reported by Ferrat *et al.* (2003). Anionic detergents at higher concentrations appeared to cause a severe alteration in the photosynthetic apparatus as is relevant by the net reduction in photosynthetic pigment observed during the study.



Mean of control = 1.277
VR (conc.) ** CD (conc.) **0.021**
VR (day) ** CD (day) **0.030**
VR (interaction) ** CD (interaction) **0.060**
[** P< 0.01 CD at 5 % level

Fig. 2. Variations in Total chlorophyll (mg g $^{-1}$ fresh weight) in *N. indica.*

Stress induced changes when expressed in terms of Chlorophyll Stability Index (CSI) showed the green pigment to remain stable at the lower concentration - 1.296 at 0.1 *g l* $^{-1}$ and 1.085 at 0.25 *g l* $^{-1}$. However, a reduction in CSI with concomitant rise in detergent concentration was seen and a maximum decrease of above 60% was observed at 1.0 *g l* $^{-1}$. The marked reduction was seen prior to decay at concentrations above 0.5 *g l* $^{-1}$ and 1.0 *g l* $^{-1}$



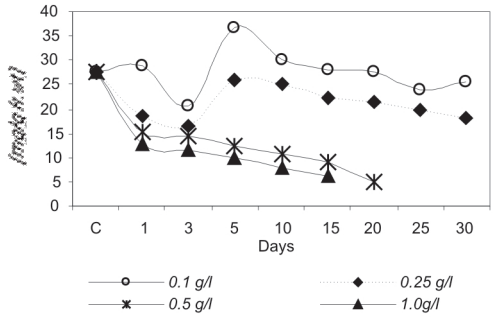
VR** CD 0.050
** P< 0.01 CD at 5 % level

Fig. 3. Chlorophyll stability index in *N. indica.*

(Fig. 3). The decrease in CSI at higher concentrations emphasizes phytotoxicity due to depletion of chlorophyll pigments and importance of the parameter in stress related studies (Praderm *et al.*, 2003).

The total protein content in the experimental plants depicted a characteristic pattern. Leaf protein content showed to decrease with increase in duration and intensity of the stress. At lower detergent levels (0.1 *g l* $^{-1}$ and 0.25 *g l* $^{-1}$) an initial linear increase was seen and further a decrease whereas at both the higher concentration a decrease was noted and this was more prominent at 0.1 *g l* $^{-1}$ (Fig. 4). Protein content in plant cells is an important indicator of their physiological state as during stress and are possible targets of oxygen radicals and its degradation is an index of oxidative stress. The degradation of proteins noticed in the present study at higher concentrations could have occurred by protein oxidative damage that inhibited absorption of nutritive materials required for synthesis as reported by Minato *et al.* (2005) or possibly due to active proteolysis resulting in degradation, accelerated catabolism during disturbances in the membrane systems and reduction in soluble protein due to reduced biosynthesis (Stoeva and Bineva, 2003). The increase in leaf protein content at lower concentrations of the detergent suggested the potential of the plant to protect their enzymes during initial stress and thereby conserve stabilization of protein synthesis as suggested by several workers (Palliwal *et al.*, 1998).

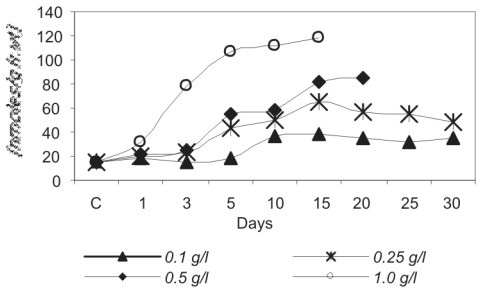
Analysis for lipid peroxidation was done in leaf and root tissues of control and detergent stressed plants. Malonedialdehyde, the lipid peroxidation



Mean of control = 27.787
VR (conc.) ** CD (trt) **0.38**
VR (day) ** CD (day) **0.540**
VR (interaction) ** CD (interaction) **1.080**
[** P< 0.01 CD at 5 % level

Fig. 4. Variations in Total protein (mg g $^{-1}$ fresh weight) in *N. indica.*

product was found significantly elevated in stressed plants when compared to control counterparts. There was a marked linear increase in MDA with increasing severity and duration of stress. Nevertheless, the root tissues were more affected as a relatively higher rate of lipid peroxidation occurred (Fig. 5 and Fig. 6). Lipid peroxidation is a natural metabolic process under normal aerobic conditions and it is one of the most investigated consequences of ROS action on membrane structure and function. Variation in malonedialdehyde (MDA) content, a lipid peroxidation product of the bio membrane system revealed that it is an indirect sensitive index to indicate the extent of detergent induced cell damage. The increase in MDA content observed in the experimental plants showed that the presence of detergents can have increased lipid peroxidation due to excessive generation of free radicals (Snell and Mullock, 1987). PUFA, the main component of membrane lipids are susceptible to peroxidation as the generated free radicals can react with the methylene groups of PUFA forming conjugated dienes, lipid peroxyl radicals and hydroperoxides as explained by Smirnoff (1995). The lipid hydroperoxides, thus formed may also have affected the membrane properties by increasing hydrophilicity of the internal side of the bilayer enhancing ion leakage.

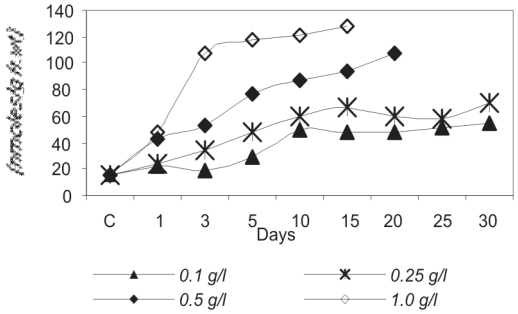


Mean of control = 14.47

VR (conc.) **	CD (conc.) **0.699**
VR (day) **	CD (day) **0.988**
VR (interaction) **	CD (interaction) **1.976**
[** P< 0.01	CD at 5 % level]

Fig. 5. Variations in Lipid peroxidation (n moles g $^{-1}$ fr. wt) in tissues of leaves in *N. indica*

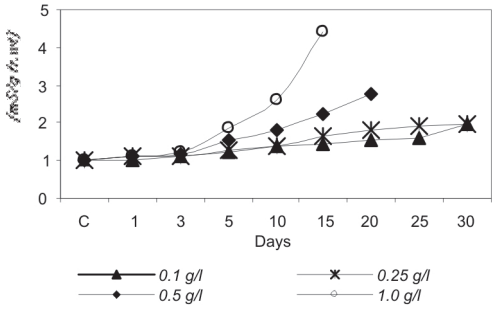
An increase in the electrolyte leakage was observed during for all concentrations of detergents when compared to the control. The degree of ion loss from leaf and root tissues was dependent on concentration and duration of detergent exposure. Relatively, a greater electrolyte leakage was noticed in 0.5 *g l* $^{-1}$ and 1.0 *g l* $^{-1}$ and the plants did not survive beyond 15 days suggesting severe membrane deterioration at higher concentrations Even at lower concentrations loss of ions into the bathing medium was noted (Fig. 7 and Fig. 8). The concentration dependent increase in tissue permeability measured as electrolyte leakage from leaves and root tissues of the experimental plants in the present study may be due to the cellular damage by detergent exposure occurring at membrane levels and promoting the inhibition of intercellular processes as reported by Morreno-Garrido et al. (2001). A similar dose and time-dependent increase in the efflux of electrolytes was found in studies by Trivedi *et al.* (2004). The tendency to stabilize the phenomenon of ion leakage observed in the macrophyte plant even at lowest concentration of stress applied (0.1 g l -1 of detergent) can be due to stress resistance imparted by the activity of ascorbic acid (Thomas *et al.*, 1992).



Mean of control = **15.274**

VR (conc.) **	CD (conc.) **0.701**
VR (day) **	CD (day) **0.992**
VR (interaction) **	CD (interaction) **1.984**
** P< 0.01	CD at 5 % level

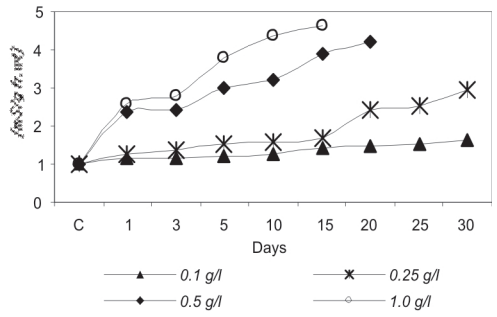
Fig. 6. Variations in Lipid peroxidation (n moles g $^{-1}$ fr. wt) in tissues of roots in *N. indica*



Mean of control = **1.010**

VR (conc.) **	CD (conc.) **0.087**
VR (day) **	CD (day) **0.123**
VR (interaction) **	CD (interaction) **0.246**
** P< 0.01	CD at 5 % level

Fig. 7. Variations in Electrolyte leakage (mS g^{-1} fresh weight) in tissues of leaves in *N. indica*

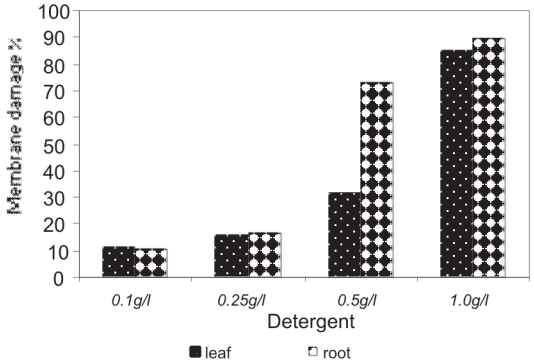


Mean of control = **1.016**

VR (conc.) **	CD (conc.) **0.105**
VR (day) **	CD (day) **0.148**
VR (interaction) **	CD (interaction) **0.297**
** P< 0.01	CD at 5 % leve

Fig. 8. Variations in Electrolyte leakage (mS g^{-1} fresh weight) in tissues of roots in *N. indica*

The decrease in membrane stability assessed as membrane injury (%) was proportional to the severity and the persistence of the detergent and membrane permeability was significantly enhanced as detergent concentration increased. It is observed that the roots in *Nymphoides* experienced higher membrane injury, than the leaves (Fig. 9). Bindu and Philip (2001) and Brandt *et al.,* (2001) inferred that exposure to surfactants caused oxidative stress and increased the lipid peroxidation products in tissues of aquatic organisms. Further, the membrane phospholipids when continually subjected to oxidant challenges may also get peroxidised, become rigid and lose selective permeability and integrity as reported by Davies (2000). The concentration dependent observations in the experimental plants can be attributed to enhanced permeability of membranes due to uncoupling



Leaf VR **	CD 9.280
Root VR **	CD 8.540
** P< 0.01	CD at 5% level

Fig. 9. Membrane damage (%) in leaf and root tissues of *N. indica*

reactions during energy metabolism, dissipation of ion gradients and ultimate leakage of essential cell constituents (Sinha *et al.,* 2005). Death of macrophytes at greater detergent concentrations can be attributed to the diffusion of water-soluble lipid peroxidation products from the membrane into other sub-cellular components creating a detrimental effect to viability of the plants.

CONCLUSION

The study revealed that *N. indica,* the rooted floating macrophyte prevalent in the surface waters in some parts of India exhibited significant changes in growth and biochemical characteristics when exposed to even low concentrations of synthetic detergents. Enhanced growth found at lower concentrations emphasizes the potential of the contaminant to promote eutrophication in natural systems. Measurements of levels of lipid peroxidation, leakage of ions and membrane damage were found comparatively more pronounced and hence serve as effective tools to predict damage inflicted by detergent stress. Roots were found more vulnerable. These growth and biochemical parameters can be used for evaluating the effect of LAS detergents in aquatic macrophytes. The greater impact observed on plant characteristics at higher concentrations is reflective of the phytotoxic nature of the contaminant resulting in death of the plants, which in turn contributes to further deterioration of our aquatic ecosystems. The results can further be related to the possible effects that could occur to other rooted floating macrophytes growing in the natural area as stress impacts are major factors limiting growth, metabolism and productivity of wetland systems.

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