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Effect of Detergents on the Growth of Two Aquatic Plants: *Azolla pinnata* and *Hydrilla verticillata*

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Abstract

The effect of three chemical formulations- two surfactants, Sodium Lauryl Sulfate (SLS) and Sodium Dodecyl Benzene Sulfonate (SDBS) and a builder Sodium Tripolyphosphate (STPP) on two aquatic plants – *Azolla pinnata* and *Hydrilla verticillata* was studied at various concentrations for a period of five months, from November to March. It was observed that while the detergents favoured the growth of plants at lower concentrations, they hampered their growth at higher concentrations. SDBS was found to be the most toxic to these plants and STPP the least, with SLS exhibiting intermediate levels of toxicity.

Keywords: Surfactants, builder, Sodium Lauryl Sulfate (SLS), Sodium Dodecyl Benzene Sulfonate (SDBS), Sodium Tripolyphosphate (STPP), *Azolla pinnata*, *Hydrilla verticillata*.

Introduction

Detergents are a wide range of formulations comprising primarily of surfactants (=surface-active agents) such as which lower the surface tension of liquids in which they are dissolved, and builders such as polyphosphates, zeolites and polycarboxylic acids which help soften the water. Together with the complexing agents, bleaching agents, optical brighteners and several other auxiliary substances, they contribute to the cleaning process (Pandey *et al.*, 2001). Surfactants are classified on the basis of their hydrophilic or solubilizing properties into anionics, non-ionics, cationics and amphoterics (Misra, 1982). Because many of them contain phosphates, their release into surface waters has been linked to the process of eutrophication and other adverse effects on the aquatic environments (Prat and Giraud, 1964, Canton and Slooff, 1982, Kaushik *et al.*, 2001). Further, both surfactants and builders are also toxic to a variety of aquatic organisms (Feisthauer *et al.*, 2001). The problem of

detergents is growing rapidly in developing countries such as India where the use of detergents has grown manifold but the facilities for sewage treatment are extremely poor. Though the biological effects of detergents have been demonstrated extensively as recently reviewed by Feisthauer et al. (2004), there is a dearth of Indian studies in this regard.

This communication reports the observations of a short-term experimental study on the effect of two surfactants and a builder on two common macrophytes, *Azolla pinnata* and *Hydrilla verticillata*.

Methods

The plants (*Azolla pinnata* and *Hydrilla verticillata*) used in this study were grown in the garden tanks on the university campus in unpolluted water. Two surfactants, Sodium Lauryl Sulphate (SLS), Sodium Dodecyl Benzene Sulphonate (SDBS) and a builder, Sodium Tripolyphosphate (STPP) were used for the toxicity tests. Five concentrations of aqueous solution of each compound were prepared in distilled water. Petri plates (10 cm diameter) were used for growing *Azolla pinnata* whereas beakers (500 ml volume) were used for the growth of *H. verticillata*. Ten equal sized fronds of *A. pinnata* were placed in each Petri plate filled with the solution and 10-cm long pieces of terminal shoots of *H. verticillata* were placed in each beaker. Ten replicates for each concentration of the three surfactants were taken for our study. The concentrations used for the detergents were as follows: SLS - 2, 5, 10, 15 and 20 ppm; SDBS - 1, 5, 10, 15, and 20 ppm, and STPP - 5, 10, 20, 50 and 100 ppm. Normal tap water served as control. The detergent solutions were added as and when required to compensate for the loss of water from the detergent solution by evaporation. Each experiment lasted for about 4 weeks and was carried out during the months of November to March.

Morphological changes (such as increase in the number of fronds and area, breaking of fronds or shoots, yellowing of fronds or shoots) were visually observed and recorded daily. Growth was measured in terms of area of fronds, number of fronds and biomass (dry weight) for *A. pinnata*. For *H. verticillata*, an increase in the length of shoot was taken as the parameter for growth; besides, browning in the shoots at different concentrations was also studied. Chlorophyll content was estimated in the beginning and at the end of the experiment by homogenization in 80% acetone and centrifugation followed by the measurement of O.D. in a double-beam spectrophotometer at 645 nm and 663 nm. Chlorophyll estimation was carried out by the following formula given by Maclachlan and Zatik, as follows (Trivedy and Goel, 1984).

$$\text{Total chlorophyll (mg/g)} = \frac{20.2 \times \text{O.D.}_{645} + 8.02 \times \text{O.D.}_{663} \times V}{d \times w \times 1000}$$

Here, O.D. = optical density, V = volume of solvent (25 ml), d = path length (cm), w = weight of plant (g)

Results and Discussion

Effect of SLS

In case of *A. pinnata*, it was observed that as the concentration of SLS increased from 2ppm to 15 ppm, an increase in the number of fronds, the area of fronds and the dry weight was observed, while at 20 ppm concentration of SLS, a decrease in the number and area of fronds, as well as the dry weight was observed. The chlorophyll content, however, was found to decrease at all concentrations except at 2 ppm, where no appreciable increase or decrease in chlorophyll content was observed. At 20 ppm concentration, the plants turned completely brown (Table 1, Figures 1-3).

In case of *H. verticillata*, the plants remained green and increased in length on exposure to concentrations of 2 ppm and 5 ppm, but turned brown at 10 ppm, 15 ppm and 20 ppm. However, the plants were observed to recover on the 24th day and small secondary branches arose at the nodes (Table 1, Figure 4).

Table 1: Effect of sodium lauryl sulfate (SLS) on *Azolla* and *Hydrilla*

Conc. (ppm)	Fresh cut (g)	Dry wt. (g)	Area (sq.cm)	No. of fronds	Chl (mg/g)	Increase in Length (cm)	Conc. (ppm)
Control (0)	0.2165	0.0245	5.4345	267	0.249	1.6	Control (0)
2	0.2654	0.026	5.7523	283	0.268	1.73	2
5	0.2797	0.028	5.8233	298	0.203	1.87	5
10	0.3587	0.0366	6.8254	309	0.165	2.23	10
15	0.3868	0.0384	9.6143	334	0.154	2.63	15
20	0.2353	0.023	5.628	279	0.105	2.06	20

* The plants had initially only 10 fronds each.

Effects of SDBS

Growth of *A. pinnata* increased with increasing concentration of SDBS from 1 to 10 ppm but decreased at 15 ppm and 20 ppm. However, even after decrease in the growth at 15 and 20 ppm, the growth was still found to be higher than that observed in the control plants ((Table 2, Figures 5-7). The chlorophyll content however, was found to decrease at all the concentrations of SDBS.

In case of *H. verticillata*, maximum growth was observed at 10 ppm concentration of SDBS and the plants turned brown at 15 and 20 ppm. No recovery was observed at any of the concentrations of SDBS (Table 2, Figure 8).

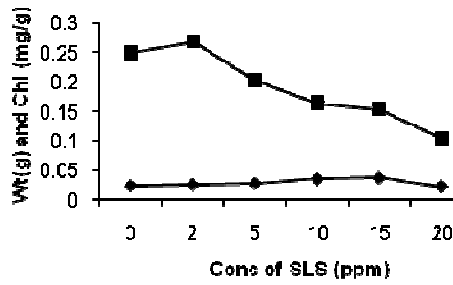


Figure 1 Effect of SLS on growth and chlorophyll content of *Azolla*

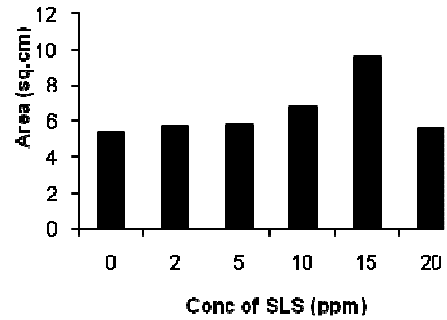


Figure 2 Effect of SLS on growth (area) of *Azolla*

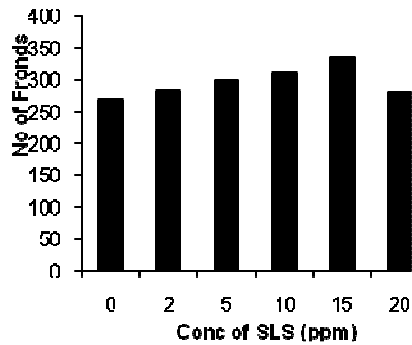


Figure 3 Effect of SLS on growth (no. of fronds) of *Azolla*

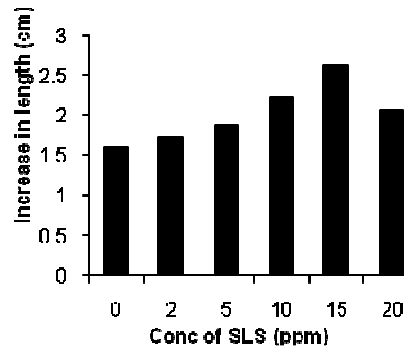


Figure 4 Effect of SLS on growth (increase in length) of *Hydrilla*

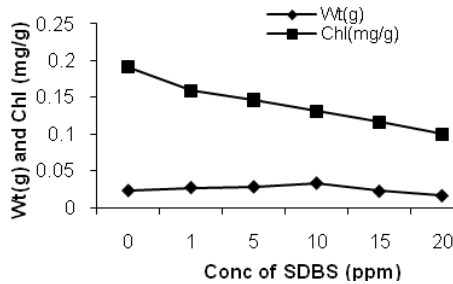


Figure 5 Effect of SDBS on growth and chlorophyll content of *Azolla*

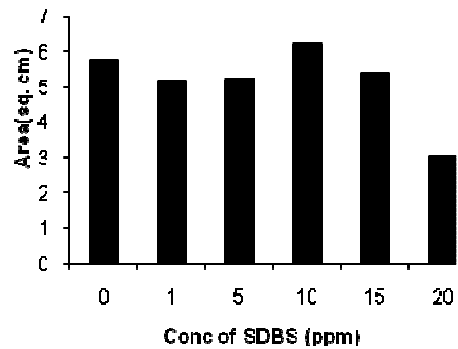


Figure 6 Effect of SDBS on growth (area) of *Azolla*

Table 2. Effect of sodium dodecyl benzene sulfonate (SDBS) on *Azolla* and *Hydrilla*

<i>Azolla</i>						<i>Hydrilla</i>
Conc (ppm)	Fresh wt (g)	Dry wt. (g)	Area (sq.cm)	No. of fronds	Chl (mg/g)	Increase in Length (cm)
Control (0)	0.224	0.023	5.758	194	0.168	1.0
1	0.2364	0.027	5.1674	244	0.132	1.1
5	0.2388	0.028	5.1886	259	0.118	1.3
10	0.2608	0.0335	6.201	275	0.098	1.6
15	0.2146	0.0228	5.3782	183	0.094	1.08
20	0.1408	0.0156	3.0364	137	0.084	0.9

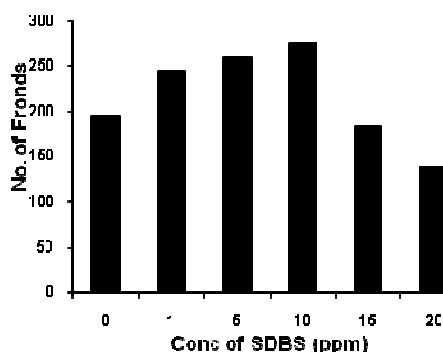


Figure 7 Effect of SDBS on growth (no. of fronds) of *Azolla*

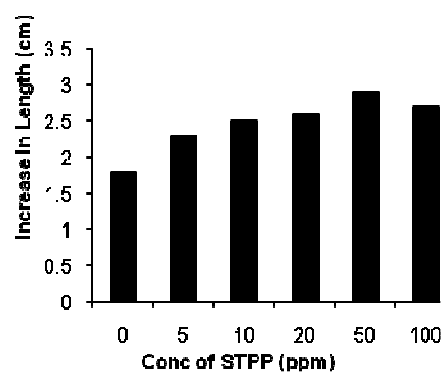


Figure 8 Effect of SDBS on growth (increase in length) of *Hydrilla*

Effects of STPP

An increase in the number and area of fronds, as well as biomass (dry weight) was observed as the concentration of STPP increased from 5ppm to 50 ppm; however, it was found that there was a decrease in the growth at 100 ppm concentration (Table 3, Figures 9-11). No browning was observed at any of the concentrations. The chlorophyll content was found to be lower as compared to the initial value, but higher than that of the control.

The shoots of *Hydrilla* also increased in length with increasing concentration of STPP from 5 to 50 ppm. At 100 ppm, a decline in the shoot length was observed (Table 3, Figure 12).

The study reveals that SDBS is most toxic to *A. pinnata* and *H. verticillata*, STPP is the least toxic and SLS exhibits intermediate levels of toxicity. For each of the three detergents, the effects were similar for the two plants at a given concentration, but the behaviour of the plants was different with respect to the type of the detergent.

In *A. pinnata*, an increase in the number of fronds was accompanied by a decrease in chlorophyll content. This implies that the plants adopted the strategy of increasing their photosynthetic area to cope up with the ecological stress produced by the detergents that resulted in reduced chlorophyll content. This effect was not observed in *H. verticillata* probably because only portions of shoots were taken for the study.

Table 3 Effect of sodium tripolyphosphate (STPP) on *Azolla* and *Hydrilla*

<i>Azolla</i>						<i>Hydrilla</i>
Conc. (ppm)	Fresh cut (g)	Dry wt. (g)	Area (sq.cm)	No. of fronds	Chl (mg/g)	Increase in Length (cm)
Control (0)	0.321	0.024	4.917	164	0.081	1.8
5	0.3522	0.0271	6.463	223	0.245	2.3
10	0.3798	0.0291	6.655	246	0.180	2.5
20	0.4094	0.3308	8.369	269	0.170	2.6
50	0.5644	0.051	12.248	319	0.144	2.9
100	0.4208	0.038	7.706	172	0.102	2.7

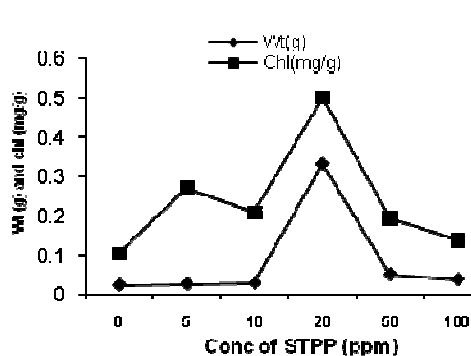


Figure 9: Effect of STPP on growth and chlorophyll content of *Azolla*

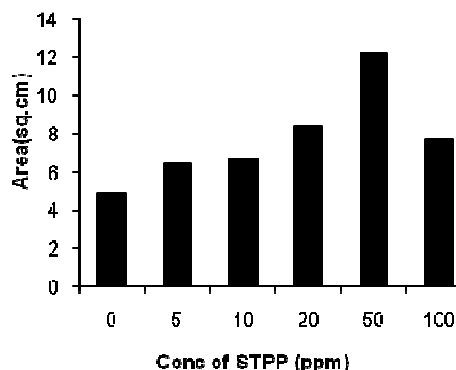


Figure 10: Effect of STPP on growth (area) of *Azolla*

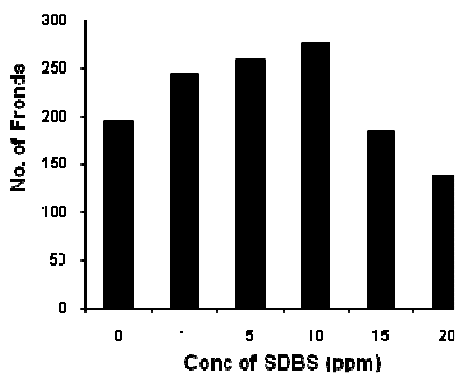


Figure 11 Effect of STPP on growth (no. of fronds) of *Azolla*

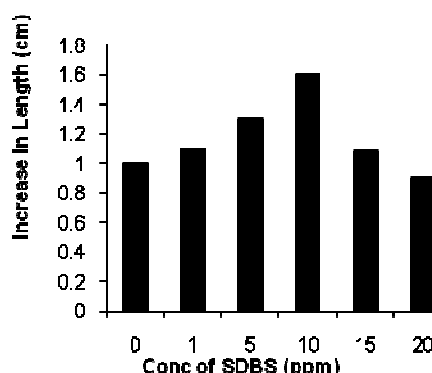


Figure 12 Effect of STPP on growth (increase in length) of *Hydrilla*

Because the experiment was conducted under laboratory conditions with only two plant species and a single detergent at a time, the extrapolation of this data to the field conditions has some limitations. Often, several detergents are discharged at a time in the field, and their concentrations vary depending upon the volume of water, mixing conditions, pH and hardness of water, and other factors. Although literatures reveal a very limited data on the environmental concentrations of surfactants in Indian surface waters, however, Mukherjee and Pankajukshi (1995) have estimated the loading rates of some formulated detergents to be 400 to 1000 mg/l, which, therefore, has the distinct potential to exceed chronic and possibly acute, levels of toxicity. This is in agreement with the studies reported by Chawla *et. al.* (1989), in which the effect of linear alkyl benzene sulfonate (LAS) on aquatic flora indicated toxicity in terms of loss of biomass and reduction in total protein and chlorophyll contents, leading to substantial changes in ultra-structural features. This indicated that the phytotoxic dose of LAS for *Lemna minor* and *Spirodela polyrrhiza* is 8.05%, *Pistia stratiotes*, *Hydrilla verticillata* and *Ceratophyllum demersum*, 0.01% and *Salvinia molesta* was found to be sensitive even to 0.005% LAS (Chawla *et. al.*, 1989).

In India, the water bodies get polluted from a variety of sources, hence, it is not easy to determine the contribution of a single pollutant. As a result, estimation of the toxicity levels of LAS becomes difficult due to potential interactions between other organic and inorganic pollutants. However, in most of the cases, the concentrations of detergents were far exceeding than those experienced in the environment. At such high levels, the effects could be due to detergent related phenomenon rather than toxicity of chemical constituents (Singh *et. al.*, 1990). Thus, it is difficult to determine the risk of LAS to aquatic environment without first knowing the usage rates and environmental concentrations of LAS in India.

Author's Contributions: Ms. Puneeta Pandey (Research Scholar) carried out the experiments, performed the calculations and prepared the manuscript and Dr. Brij Gopal (Professor) Research Supervisor had contributed in experimental design and final editing of the manuscript.

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