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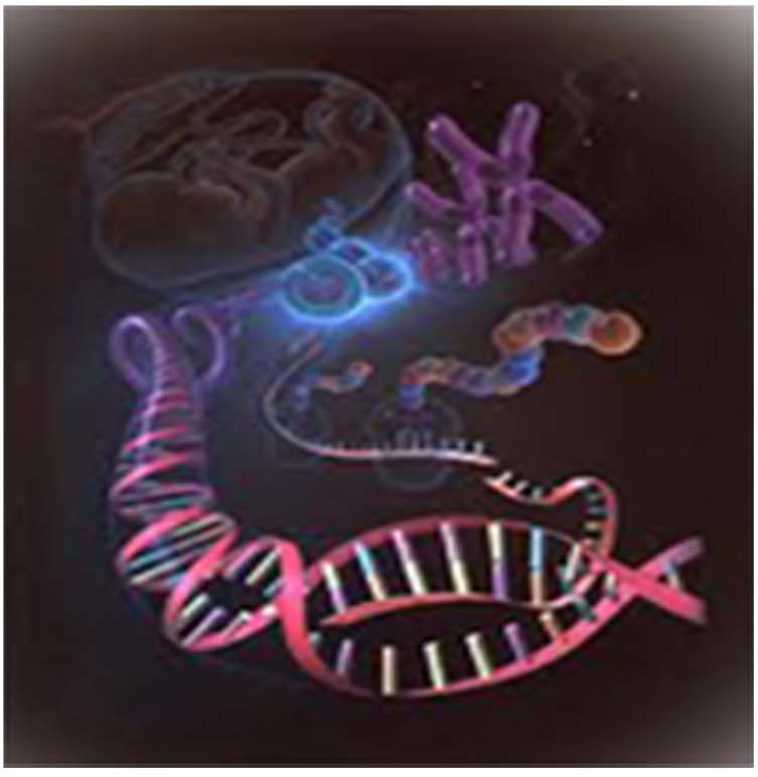

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Research Paper

EFFECTS OF SURFACTANT (SODIUM LAURYL SULPHATE) ON *HYDRILLA VERTICILLATA*

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This work highlights the effects of Sodium Lauryl Sulphate on growth and physiology of the aquatic plant (*Hydrilla verticillata*). The effects were observed at five different concentrations. The experimental set up was laid within the open space of the Department of Environmental Studies, GIS, GITAM University. The duration of the experiment was 24 days. The results showed significant effect of sodium lauryl sulphate on plant's morphology (browning of shoots, fragmentation and leaf shedding), chlorophyll and protein content. The effects on chlorophyll and protein were apparently significant at higher concentrations (4-20 ppm). There was no significant difference between plants in control set and plants exposed to 2 ppm concentration of surfactant with respect to various parameters considered in the present study.

Keywords: Surfactant, Sodium Lauryl Sulphate, *Hydrilla verticillata*, Chlorophyll, Protein

INTRODUCTION

It is imperative to balance economic progress and ecological considerations in order to ensure clean air and water for all sections of the society. Few days back, Water Day was celebrated and the theme for this year was “Water for the Cities: Responding to Urban Challenge”. In view of the current attention being given to urban water resources which include lakes, rivers and ground water, the present study was guided to investigate the impact of one of the most widely used pollutant ‘the surfactants’ on aquatic plants. Surfactants refer to the surface active agents or those that lower the surface tension of water. Surfactants are classified on the basis of their hydrophilic or solubilizing properties into anionic, non-ionic and cationic types. Alkylbenzene sulfonates are examples of anionic surfactants in which the alkylbenzene portion is lipophilic and the sulfonate portion is the hydrophilic components. A detergent is a mixture of surfactants. These are amphipathic molecules that contain both polar and hydrophobic groups. In water, the polar group forms hydrogen bonds with water molecules

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while the hydrocarbon chains aggregate due to hydrophobic interactions. These properties enable detergents to lower the surface tension, be soluble in water and also aid in the cleaning action (Adewoye, 2010).

Most of the domestic and industrial detergents contain high (up to 40%) phosphate content. These surfactant molecules enter the plants through roots or surface absorption. They are toxic to plants and cause retardation of plant growth, affect elongation of roots, photosynthetic efficiency, uptake of cations and growth of pollen tubes. They damage cell membranes owing to their hydrophobicity and alter the absorption maxima of chlorophyll pigments. They are reported to cause denaturation of proteins and inhibit enzymes in various metabolic processes. The cell membrane of a leaf, which is composed of phospho-lipids and proteins, are insoluble in water but soluble in detergent or surfactant solutions. They solubilize membrane proteins by mimicking the lipid bi-layers. Proteins that are embedded in the membrane lipid bi-layers are incorporated into these micelles via hydrophobic interactions. They are now surrounded by a layer of surfactant molecules and the hydrophilic portions are exposed to the aqueous medium (Bhairi, 2001).

The adverse effects of most pollutants (pesticides, heavy metals and industrial wastes) on aquatic plants have been reviewed earlier (Srivastav et al., 1993; Heumann, 2007; Mafald S Faria, 2007; Gabriele Alfano et al., 2009; Jomova et al., 2009) as aquatic bodies are preferred sites for the disposal of refuse, sewage and waste water from industries and domestic settlers. Surfactants have not been given due attention in view of the paucity of literature available on plants and animals. Emil Smith in 1941 demonstrated the effects of Sodium Dodecyl Sulphate (SDS) on chlorophyll and protein contained in Spinach leaves. In green leaves the chlorophyll pigment is linked chemically to proteins. SDS at lower concentrations attack different linkages wherein chlorophyll remains combined with smaller fragments of proteins while at higher concentrations was found to convert the chlorophyll into phaeophytin.

As early as 1989, Geeta Chawla demonstrated that the floating plants like *Pistia stratiote* and *Salvinia molestamitchell,* were more resistance to detergents at lower concentrations. The study was conducted on the following plants like *Salvinia molestamitchell, Hydrilla verticillata, Ceratophyllum demursum, Lemma minor, Spirodella polyrhiza, Schleid and pistia stratiotes* and the detergent selected for the study was linear alkyl benzene sulphonate. At lower concentration of LAS (0.003%), the percent decline in chlorophyll content ranged from 14% to 63% in all the plants. The percent loss of chlorophyll increased to 72% to 87% in all plants at elevated concentrations of 0.05% LAS in the experiment. The protein content was not much affected by detergents in the present experiment. The total protein of *Salvinia* was reduced to 25% of treated concentration. The plants exhibited the adverse effects of detergent after 48hrs of treatment and the symptoms included brown coloration, loss of chlorophyll and etiolation.

The response of aquatic plants to surfactants was observed in detail in the study conducted by Puneeta Pandey and Brij Gopal in 2007. They subjected the plants to different concentrations of the surfactants and also recorded the effects for the different types of surfactants [Sodium Lauryl Sulphate (SLS), Sodium dodecyl Benzene Sulphate (SDBS) and Sodium tripolyphosphate

(STPP)] used in the study. The response varied for the two aquatic plants namely *Azolla pinnata and Hydrilla verticillata* keeping the type of surfactant and concentration constant. When the plant *Azolla pinnata* was exposed to different types of surfactants (SLS, SDBS and STPP) at different concentrations, they observed an increase in growth at lower concentrations, but at higher concentrations, chlorophyll content and growth of the plant were inhibited. In case of *Hydrilla verticillata,*the response was similar to that seen for *Azolla pinnata*. Lower concentrations favored growth. Morphological changes such as browning of shoots were also recorded for different concentrations. In *Hydrilla verticillata,* the polygonal epidermal cell collapsed accompanied by a slight loss of wax layer on the axial surface. Overall, it was found that SDBS was most toxic to plants under study followed by SLS and STPP.

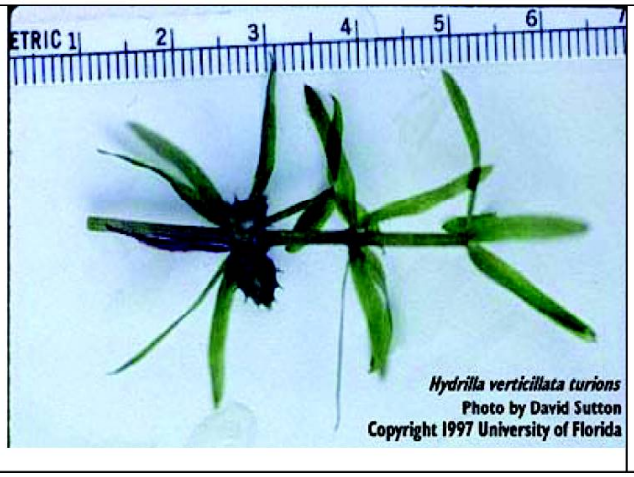
The effect of another commonly used surfactant Linear alkyl benzene sulphonate, whose concentration varies from 20mg/L in untreated waste water to 1-2 mg/L in treated waste water, was investigated on aquatic plants (Zhao Qiang, 2008). The study reported that upon exposure to these surfactants, activated oxygen species are released from mitochondria and chloroplasts. These activated oxygen species damage metabolism through oxidation of lipids, proteins and nucleic acids. Superoxide radicals are toxic byproducts of oxidative metabolism. The non-polar chain of surfactant molecules bind to various bioactive macromolecules including carbohydrate, proteins, peptides and nucleic acids and thereby affect aquatic plants.

MATERIALS AND METHODS

The plant (*Hydrilla verticillata*) used in this study was taken from a pond near to the Management block, in front of Knowledge Resource Centre, GITAM University Campus (Figure 1). *Hydrilla Verticillata* belongs to the family of Hydrocharitaceae.

H. verticillata is slender, submerged, perennial aquatic herb. Stem is much branched rooting at the nodes, usually 7.6 m long with spreading thick mats on the water surface. Leaves are about

Figure 1: *Hydrilla verticillata*



Scientific Classification-

Kingdom – *Plantae*

Order – *Alismatales*

Family–

Hydrocharitaceate

Genus – *Hydrilla*

Species – *verticillata*

1.5cm long in whorl of three to eight together. Flowers are tiny, red, pink or white in small umbels arranged in auxiliary and terminal panicles. Storage structures like turin and tuber are 4 – 15mm long off- white to near black in color. It can survive in a few centimeters of water or in depths of up to 6m.

In this study, surfactant like Sodium Lauryl Sulfate (SLS) was used to investigate its effects on plant growth. Five concentrations of detergents were maintained, i.e., 0, 4, 8, 12, 16, 20 μg/ml. Treatments containing different concentrations of surfactants were designated as T1, T2, T3, T4 and T5 respectively. Two 60 L tubs were used to maintain the stock of *Hydrilla Verticillata* in *in vitro* conditions. This experiment was carried out in two replicate sets (total 12 tubs) within the department for 24 days. Twenty five plants were randomly selected from the stock and kept in each of the 12 tubs. The amount of detergent added to each tub was calculated based on the volume of water in the tub and the corresponding concentration level required.

The parameters selected in this study to monitor the effects were protein content, chlorophyll content and morphological attributes like length of shoots, fragmentation and browning of shoots. Total protein content in the plant tissues was estimated by the method of Lowery (1951) using 10% of Trichloroacetic acid (TCA). The color that developed was read at 600nm. Chlorophyll were estimated by standard procedure using 90% acetone. The readings were taken at three wavelengths (663 nm, 645nm, 630nm). Calculation for chlorophyll was done using respective formulae (Trivedy and Goel, 1984).

RESULTS AND DISCUSSION

The results of the present experiment showed the biochemical and morphological response of aquatic plant towards the particular surfactant. Tap water was used to grow plants in this study and nutrients from external sources were not added. In view of the nutrient constraints to plant growth, the experimental duration was limited to 24 days. Geeta Chawla in 1989 rightly pointed out that nutrient deficiency, light and temperature may affect phytotoxicity results. However, the responses from the control set would account for the above mentioned factors.

MORPHOLOGICAL PARAMETERS

In this experiment, morphological parameters like length of shoots, browning of shoots, shedding of leaves and fragmentation of plants were observed for 24 days. The initial length of the plants were 15 cm and they were all green and healthy. Significant changes in length or color of the plant was not seen among the various treatments including the control set on second sampling day. However, increase in length of the shoots were recorded from third day onwards until 5 days for all the treatments except control set (Table 1). The *H. verticillata* plants did not exhibit any morphological changes up to six days in control set probably because this period was required for acclimatization to local environmental set up. After six days length increased up to 17th days while flowering began by 9th day. There was a 2.5 cm increase in length of the plant towards the end of experimental period. In the other treatments, the plants survived till 9 or 11 days (Table 1). New leaves emerged in some plants belonging to T1 and T2 treatment sets. The increase in length and emergence of new leaves may be attributed to the provision of nutrients to plants from surfactant present in solution.

Table 1: Length of *Hydrilla verticillata* Plant

No of Days	Treatments					
	Control	T1	T2	T3	T4	T5
1	15	15	15	15	15	15
2	15	15	15	15	15	15
3	15	15	15	15.5	15.5	16
4	15	15.5	16	16	16	16
5	15	15.5	16	16	16	16
6	16	16.5	16	16	16	16
7	16.5	16.5	F	F	F	F
8	16.5	16.5	F	F	F	F
9	16.5	16.5	F	F	D	D
10	16.5	16.5	F	F	D	D
11	16.5	16.5	D	D	D	D
12	16.5	16.5	D	D	D	D
13	17	16.5	D	D	D	D
14	17	17.2	D	D	D	D
15	17	17.2	D	D	D	D
16	17.6	18	D	D	D	D
17	17.6	18	D	D	D	D
18	17.6	18	D	D	D	D
19	17.6	18	D	D	D	D
20	17.6	18	D	D	D	D
21	17.6	18	D	D	D	D
22	17.6	18	D	D	D	D
23	17.6	18	D	D	D	D
24	D	D	D	D	D	D

Note: F – Fragmentation; D – Dead.

The shoots began to turn brown by 4th day for plants belonging to T4 and T5 treatment sets. Intense browning of shoots was observed by 6th day of plants of T3, T4 and T5 sets and even plants in T2 set also turned brown (Table 2). Browning of shoots and fragmentation of plant illustrates the toxic effect of surfactant molecules on *H. verticillata* plants. As they readily solubilize membrane proteins by mimicking the lipid bi-layers of biological membranes, they result in

Table 2: Browning of *H. verticillata* Shoots During the Experiment

No of Days	Treatments					
	Control	T1	T2	T3	T4	T5
1	NO	NO	NO	NO	NO	NO
2	NO	NO	NO	NO	NO	NO
3	NO	NO	NO	NO	NO	NO
4	NO	NO	NO	NO	LB	LB
5	NO	NO	NO	LB	MB	MB
6	NO	NO	LB	MB	DB	DB
7	NO	NO	MB	DB	DB	DB
8	NO	NO	DB	DB	DB	DB
9	NO	NO	DB	DB	D	D
10	NO	NO	DB	DB	D	D
11	NO	NO	D	D	D	D
12	NO	NO	D	D	D	D
13	NO	NO	D	D	D	D
14	NO	NO	D	D	D	D
15	NO	NO	D	D	D	D
16	NO	NO	D	D	D	D
17	NO	LB	D	D	D	D
18	NO	LB	D	D	D	D
19	NO	LB	D	D	D	D
20	NO	LB	D	D	D	D
21	NO	LB	D	D	D	D
22	NO	LB	D	D	D	D
23	NO	LB	D	D	D	D
24	NO	D	D	D	D	D

Note: LB – Light brown; MB – Medium brown; DB – Dark brown; D - Dead.

leaching of soluble cell components into the solution. Surfactant molecules bind to the cell membrane by partitioning the lipid bi-layer at low concentrations. However, at higher concentrations or upon prolonged exposure to surfactants, the membranes disintegrate to form mixed micelles with the surfactants molecules. The hydrophobic regions of the membrane proteins are surrounded by the hydrophobic chains of micelles (Bhairi, 2001).

After 7 days length was not recorded as plants in most treatment sets began to fragment and by 9th day plants in T4 and T5 sets were dead. Shoots turned brown in almost all sets except control and T1 set. Plants in T2 and T3 sets were dead by 11th day of observation (Table 2). The death of the plants could be due to exhaustion of nutrients in the solution and loss of permeability of cell membranes that disrupted the osmotic gradient and uptake of nutrient ions from solution. In control and T1 treatment sets, plants began to flowering by 9th day and remained healthy until 17th day when little browning of shoots was noticed. Plants in control set became weak by 24th day of the experiment owing to lack of nutrients.

DRY WEIGHT

The percent dry weight refers to the amount of dry weight measured for every 100gm of fresh weight of the plant material. There was no distinct pattern observed for changes in dry weight in the present experiment however, the dry weight recorded at the end of the experimental period was less than that noted on zero sampling day. The dry weight was found to increase until 4 days of sampling followed by which there was a distinct decline. The reported percent dry weight for plants on zero day was 11.45% while that recorded at the end of experimental period was 7-11.6% for treatments T2, T3, T4 andT5 (Figure 2). In control set and T1 set, there was a rise in percent dry weight on 9th day followed by a peak on 4th day and thereafter it continuously decreased to 9-13.7% respectively towards the end of experiment (Figure 2).

CHLOROPHYLL

In control set, the chlorophyll values continually decreased from day zero until day nine. The chlorophyll content on zero days was 0.740mg/g dry weight and on day ninth was 0.149mg/g. After nine days, the chlorophyll content increases until 17th day and thereafter declining to 0.032mg/g towards the end of the experiment. In T1 treatment, the chlorophyll content decreased from day zero to 6th day (0.740-0.209mg/g) following which the chlorophyll content began to increase. The chlorophyll content on day 11th was 0.465 mg/g (Figure 3). The chlorophyll content eventually declined to 0.081mg/g towards the end

Figure 2: Changes in Percent Dry Weight of *H. verticillata* in Different Treatments During the Experiment

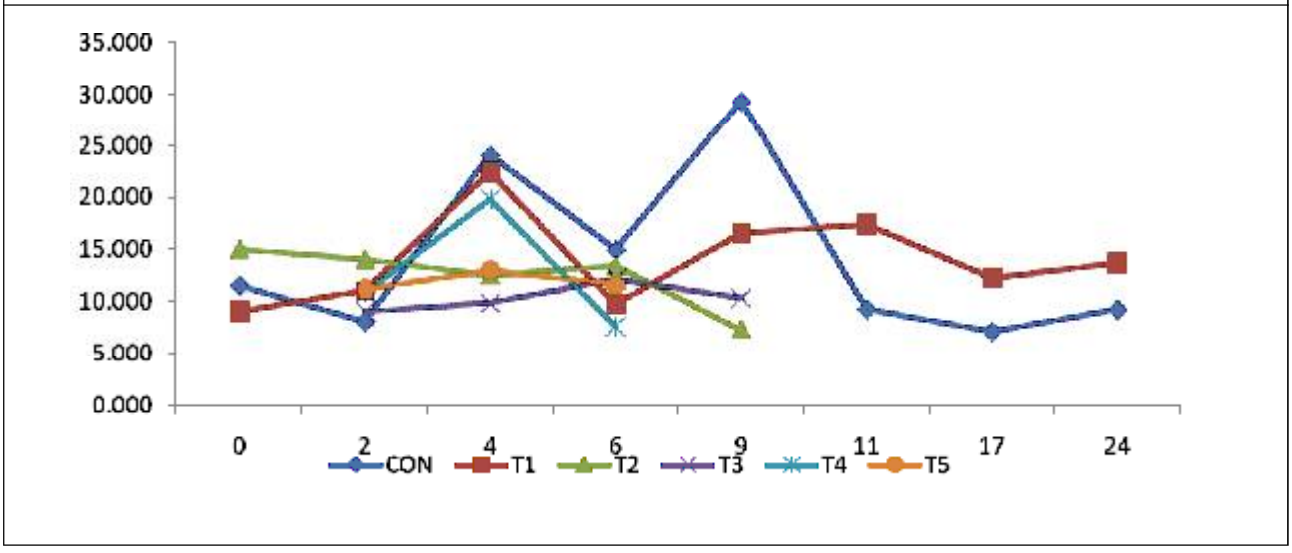
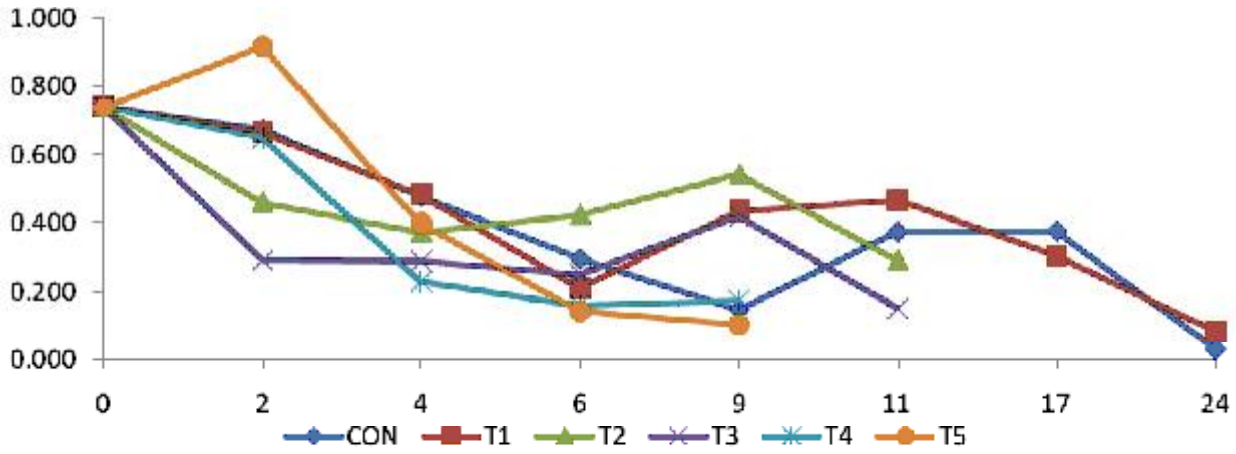


Figure 3: Changes in Chlorophyll Content of *H. verticillata* Throughout the Experimental Period in Different Treatments



of the experimental period. Thus, all nutrients being used up by the plant and all metabolic energy were being diverted towards emerging reproductive structures.

In T2 treatment, the content of chlorophyll decreased from day zero until 4th day of experiment (0.740mg/g-0.374mg/g) and thereafter, chlorophyll content increases to attain another peak by 9th day followed by a decline by 11th day of the experiment, which coincided with its death. The chlorophyll content recorded on 11th day was 0.290mg/g (Figure 3). In T3 treatment, the chlorophyll content significantly declined from zero day to 2nd day (0.740-0.290mg/g) followed by a slight decrease until day 6th from 0.290 to 0.248mg/g. There was a subsequent rise in chlorophyll content from day 6th to 9th day (0.248-0.418mg/g). The plants succumbed to detrimental impacts of detergent by 11th day by which a sharp decline in chlorophyll content was recorded (0.140mg/g). In T4 treatment, there was a considerable decline in chlorophyll content reaching a minimal value of 0.227mg/g on 4th day. These plants were dead by 11th day of the experiment (Figure 3). Contrary to the pattern observed in previous treatments, there was an immediate increase in chlorophyll content recorded for plants of T5 treatment (0.917mg/g). However, the chlorophyll content subsequently declined until 9th day (0.103,g/g) when the plants became weak and were dead.

The initial decline in chlorophyll seen in most treatments including control set can be attributed to inadequate nutrients available to support both vegetative and reproductive growth of plants. Thus, all nutrients being used up by the plant and all metabolic energy were being diverted towards emerging reproductive structures. The subsequent rise in chlorophyll can be explained by the diversion of plant's metabolism from reproductive growth to vegetative growth. This is quite apparent from morphological data when flowers appeared in control and T1 set on 9th day. Further, length of the plants increased by 1-2 cm and dry weight also showed a peak by 9th day which indicate growth in plants. On the other hand, significant difference in decline of chlorophyll content was evident between control

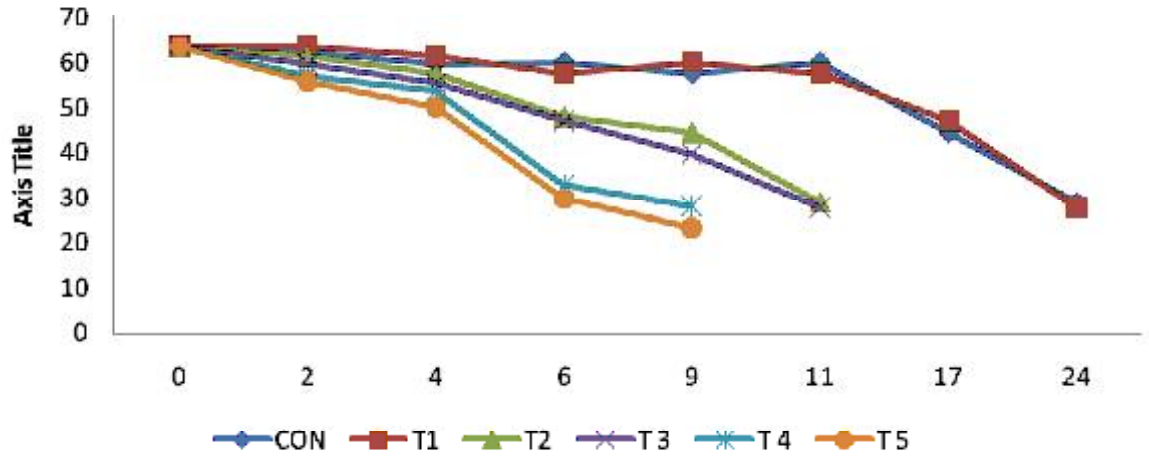
set and the remaining treatment sets (T2, T3 and T4). The presence of surfactant molecules may probably add on to the existing nutrient stress of the plants in T2, T3, and T4 treatments. Surfactant concentration was highest for T5 treatment and yet the chlorophyll content attained its peak on 2nd day which can be explained by the provision of nutrients to plants from surfactant molecules. But as surfactants are known to lyse cell membranes by disrupting their permeability, toxic effects were evident in the subsequent sharp decline resulting in plant death by 9th day of the experiment. In T4 treatment, the plants showed a steady decline in chlorophyll content while in T2 and T3 treatments, the plants exhibited the similar pattern as seen for T1, and control treatment. In previous studies (Geeta Chawla, 1989 and Pandey and Gopal, 2007), similar reduction in chlorophyll content was observed and it was attributed to the conversion of chlorophyll into phaeophytin (Smith, 1941).

PROTEIN

Changes in the pattern of protein content were found similar among different treatments even though the rate of decline varied considerably depending upon the concentration of surfactants. For control and T1 treatment, the protein content remained more or less similar until 11 days of the experiment after which a steep decline in protein content was evident. In contrast, the decrease in protein content in T2 and T3 treatment sets was nearly continuous throughout the 11 days of the experiment after which the plants were dead. A sharp decline in protein content was observed after 4 days for plants belonging to T4 and T5 treatment sets. These plants survived till 9 days. The content of protein by 9th day for T4 and T5 treatment was 28 and 23mg/g dry weight respectively, by 11th day for T2 and T3 treatment was 29 and 28mg/g dry weight respectively and by 24th day for control and T1 treatment was 29 and 28mg/g dry weight respectively (Figure 4).

The decline in protein content observed may be attributed to the release of oxidative species within cells upon exposure to surfactant molecules (Zhao Qiang, 2008). These oxygen active species cause damage to the activity and

Figure 4: Changes in Protein Content of *H. verticillata* Throughout the Experimental Period in Different Treatments



conformation of proteins as well as lipids and nucleic acids like DNA/RNA. Further, the non polar chains of surfactant molecules may bind to these bio-molecules and inactivate them. The results aptly showed that greater the concentration of surfactants, the steeper the decline in protein content. This study therefore, illustrates that the response of protein to varying concentrations of surfactants is quite positive and direct. Hence, further studies on closely monitoring the response of proteins to surfactants should be conducted to provide an insight into the plausible reasons behind the effect on metabolism of plants.

CONCLUSION

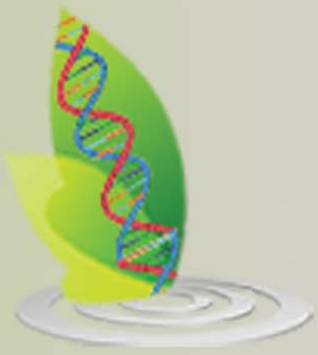
The present study documents the effects of sodium lauryl sulfate on morphology and biochemical parameters of aquatic plant *H verticillata*. Browning and fragmentation of shoots was found to correlate positively with increasing concentrations of surfactants. Further, the pattern of change protein content also explicitly shows the toxic effect of increasing concentration of surfactant molecules. Chlorophyll content is a measure of the plant's photosynthetic rate and changes in pattern of this parameter denotes the shift in plants' metabolic needs. Initially, the plant diverts a large part of its metabolic energy towards reproductive growth. This was evident from the rise in chlorophyll content after 9th day when flowers appeared on plants in control set. In future, studies should be conducted to understand the physiological response of plants over a longer duration and also to investigate the fate of surfactants in water.

REFERENCES

1. Adowoye S O (2010), "Effects of Detergents Effluent Discharges on the Aspect of Water Quality of River, Nigeria", *Agriculture and Biology Journal of North America*, Vol. 1, No. 4, pp. 731-736
2. Bhairi S M (2001), "A Guide to Properties and Uses of Detergents in Biology and Biochemistry", *Calbiochem-Novabiochem Corporation*, pp. 1-41
3. Chawla G, Virendramisra P and Viswanathan N (1989), "Toxicity of Linear Alkyl Benzene Sulphonate on Some Plants, *Water, Air and Soil Pollution*, Kluwer Academic Publishers, Vol. 43, pp. 41-51.
4. Faria S M, Nogueira A J A and Soares A M V M (2007), "The Use of Chironomus Riparius Larvae to Assess Effects of Pesticides from Rice Fields in Adjacent Freshwater Ecosystems", *Ecotoxicology and Environmental Safety*, Vol. 67, pp. 218-226.
5. Heuman H G (1989), "Effects of Heavy Metals on Growth and Ultrastructure of *Chara vulgaris*, Federal Republic of Germany", *Protoplasma*, Vol. 136, pp. 37-48
6. Jomova K and Mrovic M (2009), "Effects of Heavy Metal Treatment on Molecular Changes in Root Tips of *Lupinus luteus* L.Czech", *Journal of Food Science*, Vol. 27, pp. 368-389
7. Jena-Louis Salager (2002), "Surfactant Types and Uses, Frip Booklet # E300-A, Teaching Aid in Surfactant Science and Engineering in English", *Merida- Venezuala Version*, Vol. 2, pp. 1-49.
8. Pandey P and Gopal B (2010), "Effects of Detergents on the Growth of Two Aquatic Plants (*Azolla pinnata* and *Hydrilla verticillata*)". *Environment & We: An*

International Journal of Science and Technology, Vol. 5, pp. 107-114

9. Qiang Z, Zhenbin W U, Cheng S and Feng H E (2008), "Response of submersed macrophyte Hydrilla verticillata (L.f) Royle o Sodium Dodecyl Benzene Sulphonate stress", *Journal of Natural Sciences*, Vol. 13, No. 2, pp. 221-226
10. Smith E L and Pickels E G (1941), "The effects of detergents on the chlorophyll-protein compound of spinach as studied in the ultracentrifuge", *The Journal of General Physiology.*
11. Srivastav R K, Gupta, S K, Nigan, K D P and Vasudevan P (1993), "Treatment of Chromium and Nickel in waste water by using aquatic plants", *Water Research,* Vol. 28, No. 7, pp. 1631-1638
12. Trivedy R K and Goel P K (1984), "Chemical and Biological Methods for water pollution studies", *Environmental Publications*, Karad.



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