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EFFECTS OF DOMESTIC DETERGENTS, SEWAGE AND LEACHATE OF WATER HYACINTH DECAY ON THE GROWTH OF *SCENEDESUMS OBLIQUUS* (CHLOROPHYTA) : I. INDIVIDUAL EFFECTS

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Both detergents, Nirma and Surf, cause an acute toxicity to the *S. obliquus* growth by their low as well as high concentrations. The leachate of water hyacinth decay and domestic sewage effect different responses in the alga by their high and low concentrations. The higher concentrations (>10^5 ppm) of domestic sewage and leachate of 1 and 10 days decay stimulate the algal growth by 1.5 to 2 times. But the lower concentrations of domestic sewage (<10^4 ppm) and leachate of 10 and 35 days decay (<10^3 ppm) cause, respectively, a 2-fold and > 3-fold suppression in the algal growth. The toxicity of leachate increases considerably as the decay continues in hyacinth litter. The leachate of 1-day decay is the least toxic and that of 35-day is the most toxic to alga. The leachate of decay in the hyacinth leaf and root litter show similar patterns and amounts of effects on the algal growth in various concentrations and periods of decay.

Key words : Detergents, Sewage, Leachate of hyacinth decay, *Scenedesmus obliquus*, Toxicity test.

Most tropical freshwater wetlands, especially those located in southeast Asia, receive regular inputs of domestic detergents and sewage from the neighbouring cohorts of human population who use wetlands extensively for bathing, washing of clothes, disposal of untreated excreta and cultivation of animal and plant crops. The high amount of phosphorus in detergents and nitrogen and phosphorus in the sewage facilitates a faster eutrophication of these wetlands under the tropical climate, since both N and P are the major agents of eutrophication in freshwaters the worldover (Landner, 1976; Forsberg and Ryding, 1980; Cluis *et al.*, 1988). These wetlands thus support very high production rates and biomass of phytoplankton and macrophytes, particularly the free-floating water hyacinth (*Eichhornia crassipes* (Mart.) solms). The growing water hyacinth stands utilize inorganic nutrients at fast rates and cover the wetland's surface area almost completely, and thereby effectively antagonize the phytoplankton community (Knipling *et al.*, 1970; Reddy and DeBusk, 1985; Orth and Sapkota, 1988). The surfactants used in domestic detergents, like anionic linear alkyl benzene sulphonate and cationic octyl phenoxy polyethoxy ethanol, are known to cause an acute toxicity to many algae (Matulova, 1964; Ukeles, 1965; Nyberg, 1976; Kikuchi, 1979; Yamane, 1984) and even a substantial reduction in the density and variety of a natural phytoplankton community (Lewis, 1986).

The availability of light and nutrients to phytoplankton considerably improves during the period of water hyacinth decay when the hyacinth litter releases substantial amounts of N and P by physical leaching during the initial phase and by microbial processes during the later phase of its decomposition (Reddy and Sacco, 1981; Varghese, 1991; Gaur *et al.*, 1992). As a consequence, the phytoplankton production rates and biomass should improve during this period, however our studies reveal no such improvement (Agarkar, 1984; Sandhu, 1986; Sharma 1986). There is no information about the toxic effects of leached matter from the decaying hyacinth litter and the domestic sewage. It is also not known as to how the detergent containing both surfactant and phosphorus affect the algal growth, since the former suppresses the growth while the latter stimulates the growth. The present study thus has been designed to analyse the effects of domestic detergents, sewage and leachate of water hyacinth decay on the algal growth, using a green alga *Scenedesmus obliquus* (Turpin) Kuetzing, and to determine whether any or all of these materials can cause an acute toxicity to the alga.

MATERIALS AND METHODS

Test Materials : Nirma and surf, brand names of commonly used detergent powders by the society, have been considered in the present study. An aqueous stock solution of each detergent was prepared by dissolving 4 g detergent powder in 80 ml glass distilled water and the final volume made to 100 ml. Phosphorus concen-

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Table 1: Chemical composition of leachate of water hyacinth decay (Mean ± SD)

Days of decay	Type of litter	pH Units	PO_4-P ppm	NO_3-N ppm
1	Leaf	6.5±0.2	33±3	2.3±0.1
	Root	7.0±0.2	20±1	1.8±0.1
10	Leaf	7.8±0.1	37±2	3.8±0.2
	Root	7.4±0.3	23±1	3.4±0.2
35	Leaf	8.0±0.2	35±3	6.4±0.5
	Root	7.9±0.1	21±2	4.5±0.4

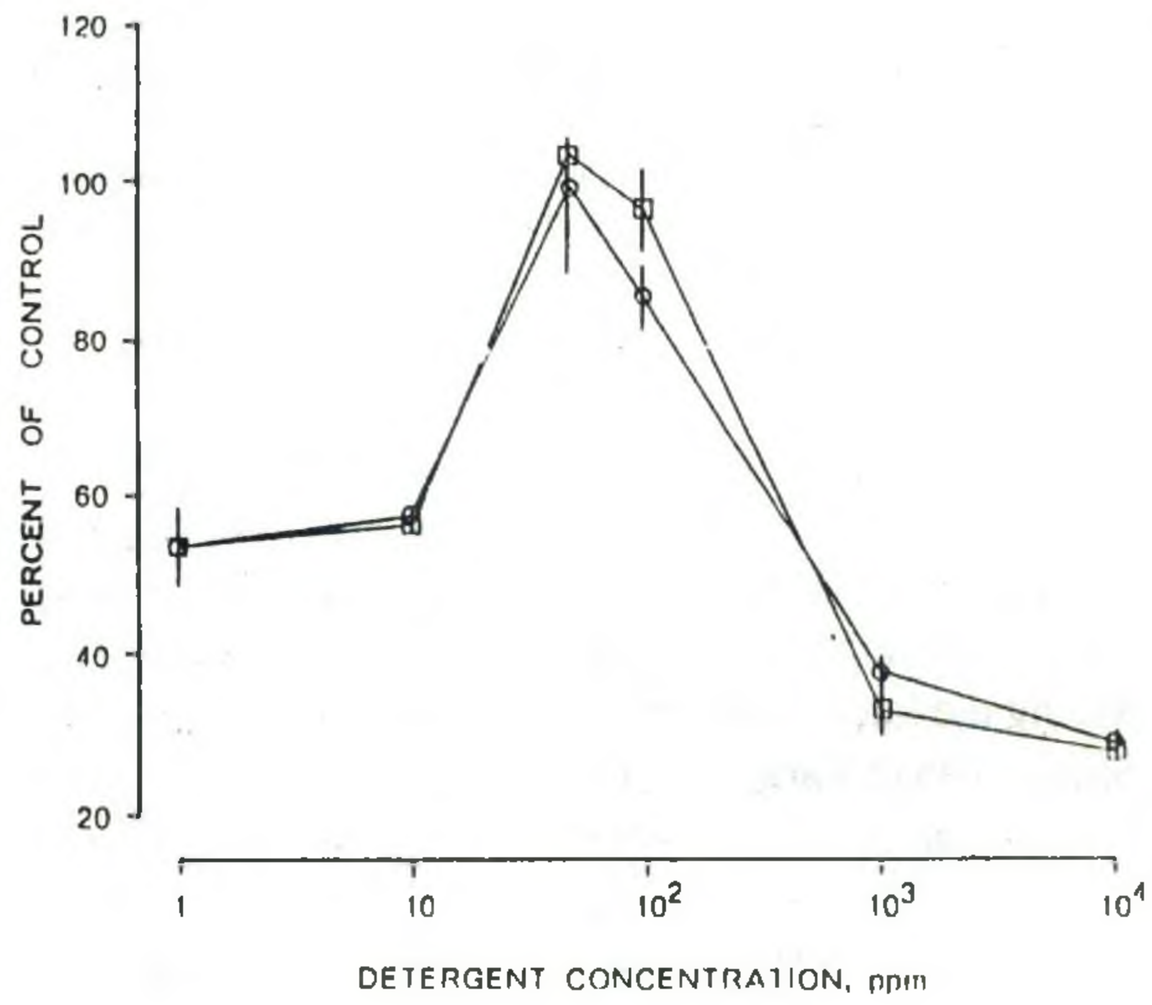

Fig. 1. Effect of Nirma (○) and Surf (□) on the growth of *S. obliquus* (Mean ± SD).

tration in detergents was 7 mg/g Nirma and 39 mg/g Surf. The domestic sewage was collected from a local nalah, filtered through whatman glass fibre filter paper (GF/C) and sterilized in a steam sterilizer at 121°C and 15 lbs pressure for 15 minutes. The sewage contained 5 ppm PO_4-P, 1.5 ppm NO_3-N and 350 ppm COD.

The leachate of water hyacinth decay was obtained from its post-bloom litter, collected from a local lake. The leaf and root portions of the hyacinth litter were separated, washed thoroughly under tap water and dried at 60°C for 3 days. The tared portions (10g) of coarse leaf and root litter were placed in separate sets of 12 polypropylene bottles (ca.1L) containing 600 ml lake water which was filtered through a 1 mm mesh sieve. The mouth of each bottle was covered by a coarse cotton cloth and the bottles were then incubated in the dark at 28±2°C. Each bottle was shaken twice a day, and its water level was maintained by weekly addition of sterile glass-distilled water. The leachate from sets

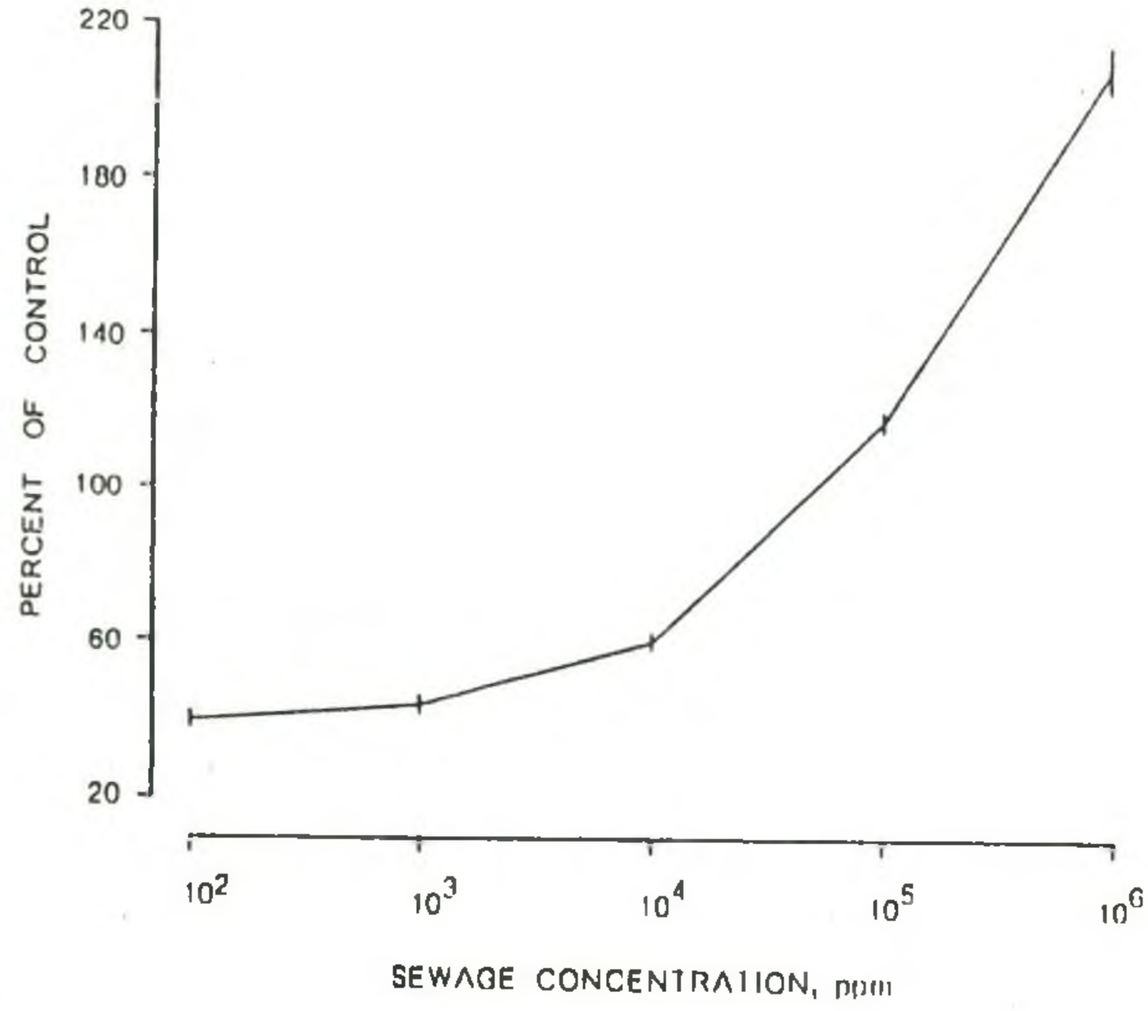

Fig. 2. Effect of domestic sewage on the growth of *S. obliquus* (Mean ±SD).

of decaying leaf and root litter was collected after 1, 10 and 35 days of decay. After each decay period, the leachate from the 3 randomly selected bottles of each set was retrieved by filtering the contents of each bottle through whatman glass fibre filter paper (GF/C). The filtrate of the 3 bottles of each set was pooled and then sterilized in a steam sterilizer for 15 minutes. The chemical composition of the two leachates is shown in Table 1.

Stock algal cultures - The algal assay procedure used was the standard methods (APHA, 1985; ISO, 1987) with certain modification. The test alga *Scenedesmus obliquus* was grown submerged in the nutrient solution (Composition in ppm: KNO_3 25; KH_2PO_4,2; $CaCl_2$ $2H_2O$,5; $NaHCO_3$, 15; Na_2EDTA, 2.61; H_3BO_3, 0.05;$MnCl_2$. $4H_2O$, 0.036; $FeSO_4$. $7H_2O$, 0.249; $ZnSO_4$. $7H_2O$, 0.004; $CuSO_4$. $5H_2O$, 0.001; Na_2MoO_4. $2H_2O$, 0.002; all analytical grade chemicals). For this purpose, the required number of 150 ml Erlenmeyer flasks with cotton caps containing 40 ml nutrient solution (pH-7.5) was sterilized in a steam sterilizer for 15 minutes. After cooling, the contents of each flask were inoculated with 1 ml cell suspension in the exponential growth phase in order to obtain the initial cell density of 10^5±10% cells ml^{-1}. The inoculated flasks were placed on a white surface protected from daylight under constant illumination from the two parallel fluorescent 25 w tubes at 28±2°C for 3 days. The cultures were shaken twice a day for 5 minutes, and

Table 2. The growth of Scenedesmus obliquus in the control sets (Mean ± SD)

Time days	Cell density 10^5 cells ml^{-1}	Chlorophyll a µg ml^{-1}	Optical density at 675 nm
0	1.24±0.2	0.020±0.002	0.018±0.003
1	2.71±0.2	0.044±0.004	0.039±0.004
2	4.23±0.4	0.064±0.007	0.056±0.006
3	6.12±0.6	0.093±0.008	0.081±0.007

Chlorophyll a (µg ml^{-1}) = $2.375 \times 10^{-3} + 1.48 \times 10^{-7}$ cell density (10^5 cells ml^{-1}) ($r^2 = 0.998$, $P<0.001$)
Chlorophyll a (µg ml^{-1}) = $-1.015 \times 10^{-3} + 1.16$ optical density ($r^2 = 0.999$, $P<0.001$)

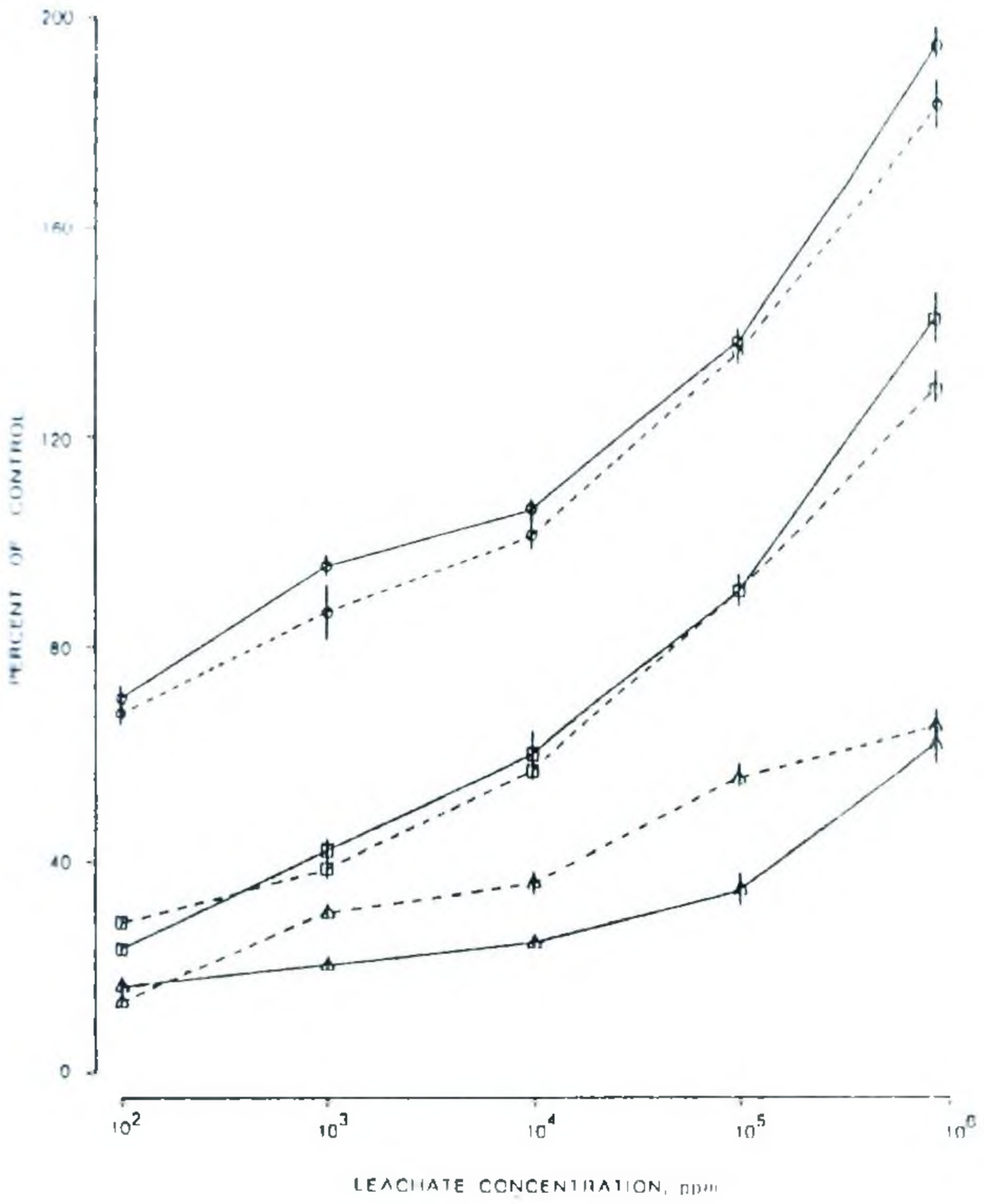

Fig. 3. Effect of leachate of 1-day decay ( ), 10-day decay ( ) and 35-day decay ( ) in the hyacinth leaf ( ) and root ( ) litter on the growth of *S. obliquus* (Mean ± SD).

the growth in each flask was measured daily. The growth of *Scenedesmus* cell suspension was measured by optical density at 675nm using a 1 cm light path on a Systronics digital spectrophotometer 106, chlorophyll a by methanol extraction (Mackinney, 1941) and cell density by using a haemocytometer cell under a compound light microscope (X600).

The three parameters of algal growth have shown a very significant positive correlation with each other and gave similar results ($Fp>0.05$; Table 2). The semi-logarithmic graphic presentation of these values revealed an exponential growth up to 3 days after begining of the incubation. This means that there was about 5-fold increase in the algal biomass within three days. The maximum specific growth rate of the alga was 0.78 d^{-1}, and its average rate on the basis of 3-day incubation was 0.5±0.06 ($r^2 = 0.97$).

Test preparations - For analysing the effects of detergents, sewage and leachate of water hyacinth decay, an appropriate aliquot of their aqueous solution was measured in separate sets of eighteen 150 ml Erlenmeyer flasks containing 4ml of the 10-fold concentration of nutrient solution. The final volume in each test flask was made to 40 ml from glass-distilled water, and the pH to 7.5, to get 10^2 to 9×10^5 ppm concentration levels of the leachate and sewage, and 1 to 10^4 ppm concentration levels of detergents.

The growth in the test and control flasks was measured after a 3-day incubation according to recent recommendations of International Standards organization (ISO, 1987). The results are expressed as mean±SD algal biomass as percentage of the control, based on three replicates of two experiments. The LC_{50} values of each test material was calculated by a best fit regression of log concentration of the test material on the algal biomass as percentage of control by the least square method.

RESULTS

Detergents - Both detergents, Nirma and Surf, caused very similar patterns and amounts of responses in the algal growth in various concentrations ($Fp>0.05$, Fig. 1). The higher as well as lower concentration levels effectively suppressed the algal growth, though the higher concentrations were more toxic compared with the lower ones ($Fp<0.01$). The medium concentration levels (50 to 100 ppm) caused a no-effect in the algal growth. The amount of suppression decreased significantly upon dilution of the higher concentration level ($Fp<0.001$) and non-significantly upon dilution of the lower concentration level ($Fp>0.05$). The LC_{50} of Nirma and Surf were 710 and 660 ppm, respectively.

Domestic sewage - The algal growth was considerably stimulated by the higher concentration levels and effectively suppressed by the lower concentration levels of domestic sewage (Fig. 2). The amount of stimulation decreased and of suppression increased upon dilution of domestic sewage. Thus, the maximum

stimulation occurred under the highest concentration ($9x10^5$ ppm) and the maximum suppression under the lowest concentration (100 ppm). The LC_{50} of domestic sewage was $1.2x10^3$ ppm.

Leachate of water hyacinth decay - The algal growth was considerably stimulated by the higher concentration levels of leachate of 1 and 10 days decay, but was effectively suppressed by the lower concentration levels of leachate of 10 days decay and by all the levels of leachate of 35 days decay (Fig. 3). The amount of stimulation decreased and of suppression increased significantly upon dilution of leachate ($Fp<0.001$), and also between leachates collected after 1 and 35 days decay ($Fp<0.01$). Thus, the concentration levels of leachate of 35 days decay were the most toxic and that of 1 day decay were the least toxic to alga. The patterns and amounts of effects of leachate on the algal growth were very similar between leaf and root litter in various concentrations and periods of decay ($Fp>0.05$). The maximum stimulation occurred under the effect of highest concentration ($9 x10^5$ ppm) of leachate of 1-day decay and the maximum suppression was recorded under the lowest concentration (100 ppm) of leachate of 35-days decay. The LC_{50} of leaf leachate of 1,10 and 35 days decay were, respectively, 5,$3.7 x 10^3$ and $6.9 x 10^4$ ppm, and that of root leachate were 9, $3.4 x 10^3$ and $6.9 x10^4$ ppm.

DISCUSSION

Higher concentrations of domestic sewage and leachate of water hyacinth decay considerably stimulate the algal growth. This is obviously due to a higher amount of nutrients in the sewage and leachate. Domestic sewage is considered generally a principal cause of high phytoplankton growth in wetlands (Cluis *et al.*, 1988; Couture *et al.*, 1985). Lower concentrations of sewage and leachate of 10 and 35 days decay in the hyacinth litter cause an effective suppression in the algal growth. This demonstrates that both sewage and leachate contain some toxicants for algal growth, and the concentration of toxicants in the leachate increases as the decay in the hyacinth litter is accomplished by microbial processes. Although not analysed in the present study, these toxicants may well be the soluble phenolic compounds and microbial metabolites. Phenolic compounds are known to act as antimicrobial agents and herbivore deterrents (Rice and Pancholy, 1974; Woodhead and Cooper-Driver, 1979; Buchsbaum *et al.*, 1984) and they leach out rapidly during the initial phase and slowly during the later phase of decomposition in lignocellulosic aquatic macrophytes (Wilson *et al.*, 1986). Toxicity estimates of detergents in the present study are similiar to the reported range of 0.05 to 500 ppm for different surfactants determined in the laboratory using various test species (Matulova, 1964; Ukeles, 1965; Nyberg, 1976; Kikuchi, 1979; Yamane, 1984; Lewis, 1986).

A considerable stimulation in the algal growth by the highest concentration and an acute toxicity by the lowest concentration of leachate of hyacinth decay and domestic sewage may denote that toxins of leachate and sewage are not able to affect the algal growth when nutrient levels are high but can do so when nutrient levels are low. The direct extrapolation of results of the present study to the phytoplankton community in a wetland may not be completely possible, since the effects are determined with one alga. However, our results provide an experimental evidence that domestic sewage and leachate of hyacinth decay cause very different response to alga by their higher concentrations in comparison with that caused by their lower concentrations. It may thus be envisaged that domestic sewage and leachate of hyacinth decay can stimulate the algal growth at their point sources, but can effectively suppress the algal growth in other parts of wetland after being diluted. Detergents will effectively suppress the algal growth at their point sources as well as in other parts of wetland.

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