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Combined effects of cadmium and linear alkyl benzene sulfonate on *Lemna minor* L.

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The effects of 0.1 ppm cadmium and 0.005% linear alkyl benzene sulfonates (LAS) on the uptake and metabolic incorporation of ^{14}C glycine by *Lemna minor* L., after 2, 24 and 48 h were studied for antagonistic/synergistic effects. Combined exposure was found to decrease the ^{14}C incorporation into proteins, DNA, RNA and phospholipids, to a greater extent than individual exposure. The presence of LAS increased the uptake of ^{109}Cd in the plants.

Keywords: cadmium; detergents; duckweed; glycine-metabolism; toxicity; water pollution

Introduction

The ecotoxicological impact of water pollution usually involves the combined effects of more than one stress factor. Even though studies with individual species and specific toxicants have led to considerable information for regulatory purposes and environmental impact assessment, investigations of combined effects are essential and few (Lewis, 1990; Potani *et al.*, 1990) have been undertaken. The ecotoxic potential of linear alkyl benzene sulfonates (LAS) detergents and common water pollutants, on diverse aquatic flora and fauna (Misra *et al.*, 1987; Chawla *et al.*, 1989) suggested the need to study their toxicity along with that of other pollutants. It was found earlier that sublethal amounts of mercuric ion could retard the biodegradation of the detergent (Misra *et al.*, 1991). A study was undertaken to determine any changes in the ecotoxicity of cadmium when there is simultaneous exposure to other factors including LAS, prompted partly by studies on the metabolic changes caused by LAS and Cd alone on aquatic flora (Chawla *et al.*, 1989; Singh *et al.*, 1991). Therefore, the combined effects of Cd and LAS on the uptake and incorporation of [^{14}C]glycine by *Lemna minor* L. were investigated. Glycine was selected in view of its reported uptake from the medium by *Lemna* (Fischer and Luettge, 1980) and its incorporation into a wide variety of compounds.

Materials and methods

Axenic cultures of *L. minor* L. propagated in the laboratory and maintained as described earlier (Chawla *et al.*, 1991) were used. For each experiment healthy young plants were

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used, and for each group four such sets were taken. [^{14}C]Glycine (21.4 mCi mmol^{-1}, BARC, Bombay, India) was diluted in 1 mM cold glycine and for each set 10 μCi were added at the beginning of the experiment. The amounts specified for LAS and Cd were on the basis of 25% of their EC_{50} values. Four groups of experimental sets were used: (1) control, (2) 0.1 ppm cadmium, (3) 0.005% LAS (Indian Petrochemicals Ltd., Baroda, India), and (4) 0.1 ppm Cd + 0.005% LAS.

After 2, 24 and 48 h of exposure, the plants were harvested, washed thoroughly with distilled water, blotted on paper, weighed and processed for the various estimations. The plants were homogenized (5% w/v) in cold water and aliquots were processed for chlorophyll and other pigments after extraction with 80% acetone (Arnon, 1949). With other aliquots, protein was precipitated by 20% TCA. From the TCA precipitate phospholipids, RNA, DNA and proteins were separated by the methods of Volkin and Cohn (1964). TCA soluble fractions were also collected, and phospholipids were extracted with methanol + chloroform (3:1), separated on silica gel G by thin layer chromatography, and identified by iodine. Phosphorus and protein contents were determined according to Fiske and Subbarow (1925) and Lowry *et al.* (1951), respectively. For radioactive counting, 1 ml aliquots were mixed with 5 ml of freshly prepared scintillation cocktail and placed in a scintillation counter (LKB Model 1215/1216). The composition of the scintillation cocktail was 52 g naphthalene, 2.25 g 2,5-diphenyloxazole, 65 mg 1,4-bis(5-phenyloxazolyl)benzene, 250 ml dioxane, 150 ml methanol, and 250 ml toluene. One way analysis of variance was done according to Tracy L. Gustafson, Round Rock, Texas, version 3 (1984).

Incorporation of ^{109}Cd was assessed to determine whether the greater toxicity of Cd in the presence of LAS was due to higher uptake of the metal from the medium by the plant. For this, 0.1 μCi ^{109}Cd mCi per μg Cd, Radiochemical Centre, Amersham, UK) was mixed in 750 ml of medium containing 0.1 ppm $CdCl_2$ alone and 0.1 ppm Cd and LAS (0.005%). These media were used for exposing *L. minor* as described above. After 2, 24 and 48 h, the plants were harvested, blotted on a paper and weighed. The emission from ^{109}Cd incorporated in each sample was recorded on an LKB ultrogamma counter for 1 min. Corrections were made for background and natural decay of isotopes during the experiment.

Results

There was a steady increase in growth in the controls (Table 1). Both Cd and LAS retarded growth, whereas when both factors were present, the decrease in biomass was synergistic at 48 h ($p < 0.01$) and growth inhibition was 26% after this time interval. Chlorophyll content increased in the control and LAS treatments over time (Table 2). Chlorophyll content was higher than in the controls ($p < 0.05$) after 48 h in case of LAS alone. Cadmium alone after 2 and 24 h had values similar to the controls but was 14% less after 48 h. Initially, Cd + LAS did not show any difference when compared with LAS alone but was higher than controls. As exposure progressed, the combined effect on chlorophyll after 48 h was slightly higher than in the controls but lower than that of LAS alone ($p < 0.01$). The LAS + Cd values were higher than Cd alone ($p < 0.01$) after 48 h, but after 24 h they were less.

The total protein content of the controls increased by almost 77% ($p < 0.01$) after 48 h (Table 3). Neither LAS nor Cd alone caused any change after 24 h but the combined

Table 1. Biomass changes in *L. minor* due to Cd and LAS exposure at four time intervals[a]

	Changes in biomass as % of 0 h time			
	0 h	2 h	24 h	48 h
Control	0.054 ± 0.004	0.056 ± 0.003	0.058 ± 0.004	0.069 ± 0.005
LAS	0.055 ± 0.004	0.054 ± 0.004	0.049 ± 0.003	0.047 ± 0.007
Cd	0.059 ± 0.005	0.057 ± 0.004	0.059 ± 0.003	0.052 ± 0.003
Cd + LAS	0.059 ± 0.006	0.055 ± 0.004	0.049 ± 0.003	0.040 ± 0.005

[a]Values are arithmetic mean ± SE of four replicates.

Table 2. Effect of Cd and LAS on total chlorophyll content at three time intervals (mg per g fresh weight tissue)[a]

	2 h	24 h	48 h
Control	0.60 ± 0.06	0.62 ± 0.13	0.65 ± 0.09
LAS	0.71 ± 0.14	0.78 ± 0.05	0.89 ± 0.02
Cd	0.60 ± 0.08	0.64 ± 0.12	0.56 ± 0.03
Cd + LAS	0.69 ± 0.60	0.53 ± 0.08	0.70 ± 0.02

[a]Values are arithmetic mean ± SE of four replicates.

Table 3. Effect of Cd and LAS on protein content of *L. minor* at three time intervals (mg per g fresh weight)[a]

	2 h	24 h	48 h
Control	56.0 ± 2.3	83.0 ± 6.2	99.5 ± 8.9
LAS	57.1 ± 5.8	81.3 ± 1.6	78.3 ± 4.5
Cd	57.3 ± 3.6	81.5 ± 4.3	67.2 ± 1.2
Cd + LAS	56.2 ± 2.5	58.3 ± 2.1	56.5 ± 7.8

[a]Values are arithmetic mean ± SE of four replicates.

system led to a 30% decrease ($p < 0.001$). In fact, in the combined system there was no increase in protein content during the study. After 48 h, LAS and Cd caused 22% and 33% decrease in protein, respectively, compared with controls. The data recorded in Table 4 show that LAS tended to enhance the Cd uptake at all the time intervals, although the statistical significance was low.

Glycine uptake by *L. minor* increased with time of exposure in the case of the controls, the values after 48 h being about 4 times that after only 2 h ($p < 0.001$) (Table 5). In the presence of Cd, uptake was higher in all the periods by 28% ($p < 0.01$), 28% ($p < 0.01$) and 124% ($p < 0.02$) after 2, 24 and 48 h, respectively, compared with controls. With LAS alone, the total counts per unit tissue were 63% ($p < 0.001$), 78% ($p < 0.01$), 85% ($p < 0.01$) of the controls after 2, 24 and 48 h, respectively. With LAS and Cd together

Table 4. Uptake of Cd by *L. minor* at three time intervals (μg per g tissue) in the absence and presence of LAS[a]

	2 h	24 h	48 h
Control	–	–	–
LAS	–	–	–
Cd	18 ± 5	49 ± 3.0	44 ± 2.0
Cd + LAS	23 ± 6	53 ± 2.0	57 ± 2.0

[a]Values are arithmetic mean ± SE of four replicates.

Table 5. Glycine ^{14}C uptake by *L. minor* in presence of Cd, LAS and Cd + LAS at three time intervals (counts min^{-1} per mg tissue)[a]

	2 h	24 h	48 h
Control	5287 ± 161	16850 ± 2159	21568 ± 2191
LAS	1948 ± 170	3639 ± 801	3185 ± 712
Cd	6773 ± 168	21497 ± 301	48201 ± 4150
Cd + LAS	545 ± 61	1656 ± 259	7217 ± 477

[a]Values are arithmetic mean ± SE of four replicates.

the counts after 2 and 24 h were much less than even with LAS alone ($p < 0.01$), but showed over 3 times the counts of controls ($p < 0.01$) after 48 h.

The content of radioactive glycine in the TCA insoluble fractions of *L. minor* are recorded in Fig. 1. In the controls, the rate of protein synthesis showed progressive increase after 24 and 48 h ($p < 0.001$). During this period, under toxic stress, the value was considerably lower at later stages, even though, as a result of the toxicants, there was a rapid initial increase after 2 h, especially with LAS alone ($p < 0.001$), compared with controls. After 24 and 48 h Cd alone caused about 44% ($p < 0.01$) and 71% ($p < 0.001$) decrease. With LAS alone, the decrease in protein synthesis amounted to 35% ($p < 0.1$) and 65% ($p < 0.001$) after 24 and 48 h, respectively, compared with controls. Cd alone was, thus, slightly more effective in reducing glycine incorporation with protein than LAS. Nevertheless, when both toxicants were present protein synthesis was less than with separate toxicants. The values after 24 and 48 h were 63% ($p < 0.01$) and 79% ($p < 0.001$), respectively, causing synergistic toxicity compared with controls.

The counts in the TCA soluble fraction showed a higher level (data not given) in Cd exposed cases compared with controls. This was more marked after 48 h, the values being about 3 times that of controls ($p < 0.001$). LAS caused a highly significant decrease in nonprotein radioactivity in all the stages. The combined exposure to Cd and LAS resulted in even lower values after 2 ($p < 0.001$) and 24 h ($p < 0.001$), showing synergistic effects compared with controls. However, after 48 h the counts were about five times that of LAS alone ($p < 0.001$). This was only about 40% of the controls ($p < 0.01$), so that an antagonistic effect seems unlikely.

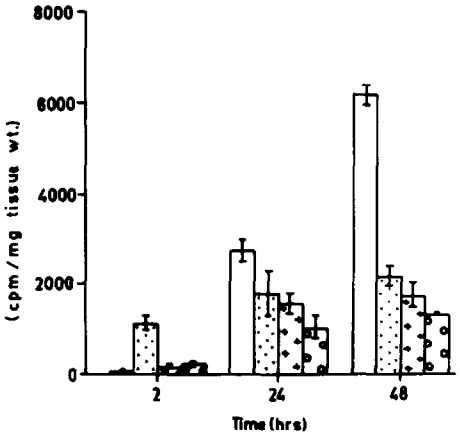

Fig. 1. Incorporation of [^{14}C]glycine into the protein fraction of *L. minor*: ☐, in presence of LAS; ⁘, Cd; ⊞ alone and Cd plus LAS; ⁘ at three different time intervals.

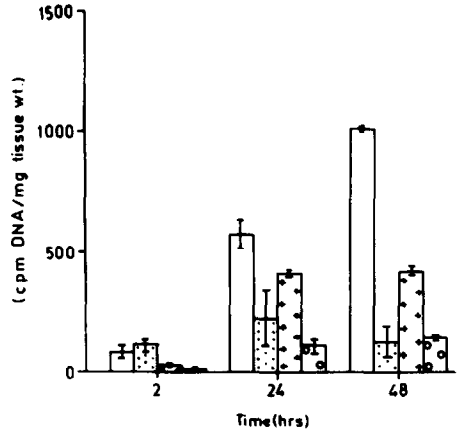

Fig. 2. Incorporation of [^{14}C]glycine into the DNA fraction of *L. minor*: ☐, in presence of LAS; ⁘, Cd; ⊞ alone and Cd plus LAS; ⁘ at three different time intervals.

In control plants, radioactivity in the DNA fraction showed progressive increase, with about 75% increase between 24 h and 48 h ($p < 0.001$) (Fig. 2). Cd alone caused 65%, 27%, 58% decrease in DNA synthesis in terms of tissue mass. After 24 and 48 h, evidence of toxic effects resulted in 61% and 88% decreases in DNA label. Simultaneous exposure to LAS and Cd was even more detrimental to DNA synthesis, with 81–87% inhibition in all the stages.

The glycine label in RNA increased by 5.7- and 8.8-fold after 24 h ($p < 0.001$) and 48 h ($p < 0.02$) compared with 2 h sample in the controls. The effect of Cd alone was a 40% decrease after 24 h ($p < 0.02$) compared with controls. As in case of DNA, LAS alone caused an initial low increase in RNA synthesis also, but after 24 and 48 h the RNA label

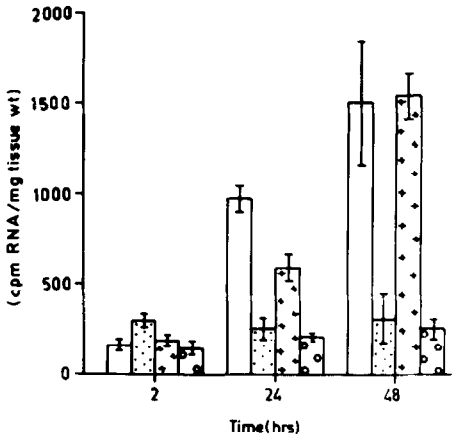

Fig. 3. Incorporation of [^{14}C]glycine into the RNA fraction of *L. minor*: ☐, in presence of LAS; ⁘, Cd; ⊞ alone and Cd plus LAS; ⁘ at three different time intervals.

decreased by 74% and 80%, respectively. LAS + Cd did not affect the RNA label after 2 h, but after 24 and 48 h caused 78% and 83% decreases, respectively (Fig. 3).

Incorporation of the glycine label into the phospholipid fraction, increased in all the cases during the study (Fig. 4). In controls, the values after 24 and 48 h were 4.7- and 8.7-fold higher than that after 2 h ($p < 0.001$). In the case of Cd treated *L. minor*, the phospholipid values were only 39%, 69% and 59%, respectively, compared with controls after 2, 24 and 48 h. LAS by itself was even more inhibitory than Cd, the corresponding values being 80%, 73% and 73% compared with controls. After 2 h, LAS + Cd treatment showed a similar value to that of Cd alone, but after 24 and 48 h, the phospholipid label was synergistically reduced. The values were only 12.7% and 14.5% of the controls. The phospholipid extract of 48 h treated plants were concentrated in a

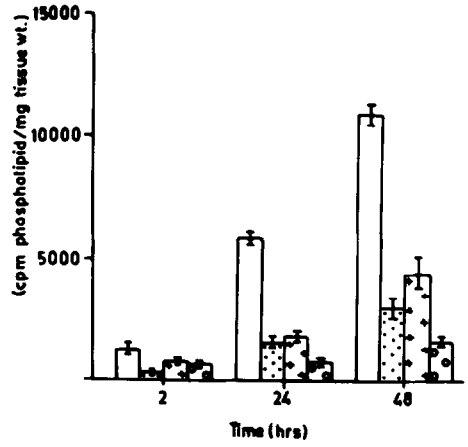

Fig. 4. Incorporation of [^{14}C]glycine into the phospholipid fraction of *L. minor*: ☐, in presence of LAS; ⁘, Cd; ⊞ alone and Cd plus LAS; ⁘ at three different time intervals.

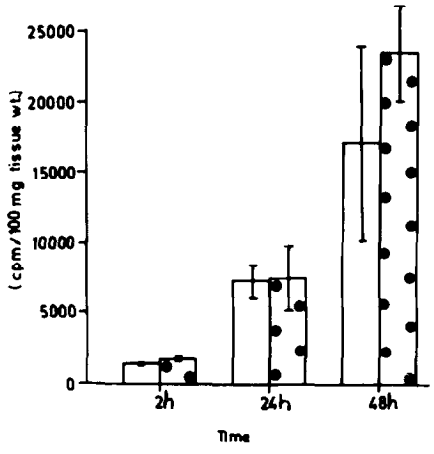

Fig. 5. Uptake of ^{109}Cd by *L. minor* in the presence of Cd ☐ and B. Cd plus LAS ⁘ at three time intervals.

vacuum, lecithin was separated on TLC plates and counted for radioactivity. The specific activities in terms of phorphorus content of the lecithin spot were 133 ± 40, 21 ± 3, 43 ± 10, and 18 ± 4 for control, LAS, Cd and LAS + Cd, respectively, indicating lecithin synthesis to be affected in toxicity.

The data on radioactive ^{109}Cd uptake indicated that even after 2 h there was detectable uptake, which increased by 5.1- and 12-fold, respectively, after 24 ($p < 0.01$) and 48 h ($p < 0.01$). In the case of Cd + LAS the pattern was similar but the uptake was slightly higher than with Cd alone. After 2, 24 and 48 h, the isotopic Cd contents were 27% ($p < 0.05$), and 37% ($p < 0.01$) higher (Fig. 5).

Discussion

In *Scenedesmus quardicauda* the uptake of radioactive amino acids and nutrients was reduced by LAS (Chawla *et al.*, 1987); in *L. minor* this could be attributed to surface morphological changes (Guilizzoni, 1991). The present results show that LAS decreased the glycine uptake and Cd tended to enhance it, especially at later stages, which could be due to tissue injury. The higher proportion of nonprotein radioactivity in Cd exposed plants after 48 h also indicates tissue injury and protein catabolism. Combined exposure to Cd and LAS showed that the biomass decrease was due to synergistic effects. In the case of chlorophyll, LAS alone slightly increased and Cd decreased the content. Cd content increased in presence of LAS. Similarly, the rate of uptake followed by ^{109}Cd uptake was also increased by the presence of LAS.

The initial uptake of Cd by *L. minor* is in agreement with the data of Polar and Kucukcezzar (1986). Simultaneous exposure to Cd and LAS reduced DNA, RNA, protein and phospholipid synthesis compared with individual exposures. In the present data it is shown that the Cd concentration (0.1 ppm) generally encountered in natural bodies of water could pose a toxic risk to aquatic macrophytes. The increase in the uptake of Cd in the presence of traces of LAS could be due to better solubilization of the material as well as to the permeability changes induced by the detergent. Earlier studies

from our laboratory showed that LAS enhances ^{45}Ca uptake from water by snails (Misra *et al.*, 1989) and ^{65}Zn uptake by *S. quardicauda* (Chawla *et al.*, 1987). Retarded macromolecular synthesis by Cd could be due to interference with enzymes, especially with functional thiols (Stobert *et al.*, 1985). Weigel (1985) studied the inhibition of CO_2 fixation in lambs lettuce by Cd in the regenerative phase of the Calvin cycle. The decrease in DNA synthesis by Cd and its synergistic increase by LAS could be the reason for growth retardation. Thus, the Cd mediated metabolic changes in *L. minor* are further aggravated by LAS exposure.

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