Assessment of Soil Microbial Toxicity on Acute Exposure of the Anionic Surfactant Linear Alkylbenzene Sulphonate

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ABSTRACT

The use of synthetic detergents is on increasing ladder due to shift from soaps to detergents. Linear Alkylbenzene Sulphonates (LAS) are one of the major anionic surfactant and used as active ingredients in household and personal care products. LAS may reach the soil environment due to its widespread use and disposal. In soil microcosm study LAS at different concentrations were added to the test soil and total bacterial, fungal and actinomycetes counts were recorded at different intervals. Bacteria showed high sensitivity to LAS exposure concentration, compared to fungi and actinomycetes. Soil dehydrogenase activity was also recorded and found that LAS treatment reduced dehydrogenase activity significantly. The correlation between heterotrophic bacterial count and dehydrogenase activity was done using a matrix of Pearson’s correlations coefficient. Though at lower concentrations a positive correlation was noted between the enzyme activity and bacterial count, at higher LAS concentrations a negative correlation was observed. Further studies are needed in order to evaluate the effects of LAS on soil quality.

Key words: Soil microcosm, Tukey multiple comparison, dehydrogenase activity, Karl Pearson’s correlation coefficient

INTRODUCTION

Surfactants, one of the major xenobiotic compounds used today, are widely used in household cleaning detergents, personal care products, textiles, paints, polymers, pesticide formulations, pharmaceuticals, mining, oil recovery and pulp and paper industries. Anionic surfactants are the earliest and the most common surfactants. One of the major xenobiotic anionic surfactant that has large-scale industrial application and thus broad environmental release is Linear Alkylbenzene Sulphonates (LAS). Many of the commercially available synthetic detergents contain alkylbenzene sulphonate (LAS) and its isomers (Dong et al., 2004).

LAS may reach the soil environment when anionic surfactants are used as emulsifying, dispersing and spreading agents in the processing of fertilizers and distribution of pesticides in agriculture. Concentrations of LAS in raw sewage sludge are very high due to its wide spread use and strong sorption on sludge during treatment. According to Mackay et al. (1996), LAS reaches the soil through sludge which is used as a fertilizer on agricultural land. The presence of surfactants in sludge may have undesirable environmental effects since the surfactant molecules may reach to groundwater contributing to its contamination. According to Jensen (1999), the load of LAS in sewage sludge may be considerable with concentrations of more than 10 g kg⁻¹ dry weight.
Pollution of the terrestrial environment with a variety of industrial products and agrochemicals necessitate better understanding of soil microbial population dynamics. Exposure of soil to xenobiotics make it hostile for microorganisms. This in turn affect the microbial enzymes and soil fertility (Zahir et al., 2001). Soil enzymes play pivotal role in ecosystem management and nutrient recycling (Makoi and Ndakidemi, 2008). Enzymes respond to soil changes well in advance compared to other soil quality indicator changes are detectable.

Soil dehydrogenase can be taken as a representative enzyme in soil fertility studies because it is closely associated with soil microbial activity (Garcia and Hernandez, 1997; Quilchano and Maranon, 2002). Dehydrogenase is responsive to soil environment (Caldwell, 2005). Hence, any fluctuation in the soil properties like aeration (Brzezinska et al., 2001), organic content (Gajananda, 2007), presence of xenobiotics (Gao et al., 2010), agricultural practices like use of pesticides and herbicides (Stepniewska et al., 2007) will influence the enzyme. It can also indicate the type and significance of pollution in soils (McCarthy et al., 1994; Pitchel and Hayes, 1990).

The presence of LAS in commercially available detergents is very important for this study. This confirms the discharge of the chemical to the environment during the day-to-day cleaning activities. In India, domestic waste water and city sewage are used for irrigating kitchen gardens and crop lands and the surfactants and other additives may affect crops or soils or both. This may ultimately cause nutritional imbalance, disorders and toxic effects on growth and metabolism of organisms. A toxicity study is becoming relevant in this scenario. Toxicity study of acute exposure of LAS on soil microbial activity was carried out. The methodology and the findings of the study are detailed below.

MATERIALS AND METHODS
LAS in detergents: LAS in detergent was analysed as follows. To 1 g of detergent, 100 mL of methanol was added and the mixture was sonicated for 10 min. Twenty microliter of the supernatant methanol was injected into the HPLC. Ten commercially available detergents were selected for LAS analysis (Nakae et al., 1981).

LAS toxicity study on soil microbial activity using soil microcosms: Soil used for experiments was collected from agricultural soil which had no previous exposure to LAS contamination. After the removal of plant residues, samples were collected at a depth of 0.20 cm using a soil core sampler. The soil was air dried immediately after collection and sieved to remove granules and plant residues. Aqueous solutions of LAS stock solution were freshly prepared for addition to soil. Triplicate soil samples were mixed with diluted LAS solutions to give the resulting LAS concentrations of 0, 2, 4, 8 and 16 g kg$^{-1}$.

Assay of potential dehydrogenase activity (Casida et al., 1964) and microbial population counts were done with sub samples from the same batches of LAS amended soil. These batches represented 100 g dry weight of soil in each of fifteen 250 mL conical flasks. Appropriate LAS solutions were added drop wise to triplicate flasks to give selected concentrations and a gravimetric water content of 16%. All soils were carefully mixed and incubated in the dark at 15°C. Microbiological and enzymatic assay were done after 7th, 14th and 21st days of incubation. Prior to subsampling, the soil was thoroughly mixed. During incubation, the soil microcosms were weighed regularly and weight loss was compensated for by addition of water.

Quantification of microbial populations in soil: Quantification of microbial population in LAS amended soil was done as per the method proposed by Alef (1995).
**Statistical analysis:** Statistical analysis of the results was done using ANOVA, Karl Pearson’s correlation coefficient and Tukey multiple comparison test by using SAS system (W32_VSHOME).

**RESULTS**

**LAS in commercial detergents:** The amount of LAS in ten commercially available detergents were analysed (Table 1). It was found that all the tested detergents contain LAS but the concentration varied. By comparing the fingerprint area in the LAS control with that of the tested commercial detergents presence of LAS in detergents but in varying concentration was confirmed. From the HPLC chromatogram major peaks of the control and the tested detergents were selected. The area of the selected peaks of the chromatogram was calculated and compared that with LAS control. Among the tested detergent D1 contained highest concentration of LAS, about 451.695 ppm and the detergent D7 was found to contain lowest concentration of LAS about 47.119 ppm.

**LAS toxicity study on soil microorganisms:** A soil microcosm study was performed to check the effect of different concentrations of LAS on soil bacteria, fungi and actinomycetes (Table 2). A control treatment without LAS was maintained throughout the study. The bacterial count of LAS amended soil treatment recorded an increase compared to control. But as the

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Total area of the chromatogram</th>
<th>Concentration of LAS g⁻¹ of detergent (ppm) compared to standard (500 ppm LAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>792056</td>
<td>451.695</td>
</tr>
<tr>
<td>D2</td>
<td>176090</td>
<td>103.276</td>
</tr>
<tr>
<td>D3</td>
<td>877846</td>
<td>51.506</td>
</tr>
<tr>
<td>D4</td>
<td>283490</td>
<td>166.318</td>
</tr>
<tr>
<td>D5</td>
<td>327628</td>
<td>191.892</td>
</tr>
<tr>
<td>D6</td>
<td>493617</td>
<td>289.402</td>
</tr>
<tr>
<td>D7</td>
<td>809061</td>
<td>47.119</td>
</tr>
<tr>
<td>D8</td>
<td>599817</td>
<td>351.957</td>
</tr>
<tr>
<td>D9</td>
<td>287588</td>
<td>108.954</td>
</tr>
<tr>
<td>D10</td>
<td>437018</td>
<td>256.412</td>
</tr>
<tr>
<td>LAS (500 ppm)</td>
<td>852792</td>
<td>55.217</td>
</tr>
</tbody>
</table>

**Table 2: Total viable count of soil microorganisms in soil microcosm study with different concentrations of LAS**

<table>
<thead>
<tr>
<th>LAS concentrations (mg kg⁻¹)</th>
<th>7th CFU mL⁻¹</th>
<th>14th CFU mL⁻¹</th>
<th>21st CFU mL⁻¹</th>
<th>7th CFU mL⁻¹</th>
<th>14th CFU mL⁻¹</th>
<th>21st CFU mL⁻¹</th>
<th>7th CFU mL⁻¹</th>
<th>14th CFU mL⁻¹</th>
<th>21st CFU mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6.672</td>
<td>5.602</td>
<td>6.693</td>
<td>3.477</td>
<td>3.301</td>
<td>3.477</td>
<td>2.602</td>
<td>2.301</td>
<td>2.301</td>
</tr>
</tbody>
</table>

CD (0.05%)  

<table>
<thead>
<tr>
<th>SOY</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>2.87</td>
<td>1.54</td>
<td>1.49</td>
</tr>
<tr>
<td>Days</td>
<td>2.33</td>
<td>1.19</td>
<td>1.15</td>
</tr>
<tr>
<td>Interaction</td>
<td>4.07</td>
<td>2.68</td>
<td>2.57</td>
</tr>
</tbody>
</table>

*Total count on 0th day Bacteria: 6.968, Fungi: 3.845 and Actinomycetes: 2.602*
Fig. 1: Multiple comparison of bacterial count with LAS concentration and incubation time (Tukey)
Fig. 2: Multiple comparison of fungal count with LAS concentration and incubation time (Tukey)

Fig. 3: Multiple comparison of actinomycetes count with LAS concentration and incubation time (Tukey)

enzyme activity as noted in the case of microbial count. The correlation between soil bacterial count and dehydrogenase enzyme activity was done using Karl Pearson’s correlation coefficient (Table 3). It was found that there was a positive correlation between bacterial count and enzyme activity in 2 and 4 mg LAS exposure concentrations. In case of higher selected concentrations i.e., 8 mg, there was no correlation and at 16 mg there was a negative correlation.
Table 3: Karl Pearson’s correlation coefficient between bacterial count and dehydrogenase activity

<table>
<thead>
<tr>
<th>LAS concentrations (mg kg⁻¹)</th>
<th>Dehydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-4.887470**</td>
</tr>
<tr>
<td>2</td>
<td>19.709940**</td>
</tr>
<tr>
<td>4</td>
<td>7.005240**</td>
</tr>
<tr>
<td>8</td>
<td>2.338554**</td>
</tr>
<tr>
<td>16</td>
<td>-0.191540 ns</td>
</tr>
</tbody>
</table>

**Significant p<0.01, *Significant p<0.05 and ns: Not significant

Fig. 4: Dehydrogenase activity of soil treated with different concentrations of LAS

DISCUSSION

Toxicity tests with soil microorganisms which are important for the nutrient cycling, are essential for an evaluation of the environmental effects of LAS (Jensen, 1999). The presence of LAS was analysed in ten commercially available detergents. It was found that LAS is a major surfactant component in all the tested detergents. The presence of such a high concentration of LAS in detergent formulations will definitely increase the chances of disposal of untreated LAS in the environment. The wide and indiscriminate use of detergents in household and industrial applications is therefore a potential source of LAS pollution. Hence, the toxicity study by taking representative organisms is very relevant.

It is necessary to quantify microbial populations in the contaminated soils. By using media with different types and levels of nutrients, the range of species identified may be completely different. Also the use of different extractants and extraction methods can give different results (Alelu, 1991). It is important that the medium used should facilitate the growth of the largest possible range of microorganisms. In this study a complex media was used for the enrichment and counting of microorganisms from LAS contaminated soil. The medium supported the growth of all the selected microorganism under study- bacteria, fungi and actinomyces. LAS concentration in natural soil is typically low, ranging between 0.7 and 1.4 mg kg⁻¹ (Carlsen et al., 2002). In sludge amended soil its concentration may reach up to 20 mg kg⁻¹ (Slobe et al., 2000). Considering these reports the LAS concentration range in this study was determined and it ranged between 0 and 16 mg kg⁻¹ of LAS.

The bacterial count in LAS amended soil showed statistically significant variation in the selected treatments on LAS exposure. In a similar study it was reported that bacterial count was higher in LAS amended soil than in control soil (Vinther et al., 2003). According to them the increase in bacterial count may be due to either proliferation of LAS degrading bacteria or desorption of bacteria from soil particles. Findings of Sanchez-Plinidad et al. (2008) supported these results. According to them proliferation of selected strains occurred in LAS containing media. Application of high concentrations of LAS exerts a selective pressure on the heterotrophic platable bacterial
diversity and it was found to be reducing. In soil bacterial diversity is more important than bacterial count. So it can be concluded that even though LAS did not decrease soil bacterial count it cannot be taken as a criteria to state that LAS is non toxic to soil microorganisms. Because fertility of an agriculture soil is intimately linked to its particular microbiota and the relationship that exist between the microbial groups involved in nutritional cycles which are essential for the normal function of soil.

The effect of bacterial count on the selected LAS concentrations was analysed statistically using Tukey test. The bacterial count obtained in 0, 2 and 16 mg kg^{-1} LAS vary significantly with all other treatments. But there was no significant variation between the bacterial count from the treatments 4 and 8 mg kg^{-1}. At the same time bacterial count of 4 and 8 mg kg^{-1} vary significantly with the other treatments.

According to Elsgaard et al. (2001), under laboratory conditions, a partial recovery of microbial parameters occurred from toxic effects of LAS on prolonged incubation. Brandt et al. (2001) also reported the effect of LAS on activity and population dynamics of heterotrophic and ammonia oxidizing microorganisms. According to them the effect of LAS on the investigated microorganisms largely occurred during the first two months. Autotrophic ammonia incubation was inhibited initially in the LAS spiked sludge and on continued incubation it was found to increase. These effects may be due to the soil recovery or resilience of soil to toxic chemicals as reported by Bentz et al. (2004). This soil reaction and the stability attained by it after exposure to a toxic compound have been considered as an important characteristic of ecological systems (Hill, 1987).

Soil enzymes are good indicators on soil microbial activity (Abdalla and Langer, 2009). The dehydrogenase enzyme and phosphatase enzyme are two commonly selected soil enzymes and are recommended as suitable indicators of soil microbial activity by many workers (Matinizeh et al., 2008; Maurya et al., 2011). In their studies, to check the soil microbial activities, Subrahmanya et al. (2011) selected soil enzyme particularly dehydrogenase enzyme activity as an index.

Dehydrogenase activity can be effectively employed for providing a reliable measurement of microbial activity in conjunction with biodegradation of xenobiotics. As part of the characterisation of Polycyclic Aromatic Hydrocarbon (PAH) degrading soil microbes Srujana and Khan (2012) considered this enzyme and studied its activity in the presence of PAH. Onweremadu and Nwufo (2009) investigated the effect of different levels of chromium on selected soil microbial parameters and came to the conclusion that soil microbial activity decline to 17.07% in contaminated soils when compared with soils of control experiments. Venkatesan and Senthurpandian (2006) reported significance inhibition on the soil enzyme activity was reported due to application of trace elements.

Dehydrogenase enzymes are considered to be inactive in extracellular form and the potential dehydrogenase activity is, therefore, widely used as a measure of microbial biomass and activity (Morra, 1997). Linu et al. (2009) correlated the soil dehydrogenase activity and microbial activity and stated that the soil dehydrogenase activity increases with microbial inoculation. Elsgaard et al. (2001) reported that the dehydrogenase enzyme activity was recovered on prolonged incubation. But in this study such a recovery of the enzyme activity was not detected even though the microbial count was increased.

Meliani et al. (2011) employed Pearson’s correlation coefficient to study the relationship between P. fluorescens abundance and soil physiochemical properties and demonstrated that the abundance of the P. fluorescens is influenced by soil abiotic and biotic factors. In this study the correlation between heterotrophic bacterial count and dehydrogenase enzyme activity was done
using a matrix of Pearson’s correlation coefficient. At lower concentrations a positive correlation was observed between the bacterial count and enzyme activity. This may be due to the fact that LAS cause desorption of bacterial cells adsorbed on clay particles. At higher LAS concentration a negative correlation was noted between bacterial count and dehydrogenase activity.

Sanchez-Peinado et al. (2009) reported that the continuous application of the anionic surfactant LAS to the soil increased the acid and alkaline phosphatase activity and arylsulphatase activity. But the soil dehydrogenase activity was decreased on continuous LAS exposure, showing statistically negative correlation between bacterial count and dehydrogenase activity. They suggested that the negative correlation may be due to the inhibition of cell dehydrogenase activity in response to increasing LAS concentration.

CONCLUSIONS

Linear Alkylbenzene Sulphonate (LAS) are the most commonly employed anionic surfactants. Large quantities of detergents and their components are released into aquatic and terrestrial environments after use. The acute exposure of LAS due to the huge volume disposed to soil affects soil microbial activity. The soil microbial activity is a reflection of the soil fertility and LAS in turn reduce fertility of soil. So it is high time to check the environmental impact of LAS. Soil enzyme activities, in particular dehydrogenase activity, have been suggested as appropriate parameters for the monitoring of soil contamination. Using soil microcosm study, we found out that LAS application significantly influence soil bacterial count and dehydrogenase activity. At high concentrations of LAS a statistically negative correlation was observed between the bacterial count and dehydrogenase activity. Further studies are needed in order to evaluate the effects of LAS on ecological sustainability.

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