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# Phytotoxic Effects of Gray Water Due to Surfactants

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Abstract. **Recycling wastewater containing soaps and detergents for plant growth is highly desirable when fresh water is limited. This is especially true during times of drought and is imperative in some specialized situations such as a regenerative space habitat. To regenerate food, water, and air, the National Aeronautics and Space Administration's Controlled Ecological Life Support System (CELSS) must recycle wastewater commonly known as gray water. The anionic surfactant Igepon is the principal ingredient of many detergent formulations and soaps and is a prime candidate for use in a space habitat. To determine if gray water would have phytotoxic effects on crops grown in a CELSS, 'Waldmann's Green' lettuce (*Lactuca sativa* L.) was grown in nutrient solutions containing varying concentrations of Igepon TC-42. Igepon concentrations of 250 mg·L$^{-1}$ or higher in nutrient solutions resulted in phytotoxic effects in lettuce. Thus, the toxic threshold of Igepon is <250 mg·L$^{-1}$. Toxicity symptoms include browning of the roots within 4 hours of exposure to Igepon followed by suppression of root dry mass within 24 hours. Plant death never resulted from exposure to Igepon used in these experiments, although roots were killed. The phytotoxic effect of Igepon was not persistent; plants initially displaying acute toxicity show clear signs of recovery within 3 days of initial exposure. Further, when fresh plants were exposed to these same nutrient solutions 3 days or more following initial Igepon addition, no phytotoxic effect was observed. The elimination of the phytotoxicity was associated with a decrease in fatty acid components in the nutrient solution associated with Igepon. The degradation of phytotoxicity appears to be associated with microbes present on the surface of the roots and not directly due to any plant process or instability of the surfactant.**

The National Aeronautics and Space Administration (NASA) is developing Controlled Ecological Life Support Systems (CELSSs) for long-duration space missions. The success of CELSSs depends on the ability to reduce significantly the resupply requirements to a space habitat; this largely depends on the efficient recovery and recycle of resources from waste streams. Water represents the largest mass in the waste stream of a human-occupied habitat. Recovering this resource is essential, as the cost of launch to low-earth orbit has been estimated to be as much as $22,000/kg (Moses et al., 1989). With an estimated daily water use of 27 kg·d$^{-1}$ per person, resupply will be highly costly and displace scientific and other critical payloads.

Wastewater streams resulting from shower, laundry, and dishwashing activities contain solutes in low concentrations. Soaps and detergents (surfactants) are the major chemical contaminants; such wastewater is commonly referred to as gray water. These gray water sources make up the largest single waste stream, estimated at 75% to 95% of the total of liquid and solid wastes. Recovering gray water for potable and hygiene reuse is essential for space habitation and self-sufficiency in other remote and difficult-to-resupply human encampments. Reusing gray water in the domestic community is becoming more important as current water sources are becoming insufficient to meet increased demand in areas such as California. Water supplies are also being reduced as groundwater and surface water sources become polluted. Domestic reuse of gray water is mainly for landscape irrigation; however, there are small but increasing efforts to reuse gray water as hygiene and flush water.

Plants are the primary component of a CELSS. They represent the only practical method to produce food and play are critical in revitalizing air, purifying water, and processing waste (Bubenheim, 1991). In our current investigations, we have grown lettuce in controlled-environment, hydroponic culture with an anionic surfactant, Igepon TC-42 (a linear alkyl taurine sulfonate), added to the nutrient solution to simulate gray water. Igepon TC-42 is the principal ingredient of many detergent formulations and soaps and is a prime candidate for use in space habitats. The presence of surfactants in the root zone is of concern, as surfactants can disrupt membrane integrity allowing access to intercellular components (Quinn, 1976). Previous workers have reported growth stimulation and inhibition in higher plants and algae after exposure to linear alkyl benzene sulfonates (LABSs). Growth of the algae, *Scenedesmus quadricauda*, was stimulated at levels up to 500 mg·L$^{-1}$ LABSs and inhibited at concentrations >1000 mg·L$^{-1}$ LABSs (Chawla et al., 1986). Rinallo et al. (1988) showed that root growth was suppressed more than leaf elongation when wheat (*Triticum durum* Desf.) seedlings were exposed to LABSs. Since LABSs are very similar in chemical structure to Igepon, we anticipated similarities in the dose response. Thus, we initially focused on characterizing the dose response and toxicity threshold for plants exposed to Igepon via the nutrient solution. In addition, the effects of long-term exposure and the fate of Igepon in hydroponic nutrient solutions were also investigated.

## Materials and Methods

**Hydroponic system.** The recirculating hydroponic system consisted of four identical, physically separated units to provide individual treatments. Figure 1 shows a schematic of the system.

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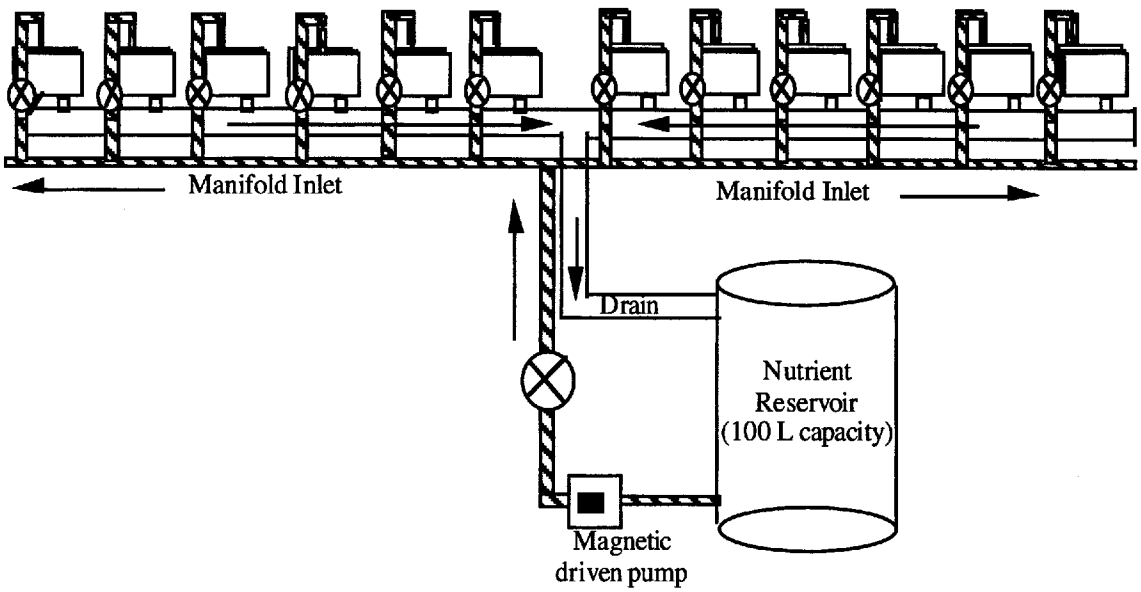


Fig. 1. Schematic drawing of a single line of the recirculating hydroponic system; one of four identical units pictured here.

Each unit consisted of three major components:

1. A 100-L capacity reservoir for nutrient solution and a magnetic drive pump with polypropylene coated impeller and pump body for solution distribution.
2. Twelve 6.4-L, plant-growing containers with a growing surface of 28 × 18 cm. Each container had six 1.5-cm-diameter holes to accommodate the lettuce plants.
3. A nutrient solution recirculation system with a manifold to distribute nutrient solution from the reservoir to each container and a drain from each container for solution return to the reservoir.

**Experimental design.** The four hydroponic systems were arranged on two benches in a greenhouse. Each hydroponic system included six growing containers on each of the two greenhouse benches. A randomized, complete-block, experimental design was used, with each greenhouse bench representing a block.

**Nutrient solution.** Seeds were germinated under fluorescent lamps in a germination tray containing one-fifth-strength nutrient solution (Table 1). On day 3, nutrient levels were increased to one-third strength. On day 5, uniform seedlings were transferred to the greenhouse production system containing the same strength nutrient solution. Two days later, the nutrient solution was increased to full strength. The pH of the nutrient solutions was maintained between 5.7 to 6.3 and increases in pH were compensated for by adding 0.1 M $HNO_3$. Electrical conductivity was monitored and nutrient stocks were added daily as required to maintain conductivity at 980 $\mu S \cdot cm^{-1}$. Water lost from the systems due to transpiration was replaced with deionized water.

**Environmental conditions.** Temperature in the greenhouse was maintained between 21 and 24 °C. Naturally occurring photoperiod was not altered and ranged from a maximum of 14 h in summer to a minimum of 11 h in fall and spring.

**Determining Igepon phytotoxicity.** Igepon phytotoxicity was studied in four separate groups of experiments. In all experiments, Igepon was added 17 d after planting (DAP). The goals of Expts. 1 and 2 were to 1) define the dose response curve and 2) identify the toxicity threshold of Igepon. In Expts. 1 and 2, the treatments were 0, 250, 500, and 1000 $mg \cdot L^{-1}$ Igepon and 0, 125, and 250 $mg \cdot L^{-1}$ Igepon, respectively. Whole-plant, destructive harvests were made at 17, 20, 27, and 35 DAP. At each harvest, six plants were randomly selected and removed from each treatment. Fresh and dry mass of leaves, stems, and roots were determined for each plant. Stem and leaf mass were combined and expressed as shoot mass.

The goals of Expt. 3 were to 1) characterize the growth suppression and recovery resulting from a single toxic dose of Igepon, 2) quantify the duration of the suppression, and 3) quantify the long-term effects of Igepon on growth. Lettuce plants were grown in nutrient solution containing 0 (control) or 250 $mg \cdot L^{-1}$ of Igepon, and whole-plant harvests were conducted daily to characterize the

Table 1. Nutrient solution composition: nutrient source and solution concentration.

| Nutrient source | Solution concn |
|---|---|
| Macronutrient | (mM) |
| $KNO_3$ | 5.0 |
| $Ca(NO_3)_2$ | 2.5 |
| $MgSO_4$ | 1.5 |
| $K_2HPO_4$ | 0.5 |
| $KH_2PO_4$ | 0.5 |
| Micronutrient | (μM) |
| $H_3BO_3$ | 46.6 |
| $MnSO_4$ | 4.5 |
| $ZnSO_4$ | 0.38 |
| $(NH_4)_6Mo_7O_{24}$ | 0.05 |
| $CuSO_4$ | 0.15 |
| $CoCl_2$ | 0.08 |
| $Fe^{3+}$ (Sequestrene 330) | 96.0 |

recovery pattern of plants after a phytotoxic dose of Igepon. Three plants were randomly selected and harvested from each treatment daily for 11 consecutive days after introducing Igepon, and fresh and dry masses were determined for shoots and roots.

**Persistence of Igepon phytotoxicity.** The goal of Expt. 4 was to determine whether the rapid recovery of plants after an acute exposure to a phytotoxic concentration of Igepon was the result of 1) plant adaptation, 2) chemical instability of Igepon in solution, 3) a direct action by the plant, or 4) other action associated with the hydroponic culture of the plant. Igepon treatments were presented in three ways, each at a concentration of 250 mg·L$^{-1}$ and added to the growing system 17 DAP. The treatments were 1) fresh Igepon added to one hydroponic system; 2) Igepon dissolved in deionized water, aged for 3 d, and added to a second hydroponic system; and 3) Igepon dissolved in nutrient solution, aged for 3 d, and added to a third hydroponic system. To determine the persistence of Igepon phytotoxicity, some plants were transferred from the control treatment (no Igepon) to systems containing the three Igepon treatments. Three plants were randomly selected and harvested from the control and each Igepon treatment for 6 consecutive days after introducing Igepon. Transferred plants were harvested from each Igepon treatment for 3 consecutive days after the transfer. Thus, all plant harvests ended on the same day, 23 DAP. Dry and fresh mass were determined for shoots and roots at each harvest.

The presence of Igepon in the nutrient solution was monitored using gas chromatography–mass spectrophotometry analysis for fatty acids characteristic of Igepon in the nutrient solution (Belisle, 1993).

**Statistical analysis.** To define the dose response, where two experiments were required to span the relevant Igepon concentrations, data were pooled for the two experiments and results presented in Fig. 2. Standard errors (n = 6) for each mean are included in Fig. 2; five Igepon treatments and multiphasic response did not permit a regression analysis. In Expt. 3, data were analyzed using exponential regression analysis for each phasic response (Figs. 3 and 4).

## Results and Discussion

**Igepon phytotoxicity.** Igepon concentrations of 250 mg·L$^{-1}$ and greater in the hydroponic nutrient solution resulted in acute phytotoxic effects in lettuce (Fig. 2). Growth was suppressed by 33%, 83%, and 85% at concentrations of 250, 500, and 1000 mg·L$^{-1}$ when measured 6 d after adding Igepon. Paired analysis of variance for Expts. 1 and 2 using a *t* test showed no significant differences, with *P* values ranging from 0.18 to 0.55; thus, data of the two experiments were pooled to produce Fig. 2. Comparing control treatment with the lowest Igepon concentration (125 mg·L$^{-1}$) gave an F value of 2.365 for df = 1, giving a nonsignificant *P* value of 0.145. Comparing the higher Igepon concentrations with the control treatment or 125 mg·L$^{-1}$ Igepon showed significant differences.

A lettuce seedling bioassay conducted in our laboratory supported these whole-plant findings, showing an acute toxicity threshold value for 200 mg·L$^{-1}$ Igepon (Greene et al., 1992). The dose-response curve identified for Igepon in the lettuce seedling bioassay was used to select the treatment concentration, and the results presented in Fig. 2 follow that curve. Short-term, whole-plant studies (data not shown) and the long-term studies reported here mimicked the bioassay response curve: key points on the curve were expressed at the same absolute concentrations for the seedling bioassay and whole-plant studies.

Root growth appears to have been almost totally stopped at 500 mg·L$^{-1}$ or higher. The growing tips of the roots were killed, but Igepon toxicity appeared to decrease with time. Plant death did not result from any Igepon treatment in any of our studies. In all cases, after initial root growth suppression (including root loss at high concentrations), new roots were initiated and all plants recovered from the damage and resumed a growth rate similar to that of the control.

The initial visual symptoms of Igepon toxicity, browning of roots, was observed within 4 to 6 h after Igepon exposure. Suppression of plant growth and tissue damage were evident within 24 h. The damage to the root system in response to an acutely toxic concentration of Igepon (250 mg·L$^{-1}$) is reflected in a decrease in

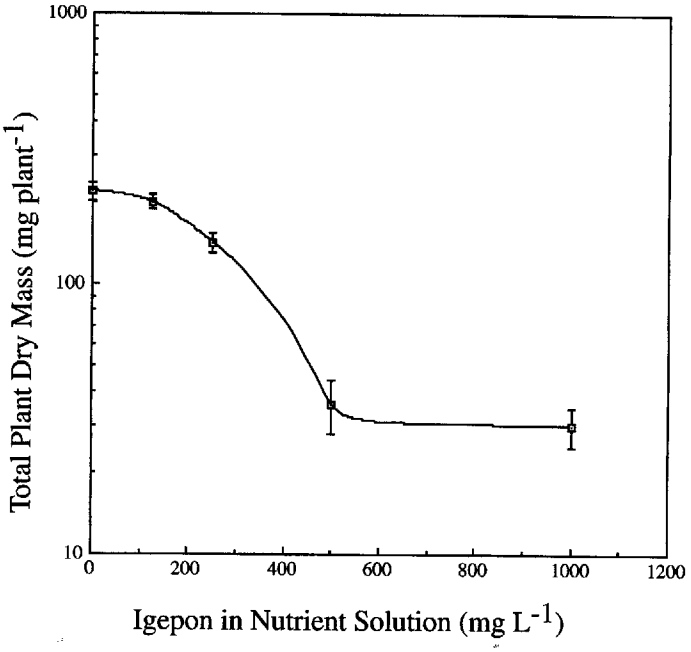


Fig. 2. Dose response of lettuce to Igepon. Dry mass of 23-d-old plants exposed to various Igepon concentrations in the hydroponic nutrient solution for the previous 6 d. Bars indicate ±1 SE, n = 6.

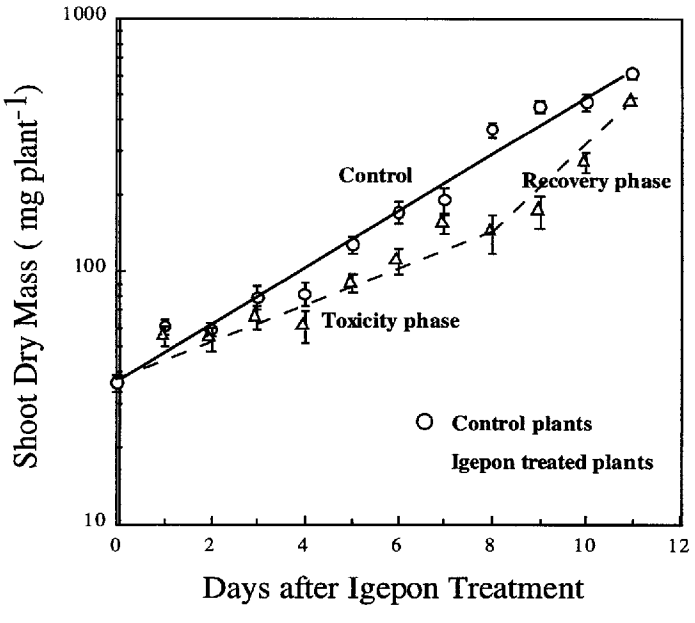


Fig. 3. Root dry mass of lettuce over an 11-d period after exposure to an acutely toxic concentration of Igepon (250 mg·L$^{-1}$) at 17 d after planting. Regression equations: Control, y = 6.514 × 10(0.117x), $R^2$ = 0.93; Igepon recovery phase, y = 2.30 × 10(0.145x), $R^2$ = 0.96

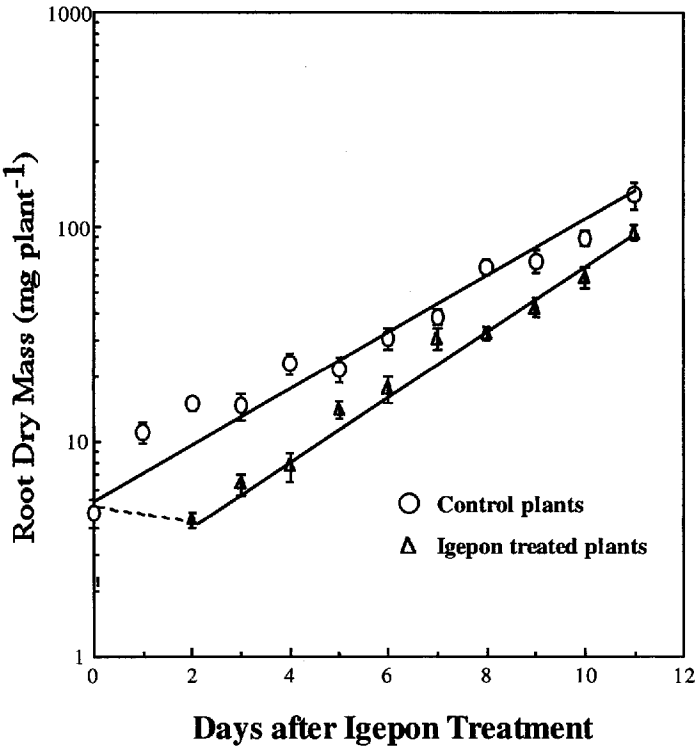


Fig. 4. Shoot dry mass of lettuce over an 11-d period following exposure to an acutely toxic concentration of Igepon (250 mg·L$^{-1}$) Igepon at 17 d after planting. Regression equations: Control, y = 36.60× 10(0.112x), $R^2$ = 0.96; Igepon toxicity phase, y = 37.65 × 10(0.077x), $R^2$ = 0.79; Igepon recovery phase, y = 2.30 × 10(0.181x), $R^2$ = 0.81.

root mass the first day after treatment (Fig. 3). Growth of new, white roots was observed 2 d after Igepon exposure. Leaf growth resumed 9 d after exposure (Fig. 4). Root regeneration before resumption of leaf growth supports the concept that energy is prioritized to root growth under stress (Brouwer and DeWitt, 1969).

**Phytotoxicity persistence.** When lettuce plants were transferred from the control nutrient solution to treatments where plants and Igepon had been in association for 3 d, no phytotoxicity was observed. The growth of the transferred plants was identical to that of the plants remaining in the control nutrient solution. However, if plants grown in control nutrient solution were transferred to treatments containing nutrient solutions with Igepon where there were no plants previously, phytotoxicity occurred regardless of how the Igepon had been pretreated. Igepon dissolved in water or nutrient solution and aged for 3 d before introducing plants resulted in acute phytotoxic effects equivalent to fresh Igepon with growth suppression in the range of 30% to 35% (data not shown). These results demonstrate the short-lived nature of Igepon phytotoxicity provided that plants were present in the hydroponic solution. Loss of the phytotoxicity is not due to chemical decomposition or reaction with the nutrient solution.

Monitoring the fatty acid content in the nutrient solution associated with Igepon was an excellent indicator of the phytotoxic potential. Ageing Igepon in any manner tested resulted in only slight decreases in fatty acid content if lettuce plants were not present (Table 2). Three days after 250 mg·L$^{-1}$ Igepon had been added to nutrient solutions in which lettuce was growing, the fatty acid content was reduced to 0.5% of the original value. Igepon degradation, particularly as indicated by fatty acid decrease, can

Table 2. Fatty acid (FA) compositon of nutrient solution with 250 mg·L$^{-1}$ Igepon and fatty acids remaining in nutrient solution after 3 days in the presence and absence of lettuce plants.

| FA | Nutrient solution containing Igepon (mg·L$^{-1}$) | Plants | No plants |
|---|---|---|---|
| | | FA (%) remaining after 3 d | |
| Lauric acid (C12:0) | 141.5 | 0.5 | 85.1 |
| Myristic acid (C14:0) | 61.3 | 0.7 | 81.3 |
| Palmitic acid (C16:0) | 29.3 | 0.7 | 80.7 |
| Stearic acid (C18:0) | 8.0 | 0.0 | 73.0 |
| Oleic acid (C18:1) | 9.0 | 0.0 | 65.3 |
| Total | 249.1 | 0.5 | 80.8 |

best be attributed to microbial activity associated with the plant roots. Microorganism responsibility for phytotoxicity degradation is supported by Wisniewski and Bubenheim (1993), in which the microbial population from lettuce roots growing in nutrient solution containing Igepon was cultured in a bioreactor and maintained separately from the plants. When gray water containing Igepon was introduced to this bioreactor, the same pattern of fatty acid decomposition as observed with the hydroponic nutrient solution–plant association was evident.

Rapid degradation of surfactants by microbes associated with plant detritus (Federle and Ventullo, 1990) and degradation of anionic surfactants by bacteria and fungi associated with higher plants have been reported (Alexander, 1981; Cooke and Matsuura, 1963; Lee, 1970; Hrsak and Grbic-Galic, 1995). Biological degradation of Igepon TC-42 has previously been demonstrated (Cordon et al., 1970; Ryckman and Sawyer, 1957; Winter, 1962). Our results show that the loss of Igepon phytotoxicity is concomitant with the decrease of component fatty acids. The mechanism of Igepon biodegradation has not been fully determined, but two potential pathways have been proposed, both resulting in oxidation of the fatty acids (Cordon et al., 1970; Sheers et al., 1967). Our findings indicate that microorganisms associated with lettuce in hydroponic culture could degrade Igepon TC-42 and eliminate phytotoxic effects. This suggests that the phytotoxicity of gray water resulting from the surfactant contaminants can be mitigated using biological organisms.

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