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The Biodegradability of Detergents and the Effects of Biodegradable Detergents upon Root Elongation of Certain Plants

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THE BIODEGRADABILITY OF DETERGENTS AND THE EFFECTS OF BIODEGRADABLE DETERGENTS UPON ROOT ELONGATION OF CERTAIN PLANTS

by

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THE BIODEGRADABILITY OF DETERGENTS AND THE EFFECTS OF BIODEGRADABLE DETERGENTS UPON ROOT ELONGATION OF CERTAIN PLANTS

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The effects of three biodegradable detergents upon root elongation of Cucumis sativus and Avena sativa was determined. All three detergents inhibit root elongation; the degree of inhibition depends on the brand of detergent and the species tested. Contact of the detergents with a sandy-loam results in reversal of inhibition of root elongation; the degree of reversal varies with detergent and species. However, reversal after 48 hours contact with soil does not appear to be due to degradation by microorganisms. Effects of aeration and of pH on test solutions are discussed.

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INTRODUCTION

Public and governmental concern about the presence of synthetic detergents in our water supplies first came about in the early 1950's with the appearance of foam on the nation's waterways, in drinking water of certain areas and in sewage treatment plants where the foam interfered with plant operations. The agent responsible for this foaming was determined to be the wetting agent (surfactant) alkylbenzene sulfonate (ABS) which resisted breakdown by microorganisms. Government hearings were held and legislation passed which required detergent manufacturers to find a substitute for ABS. This substitute had to be one which microorganisms could break down i.e. a biodegradable material. Such a material was developed and since June, 1965, all detergent products on the market contain biodegradable surfactants, the most common of which is linear alkylbenezene sulfonate (LAS).

Detergents as pollutants in water supplies have received much publicity by governmental agencies and the various news media. Such publicity led us to our concern about the effects of detergents upon certain living organisms. Although some preliminary work has been carried out in this laboratory on the effects of certain detergents on goldfish and snails, more extensive investigations concern the effects of detergents upon the seedlings of

cucumber (Cucumis sativus) and oats (Avena sativa).

Correspondence with various detergent companies determined something about the chemical composition of the detergents with which we desired to work. In all cases detergents were reported to be a mixture of many chemicals. A typical formulation, that of Tide XK follows: linear alkylbenzene sulfonate, alkyl sulfate, inorganic salts such as phosphates, silicates, borates; also chemically unidentified special ingredients which are reported to act as anti-redeposition agents, suds modifiers, optical brighteners and stain removers (41). It is such complexes of many chemicals which may enter water supplies as pollutants.

Several questions were formulated at the outset of this work. First of all we sought to know if selected detergents are inhibitory to root elongation of certain plants and if so, what concentrations cause such inhibition. Other questions were directed at manufacturers' claims that their products are biodegradable and if so, under what set of conditions and period of time. Also if such degradation does occur would fragments be produced which in themselves are toxic. Answers to such questions as these became focal points of these studies.

Initial work was performed to determine if selected detergents were inhibitory to root elongation of cucumber and oat seedlings. Once it was established that such inhibition occurred and at what concentrations, experiments were performed to ascertain if such inhibition could be reversed by standing detergent solutions

against soil under various conditions.

It appears unlikely that the inorganic components of detergents such as phosphates, borates and silicates are inhibitory to elongation of roots of seedlings at their low concentrations. Rather, it is more probable that the inhibitory component is organic in nature i.e. the linear alkylbenzene sulfonate, the alkyl sulfate, a brightener or some other such constituents.

Surfactants have been suspect for some time. If one assumes that surfactants are inhibitory to seedlings, their biodegradation should reverse root inhibition. The experimentation that followed was directed by the above assumptions.

HISTORICAL BACKGROUND

In the mid 1930's the first synthetic detergents became available to the public. At this time soap was in widespread use and the detergents were considered a speciality item.

The advent of World War II brought about a scarcity of the ingredients that go into the making of soap (tallow, fats, oils) and suppliers and manufacturers began to look for other more readily available raw materials (44).

When automatic washing machines became available to consumers during the post war boom of 1946-1950, a demand for a better cleaning agent than soap was created by consumers, especially those that lived in hard water areas. Procter and Gamble Company met this demand by producing the first heavy duty detergent in 1946. It had as its active ingredients the surfactant alkyl sulfate and the builder[1] sodium tripolyphosphate. It was marketed under the trade name of Tide. By 1949 this detergent was in national distribution (41).

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[1]A builder is the phosphate portion of a detergent which enables the surfactant to perform more efficiently in getting out dirt; it also softens the wash water and helps prevent redeposition of dirt on clothes.

Other synthetic detergents were subsequently developed by Procter and Gamble and also by other companies. By 1962 ninety percent of all cleaning products used in household dishwashing and laundering were synthetic detergents (7).

As early as 1950, long before the sale of detergents reached its peak on the market, stories of foaming incidents occurring in rivers, streams and sewage treatment plants appeared in the nation's press.

In 1951 the Soap and Detergent Association set up the Technical Advisory Council, and research programs were developed by various agencies to study the effect or lack of effect of detergent product constituents on water and sewage treatment processes and on aquatic life and water resources (44).

By 1956 it was known that alkylbenzene sulfonate (ABS), the surfactant used in most household detergents, was responsible for the foam problem attributed to detergents (44).

Laboratory studies showed that 2-4 percent removal of ABS was obtained when sewage was put through primary treatment; removals of 50 percent were obtained in the conventional activated sludge[2] treatment, and when conditions were selected with the objective of increasing ABS oxidation, removals as high as 80

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[2]In this process aerobic bacteria and other microorganisms that use waste material as a source of food are suspended in the sewage by violent air agitation. In this way the organisms are brought into very close contact with their source of food.

percent could be obtained. However, such residues from this treatment were highly resistant to further biodegradability (7). Clearly there was a need to develop a surfactant to replace ABS. The criteria for choosing the new surfactant were: 1) the material must have a high potential for biodegradability when subjected to adequate sewage conditions or when subjected to proper natural conditions 2) the material must show proper performance characteristics in use, i.e. superiority as a wetting agent. Initial research in 1956 showed that a straight-chain surfactant, later named linear alkylate sulfonate (LAS) met the two criteria stated above. In contrast to the branch chain molecular structure of ABS, the LAS material is more readily attacked by microorganisms (44).

A technological breakthrough in 1962-1963 permitted the large scale commercial production of the LAS type surfactant. Detergent industry spokesmen announced to concerned legislative bodies and to the public that all ABS-based products would be converted to LAS. By June 30, 1965 all washing and cleaning products manufactured for household and industrial use were based on LAS and other "soft" or biodegradable materials (44).

Detergent pollution problems may still arise, however, in areas in which homes are not linked with public sewage facilities.

These homes also obtain their drinking water from private wells. In some instances the household wastes, including detergents may not have enough time or proper conditions to degrade before reaching the water table and these wastes tend to show up in well water (6).

Suffolk County, Long Island, New York is a good example of such a pollution problem. In fact the first published report of ground water contamination in which detergents were involved occurred in this county in 1958 (7). Homes in this county use cesspools which are inadequate in this sandy area with its shallow wells.

Theodore Brenner, research director of the Soap and Detergent Association, states that an LAS detergent degrades better than an ABS type detergent only if a cesspool or septic tank system is operating under proper conditions, i.e. in soil that has adequate absorptive capacity. Sewers and public water supplies are the only way to avoid a water pollution problem involving detergents or other soluble organic wastes (6).

As long as inadequate sewage treatment conditions exist in some areas of the United States, we must be concerned about the effects of detergents in our water supplies.

Literature Review

In reviewing the literature one finds numerous reports on the effects of detergents on bacteria and other microorganisms (8,17,21,30,35,36,37). Work has also been done on invertebrates such as snails, clams and oysters (4,5,19). These studies utilized ABS, LAS and nonionic[3] type detergents.

Considerable work has been reported on the effects of detergent solutions on fish. In most of this work, concern has been with the older type (ABS) detergent which is no longer on the market in the United States (1,9,10,20,31,46). However, more recent work is concerned with the effects of LAS and nonionic types of detergents presently in use (14,47).

Very little work has been reported on the effects of detergents on plants. Of that reported, most concerns the "hard" detergents; those resistant to biodegradation. Some work reported concerns the effects of nonionic surfactants or detergents and cationic[4] detergents on plants.

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[3] A nonionic detergent is one that does not break down into charged particles in aqueous solution.

[4] A cationic detergent is one that forms a positively charged group or cation in aqueous solution. These detergents are used as disinfectants.

Spurrier and Jacobs (45) showed that germination and plant growth were reduced by the application of 1000 ppm[5] of an anionic[6] sodium sulfonate type of surfactant.

Roberts and Kerridge (43) treated rooted water plants Ranunculus aquatilis, Potamogeton pectinatus and Potamogeton densus with an alkyl aryl sulfonate detergent and found a range of tolerances from 1-5 ppm depending on the species of plant tested.

Bing (3) conducted experiments in which solutions of alkylbenzene sulfonate were used to water the soil of bench and pot plants for time periods ranging from 20 days to 5 months. No consistently adverse effects were found on the growth of plants treated continuously with the ABS solutions in concentrations up to 50 ppm.

Several workers have tested the effects of synthetic detergents in waste water upon certain crop plants. Dulk (11) found that all but one of eight truck garden plants tested suffered severe growth depression when irrigation water contained an alkyl aryl sulfonate type detergent. Klein, Jenkins and McGauhey (23) compared the

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[5]Generally speaking, the concentration of a washwater solution will be in the range of 1000-3000 ppm total product. Depending on the brand used, a washwater solution with this concentration of complete product would contain about 200-600 ppm or more surfactant (7).

[6]An anionic detergent breaks down in aqueous solution giving a negatively charged group or anion.

effects of ABS in water culture to that of soil. Test plants included sunflowers, barley and white lupine. Although ABS caused severe growth inhibition of sunflower and barley in water culture, only one species of test plant (sunflower) of the three grown in soil was adversely affected. Moreover, irrigation with sewage proved beneficial to plants in spite of the presence of ABS. Knauth (24,25) studied the effects of detergent containing irrigation water upon garden vegetables and sugar beets. No adverse effects to plants were noted in concentrations of detergent up to 1000 milligrams per liter. The effects of alkyl sulfonate and alkylbenzene sulfonate detergents upon tomato plants grown in hydroponic culture was studied by Popa et al. (39). Concentrations of detergent up to 1000 milligrams per liter did not affect crop quantity or quality but a concentration of 5000 milligrams per liter reduced crop production 50 percent. Wesche (50) tested the effects of an alkylbenzene sulfonate and a tetrapropylenebenzene sulfonate detergent in waste water upon various soils and plants. Plant growth was not influenced by short treatment and detergent concentrations of 6 milligrams per liter. Higher concentrations caused severe damage and bald spots. Damage with the former was only half that with the latter. Mosses were more sensitive to detergent solutions than grasses.

The effects of various detergents or surfactants upon different species of algae has been reported in the literature. Wurtz-Arlet (54), Matulova (32,33) and Ukeles (48) each showed the inhibition

of growth of algae when cultured with anionic, cationic and nonionic type detergents. The degree of inhibition in each case depended upon the type of detergent used and the species of algae tested. The ubiquitous alga Cladophora glomerata was shown to be sensitive to an anionic ABS type of detergent by Whitton (53). Hicks and Neufold (18) studied C^{14} assimilation of two species of algae, Vaucheria and Cladophora, the algae being cultured with varying concentrations of an ABS type detergent. C^{14} assimilation decreased with increasing concentration of ABS and also decreased with time exposure to ABS. A slight stimulation of C^{14} uptake seemed to occur at abbreviated exposures to low concentrations of ABS.

Synthetic detergents are not always toxic to algae. Wurtz-Arlet (55) determined that three species of filamentous algae were not much affected by the action of three anionic type detergents although the cultures were somewhat slowed down as compared to controls. Her studies also indicated that the algae present appeared to promote detergent disappearance from culture, the time of the disappearance being a factor of initial detergent concentration and also of the chemical composition of the detergent. Maloney (29) showed that the unicellular green algae, Chlorella pyrenoidosa was capable of growing on cultures in which a detergent, an ABS type, served as the sole phosphorus source. In fact, the detergent stimulated the growth of this alga.

There are numerous reports in the literature on the effects of nonionic detergents on plant growth. No reference is made in these reports as to whether or not these detergents are biodegradable. Work prior to 1965 probably involves detergents resistant to break down by microorganisms while that after 1965 may involve biodegradable products. However, this is uncertain since foreign countries presently continue to market the "hard" type detergents.

A nonionic detergent at concentration of 0.007-0.01 percent was shown to stop protoplasmic streaming in algae and certain plant cells after exposure of 1-3 hours. Plasmolysis of beet disks and epidermis of Rhoeo discolor was shown to occur in the presence of 0.01-0.05 percent detergent solutions (13).

Prill, Barton and Solt (40) and Ghillini (12) have used wheat as a tool in determining the effect of nonionic surfactant upon plant growth. Prill et al. found that certain nonionic surfactants had little inhibitory effect on wheat roots while the four anionic and three cationic surfactants tested were inhibitory at concentrations of 20 ppm. Ghillini tested several nonionic surfactants by applying them to wheat plants in mineral nutritive solutions. Some surfactants had no effect upon plant growth; others inhibited growth at high concentrations but stimulated growth at low concentrations.

Vieitez et al. (49) and Mendez et al. (34) tested the effects of three nonionic surfactants (trade names Tweens 80, 40 and 20)

on the growth of Avena coleoptile sections. Vieitz determined that Tween 80 was stimulating in itself and in combination with IAA, slightly enhanced coleoptile growth. Tween 40 had no effect on growth and Tween 20 markedly inhibited growth of coleoptile sections. Mendez also determined Tween 20 to be inhibiting to Avena coleoptile section elongation while Tween 40 gave erratic results. In this work, however, Tween 80 was shown to have no direct effect on cell elongation.

Additional work on determining the effects of nonionic surfactants on root growth has been carried out by MacDowall (28) and Parr and Norman (38). MacDowall showed that Tween 20 when present in culture media at 0.001 percent occasionally stimulated growth and/or nicotine synthesis of isolated roots of Nicotina rustica and whole plants of Nicotina tabacum. It also stimulated root respiration and the succinoxidase activity of root mitochondrial fractions. Parr and Norman tested 22 representative nonionic surfactants. Lower concentrations repressed the elongation of the primary root of cucumber seedlings. Higher concentrations of selected surfactants were more inhibitory and depressed root hairs and lateral roots. Certain surfactants also significantly interfered with potassium uptake.

When the nonionic detergent, Tween 60, was added to the nutrient solution of the green alga, Enteromorpha linza, an increase in growth was noted (2). Berglund concluded that this effect depended upon the lowered surface tension of the culture

media.

Of the remaining reports found in the literature, only one definitely deals with a biodegradable detergent. The other report may deal with biodegradable detergents; the reference is made to "new" detergents in any case and the work was published after 1965.

Lichtenstein et al. (27) studied the effects of the biodegradable detergent LAS on the uptake of certain insecticides and on plant growth. He found that the LAS did not affect the penetration of the insecticides Lindane or Aldrin into roots but significantly reduced the amounts of Parathion in the root system.

Finally Hartmann (16) tested the toxicity of 12 new detergents against the seedlings of oats and barley and on a mixed culture of autotrophic organisms. Low concentrations of detergents stimulated the growth of the oats and barley but toxic action was noted with increasing concentration. This latter effect varied greatly with different detergents.

METHODS AND MATERIALS

Plant Material

Cucumber seeds (Cucumis sativus L., var. Straight-eight) and oat seeds (Avena sativa L., var. Coachman) used in experimental work were purchased from Farm Bureau Services, Lansing, Michigan.

Detergents

Two of the detergents tested were purchased at a local supermarket in the summer of 1968. One of these, marketed under the trade name of Living, is manufactured by Haviland Products Company, Grand Rapids, Michigan. The exact chemical nature of this detergent is unknown; correspondence with the company states that this laundry powder is a blend of inorganic detergents, deflocculants, optical dye, water conditioners, anti-redeposition agents and an ethoxylated derivative of coconut oil. Living is advertised as a "low-sudser" and as containing "biodegradable cleaning compounds". The other product, marketed under the trade name of Tide XK, is manufactured by Procter and Gamble Company, Cincinnati, Ohio. Tide, according to Procter and Gamble, contains a mixture of two surfactants, both of which are biodegradable; they are linear alkylbenezene sulfonate and alkyl sulfate. These surfactants are anionic in nature. Tide also

contains substantial quantities of inorganic salts that are reported to act as water softeners and detergency builders. The third detergent tested is SA-8 manufactured by Amway Corporation, Ada, Michigan, and must be purchased from a salesman; it cannot be purchased in any store. The company identifies SA-8 as a nonionic, alkaline detergent with a linear alcohol ethoxylate as a primary active ingredient. It is a "low-sudser" and is described by the company as biodegradable. The SA-8 used in our experimental work was supplied by the Amway Corportion.

Soil

Soil used in experiments is a sandy-loam obtained directly from the potting shed, Western Michigan University greenhouse. When necessary, soil was sterilized by autoclaving at 15 psi and 250° F (15).

Determination of Inhibiting Concentrations

An inhibiting concentration of any detergent for the purposes of this work is defined as that concentration which permits good germination (90 to 100 percent) but which inhibits root elongation to about one-half of control.

The inhibiting concentration of each detergent upon oat and cucumber seeds was determined as follows: A stock solution of 10,000 ppm detergent was prepared using tap water heated to 70° C. This temperature approximates that of the hot water cycle used in

laundromats (44). These were allowed to cool overnight before diluting to volume. Suds were allowed to disperse during the procedure so that greater accuracy of dilution could be obtained. Concentrations of detergent ranging from 200 to 3000 ppm were prepared from stock solutions. A modification of the Ready-Grant root elongation test was used to assay detergent toxicity (42). Thirty-five plastic dishes[7], 6 inches in diameter and 2½ inches deep with lids, were prepared using Armstrong number 6 filter paper[8]. Dishes were set up in series of five. Fifteen milliliters of either tap water or detergent solution were added to each dish. Seeds of either cucumber or oats were placed in the dishes, twenty per dish. Finally all dishes were placed in the dark at 23-25° C. At the end of 120 hours, seedlings were removed and root length measured. In cucumber seedlings, the primary root was measured; in oats, the longest root was measured.

Determination of Biodegradability

Experiments were performed to determine if contact of detergents with a sandy-loam would reverse inhibition of root length.

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[7]Plastic containers were obtained from Bradley Industries, Inc., Franklin Park, Illinois.

[8]This filter paper is manufactured by Armstrong Cork Co., Lancaster, Pennsylvania.

It was assumed that such reversal by standing against soil would be due to degradation by microorganisms and of biodegradable components.

The following series using 5½ inch diameter and 5½ inch deep plastic dishes was set up for each detergent tested:

Dish 1: 250 grams autoclaved sandy-loam in one liter of tap water, no aeration

Dish 2: 250 grams autoclaved sandy-loam in one liter of tap water, with aeration

Dish 3: 250 grams untreated sandy-loam in one liter of tap water, no aeration

Dish 4: 250 grams untreated sandy-loam in one liter of tap water, with aeration

Dish 5: One liter inhibiting concentration of detergent, no aeration

Dish 6: One liter inhibiting concentration of detergent, with aeration

Dish 7: One liter inhibiting concentration of detergent plus 250 grams autoclaved sandy-loam, no aeration

Dish 8: One liter inhibiting concentration of detergent plus 250 grams autoclaved sandy-loam, with aeration

Dish 9: One liter inhibiting concentration of detergent plus 250 grams untreated sandy-loam, no aeration

Dish 10: One liter inhibiting concentration of detergent plus 250 grams untreated sandy-loam, with aeration

All dishes were vigorously stirred at the beginning of the experiment and again after 24 hours. All dishes were covered. Air was filtered through glass wool before entering the test solution. The pH of test solutions was recorded at 24 and 48 hours. A typical set up is shown in Figure 1.

At 24 and 48 hour intervals, fifteen milliter samples were taken from each dish and tested against cucumber or oat seeds. After 120 hours in the dark at 23-25° C, germination and root length measurements were recorded using the procedure previously employed in determination of biodegradability.

Figure 1. Typical set-up used in determination of biodegradability

A, air source; G, glass wool filter; T, glass tubing; R, rubber tubing; D, 250 grams soil; S, one liter test solution.

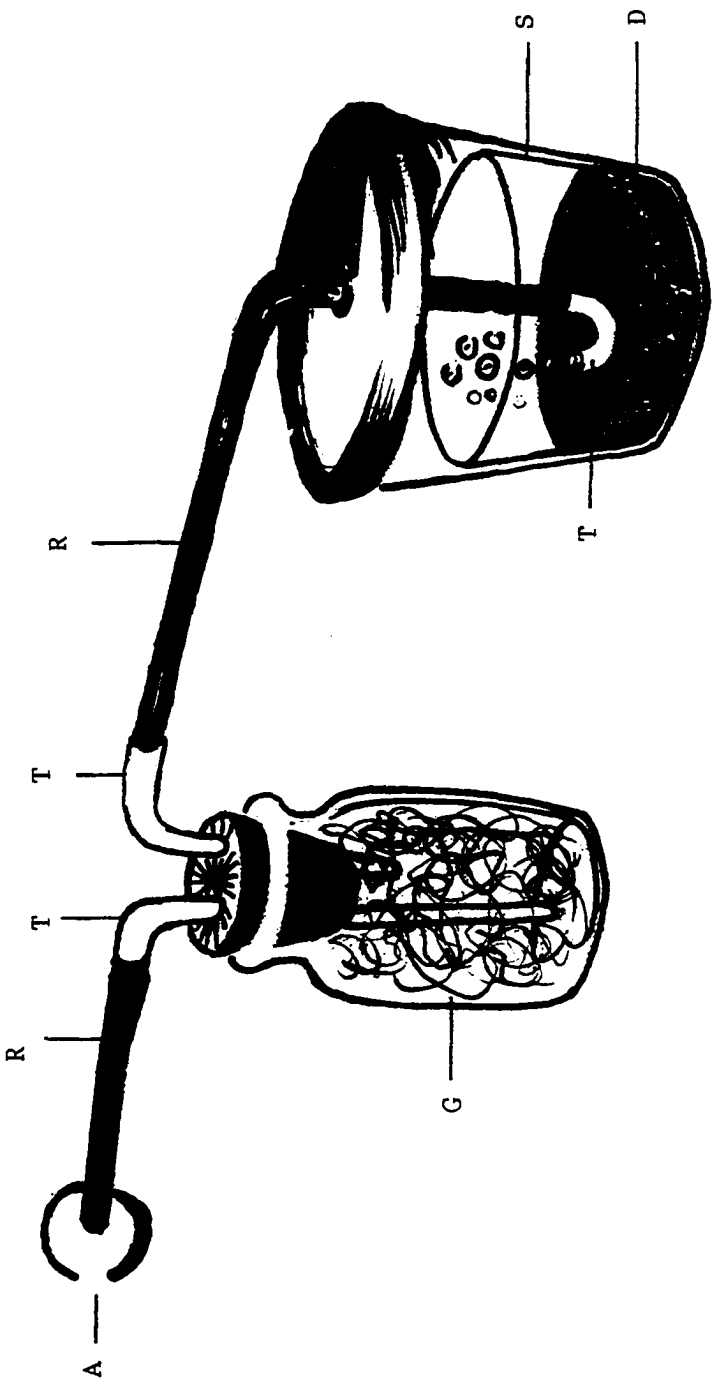

Figure 1

RESULTS

Determination of Inhibiting Concentration

The inhibiting concentration of the detergent Living upon Cucumis sativus seeds was determined. In these experiments efforts were directed towards finding that concentration which while permitting high initial germination, inhibited root elongation as measured after 120 hours to about one-half that of controls. Such a concentration was labelled "inhibiting concentration" for the purposes of our studies. Results are shown in Table 1.

As shown in Table 1, the concentration which permits good germination but which inhibits root elongation to approximately 50 percent that of controls is 2500 ppm. This concentration was used in further studies of Living on Cucumis.

The inhibiting concentration of Living upon Avena sativa was determined. Results are shown in Table 2. 1000 ppm was chosen for further studies on Avena.

The inhibiting concentration of Tide XK upon Cucumis sativus was determined. Results are shown in Table 3. 1500 ppm was chosen as the inhibiting concentration of Tide XK for further studies on Cucumis.

The inhibiting concentration of Tide XK upon Avena sativa was determined. Results are shown in Table 4. 500 ppm was selected

to be the inhibiting concentration of Tide XK upon Avena sativa to be employed in further studies.

The inhibiting concentration of SA-8 was determined for Cucumis sativus. Results appear in Table 5. 2000 ppm was selected as the inhibiting concentration of SA-8 upon Cucumis sativus to be used in further studies.

The inhibiting concentration of SA-8 was determined for Avena sativa. Results are shown in Table 6. As shown in Table 6, that concentration which inhibited root elongation about 50 percent that of control and permitted good germination is approximately 500 ppm. This concentration was employed for further studies of SA-8 on Avena.

A summary of inhibiting concentrations for each detergent upon Cucumis and Avena appears in Table 7.

Determination of Biodegradability

Inhibiting concentrations of the three detergents being tested, Living, Tide XK and SA-8, were treated in various ways. Treatment consisted of standing the detergent in contact with a sandy-loam for a period of 48 hours. In some cases, the sandy-loam had been autoclaved and therefore had a considerably lower number of microorganisms when compared to untreated sandy-loam. Treatments were carried out both under conditions of aeration and nonaeration to take into consideration the oxygen demands of aerobic

microorganisms when present. pH values of all solutions were recorded at the end of 24 and 48 hours.

After treatment i.e. standing against soil, samples of each detergent taken at 24 and at 48 hours were tested against cucumber or oats. Root elongation measurements were made at the end of 120 hours.

An inhibiting concentration of Living upon Cucumis sativus (2500 ppm) as previously determined was allowed to stand against soil and was tested against cucumber seeds at the end of 24 and 48 hours. Results are shown in Table 8.

An inhibiting concentration of Living upon Avena sativa (1000 ppm) as previously determined was allowed to stand against soil and was tested against oat seeds at the end of 24 and 48 hours. Results appear in Table 9.

An inhibiting concentration of Tide XK upon Cucumis sativus (1500 ppm) as previously determined was allowed to stand against soil and was tested against cucumber seeds at the end of 24 and 48 hours. Results are shown in Table 10.

An inhibiting concentration of Tide XK upon Avena sativa (500 ppm) as previously determined was allowed to stand against soil and was tested against oat seeds at the end of 24 and 48 hours. Results are shown in Table 11.

An inhibiting concentration of SA-8 upon Cucumis sativus (2000 ppm) as previously determined was allowed to stand against

soil and was tested against cucumber seeds at the end of 24 and 48 hours. Results appear in Table 12.

An inhibiting concentration of SA-8 upon Avena sativa (500 ppm) as previously determined was allowed to stand against soil and was tested against oat seeds at the end of 24 and 48 hours. Results are shown in Table 13.

Table 1. Effect of Various Concentrations of Living on Root Elongation and Germination of Cucumis (measurements shown are averages of 100 seeds).

Concentration	Root Length (mm)	Percent Germination
Control	82	97
1000 ppm	59	95
1500 ppm	59	95
2000 ppm	47	91
2500 ppm	41	91
3000 ppm	29	90

Table 2. Effect of Various Concentrations of Living on Root Elongation and Germination of Avena (measurements shown are averages of 100 seeds).

Concentration	Root Length (mm)	Percent Germination
Control	100	96
500 ppm	75	81
1000 ppm	34	81

Table 3. Effect of Various Concentrations of Tide XK on Root Elongation and Germination of Cucumis (measurements shown are averages of 100 seeds).

Concentration	Root Length (mm)	Percent Germination
Control	87	95
500 ppm	64	97
1000 ppm	60	99
1500 ppm	29	99

Table 4. Effects of Various Concentrations of Tide XK on Root Elongation and Germination of Avena (measurements shown are averages of 100 seeds).

Concentration	Root Length (mm)	Percent Germination
Control	93	95
300 ppm	58	45
400 ppm	37	36
500 ppm	38	37
600 ppm	33	43
700 ppm	29	51
800 ppm	22	33
900 ppm	21	48

Table 5. Effects of Various Concentrations of SA-8 on Root Elongation and Germination of Cucumis (measurements shown are averages of 100 seeds).

Concentration	Root Length (mm)	Percent Germination
Control	89	97
500 ppm	77	98
1000 ppm	61	99
1500 ppm	54	98
2000 ppm	44	92
2500 ppm	36	96
3000 ppm	24	91

Table 6. Effects of Various Concentrations of SA-8 on Root Elongation and Germination of Avena (measurements shown are averages of 100 seeds).

Concentration	Root Length (mm)	Percent Germination
Control	96	94
200 ppm	76	86
500 ppm	46	70
1000 ppm	21	42

Table 7. Summary of Inhibiting Concentrations of Three Detergents upon *Cucumis* and *Avena*.

Detergent	Seed	Inhibiting Concentration
Living	*Cucumis*	2500 ppm
Living	*Avena*	1000 ppm
Tide XK	*Cucumis*	1500 ppm
Tide XK	*Avena*	500 ppm
SA-8	*Cucumis*	2000 ppm
SA-8	*Avena*	500 ppm

Table 8. The Effects of Standing Against Soil of an Inhibiting Concentration of *Living* on the Root Elongation of *Cucumis* (measurements are averages of 100 seeds).

Treatment	Root Length (mm)		pH	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Autoclaved soil w/ aeration	76	75	6.1	7.1
Autoclaved soil w/o aeration	67	73	6.2	6.9
Untreated soil w/ aeration	78	78	6.7	6.9
Untreated soil w/o aeration	79	85	7.1	7.2
2500 ppm *Living* w/ aeration	28	27	10.2	10.1
2500 ppm *Living* w/o aeration	27	28	10.3	10.3
2500 ppm *Living* autoclaved soil w/ aeration	40	45	9.9	9.9
2500 ppm *Living* autoclaved soil w/o aeration	41	51	9.3	9.6
2500 ppm *Living* untreated soil w/ aeration	40	43	9.7	8.7
2500 ppm *Living* untreated soil w/o aeration	41	48	9.9	9.0

Table 9. The Effects of Standing Against Soil of an Inhibiting Concentration of *Living* on the Root Elongation of *Avena* (measurements are averages of 100 seeds).

Treatment	Root Length (mm)		pH	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Autoclaved soil w/ aeration	121	126	7.4	7.8
Autoclaved soil w/o aeration	129	124	7.1	7.1
Untreated soil w/ aeration	119	122	7.2	7.5
Untreated soil w/o aeration	128	125	6.7	6.8
1000 ppm *Living* w/ aeration	40	38	9.6	9.3
1000 ppm *Living* w/o aeration	40	36	9.7	9.5
1000 ppm *Living* autoclaved soil w/ aeration	76	105	8.1	7.9
1000 ppm *Living* autoclaved soil w/o aeration	67	89	8.8	7.7
1000 ppm *Living* untreated soil w/ aeration	71	112	8.3	8.2
1000 ppm *Living* untreated soil w/o aeration	61	81	8.2	8.0

Table 10. The Effects of Standing Against Soil of an Inhibiting Concentration of Tide XK on the Root Elongation of Cucumis (measurements are averages of 100 seeds).

Treatment	Root Length (mm)		pH	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Autoclaved soil w/ aeration	94	90	7.4	7.0
Autoclaved soil w/o aeration	85	95	7.5	7.0
Untreated soil w/ aeration	92	86	7.6	7.6
Untreated soil w/o aeration	95	90	7.0	7.0
1500 ppm Tide XK w/ aeration	38	33	8.8	8.9
1500 ppm Tide XK w/o aeration	39	40	8.8	8.8
1500 ppm Tide XK autoclaved soil w/ aeration	59	68	7.3	7.7
1500 ppm Tide XK autoclaved soil w/o aeration	48	58	7.3	7.4
1500 ppm Tide XK untreated soil w/ aeration	57	73	7.4	7.3
1500 ppm Tide XK untreated soil w/o aeration	54	64	7.3	7.3

Table 11. The Effects of Standing Against Soil of an Inhibiting Concentration of Tide XK on the Root Elongation of Avena (measurements are averages of 100 seeds).

Treatment	Root Length (mm)		pH	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Autoclaved soil w/ aeration	95	91	7.4	7.0
Autoclaved soil w/o aeration	90	93	7.5	7.0
Untreated soil w/ aeration	129	128	7.6	7.6
Untreated soil w/o aeration	130	136	7.0	7.0
500 ppm Tide XK w/ aeration	36	38	8.0	8.1
500 ppm Tide XK w/o aeration	36	38	7.7	7.6
500 ppm Tide XK autoclaved soil w/ aeration	116	128	7.4	7.8
500 ppm Tide XK autoclaved soil w/o aeration	92	111	7.4	7.1
500 ppm Tide XK untreated soil w/ aeration	110	123	7.9	7.9
500 ppm Tide XK untreated soil w/o aeration	102	121	6.9	6.7

Table 12. The Effects of Standing Against Soil of an Inhibiting Concentration of SA-8 on the Root Elongation of Cucumis (measurements are averages of 100 seeds).

Treatment	Root Length (mm)		pH	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Autoclaved soil w/ aeration	104	109	7.9	7.2
Autoclaved soil w/o aeration	100	106	8.6	7.5
Untreated soil w/ aeration	116	116	8.6	7.5
Untreated soil w/o aeration	114	111	7.6	7.3
2000 ppm SA-8 w/ aeration	52	59	9.1	9.1
2000 ppm SA-8 w/o aeration	48	54	9.6	9.6
2000 ppm SA-8 autoclaved soil w/ aeration	76	90	8.9	8.7
2000 ppm SA-8 autoclaved soil w/o aeration	73	86	9.2	8.7
2000 ppm SA-8 untreated soil w/ aeration	68	79	9.0	8.6
2000 ppm SA-8 untreated soil w/o aeration	64	72	9.4	9.1

Table 13. The Effects of Standing Against Soil of an Inhibiting Concentration of SA-8 on the Root Elongation of Avena (measurements are averages of 100 seeds).

Treatment	Root Length (mm)		pH	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Autoclaved soil w/ aeration	111	115	7.6	7.8
Autoclaved soil w/o aeration	112	118	6.7	7.1
Untreated soil w/ aeration	112	97	8.0	7.9
Untreated soil w/o aeration	109	113	7.3	7.2
500 ppm SA-8 w/ aeration	36	38	9.0	8.8
500 ppm SA-8 w/o aeration	46	44	9.1	8.8
500 ppm SA-8 autoclaved soil w/ aeration	111	113	7.4	7.8
500 ppm SA-8 autoclaved soil w/o aeration	86	105	7.8	7.4
500 ppm SA-8 untreated soil w/ aeration	108	113	8.1	7.9
500 ppm SA-8 untreated soil w/o aeration	96	104	8.1	7.8

DISCUSSION

Determination of Inhibiting Concentration

In determining the inhibiting concentrations of each detergent upon cucumber and oats, it was not always possible to select a concentration that inhibited root elongation to exactly one-half of controls. In cases where a choice existed between two concentrations, the stronger concentration was chosen arbitrarily.

It was found that 500 ppm of the detergent Living, inhibited root elongation of Avena to three-fourth's of control while a concentration of 1000 ppm inhibited root elongation to about two-fifths of control, less than one-half (Table 2). The inhibiting concentration of Living upon Avena was chosen arbitrarily in this instance as 1000 ppm.

Again, 500 ppm and 1000 ppm Tide XK both inhibited root elongation of Cucumis to about two-thirds of control while a concentration of 1500 ppm inhibited root elongation to about one-third of control (Table 3). 1500 ppm was chosen arbitrarily as the inhibiting concentration.

Tide XK at 300 ppm inhibited root elongation of Avena to about two-thirds of control while 400 and 500 ppm both inhibited root elongation of Avena to less than one half of controls. Since

there is no significant difference between these two concentrations in terms of root elongation measurements and percent germination, the higher concentration of 500 ppm was chosen arbitrarily as the inhibiting concentration (Table 4).

Avena was found to be more sensitive than Cucumis both in terms of percent germination and in root elongation to Living, Tide XK and SA-8. Inhibiting concentrations of each detergent upon Avena are in each case lower than those for Cucumis (Table 7). Of particular interest are the differences observed in percent germination. In all instances Avena germination is lower than that of Cucumis. Tide XK is especially toxic to Avena. Percent germination is only approximately 33-51 percent of control (Tables 1-6).

Determination of Biodegradability

Once it was established that certain biodegradable detergents inhibit root elongation of cucumber and oat seedlings, experiments were performed to determine if such inhibition by these detergents could be reversed by contact with a sandy-loam. Since all detergents on the market must by law contain components which are described as biodegradable, it was assumed in these studies that microorganisms in soil would attack and degrade such components. Furthermore such degradation would result in the reversal of root inhibition.

Since microorganisms present in soil may or may not need

conditions of high oxygen tension, treatments were carried out both under conditions of aeration and nonaeration. No significant differences are observed in root elongation of either cucumber or oats grown in either aerated or nonaerated solutions.

Also, in certain treatments autoclaved sandy-loam was used. Since this soil has considerably fewer microorganisms present than untreated soil, it was assumed that less degradation of the detergent would occur. Differences would, of course, be observed in root elongation of seeds grown in solutions from such treatments. However, results point out that if any reversal of root inhibition occurs it is not due to the presence of microorganisms in the soil. It is observed that there are no significance differences in length of roots grown in solutions taken from treatments in which autoclaved soil was employed when compared to roots of seeds grown in solutions taken from treatments in which untreated soil was employed (Tables 8-13).

Detergent solutions are alkaline, sometimes highly so, but once these solutions are in contact with soil, either untreated or autoclaved, the pH drops by a factor of 10 (Tables 8-13). This pH drop is accompanied by some reversal of inhibition of root elongation in cucumber and oats. Although the purpose of these studies was not to determine the reasons why detergents cause growth inhibition, the highly alkaline nature of the detergents may explain in part their toxicity.

However, in at least one case, Tide XK solutions (1500 ppm) after standing against soil for 48 hours had pH values ranging from 7.0-7.7. Control solutions of tap water and soil had values ranging from pH 7.0 to pH 7.6 in the same time period, and yet Tide XK solutions were still significantly inhibiting to root elongation of cucumber (Table 10). Further work is necessary on the relationship between high pH and detergent toxicity.

A solution containing an inhibiting concentration of Living (2500 ppm), after standing against soil for 48 hours is significantly less inhibiting to roots of cucumber than one which is merely allowed to stand alone for 48 hours (Table 8). Similar results are observed in oats (Table 9). But since there is no significant difference between measurements in those treatments using untreated soil and those using autoclaved soil, some other factor must explain the observed reversal of inhibition of root elongation. Perhaps some portion of the detergent is adsorbed on the soil. Klein et al. (22) have shown that such adsorption of synthetic detergents occurs in most soils.

Data in Table 10 show that Tide XK (1500 ppm) solutions standing alone for 48 hours greatly inhibit root elongation of cucumber. Solutions which were in contact with soil for 48 hours were less inhibitory but measurements were significantly less than controls.

That differences in response to detergents varies from species to species and from detergent to detergent is again

shown in Table 11. A solution containing an inhibiting concentration of Tide XK (500 ppm) no longer inhibits Avena root elongation after standing against soil for 48 hours.

Table 12 shows that solutions of SA-8 (2000 ppm) standing alone for 48 hours inhibit root elongation of cucumber seedlings to about one-half of control. Root elongation measurements taken of cucumber seedlings after being tested against SA-8 solutions which had been in contact with soil for 48 hours show some reversal of root inhibition.

Table 13 shows that solutions of SA-8 (500 ppm) standing alone for 48 hours inhibit root elongation of Avena to less than one-half of control. SA-8 solutions which had been in contact with soil for 48 hours show little if any inhibition of root elongation in Avena.

Both experiments in which a complete reversal of inhibition of root elongation is observed i.e. Tide XK-Avena; SA-8-Avena, deal with an inhibiting concentration of 500 ppm. Since there is no significant difference in root measurements between treatments using untreated soil and those using autoclaved soil, biodegradation of the detergent by soil microorganisms at least in the first 48 hours is probably not a factor in this reversal. (Pilot experiments of other students in our laboratory indicate that little difference if any occurs when a detergent stands against soil, autoclaved or untreated, even after 96 hours). In view of these findings

it is proposed that the soil is able to completely tie-up a low concentration of detergent (500 ppm) by adsorption, thus rendering it harmless to roots. Additional work must be done, however, to confirm this hypothesis.

SUMMARY

It has been shown that the detergents Living, Tide XK and SA-8 inhibit root elongation of Cucumis sativus and Avena sativa, the inhibiting concentrations ranging from 500 ppm to 2500 ppm. The inhibiting concentration varies with the brand of detergent used and the species of plant tested.

Aeration of control and test solutions has no significant effect on the root elongation of Cucumis or Avena. High pH of detergent solutions may play some role in the inhibition of root elongation but such inhibition has been demonstrated when Tide XK detergent solutions had pH values comparable to controls.

Reversal of root inhibition has been demonstrated when detergent solutions are left in contact with soil for 48 hours. In most experiments this reversal is slight; in other experiments, complete reversal has been demonstrated. Since such reversal appears in treatments involving autoclaved soil as well as untreated soil, biodegradation of detergent by soil microorganisms is probably not occurring at least in the first 48 hours.

It is proposed that the soil adsorbs the detergent, thereby lessening its toxic effects. When the inhibiting concentration is low, on the order of 500 ppm, as in the case of Tide XK and SA-8 on Avena, the soil is able to tie-up all of the detergent (or at least the portion toxic to roots) and complete reversal of root inhibition is then possible.

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