THE CHEMICAL CONTROL AND THE UTILIZATION OF *ANACHARIS DENSA*

by

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INTRODUCTION

In certain lakes of Southwestern Oregon, an aquatic weed has become so well established that it prohibits boating, swimming, angling, and the moving of log rafts. This plant is *Anacharis densa* (Planchon) Victorin.

The generic name of Elodea has become increasingly more popular with the taxonomists as the proper name for this plant. (8, p.94) However, because this plant is commonly called Anacharis throughout this area of Oregon, it will be referred to as such throughout this discussion.

Anacharis was introduced into this country from South America many years ago. The plant that was introduced had staminate flowers only; therefore, the spread of this plant has been by vegetative methods only. It was brought from the eastern part of the United States into Oregon, it is thought, sometime in the 1930's. The phenomenal spread of this plant by vegetative propagation illustrates its vigor. At the present time, Tahkenitch, Tenmile, Loon, and Siltcoos Lakes are moderately to heavily infested with Anacharis.

In order to restore some of the original usefulness of these lakes and to prevent the weed from spreading, a study of the plant was initiated. This study represents a portion of that program.

One phase of the problem embraced in this study was an attempt to find a chemical which would be particularly toxic to Anacharis and thereby aid in its control. The second phase is concerned with the utilization of vegetation produced by Anacharis. Anacharis does make a very dense growth which often covers an extensive area. This phase of the problem, in other words, is to find a use for such a large amount of useless vegetation. It is useless, that is, from the standpoint that it hinders man's activities; but ecologically speaking, it may be serving a useful function in plant succession.

REVIEW OF LITERATURE

The literature reviewed will be presented for the chemicals and toxicity first. The review on the utilization of plants and plant wastes will be presented last.

Chemicals and Toxicity:

As one might expect, there are no direct references to the control of Anacharis densa in the literature. There are general recommendations such as that given by Machenthum (20, p.1066) on the use of sodium arsenite to control various aquatics but not Anacharis. Consequently, the best approach appears to be the development of a knowledge of general toxicity. On this basis, one could more nearly predict or explain the action of a chemical.

Trim gives four stages of drug interaction with the organism. (33, p.115) These stages are as follows:

(1) Interaction of the drug with the environment.
(2) Interaction of the drug with the boundary between the organism and environment, i.e. adsorption phenomena.
(3) Passage of the drug through this boundary, i.e. absorption and diffusion.
(4) Intracellular action of the drug.

The vital processes which sustain life in any organism are largely controlled by enzymes. It has been suggested that the enzyme controlling growth in plants is of a sulfhydryl nature. (33, p.299) Those compounds which have the ability to tie up the sulfhydryl group, such as the arsenite compounds and the phenylmercuric salts, will inhibit growth. Sexton (27, p.42) states that thiol groups in apoenzyme or coenzyme are one of the more reactive groups in biological systems. Also, oxidizing agents will inhibit thiol dependent reactions by converting the thiol group to a disulfide group. (27, p.43)

Sexton further advances two of the simplest mechanisms which operate in competitive inhibition of enzymes. (27, p.50) These mechanisms are: (1) The foreign substance acts as a rival substrate for a given enzyme reaction such as malonic or succinic acid, and (2) The foreign substance interferes with the synthesis of the enzyme from its precursor as in the example of sulfonamides substituting for p-amino benzoic acid. The antimetabolite aminopterin was found to interfere with mitosis. (16, p.825) Overbeek states that the growth hormone, heteroauxin, catalyzes a respiratory process involving the four carbon acids. (24, p.642) However, Slade, Templeman, and Sexton show that at higher concentrations; the growth hormones are toxic. (31, p.492)

Thus far, the discussion has hinged around the high degree of specificity of enzymatic reactions. In addition, there are species differences in regard to the toxic action of a chemical. for instance, lipase from castor bean is 400 times more susceptible to 2,4-D than lipase from wheat germ. (3, p.646)

Adsorption phenomena are controlled by surface activity. They are also affected by several factors. The pH affects the charge on any biological surface in contact with an aqueous phase. The charge also alters the degree of dis-association of ionizable drugs. (33, p.117) Most biological surfaces are negatively charged; therefore, drugs giving an organic anion should be more effective in the unionized form. (33, p.125) One exception exists in the case of the acridine compounds in which ionization is essential for toxicity. (1, p.84) Furthermore, the addition of soap to a phenol solution will reduce the interfacial tension to a minimum. At this minimum, biological activity will be the highest.

Blackman (3, p.642) and Crafts (6, p.86) shed some light on the absorption and diffusion of herbicides. They state that non-polar substances almost always enter by simple solution or diffusion through the lipoidal component of the cuticle and epidermal cell ealls. Water soluble or polar compounds probably enter by the way of the pectinacious and allied hydrophylic substances which have been shown to form a continuous path through the cuticle and epidermal cell walls of apple leaves.

Sugars added to spray solutions apparently aid in the translocation of chemicals out of the leaf. Starved plants do not translocate chemicals readily. (3, p.644) Furthermore, the chemical nature of a compound influences the passage of the chemical through the surface of a leaf. The formation of quaternary compounds from the 2-phenoxy ethyl amines markedly increases the activity of these compounds against oats. (18, p.149)

Jones et al (17, p.114) state that the biological activity of a compound depends upon having the correct degree of chemical reactivity of the toxiphore group balanced with physical properties which insure maximum ease of penetration to the site of action. For instance, the toxicity of a compound and its toxiphore can be influenced greatly by the nature of secondary substituent groups. The substitutions on a nitrogen atom of a compound, which give rise to quaternary salts, produce surface active compounds often showing toxicity. (17, p.110)

Sexton advances two postulates as to the effects of structural variation on the toxicity of organic chemicals. (28, p.2)

> "The biological activity of a substance is due to its combination with some substance or substances in the organism, and this combination is either responsible for maintaining the normal balance of the dynamic chemical processes of the organism, or in the case of drugs, results in upsetting these processes."
> "The combination of a biologically active molecule with a cell constituent may be modified to varying degrees by the variation of the physico-chemical properties of the active molecule. Structural alternation of an active molecule can alter the chemical reactivity or its capacity to react with specific substituents, and alternations can prevent or facilitate access of the molecule to the cell constituents."

Ferguson points out that the toxic concentration in a homologous series exhibits the same type of relationship as do the physical constants in the series. (27, p.57) and (1, p.29) These constants express distribution between heterogeneous phases. Therefore, the values of the toxic concentrations result from an equilibrium distribution between phases. These measured concentrations are not the concentrations at the site of action but in the surrounding medium. Ferguson's principle of structural non-specificity states that substances which are present at the same proportional saturation in a given medium have the same degree of biological activity. In line with this theory, one often finds a peak of biological activity as a homologous series is ascended. (17, p.111)

It must be remembered, however, that the line of demarcation between a physical interaction and a chemical reaction is not always easily drawn. (27, p.61) Nevertheless, the physical properties of a compound are most often dependent on the chemical structure of that compound. (1, p.25) Also, a chemical may or may not show biological activity purely on a stereochemical basis. The alkaloids, sugars, and amino acids illustrate this fact in the differences of activity of d and l forms. Similarly, the cis-trans configuration affects the biological activity.

The inherent toxicity of a substance which does not disassociate in solution is but little affected by pH. pH may greatly influence ionizable substances. (30, p.253) The effectiveness of penetration of compounds through cell membranes is influenced by pH. Cell membranes are considered to consist of two monolayers of lipoids surrounded on either side by a monolayer of protein. (1, p.75) pH affects the charge on both the cell membrane and the compound. An ion will, therefore, have a charge which is either similar to the portion of the protein surface which it approaches resulting in repulsion, or it is opposite resulting in fixation by adsorption. Thus, it follows that at a certain concentration of unionized molecules, optimum biological activity will result. A general principle has evolved from this fact through the use of the pK value. (29, p.343) and (30, p.253) For substances with an acidic group, pH has no effect on the toxicity of a compound when the pH is two or more pH units below the pK value. Above the pK value the total concentration has to be raised to obtain the same degree of response from the organism. For weak bases the reverse of the above relationship holds true. This applies principally to organisms in a solution or media.

Albert has discussed extensively the use of chelation in the selection of toxic agents. Metallic ions may be used to affect the balance of the trace elements in an organism through competition with chelating agents. Conversely, the use of strong chelating agents can be used to remove essential trace elements. (1, p.121)

In general, one may expect certain compounds to exhibit toxicity. The phenols act as protein precipitants. Also, the chlorates are not toxic per se, but their reduction products, chlorite and hypochlorite, produced by the nitrate reduction mechanism are toxic. (3, p.649) As has been pointed out previously, copper, arsenic, and organic mercurials are generally toxic if they are available to react within the cell. (34, p.299) Veldstra cites many examples in which the unsaturated lactones have toxic and inhibitory effects. (35, p.379) The dicumarol known as Warfarin is an unsaturated lactone.

It has been suggested that the 3 and 4 positions in the coumarin derivatives are responsible for most of the biological activity. (10, p.224) Veldstra suggests that the activity of the unsaturated lactones is due to a particular equilibrium between lypophylic and hydrophylic phases of the cell membrane.

Another similar group of chemicals having a wide, general biological activity are the quinones and quinoid derivatives. The broad classes of quinones consist of the benzoquinones, naphthoquinones, and the anthraquinones. (27, p.189) The heterocyclic nitrogen compounds also exhibit toxicity in many instances. This group consists of pyridine, quinoline, and acridine. By the inclusion of another nitrogen in the ring as in the case of acridine, the azine compounds are formed. Phenosafranine is an azine compound which exhibits toxicity. (27, p.205)

<u>Utilization of Plant Materials:</u>

There are many examples of utilizing plants and plant wastes. The extraction of bromine and iodine from seaweed is a classic example. Allison (2, pp.1-18) has discussed extensively the use of sawdust for mulches. Nelson has discussed the possibilities of using <u>Anacharis canadensis</u> as a fodder and a fertilizer. (21, pp.27-31) In as much as this problem on the utilization of <u>Anacharis densa</u> will be restricted primarily to mulches and fertilizers, the following discussion will deal primarily with the same.

Hutchinson (12, pp.414-415) and Schery (26, p.169) give the following characteristics and values that a substance should have if it is to be of value as a mulch.

(1) Mulches tend to save labor from watering and weeding.
(2) Mulches absorb and check run-off of water from rain and sprinkling.
(3) Mulches protect the soil against puddling
(4) They ameliorate fluctuating soil temperatures.
(5) Mulches tend to improve soil structure and increase aggregation.
(6) They may improve the nutrition of the crop.

One of the main values obtained from a mulch is its residual effects. (12, p.415)

Johnson (15, p.288) has found that there is no significant difference in pH after an area has been mulched for a long time. They did not report on peat moss which is usually considered to be an acid mulch. Boller and Stephenson report that some mulches may produce both acidic and toxic substances. (4, p.27)

Nelson brings forth the idea that the utilization of fresh-water aquatic plants would be a method of reclaiming lost natural resources. Wind, run-off water, and wave action remove the richest of the surrounding land and deposit it in streams and lakes. (21, p.29)

There is an increasing tendency to investigate aquatic vegetation for sources of food and raw material. Rose has investigated five seaweeds common to Canadian seashores and has found them to be good sources of alginates. (25, pp.18-28) In the British Isles there has developed a new industry from the utilization of the coastal seaweeds. Alginic acid is extracted from these algae which is used in medicine, research, cosmetics, and flavorings and thickenings. (22, pp.184-185)

Evidently the natural waters of the world have the capacity to produce abundantly. Jackson states that 20 tons per acre may be harvested from the sub-littoral seaweed areas. (14, p.348)

EXPERIMENTAL METHODS

The chemical screening tests were started January of 1951, and they were conducted in the laboratory building of the fur farm operated by the Fish and Game Department. The utilization experiments were conducted in the weed greenhouse of the Farm Crops Department.

Chemical Screening Tests:

All of the chemical screening tests were conducted according to one procedure which is discussed below. The only differences between the different tests are that the concentration of the chemical and the time of taking the data were varied.

Five gallon jars were calibrated to the 15 liter mark. These jars were set on a picnic table in the laboratory. Thirty six jars were usually set up at each time of treatment. A battery of fluorescent lights was placed along each side and over the top of the table. These lights were operated on a twelve hour day by a time clock. During the winter of 1951-52, an oil stove was kept burning to maintain the temperature around 55°F. The method of treatment consisted of filling the jars with water, adding the Anacharis, and then adding the chemicals which were well mixed with the water. The quantity of Anacharis used would easily fill a 600 milliliter beaker though it is doubtful if the quantity ever exceeded this amount. If the chemical or its salt was not soluble in water, acetone was used as the solvent. The chemicals were prepared by making solutions of them. One gram of chemical was added to fifty milliliters of solvent except where the solubility was too low. Then, the amount of solvent was increased accordingly. Each treatment was run in duplicate. At the end of the specified number of days, the data were taken. The data consisted of a rating given to each chemical according to the following scale.

1----A complete kill
2----All original plants nearly dead but new buds forming
3----Plants slightly softened or chlorotic but will not die
4----No noticeable affect

<u>Qualitative Screening Tests</u>: The purpose of this test was to eliminate those chemicals which did not exhibit marked toxicity toward Anacharis. The chemicals were applied at the rate of 25 parts per million based on the 15 liters of water in the jars. At the end of ten days from the time of treatment, the data were taken according to the scale set up above. Those chemicals which ranked 1 or 2 in this test were marked for further experimentation.

Quantitative Screening Tests: This test was designed to bring forth those chemicals which were toxic at low concentrations. Only those chemicals which were rated as 1 or 2 in the qualitative tests were used in this test. They were applied in a series of concentrations consisting of two, four, eight, and sixteen parts per million. At the end of seven days, the data were taken and each concentration of the chemical was given a rating.

The reason that the chemicals which ranked as number 2 were chosen was to determine if their toxicity decreased precipitously or gradually as the concentration was decreased. By limiting the length of the tests to seven days, the chemicals were limited in the time that they could exert their influence on the Anacharis. Under actual conditions, a chemical would also have a limited time to act upon Anacharis because of the shifting of currents and dilution of the effective concentration.

Combination of Chemicals Test: From the quantitative tests were chosen those chemicals which received the rating of 1 at the four parts per million concentration. The purpose of this test was to arrange these chemicals according to their toxicity, that is, what chemical is the most toxic. Each chemical was applied at one part per million with every other chemical. The resulting concentration in each of the treatments was then two parts per million. At the end of seven days the data were taken and each combination of chemicals were given a rating. Each combination was conducted in duplicate. To facilitate the making of finer distinctions in the rating of a chemical, the ranking system previously described was modified to include values to one half. For instance, a rating of 2.5 would be half way between 2 and 3.

Utilization Experiments:

All of the experiments on utilization were conducted in the greenhouse except the water holding test which was conducted in the chemistry laboratory. Due to unforeseen difficulties, sufficient quantities of Anacharis could not be obtained to conduct properly designed field tests. Some of the Anacharis was ground in a hammer mill, but the more coarsely ground material was processed by hand through a small grain grinder. The stems in the coarsely ground material measured from 3/8 to 1/2 inch in length.

To preserve the continuity of the material presented, the details of each experiment are given with the data in the next section.

EXPERIMENTAL RESULTS

First, the data from the chemical screening tests will be presented. The data from the utilization experiments will be presented last.

Chemical Screening Tests:

The data from the screening tests are presented in tabular form. The chemicals and their individual ratings are given with the most toxic chemicals in each type of test being placed first in the tables.

Qualitative Tests: Table I shows that acetone was suitable as a solvent for most of the chemicals, as it was non-toxic at 100 parts per million which is in excess of the concentrations used. Furthermore, this table shows that the wide array of chemicals used on land weeds are relatively non-toxic to Anacharis. Only after a prolonged treatment of twenty days did 2,4,5-T eventually kill the plant.

Early in the process of screening two of the acridines were tried. It was noted that 9-amino acridine was toxic whereas acridine was not. Concomitantly, safranine O was also tried. The safranine O, an azine compound, was also toxic. The structural similarity between the 9-amino acridine and the azine nucleus of safranine dye suggested that the azine nucleus might have marked toxicity for Anacharis. The safranine compounds are often dyes; therefore, a number of dyestuffs were tried along with all of the available safranine compounds. Those compounds marked with an asterisk in Table I are dyes. However, it should be noted that only the safranine compounds proved to be toxic. These compounds are safranine O, phenosafron, safranin cone, and safranine crystals.

Methylene green was also found to be toxic. The nucleus of this compound is called the thiazine nucleus which differs from the azine nucleus by having a sulfur atom in place of one of the nitrogen atoms. Another type of compound which is structurally similar to the azine compounds is the oxazine compounds. Here one of the nitrogen atoms of the ring is replaced by an oxygen atom. An example of this type of compound is gallocyanine which proved to be non-toxic.

The presence of the nitrogen in the azine and thiazine compounds suggests that it may play a part in disturbing the oxidation-reduction mechanisms of Anacharis. These nuclei are similar to the nucleus of thiamine which plays an important role in the process of dehydrogenation in biological systems.

On the basis of the data presented, there appears to be an inherent toxicity in the azine and thiazine nuclei for the Anacharis plant.

TABLE I

A Tabulation of the Chemicals and Their Affect on Anacharis at 25 Parts Per Million. The Rating of 1 Signifies a Complete Kill; A Rating of 4 Signifies No Affect

Chemical	Rating	Remarks
p-nitrobenzoic acid	1	Bleached and softened
Pentachlorophenoxy acetic acid	1	
Mercuric bromide	1	
a-naphthalene acetamide	1	Severely bleached
2,4-dinitrophenyl hydrazine	1	
p-aminodimethyl aniline	1	Plants dyed black and softened
4,4'-dibromo diphenyl	1	Quickly toxic
Metoquione	1	
Heptachloropropane	1	Apparently toxic to buds
2,6-dichloro-4-nitrophenol	1	Plants very soft; slowly toxic
3,4-dichloro benzene	1	
2,4-dichloro quinone	1	
Copper diphenyl acetate	1	
5-benzol rhodamine	1	Plants soft and green
p-thiocresol	1	Quickly toxic
p-hydroxy diphenyl	1	
2,4-dibromophenol	1	
p-bromophenacyl bromide	1	
Dibromo barbituric acid	1	
p-benzylphenol	1	

TABLE I (continued)

Chemical	Rating	Remarks
o-benzylphenol	1	
Sodium pentachlorophenate	1	Quickly toxic
1,3-diphenyl guanidine	1	
2-amino-5-diethyl amino toluene mono HCl	1	
p-amino dimethyl aniline hydrochloride	1	
Azoxybenzene	1	
2,4,5-T (20 day treatment)	1	
Allyl-B-naphthyl carbamate	1	
1-{2-(t-dodecyl mercapto) ethyl} pyridinium chloride	1	Very toxic to buds
Dinitro-o-isopropyl phenol	1	
Fumaronitrile	1	
9-amino acridine	1	
4-chloro-3-methyl-2-nitro phenol	1	
Dimethylchloro fumarate	1	
Nitroso beta naphtol	1	Quickly toxic
Dinitro-o-isopropyl phenol	1	
Menadione	1	
(Alkylbenzyl) trimethyl ammonium chloride	1	
2,4,6-trichlorophenol	1	
2,4-dichlorophenotole	1	
Rhodamine B*	1	

TABLE I (continued)

Chemical	Rating	Remarks
Diphenyl sulfone-2,4-dinitrophenylhydrazone	1	Buds appear very soft
Beta methylumbelliferone	1	
Fungicide p-162 (Julius Hyman & Co.)	1	
Safranine O*	1	
Methylene green*	1	
Phenosafron*	1	Dye penetrated tissues
Safranin conc*	1	Dye penetrated tissues
Safranine crystals*	1	Dye penetrated tissues plant crisp
Rhodamin*	1	
Diisopropyl dixanthogen	1	
2,4,6-triamino benzoic acid trihydrochloride	1	Quickly toxic to older growth
Sodium dihydrobenzoyl acetate	2	Soft & breaking; new buds forming
Beta benzoyl acrylic acid	2	Older growth dead; new buds forming
a-naphthalene acetic acid	2	Plants softened and breaking
p-hydroxy benzophenone	2	Plants soft & breaking
Phenyl-B-naphthamine	2	
a-naphthyl amine	2	Older growth tough
3,5-dinitro-o-cresylate (Na)	2	Quickly toxic
4,4',4"-hexamethyl triamino triphenyl methane	2	
2,4-dinitrochloro benzene	2	Very little regrowth of buds
2,6-dibromo-4-nitrophenol	2	
Dibromo flouresein	2	Plants chlorotic; new growth non-vigorous

TABLE I (continued)

Chemical	Rating	Remarks
1,4-diphenyl semicarbazide	2	Buds stopped growing
Isopropyl-N-phenyl carbamate	2	Internodes not growing; buds form a ball
Diethylammonium diethylthio carbamate	2	
S-diphenyl urea	2	Plants apparently not growing
Latex 620	2	
Butyl-2,4-dichlorophenoxy acetate	2	
1-aminoanthraquinone	2	
p-aminodimethyl aniline	2	
p-amino phenol	2	
Mucochloric acid	2	
4-amino antipyrine	2	
2,4-dichlorophenol	2	Buds are softened
Mixed ethyl & dimethyl mercaptothiazoles	2	Plants soft; few new buds
a-chloro-B-naphthol acetic acid	2	
Acetone semicarbazone	2	
Acrolein	2	
Indole-3-acetic acid	2	
Rhodamine S*	2	
Phenolphthalein (Na salt)	2	
Methyl-o-nitrobenzoate	3	Older portions chlorotic
2,4-dinitrobenzoic acid (17 ppm)	3	
2,4-dichloro-1-naphthoxy acetic acid	3	

TABLE I (continued)

Chemical	Rating	Remarks
Lead chromate	3	Plants very crisp and turgid
Bismuth subnitrate	3	Plants slimy & softened new buds forming
Cadmunium nitrate	3	Plants chlorotic but new buds forming
Monoethyl homophthalate	3	
p-chlorophenoxy acetic acid	3	New buds tending to abcise easily
2,4-dinitrophenyl acetic acid	3	
p-nitrophenyl acetic acid	3	
Beta naphthalene acetic acid	3	
2-methyl-4-chlorophenyl thioglycolic acid	3	Poor vigor
Hexamethylene tetraamine	3	
p-dimethylamino azophenyl arsonic acid	3	Buds vigorously growing
Tetraethyl diamino benzophenone	3	
Phenylbenzothioazole	3	Leaves have some burning
4-dimethylamino benzene-1-azo-1-naphthalene	3	New growth forming with good root growth
p-aminoazobenzene hydrochloride	3	Slow growth
1,3-dimethyl indole	3	
N,N'-diethyl-N,N'-diphenyl urea	3	
p-dimethylamino azobenzene	3	Insoluble
Beta hydroxy ethyl-o-toluidine	3	New growth non-vigorous
2,5-dichloroquinone	3	
Indophenol	3	
1,5-dihydroxy naphthalene diacetate	3	

TABLE I (continued)

Chemical	Rating	Remarks
2,4-dinitro benzene sulfonic acid	3	
Diphenyl-o-chlorophenyl phosphate	3	Slight bleaching
2,4-dinitro phenetole	3	Slow growth
p-chlorophenetole	3	Non-vigorous
Ethylene thiocyanate	3	
Flourene	3	Plants slightly bleached
2-hydroxy-4-methyl quinoline	3	
Quinalizarin	3	New growth non-vigorous
Geraniol	3	
Eugenol	3	
Isoamyl bromide	3	Plants tough
Di-o-cresyl carbonate	3	
Ethyl-N-n-butyl carbamate	3	
3-chloro-IPC	3	Plants non-vigorous
Ethylene diacetate	3	Older growth necrotic
p-flouroanisole	3	Older leaves bleached
2-aminoanthraquinone	3	
Maleic hydrazide	3	Plants crisp & healthy but not growing
2,3-dichloropropylidine dibutyrate(herbicide 1700)	3	
Benzal-iso-thiourea	3	Older growth bleached; new growth normal
p-iodo aniline	3	Much bacterial growth in culture
Aniline hydrochloride	3	
Emulsifyer R-400	3	Buds becoming large

TABLE I (continued)

Chemical	Rating	Remarks
3,3'-diamino-4,4'-dihydroxy diphenyl sulfone	3	
2-pyridinium IPC	3	
6-chloro-2-aminotoluene	3	
p-benzylamino phenol	3	
p-amino dimethyl aniline sulfate	3	
Thianthrene	3	
Di-chlor-sulphamine benzoate of soda	3	
Triphenylchloro methane	3	
Acridine	3	Buds necrotic, bleached and elongated
Di-n-heptyl acetic acid	3	
p-chloro ethyl-2,4,5-tri chlorophenyl ether	3	
2-amino-4-phenylphenol	3	
p-aminoacetophenone-2,4-dinitrophenyl hydrazone	3	
o-iodoanisole	3	
Hematoxylin*	3	
Orange G*	3	
Alizarin Red S*	3	
Erythrosin B*	3	
Carmine*	3	
Fast green FCF*	3	
Neutral violet*	3	
Diamine gel*	3	
Diamine fast brown*	3	

TABLE I (continued)

Chemical	Rating	Remarks
Diamine black*	3	
Diamine rose*	3	
Diamine red*	3	
Crysamine G*	3	
Methyl-o-bromobenzoate	4	
Acetone (100 ppm)	4	
Triethanolamine	4	
p-hydrazino benzoic acid (17 ppm)	4	
p-flourobenzoic acid (17 ppm)	4	
3,5-dinitro-2-methyl benzoic acid (17 ppm)	4	
Sodium-o-benzoic sulfimide	4	
Glycerol tribenzoate	4	
Methyl-p-nitrobenzoate	4	
2,5-dinitrobenzoic acid	4	
Ethyl-p-aminobenzoate	4	
2-bromo-3-nitro benzoic acid	4	
2,4,5-trichlorophenoxy acetic acid	4	
2,4,6-trichlorophenoxy acetic acid	4	
2-(4'-chlorophenyl) phenoxy acetic acid	4	
2-chloro-4-t-butyl phenoxy acetic acid	4	
2-(2'-chlorophenyl)phenoxy acetic acid	4	
Stannous chloride	4	

TABLE I (continued)

Chemical	Rating	Remarks
Chromium sulfate	4	
Barium nitrate	4	
Ethyl benzoate	4	
p-naphthyl benzoate	4	New buds growing vigorously
Potassium hydrogen-m-sulfabenzoate	4	
Tetrahydrofurfuryl benzoate	4	
L-propyl carbanilate	4	
Ethyl benzoyl acetate	4	
Potassium antimonate	4	
2,4-dinitro-1-naphthol-7-sulfonic acid	4	
Diphenyl-p-p'-disulfonic acid	4	
8-hydroxy quinoline-5-sulfonic acid	4	Very good growth
2,3-dichlorodioxane	4	
Diphenyl sulfoxide	4	
2,4-dichlorophenyl-4-toluene sulfate	4	
2,4-dinitro anisole	4	
2,5-dichloro benzene	4	
2-ethyl mercapto benzo-azote	4	
1-benzoyl-2-thiohydantoin	4	
2,4-dinitro toluene	4	
gallocyanine	4	
Ethyl acetanilide	4	
Dicyandiamide sulfate	4	

TABLE I (continued)

Chemical	Rating	Remarks
Dithiooxamide	4	
Betaine hydrochloride	4	
Hydatoin	4	
Phenoxyacetamide	4	
p-iodoacetanilide	4	
Ethyl-S-ethylxanthate	4	
Diphenyl thiocarbazide	4	
p-chlorophenyl dimethyl urea	4	
Sodium-2-naphthol-7-sulfonate	4	
L-propyl carbanilate	4	
Ethyl benzoyl acetate	4	
Potassium antimonate	4	
Lithium nitrate	4	
o-phenylene diacetonitrile	4	
2,4-dichloro phenoxyethyl alcohol	4	Plants growing vigorously
o-phenylene acetic acid	4	
Di-o-cresyl carbonate	4	
p-methoxy benzophenone	4	
Acetophenone	4	
Benzil	4	
2,3-dimethoxy benzaldehyde	4	
p-hydroxy phenyl arsonic acid	4	
Anisaldehyde	4	

TABLE I (continued)

Chemical	Rating	Remarks
Chloranil	4	
n-heptaldoxime	4	
Tert-amyl urea (mono)	4	
Dephenylnitroso amine	4	
Dimethyl-p-toluidine	4	
2,4-dichloro aniline	4	
S-diphenyl thiourea	4	
2,4-dinitro resorcinol (Na)	4	
9,10-dibromo anthracene	4	Insoluble
Diphenyl sulfone	4	
Ethylene diformate	4	
1,8-dihydroxy disulybenzene	4	
Diphenyl formamidine	4	
Salicylacetic acid	4	
Phenylchloroacetate	4	
t-butyl urea	4	
2-hydroxy-4-methyl quinoline	4	
4-hydroxy-1,3-dimethyl benzene	4	
4-hydroxy-1,2-dimethyl benzene	4	
5-hydroxy-1,3-dimethyl benzene	4	
2,4-dichloro benzoic acid	4	
6-chloro-2-toloxy acetic acid	4	
3,4-dichloro benzoic acid	4	

TABLE I (continued)

Chemical	Rating	Remarks
p-bromo aniline	4	
Di-p-carbonate	4	
Di-p-cresyl carbonate	4	
a,B-dibromo butyric acid	4	
Beta dithiodiglycol	4	
p-chloroaniline	4	
2-chloro-5-hydroxy toluene	4	
o-bromophenol	4	
o-hydroxy benzyl alcohol	4	
Phenylthio urea	4	
Disodium endoxohexahydro-phthalate (endothal)	4	
7-iodo-8-hydroxy quinoline sulfonic acid	4	
Methyl-p-iodo benzoate	4	
4-4'-dihydroxy diphenyl sulfone	4	
Dihydroxy diphenyl sulfone	4	
2,4-dihydroxy diphenyl sulfone	4	
4-4'-dichloro-3-3'-dinitro diphenyl sulfone	4	
4-4'-diaminodiphenyl sulfone	4	
3-aminophthal hydrazide	4	
2-amino benzo thiazole	4	
Acetyl-a-naphthalamine	4	
4-amino-1,3-dimethyl benzene	4	

TABLE I (continued)

Chemical	Rating	Remarks
Tetramethyl diamino benzophenone	4	
p-amino acetanilide	4	
Thioacetanilide	4	
2-chloroethyl-2-nitro carbanilate	4	
p,p'-diamino diphenyl methane	4	
o-isopropyl-N-2,4-dichloro phenyl carbamate	4	
a-naphthyl isopropyl carbamate	4	
o-isopropyl-N-bromophenyl carbamate	4	
2-chloro-N-N-diethyl-ethylamine hydrochloride	4	
o-isopropylaniline carbamate	4	
Furfuryl carbanilate	4	
Benzoyl acrylic acid	4	
Ammonoim rhodanilate	4	
2-methoxyethyl carbanilate	4	
8-amino-6-methoxyquinoline	4	
2-aminopyridine	4	
3-aminopyridine	4	
Sodium tetrabrome flurescein	4	
Orcinol	4	
B-naphthol isocyanate	4	
Ethyl-2,4-dichlorophenyl formate	4	
p-aminoacetophenone	4	

TABLE I (continued)

Chemical	Rating	Remarks
m-phenetidine	4	
Hydrazine sulfate	4	
Phenyl ethyl acetic acid	4	
Ethylene thiourea	4	
3,t-demethyl phenoxy acetic acid	4	
2,4-dichloro-o-phenyl-gamma-chloro propyl ether	4	
2,4-dichlorophenoxy ethyl alcohol	4	
Sodium-2,1,3-benzo triazyl acetate	4	
Sodium-1,2,3-benzo triazyl acetate	4	
Sodium-2,3,5-triiodo benzoate	4	
Furfural 2,4-dinitro phenyl hydrazone	4	
Furfural semicarbazone	4	
Acetone-2,4-dinitro phenyl hydrazone	4	
Methyl isobutyl ketone semicarbazone	4	
Acetophenone 2,4-dinitro phenyl hydrazone	4	
Nicotinic acid	4	
2-amino anthraquinone	4	Insoluble
Pyridoxine	4	
Sodium aconitate	4	
Azelaic acid (Na salt)	4	
Sodium fumarate	4	
p-amino acetophenone semicarbazone	4	

TABLE I (continued)

Chemical	Rating	Remarks
Diphenyl sulfone semicarbazone	4	
Anthranilic acid	4	
Alloxan	4	Plants very turgid
n-heptaldoxine 2,4-dinitro phenylhydrazone	4	
Abietic acid	4	
Hercolyn	4	Some of chemical adhered to leaves
2-amino-4-(p-diphenyl) thiazole	4	
Phenamine blue R*	4	
Triamine blue*	4	
Azomauve B*	4	
Azofuchsin*	4	
Diphenyl black*	4	
2-amino-1,4-dimethyl benzene hydrochloride	4	
Diamine dark blue*	4	
Diamine orange D*	4	
Cochineal red*	4	
Violamine*	4	
Cerasine*	4	
Nitrosamine red*	4	
Chlorophenine orange R*	4	
Naptamine blue*	4	
2-amino-p-cymene	4	

TABLE I (continued)

Chemical	Rating	Remarks
Sodium N-1-naphyhyl phthalamate	4	
N-2-chlorophenyl phthalamic acid (10 ppm)	4	
Sulfapyridine	4	
Sulfamerizine	4	
Sulfaguanidine	4	
Sulfadiazine	4	
2-hydroxy-1,4-dimethyl benzene	4	

<u>Quantitative Tests</u>: The data from the quantitative tests are arranged in Table II. The more toxic of the chemicals appear first in the list. Here the safranine compounds and quaternary ammonium compounds stand out as the types of compounds that are the most toxic. This test again points out that the azine nucleus has marked toxicity toward Anacharis. Also, it should be noted that nitrogen appears in the ring of all of these compounds except the (alkylbenzyl) trimethyl ammonium chloride.

The fungicide p-162 is a compound having a high chlorine content. The structure and name of the compound is confidential. It is a volatile soil fungicide still in the experimental stages.

On the basis of solubility, the following chemicals could be grouped as water soluble: Safranine conc, Safranine crystals, Phenosafron, 1-{2-(t-dodecylmercapto) ethyl} pyridinium chloride, Rhodamine B, Rhodamine S, (alkylbenzyl) trimethyl ammonium chloride, and p-amino-dimethyl aniline hydrochloride. It should be noted that this group is largely situated at the beginning of the table among the most toxic chemicals.

<u>Combination of Chemicals Test</u>: This experiment was designed to give the order of toxicity of the most toxic chemicals thus far. Those chemicals which were ranked 1 at the four parts per million concentration in the

TABLE II

The Ratings of Chemicals as Determined at Various Concentrations. Rating 1 Signifies a Complete Kill and 4 Signifies No Affect

Chemical	Concentration in ppm 16	8	4	2
Safranine cone	1	1	1	1
Fungicide p-162 (Julius Hyman Co.)	1	1	1	1
2-methyl-naphthoquinone (menadione)	1	1	1	2
Safranine crystals	1	1	1	2
Phenosafron	1	1	1	2
p-bromo phenacylbromide	1	1	1	2
1-{2-(t-dodecylmercapto)ethyl} pyridinium chloride	1	1	1	3
(alkylbenzyl) trimethyl ammonium chloride	1	1	1	4
Heptachloro propane	1	1	2	3
Sodium dehydrobenzoyl acetate	1	1	2	4
5-benzal rhodamine	1	1	2	4
p-thiocresol	1	1	2	4
2,6-dichloro-4-nitrophenol	1	1	3	4
Rhodamine B	1	1	3	4
p-naphthylamine	2	2	3	4
Mixed ethyl and dimethyl mercapto thiazoles	2	2	4	4
4-chloro-3-methyl-2-nitro phenol	1	3	4	4
3,4-dichloro nitrobenzene	1	3	4	4
3,5-dinitro-o-cresol	2	3	4	4
Rhodamine S	2	3	4	4
Beta-methyl umbelliferone	2	3	4	4

TABLE II (continued)

Chemical	Concentration in ppm 16	8	4	2
p-aminodimethyl aniline hydrochloride	2	3	4	4
2-amino-5-diethylaminotoluene	2	3	4	4
Nitroso beta naphtol	2	3	4	4
5-amino-1,3-dimethyl benzene	3	3	4	4
2,4-dichloro phenetole	2	4	4	4
p-hydroxy diphenyl	2	4	4	4
Azoxybenzene	2	4	4	4
o-naphthylamine	2	4	4	4
a-naphthalene acetic acid	3	4	4	4
Beta benzoyl acrylic acid	3	4	4	4
o-benzyl phenol	3	4	4	4
2,4,6-trichlorophenol	3	4	4	4
p-amino acetophenone	4	4	4	4
pentachloro phenoxy acetic acid	4	4	4	4
1-amino anthraquinone	4	4	4	4
Acetone 2,4-dinitro phenyl hydrazone	4	4	4	4
4,4',4"-hexamethyl triamino triphenyl methane	4	4	4	4
tert-butyl urea	4	4	4	4
o-hydroxy diphenyl	4	4	4	4
4,4'-dibromo diphenyl	4	4	4	4
o-nitrobenzoic acid	4	4	4	4
1,3-diphenyl guanidine	4	4	4	4
2,4,6-triamino benzoic acid	4	4	4	4

quantitative tests were chosen for this test. The data from this experiment are presented in Table III. Due to the difficulty of including the long names of the chemicals in the table, they are designated by a number. Table IV is a list of the chemicals and their corresponding numbers from Table III. These chemicals are arranged according to the mean of their rank. The mean of each chemical is based on the grand total. The grand total is the sum of the totals obtained from adding down the table and from adding across the table for each chemical.

In Table IV it should be noted that the safranine compounds are the most toxic. This tends to support the suggestion that the azine nucleus might have certain properties that cause it to be particularly toxic to Anacharis.

TABLE III

Data on the Interactions of Chemicals Applied at 25 PPM. The Values are Ratings on a Scale of 1 to 4 in which 1 is the Most Toxic

Chemicals	1	2	3	4	5	6	7	8	Totals
1	4.0								4.0
2	2.0	2.5							4.5
3	3.0	2.5	2.0						7.5
4	2.5	2.5	3.5	2.0					10.5
5	2.0	1.5	2.5	2.0	2.0				10.0
6	2.5	3.0	2.5	3.0	2.5	2.5			16.0
7	4.0	3.0	3.0	3.0	2.5	4.0	4.0		23.5
8	4.0	2.5	4.0	3.0	2.0	4.0	4.0	4.0	27.5
Totals	28.0	22.0	24.0	23.5	19.0	26.5	31.5	31.5	
Averages	3.1	2.4	2.6	2.6	2.1	2.9	3.5	3.5	

TABLE IV

Chemicals which Are the Most Toxic to Anacharis Arranged According to Their Toxicity. The Numbers are Those Used in Table III.

Number	Chemical	Average Rank
5	Safranine conc	2.1
2	Phenosafron	2.4
4	Safranine crystals	2.6
3	2-methyl naphthoquinone	2.6
6	Fungicide p-162	2.9
1	1-{2(t-dodecylmercapto) ethyl} pyridinium chloride	3.1
8	(alkylbenzyl) trimethyl ammonium chloride	3.5
7	p-bromophenacyl bromide	3.5

Results on the Utilization of Anacharis:

Before any material can be used for biological experiments, a chemical analysis should be made of the material. Therefore, a list of the comparative chemical analyses of *Anacharis densa*, *Anacharis canadensis*, and alfalfa is given in Table V. The data for *Anacharis densa* was determined in the Agricultural Chemistry Department at Oregon State College, and the data for *Anacharis canadensis* and alfalfa was obtained from Nelson. (21, p.27)

TABLE V

A Comparison of the Nutritive Value of *Anacharis densa*, *Anacharis canadensis*, and Alfalfa. Values are Expressed as Percentages of Dry Weight of Plant

Materials	A. densa	A. cana.	Alfalfa
Dry matter	8.25	7.52	29.39
Ash	20.00	21.80	7.72
Crude protein	20.00	26.80	17.20
Ether extract	1.21	3.53	1.88
Crude fiber	15.42	15.39	35.60
N-free extract	30.00	32.40	37.40
Calcium	1.22	2.80	1.44
Silicon dioxide	8.77	6.28	0.10
Phosphorus	0.39	0.57	0.15
Manganese (ppm)	3360.00	3310.00	47.00
Cobalt (ppm)	10.00		

From the above table, one may see that *Anacharis densa* compares favorably with alfalfa as a forage from a chemical analysis standpoint. The table also shows that Anacharis will add nutrients to the soil if it is used as a fertilizer or a mulch.

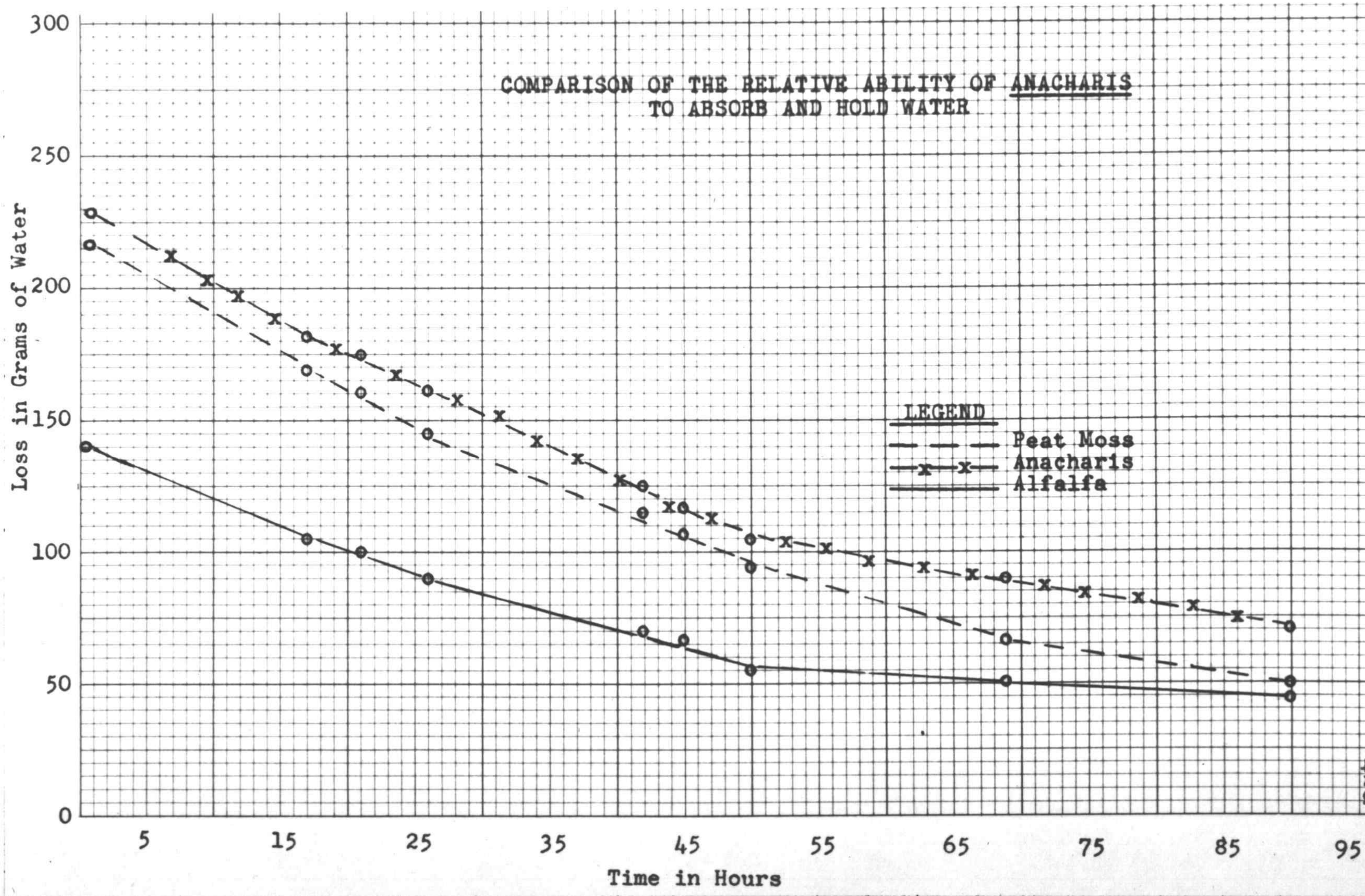
Ability of Anacharis to Absorb and Hold Water: As one of the functions of a mulch is to conserve moisture, this experiment was conducted to determine the relative ability of Anacharis to absorb and hold water.

Twenty grams of coarsely ground Anacharis, peat moss, and alfalfa were put into individual beakers. To each was added about 300 milliliters of water, and they were then allowed to set for 24 hours. After this time the beakers were inverted over burner screens fastened to ring stands. After 30 minutes the materials had stopped dripping, and the first weighings were made. These weighings were continued for four days at four hour intervals.

The data were plotted as grams of water lost. This graph appears in the following Plate I. It was noticed that Anacharis would swell up to two and one half times its dry volume when sufficient water was added to it. The other materials did little or no swelling. Although peat moss is known to have the ability to absorb much water, Anacharis appears to be equally efficient or better.

Plant Response Test: Now that the picture of the nutrient value and the water holding capacity of Anacharis has been presented, the question arises as to the plant reaction to the material. Accordingly, an experiment was conducted to determine the fertilizing value of Anacharis in relation to the length of time required for decomposition.

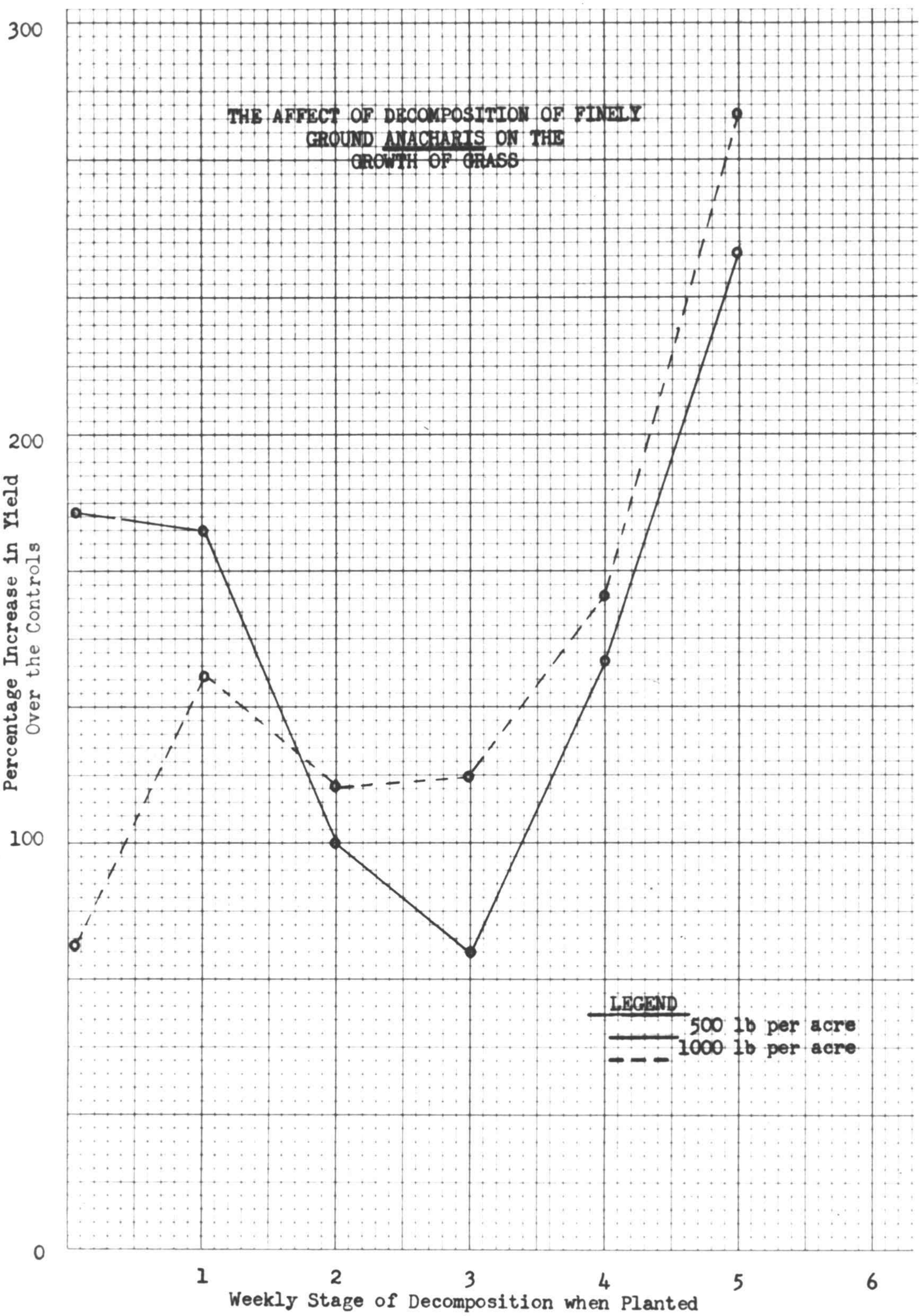
PLATE I


COMPARISON OF THE RELATIVE ABILITY OF ANACHARIS
TO ABSORB AND HOLD WATER
LEGEND
Peat Moss
Anacharis
Alfalfa
Loss in Grams of Water
300
250
200
150
100
50
0
5
15
25
35
45
55
65
75
85
95
Time in Hours

For this experiment, number 10, gallon-size cans were used as containers and were filled with Newburg silty clay loam. In six of the cans, finely ground Anacharis was mixed with the soil at the rate of 500 pounds per acre and to another six at the rate of 1000 pounds per acre. These were carried on in duplicate with common rye grass used as the indicator plant. Plantings were made in these cans at weekly intervals from zero to five weeks after the addition of the Anacharis. At each planting time, two untreated cans were also planted for controls. About one month after each planting time, the grass was harvested and the data recorded as grams of green weight.

Each of the two rates and the controls were averaged and the difference between the treatment and the control was divided by the control. These values were then plotted on a graph shown in Plate II. The data shows the percentage increase of weight of grass over the controls for each time of planting. It can be seen that in the Anacharis treated soil there is a great decrease in the rate of growth at the three week stage of decomposition

Comparison of Anacharis with Organic Nitrogen Fertilizers: On the basis of the preceding experiment, the question now arises as to how Anacharis will compare with other materials often used as organic nitrogen fertilizers. To answer this question, an experiment was conducted in the



THE AFFECT OF DECOMPOSITION OF FINELY GROUND ANACHARIS ON THE GROWTH OF GRASS
300
200
100
0
Percentage Increase in Yield
Over the Controls
1
2
3
4
5
6
Weekly Stage of Decomposition when Planted
LEGEND
500 lb per acre
1000 lb per acre

PLATE II

greenhouse using plastic flower pots as the containers. Astoria bentgrass was used as the indicator plant. All materials were well mixed with the surface two inches of the soil, and they were applied at the rate of 30 pounds of nitrogen per acre. All the materials were finely ground. Unfortunately, using the finely ground Anacharis was not the best manner in which to compare Anacharis with the other fertilizers for reasons which will be pointed out later.

The experimental design was a split plot with three replications and three times of harvest. The data were taken as grams of green weight. The materials used and the nitrogen percentage of each are listed below.

Anacharis	3.0
Fish meal	11.5
Cotton seed meal	6.8
Digester tankage	11.1
Linseed meal	5.6
Soybean meal	6.4

The data are recorded in Table VI. Table VII is an analysis of variance of this experiment. It should be noticed that in Table VI the Anacharis is the lowest yielding treatment of any. By applying the L.S.D. of 0.380 grams, the Anacharis is shown to be significantly lower than the control. But, when the totals for the individual times of harvest are compared; it can be seen that this low yield of the Anacharis treatment can be

TABLE VI

The Yield in Grams of Green Weight from Various Fertilizer Materials taken on Three Consecutive Dates

Materials	Time of Harvest 1: Replications 1	2	3	total	Time of Harvest 2: Replications 1	2	3	total	Time of Harvest 3: Replications 1	2	3	total	Ave.
Anacharis	4.5	3.9	4.8	13.2	1.4	1.6	1.5	4.5	3.6	2.2	2.0	7.8	2.83
Controls	3.3	8.5	6.9	18.7	3.3	2.0	1.1	6.4	1.4	1.4	1.6	4.4	3.27
Cotton seed m.	9.2	8.4	8.5	26.1	2.4	1.7	1.4	5.5	1.0	2.3	1.6	4.9	4.05
Tankage	8.3	9.7	9.8	27.8	1.1	1.9	1.7	4.7	1.6	1.9	1.8	5.3	4.20
Fish meal	7.3	9.5	10.8	27.6	2.3	2.2	1.3	5.8	2.0	2.0	1.8	5.8	4.35
Linseed m.	11.3	10.2	8.7	30.2	1.4	1.4	2.0	4.8	1.8	2.1	1.7	5.6	4.51
Soybean m.	8.1	10.4	11.0	29.5	2.9	1.7	1.6	6.2	2.2	1.9	2.0	6.1	4.64

L.S.D. equals 0.389 at (.05) level

accounted for in the first time of harvest. The subsequent times of harvest show that Anacharis compares favorably with the other materials. This experiment shows that it is during the early stages of decomposition of Anacharis that the growth of grass is inhibited. The data indicates that Anacharis will compare favorably with the other organic nitrogen fertilizers if it is given sufficient time to decompose past the toxic stage.

TABLE VII

The Analysis of Variance of the Yield of Various Fertilizer Materials from a Greenhouse Experiment.

Variation Due to:	Sum of Squares	Degrees of Freedom	Mean Square	F
Replications	1.01	2	.505	1.05
Materials	24.54	6	4.09	8.52*
Reps X Mater.(error a)	5.79	12	.48	
Time	571.83	2	285.91	277.58*
Time X Mater.	59.49	12	4.95	4.8
Time X Reps	7.63	4	1.84	1.78
Time X Mater. X Reps(error b)	24.83	24	1.03	
Total	694.85	62		

*Significant at the (.05) level

Determination of Toxic Substances in Anacharis: The purpose of this experiment is to determine definitely if there is a substance in Anacharis that is toxic to grass. Not knowing what type of substance might be present, both ether and water were used as solvents.

To each ten grams of finely ground Anacharis in a beaker was added 75 milliliters of ether. This was stirred occasionally and let stand for two hours. The solution was then filtered through a buchner funnel and the residue washed with an additional 25 milliliters of ether. The residue of Anacharis was dried and saved for future use. The filtrate was evaporated by putting it in a vacuum desicator. The residue left behind after the evaporation was a viscous, amber liquid having acrimonious properties. This extract was then mixed with a pyrophylite dust in such proportions that one gram of dust contained the extract from 2.5 grams of Anacharis.

For the water extract about 150 milliliters of water had to be added to each ten grams of finely ground Anacharis. The Anacharis has a marked tendency to swell when wetted. The mixture was let stand for two hours and filtered through a buchner funnel. The residue was washed with an additional 50 milliliters of water, and then the residue was dried and saved for future use. The filtrate was evaporated from a large clock glass under a red heat lamp. The residue left from this evaporation was mixed with sufficient 80 per-cent ethanol to facilitate making a pyrophylite dust. The proportions of the dust were such that one gram of the dust contained the extract from 1.25 grams of Anacharis.

To determine if these extracts were toxic, the dusts were mixed into the surface of soil. The containers for this soil were plastic flower pots. The amount of dust added to each pot was equivalent to 110 pounds of Anacharis per 1000 square feet of area. Astoria bentgrass was used as the test plant. The treatments were conducted in duplicate and consisted of the following:

1. Control (only pyrophylite added)
2. Anacharis extracted with water
3. Water extract from Anacharis
4. Anacharis extracted with ether
5. Ether extract of Anacharis
6. Untreated, finely ground Anacharis

Quantitative data were not taken on this experiment. The water extract from Anacharis and the Anacharis minus its water extract allowed the grass to grow as well as the untreated Anacharis. There was a striking difference in regard to the ether extract of Anacharis, however. Figure I shows the relative differences caused by extracting the ether soluble substances from Anacharis. On pot C the ether extract was applied which caused an almost complete inhibition of growth. The Anacharis from which

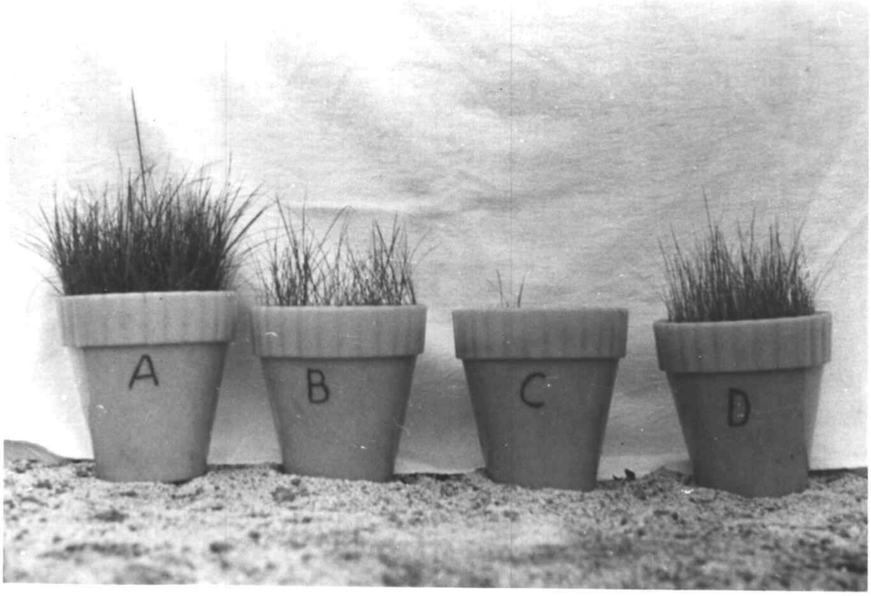


Figure 1. The toxicity of the ether extract of Anacharis. The extract was applied to pot C. The Anacharis from which the extract was taken was applied to pot A. Pot D has unaltered Anacharis applied to it and pot B is the control with pyrophylite added.

this extract was taken was applied to pot A. Notice the large increase of growth in pot A over pot D which had untreated Anacharis. The pot B is the control with only pyrophylite added.

Test of Coarsely Ground Anacharis: In the previous utilization tests, the Anacharis was finely ground. Also, the toxicity test indicates strongly that a toxic substance is present. It was thought that if the Anacharis was applied to the soil in a coarsely ground condition, this toxicity might not be as readily available and consequently not inhibit growth to an appreciable extent. To have comparable results, an experiment was set up in the exact manner as the plant response test mentioned earlier. Only one rate of Anacharis was used and that was 500 pounds per acre of coarsely ground Anacharis. The data were plotted as the percentage of increase of weight over the controls. This graph appears in Plate III following this page. Notice that at the three week stage of decomposition, the percentage increase over the check is the converse of the results obtained in the plant response test. However, instead of continuing to rise as in the plant response test, the percentage increase dropped. This might have been caused by delayed release of the toxic material by bacterial decomposition because of the Anacharis being coarser. It might also have been caused by the environmental conditions.

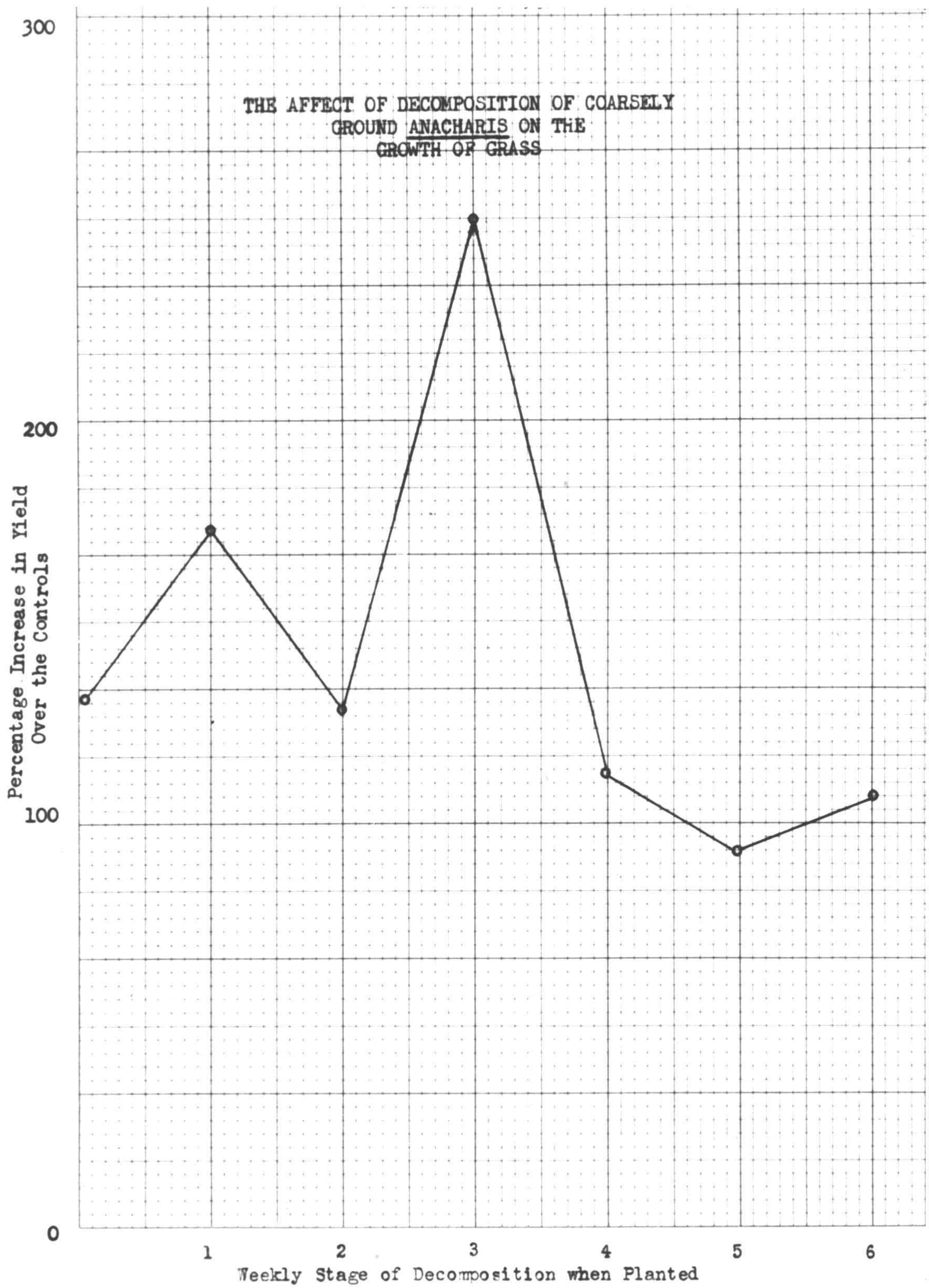

300
200
100
0
THE AFFECT OF DECOMPOSITION OF COARSELY GROUND ANACHARIS ON THE GROWTH OF GRASS
Percentage Increase in Yield Over the Controls
1
2
3
4
5
6
Weekly Stage of Decomposition when Planted

PLATE III

Comparison of Peat Moss and Anacharis as a Mulch: The final test for the determination of the agronomic use of Anacharis is to determine its value as a mulch. It was decided to compare Anacharis with peat moss which is good mulch commonly used. Because mulches are often used in establishing new lawns, Astoria bentgrass was used as the test plant.

Greenhouse flats having one and one half square feet were used as the soil containers. These flats were divided into halves which provided for two treatments. The grass was grown in a sandy loam from a river bank. After planting, the materials were applied as a mulch. The amount of water needed by the plants was judged by the controls. When the controls needed water, an equivalent amount of water was given to each of the mulch treatments. This experiment was designed as a randomized block with seven treatments and two replications. The treatments and the data, which are grams of green weight, are given in Table VIII. The rates are expressed as fractions, i.e. 100/1000. This means 100 pounds of material per 1000 square feet of area. In Table IX is an analysis of variance of the experiment. The treatments were significantly different at the one per-cent level.

It can be seen from the data that Anacharis was far superior to peat moss for mulching purposes in this experiment. Although there is considerable variation between the different rates, even the lightest rates of Anacharis fostered a good growth of the grass. Figures 2 and 3 graphically portray the differences between the treatments.

TABLE VIII

Green Weight in Grams of Astoria Bentgrass Mulched with Anacharis and Peat Moss

Material & Rate	Rep 1	Rep 2	Total	Average
Anacharis 100/1000	23.50	21.57	45.07	22.53
100/1300	9.10	20.80	29.90	14.95
100/1600	16.60	32.92	49.52	24.76
Peat moss 100/1000	3.30	3.90	7.20	3.60
100/1300	3.87	3.52	7.39	3.69
100/1600	14.40	8.76	23.16	11.58
Controls	3.72	3.45	7.17	3.58

L.S.D. equals 8.47 at (.01) level

TABLE IX. Analysis of Variance of the Yield from Anacharis and Peat Moss Mulches.

Variation Due to:	Sum of Squares	Degrees of Freedom	Mean Square	F
Replications	29.81	1	29.81	1.75
Materials	973.95	6	162.32	9.57*
Error	101.81	6	16.96	
Total	1,205.57	13		

Significant at (.01) level

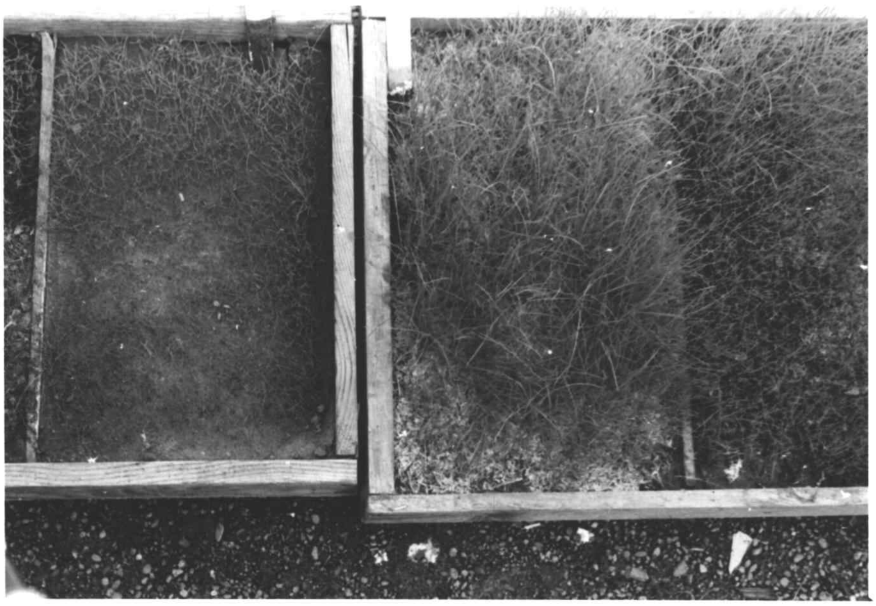


Figure 2. The use of Anacharis densa as a mulch. The control is on the left and the Anacharis mulch is on the right. Astoria bentgrass is the indicator plant.

Figure 3. A comparison of Anacharis and peat moss as a mulch for establishing grass seedings. The Anacharis mulch is on the left. Both materials were applied at the rate of 100 pounds per 1300 square feet of area.

DISCUSSION

The 25 parts per million concentration used in screening the chemicals was an arbitrary amount. It appears logical to began testing at a low concentration, however. Assume that it is desirable to treat a lake, one mile on a side and ten feet deep, with a chemical at four parts per million. Furthermore, assume that this chemical costs ten cents a pound--a very cheap chemical. We have, then, 6,400 acre feet of water, and each acre foot weighs very nearly 2.7 million pounds. Thus, at four parts per million, it would require 10.8 pounds to treat an acre foot; and it would require 64,000 pounds of chemical to treat the lake. At ten cents a pound, this would mean an expenditure of 6,400 dollars to treat this lake. In this case the lake is fairly small; the chemicals are cheap, and the concentration of chemical used is low. The reader can now imagine the expense of actually treating a real lake. This brings forcibly to mind the necessity of finding a chemical that is very toxic at low concentrations as well as being cheap. With the practical control of Anacharis by chemicals in mind, one would logically start screening at low concentrations.

If the chemical was not soluble in water, there would be the added expense of the other solvent and an emulsifier.

Also, the concentration of the chemical would probably have to be higher than that indicated by experiments in the laboratory. The chemicals used in the laboratory were used under more nearly optimum conditions than would be found in the field. Furthermore, the shifting of currents would affect the time of contact of the chemical with the weed as well as the concentration.

The question arises as to how the chemicals would affect the fish. This could only be determined by specific tests on fish with the chemical in question. However, the important thing to remember is that if a large volume of aquatic weeds were killed by a chemical, the added load of decomposing organic matter in the water would take most of the oxygen out of the water. It is likely, therefore, that many fish would die from the lack of oxygen even if the chemical were non-toxic to them.

Even though it appears that chemical control of Anacharis is impractical on a lake basis, the screening of chemicals on Anacharis could have much practical value. For high value waterways such as in irrigation systems and small ponds; the chemical control of Anacharis may be economically feasible. Also, it is reasonable to assume that these chemicals would be toxic to many other submerged aquatics which often are troublesome.

These chemicals may also have a value in the field of weed control on the land. The fungicide p-162 is reported to have been used as a pre-emergent at the rate of 40 pounds per acre.

This work on the screening of chemicals has brought out the importance of the physical properties of chemicals in relation to their use as a herbicide. Most of the chemicals toxic to Anacharis were water soluble. This signifies that the chemicals were polar compounds. This may indicate that the more polar compounds have greater ability to penetrate the plant.

It was found that the vapor pressure of a compound affects its properties as a herbicide. The first tests with the volatile soil fungicide showed that this compound was very toxic. This was during the winter month of January. When later tests were conducted during the warmer months, the compound did not appear as toxic. The odor of the compound was not nearly as noticeable during the warmer months. The higher temperatures caused the fungicide to dissipate and lower the effective concentration.

It was brought out in the review of literature that the quaternary salts are surface active compounds often showing toxicity. (17, p.110) The pyridinium and ammonium chlorides found to be toxic in this study are examples of this type of compound. The safranine dyes were also found to be particularly toxic to Anacharis. It may be that the azine nucleus of the safranine compounds is specifically toxic to Anacharis. The field of selective toxicity is based on slight physical or physiological differences between species. That is, the undesirable species is more susceptible to a drug than the desirable species. In the case of Anacharis, we are looking for susceptible spot in its chain of biological processes. The above mentioned compounds may fit into this susceptible spot in Anacharis. It is suggested that these compounds may well serve as a basis for future studies in the search for chemicals toxic to Anacharis.

The data accumulated in this study are not sufficient to formulate a theory as to why these chemicals are toxic. However, there are indicative points that should be brought out. The surface active quaternary compounds may act through adsorption on the cell membranes, or they may have marked ability to penetrate the cell membranes. The nitrogen in the azine nuclei of the safranine compounds may take part in oxidation-reduction processes. The safranine compounds are fairly easy to oxidize and reduce. Depending on the constituents on the azine nucleus, it would be possible that safranine compounds could also act through chelation and thereby tie up some of the essential minerals in the plant.

Judging from the low concentrations of two and four parts per million at which these chemicals were used, they appear to function as antimatabolities. That is, they may interfere directly with some enzymatic function in the plant. Notice that the 2-methyl-naphthoquinone is one of the K vitamins, but it has a high toxicity toward Anacharis. Although vitamin K does not have a known function in plants at this time, this compound may interfere with a function in the plant requiring one of the K vitamins.

The exact structure or name of the safranine dyes used in this study is not known exactly. The names used are those which were on the containers from which they were obtained. It is thought that the phenosafron used in this study is Diamino-phenyl-diphenazonium chloride. Phenosafranine is reported by Sexton (27, p.205) to exhibit toxicity. This is probably the same compound as the phenosafron reported in this study.

One of the difficulties in studying biological material is the obtaining of quantitative data. The screening of chemicals offers the same difficulty. Gustafson analysed 17 compounds by seven different tests. Each of the 17 compounds reacted differently in the seven tests. (11, p.653) No one test is sufficient to classify a compound. The test should be chosen in accordance with the information desired. Offord describes a method of estimating the phytocidal action of chemicals. (23, p.475) However, this method utilized visual characteristics each of which were given a rating. In this study, the writer found that the indications and reactions given by the plant itself was the most practical basis on which to take data.

The finding of a toxic substance in Anacharis when it is used as a nitrogen fertilizer may limit the use of it in the early stages of its decomposition. This toxicity does not appear when the plant is used as a mulch, however. On this basis, one might assume that the toxicity is manifested only when the material is under conditions which favor rapid bacterial action as is found in the soil. Decomposition can take place more rapidly if the material is finely ground which will result in a sudden release of the toxic substance. Thus, the more coarsely ground substance should exhibit less toxicity towards grass.

Another factor that appears to affect the release of toxicity, besides the stage of decomposition, is the rate of application of Anacharis. Plate II shows that the 1000 pound rate of application did not suppress the growth of grass as much as the 500 pound rate. It is not known whether this is within the limits of experimental error or is a real difference caused by other factors. It is suggested that a more complete experiment be conducted with

Anacharis using it in various stages of mechanical subdivision and various rates.

It is realized that Anacharis could not be used extensively as a nitrogen fertilizer as it is quite low in nitrogen. But, when it is used as a mulch; its nitrogen content cannot be overlooked. At the rate of 100 pounds of Anacharis per 1600 square feet, close to 80 pounds of nitrogen per acre is added. On the basis of the water-holding capacity of Anacharis and the results of the mulching experiment, Anacharis compares favorably with peat moss as a mulch. Thus, it would be economical to pay a slightly higher price per unit of Anacharis than a unit of peat moss. A new lawn seeding could be mulched and fertilized at the same time.

As has been stated previously, Anacharis swells when it is wetted. When the Anacharis is too finely broken into smaller particles, it tends to cake and crust over. It is believed that this wetting and swelling of the Anacharis in a finely ground condition aggravates the tendency. Under actual practice, however, this would not be a factor of any consequence. The more nearly the Anacharis could be left in a whole condition, the easier it would be to package the material.

Because of the present apparent impracticality of controlling Anacharis by chemicals, utilization of this material appears to be the best method of relieving the congestion in the infested lakes. Instead of an expenditure which would be a total outgo as in a herbicidal treatment, utilization would at least help pay its own expense.

As Nelson has pointed out, (21, p.29) the utilization of aquatic vegetation in lakes is a method of reclaiming those resources that are lost from the land by erosion. Also, because of more uniform environmental conditions, water has a tremenduous capacity to produce. Because of the constant yearly source of run-off water, lakes probably maintain their fertility more readily than a comparable acreage of land.

It is regrettable that results from properly designed field trials are not available to include in this study. There was not sufficient material with which to conduct a good field trial. Before definite recommendations can be made, field trials should be conducted to determine how Anacharis will react under actual conditions.

There was, however, a limited application of Anacharis on a new lawn seeding under conditions which permitted a comparison with peat moss. It was not possible to obtain quantitative data, but it was possible to make some observations. These observations tended to confirm the results obtained in the greenhouse.

Before the practicality of using Anacharis is fully established, the possibilities of having an adequate supply of the plant should be investigated. The outlook for this aspect of the problem also appears favorable. At the present time, another thesis is being prepared on the aspect of the economic and engineering problems of harvesting Anacharis from the lakes. This phase of the problem is being conducted under the Agricultural Engineering Department at Oregon State College. The preliminary results from this study indicate that Anacharis can be cheaply harvested.

There appears to be an adequate supply of Anacharis in the lakes to make harvesting practical. It has been estimated by persons closely associated with the problem that an average of 14 tons of cured Anacharis could be obtained from an acre of water ten feet deep. Due to the vigor of the plant, a second harvesting could easily be made during the same season.

SUMMARY AND CONCLUSIONS

Anacharis densa, a submerged aquatic weed in Southwestern Oregon, has become so well established in certain lakes that it prohibits swimming, boating, angling, and the moving of log rafts. A study was conducted on the chemical control and agronomic utilization of this plant. The purpose in mind was to relieve the congested condition in these lakes and at least partially restore them to the condition in which they could be useable to man. Through chemicals, one might be able to control this weed and to eradicate it if it should spread to new locations. There is also the possibility of utilizing this existing heavy infestation to good advantage for agronomic purposes.

There were approximately 345 chemicals tested at 25 parts per million on Anacharis to determine their toxicity. From this group were chosen the most toxic chemicals, and they were further screened to determine their lowest effective concentration.

Various experiments were conducted to determine the value of Anacharis as an organic nitrogen fertilizer and also as a mulch for new lawn seedings.

The following conclusions and recommendations are given from the results of this study:

(1) The most toxic chemicals found in this study are safranine cone, phenosafron, safranine crystals, 1-{2(t-dodecylmercapto)ethyl} pyridinium chloride, 2-methyl naphthoquinone, fungicide p-162 (Julius Hyman & Co.), p-bromephenacyl bromide, and (alkylbenzyl) trimethyl ammonium chloride.

(2) It has been shown that the treatment of an ordinary lake with chemicals would be very expensive.

(3) It is recommended that the above named chemicals serve as a basis for the study and development of chemicals having a higher toxicity which would subsequently lower treatment costs.

(4) Anacharis does not perform well when mixed into the soil as an organic fertilizer. There apparently is a toxic substance present.

(5) An ether-soluble toxic substance can be extracted from Anacharis which inhibits the growth of grass.

(6) On the basis of the data obtained, it appears that further experiments should be conducted to determine the relationship of bacterial decomposition of Anacharis to the release of this toxic substance.

(7) Anacharis appears to absorb and hold water equally as well or better than peat moss.

(8) Anacharis appears to equal peat moss in value for use as a mulch for establishing lawns.

(9) Anacharis would be worth slightly more, economically, than peat moss because of the large amount of nitrogen it contains when used as a mulch.

(10) There are indications that the supply of Anacharis is plentiful and that it can be harvested at a reasonable cost.

BIBLIOGRAPHY

1. Albert, Adrien. Selective toxicity. New York, Wiley and Sons, 1951. 288p.

2. Allison, F. E. and M. S. Anderson. The use of sawdust for mulches and soil improvement. Bureau of plant industry, soils, and agricultural engineering. U. S. Dept. of Agriculture circular 891, 1951.

3. Blackman, G. E. The principles of selective toxicity and the action of selective herbicides. Science progress 38:637-651. 1950.

4. Boller, C. A. and R. E. Stephenson. Some effects of mulches on soil properties. Proceedings of the American society for horticultural science 50:23-30. 1947.

5. Bonner, James and Arthur W. Galston. Toxic substances from the culture media of guayule which may inhibit growth. Botanical gazette 106:185-198. 1945.

6. Crafts, A. S. A theory of herbicidal action. Science 108:85-86. 1948.

7. Evenari, Michael. Germination inhibitors. The botanical review 15:153-194. 1949.

8. Fernald, M. L. Gray's manual of botany. 8th ed. New York, American Book Company, 1950. 1632p.

9. Freeland, R. O. Effects of 2,4-D and other growth substances on photosynthesis and respiration in Anacharis. Botanical gazette 111:314-324. 1950.

10. Goodwin, Richard H. and Carolyn Taves. The effect of coumarin derivatives on the growth of Avena roots. American journal of botany 37:224-231. 1950.

11. Gustafson, Felix G. A comparative study of different methods of determining activities of growth-promoting substances. American journal of botany 30:649-654. 1943.

12. Hutchinson, A. E. Mulching your garden. Horticulture 28:414-415. 1950.

13. Immer, Forrest R. Applied statistics. Minneapolis, Burgess Publishing company, 1950. 157p.

14. Jackson, Philip. Utilization of marine algae--effective harvesting remains the chief problem. The chemical age 61:347-349. 1949.

15. Johnson, W. A. and L. M. Ware. Effect of different mulches on soil acidity. Proceedings of the American society of horticultural science 55:285-288. 1950.

16. Jokaweska, Sophie, R. F. Nigrelli, and E. D. Goldsmith. Plant growth regulating effects of aminopterin. American journal of botany 36:825. 1949.

17. Jones, R. L. et al. The relationship between the constitution and the effect of chemical compounds on plant growth 2. Quarternary ammonium salts. The biochemical journal 47:110-114. 1950.

18. __________ T. P. Metcalfe, and W. A. Sexton. The relationship between the constitution and the effect of chemical compounds on plant growth 1. Phenoxyethylamine derivatives. The biochemical journal 45:143-149. 1949.

19. Latimer, L. P. and G. P. Percival. Comparative value of sawdust, hay, and seaweed as a mulch for apple trees. Proceedings of the American society for horticultural science 50:23-30. 1947.

20. Machenthum, Kenneth M. Aquatic weed control with sodium arsenite. Sewage and industrial wastes 22:1062-1067. 1950.

21. Nelson, J. Wesley, et al. Nutritive value and chemical composition of certain fresh-water plants of Minnesota. University of Minnesota, Agricultural experiment station. Technical bulletin 136. March 1939. 47p.

22. Nettleton, Arthur. Seaweed--the new provider. Compressed air magazine 55:184-185. 1950.

23. Offord, H. R. Rapid estimation of the phytocidal action of chemicals. Science 103:474-476. 1946.

24. Overbeek, J. Van. Growth regulating substances in plants. Annual review of biochemistry 13:631-666. 1949.

25. Rose, R. C. Extraction of alginates from Canadian seaweeds. Canadian journal of technology 29:18-28. 1951.

26. Schery, Robert A. Mulches for the home garden. Missouri botanical garden bulletin 39:165-179. 1951.

27. Sexton, W. A. Chemical constitution and biological activity. London, E. and F. N. Spon Ltd, 1949. 412p.

28. ____________ In Selective toxicity and antibiotics. New York, Academic press, 1949. pp. 1-13.

29. Simon, E. W. Effect of pH on the biological activity of weak acids and bases. Nature 166:343-346. 1950.

30. ____________ and G. W. Blackman. In Selectivity toxicity and antibiotics. New York, Academic press, 1949. pp. 253-265.

31. Slade, R. E., W. G. Templeman, and W. A. Sexton. Differential effect of plant-growth substances on plant species. Nature 155:497-498. 1945.

32. Stoloff, Leonard. Irish moss--from an art to an industry. Economic botany 3:428-435. 1949.

33. Trim, A. R. and A. E. Alexander. In Selectivity toxicity and antibiotics. New York, Academic press, 1949. pp. 111-141.

34. Thiman, K. V. Plant hormones, growth, and respiration. Biological bulletin 96:296-306. 1949.

35. Veldstra, H. and E. Havinga. On the physiological activity of unsaturated lactones. Enzymologia 11:373-380. March 1943 to Sept. 1945.