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In recent years, new strict environmental laws have required improved and cost-effective water purification methods by Air Force complexes. Naturally assisted primary units (microbiological) and secondary units (macrophyte) could bring waste treatment systems into tighter compliance. Aquatic macrophytes which have rapid growth rates and absorb large quantities of nutrients could provide a practical and economic method for more complete wastewater maintenance, hazardous waste clean-up or river, lake and ground waster purification. This work has shown that Lemna minor, or Common Duckweed, can successfully and thoroughly accumulate organics and metals from Air Force wastewaters.

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The MINIMIZATION OF ORGANIC AND METALLIC INDUSTRIAL WASTE VIA *LEMNA MINOR* CONCENTRATION.

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PREFACE

This progress report was prepared by Technical Research Associates, Inc. (TRA), for USAF, AFSC, Air Force Office of Scientific Research, Building 410, Bolling AFB DC 20332-6448, under contract F49620-91-C-0076. The technical monitors were Lt. Col. Jan Cerveny (AFOSR/NL) and Dr. William Berry.

This report covers the effort during the period from September 1, 1991 to December 1, 1992 by personnel at TRA. Mrs. Gail Armstrong Bowers-Irons was the Principal Investigator. Mr. Ronald Nelson was the additional researcher. Both are U.S. Citizens.

LIST OF ABBREVIATIONS

%	Percent
<	less than
≥	greater than or equal to
+	plus or greater than
°C	degree Centigrade
µL	microliter
µm	micron
AF	Air Force
AFOSR	Air Force Office of Scientific Research
Al	aluminum
As	arsenic
ASD	Air Systems Command
ATCC	American Type Culture Collection
atm	atmosphere
Br	bromium
Ca	calcium
$Ca(NO_3)_2 * 4H_2O$	calcium nitrate
Cl	chlorine
cm	centimeter
CO_2	Carbon Dioxide
CO_3	carbonate
Cu	copper
e.g.	for example
Fe	iron
Ga	gallium
Ge	germanium
gm(s)	gram(s)
H_2O	Water
HAFB	Hill Air Force Base
KH_2PO_4	Potassium Monobasic Phosphate
KNO_3	Potassium Nitrate
MEK	Methyl Ethyl Ketone
Mg	magnesium
$MgSO_4 * 7H_2O$	magnesium sulfate
MIK	Methyl Isobutyl Ketone
ml	milliliter
mm	millimeter
$MnCl_2$	manganese chloride
Mo	molybdenum
Na	sodium
Ni	nickel

LIST OF ABBREVIATIONS CONTINUED

nm	nanometer
NO_2	nitrite
NO_3	nitrate
NTIS	National Technical Information Service
Pb	lead
PO_4	phosphate
Sb	antimony
SBIR	Small Business Innovative Research
Se	selenium
Si	silicon
Sn	tin
SO_4	sulfate
TRA	Technical Research Associates, Inc.
U	uranium
US	United States
USAF	United States Air Force
UV-Vis	Ultraviolet-Visible Spectroscopy
YSI	Yellow Springs Instruments
Zn	zinc
$ZnSO_4$	zinc sulfate

INTRODUCTION

In recent years, the quantity and type of waste as well as new strict environmental laws have required improved and cost-effective water purification methods by Air Force complexes. Air Force Base treatment often dictates both primary and secondary sterilization facilities. These treatment centers remove much of the primary-bypass organic matter, 30 percent (%) of the phosphorous and 20 percent (%) of the nitrogen but often none of the metal oxides pesticides or other toxins. Thus, the conventional Air Force treatment plant could release high concentrations of nutrients into surface waters. Downstream water could therefore be contaminated with industrial and hazardous wastes. These wastes could include metal oxides, metalloids, alkaline and rare earths, soap and free Cl, fertilizers, pesticides, oil, human and animal wastes, food and other organic refuse.

Naturally assisted primary units (microbiological) and secondary units (macrophyte) could bring waste treatment systems into tighter compliance. Aquatic macrophytes which have rapid growth rates and absorb large quantities of nutrients could provide a practical and economic method for more complete waste water maintenance, hazardous waste clean-up or river, lake and ground water purification. Preliminary work has shown that *Lemna minor*, or Common Duckweed, can successfully and thoroughly accumulate organics and metals from municipal and industrial waste waters.

Duckweed is a floating, widespread and fast-growing plant. It is small, easy to cultivate and highly sensitive to surrounding factors. Many workers have studied Duckweed as indicators, monitors and metal accumulators. Sutton, et al., chose *Lemna* to indicate heavy metals and other water pollutants. He confirmed that Duckweed grown in 75% and 100% sewage effluent concentrations contained approximately 3 times as much crude protein and 4 times as much phosphorous as compared to plants grown in pond water. He also noted that aquatic macrophytes in sewage effluent were studied by Boyd, Burgess and Mackenthun.

Wang considered Duckweed an ideal candidate for aquatic toxicity testing and stated that "the strength of Duckweed assay is that it is simple, inexpensive and sensitive. It can be used for screening or monitoring aquatic toxicity."

Nasu, et al., indicated that *Lemna* has a potential as an indicator and purification element of water pollution. Hillman and Takimoto, explained that " The high sensitivity of *Lemna* to heavy metals seems to be due to the rapid absorption of metal ions in the plant body and confirmed that among the heavy metals tested, the most toxic for *Lemna* are copper and cadmium."

Clark, et al., studied the depuration of metals by *Lemna perpusilla*, exposed to heavy ash basin effluents, *in situ*. They studied eight metals: Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn and monitored alkalinity, hardness, dissolved oxygen, pH and sulfate. The study indicated that "Duckweed macrophytes demonstrated a significant potential for accumulation of heavy metals. The accumulation of metals in the plant tissues provides a mechanism of biological removal of these potential toxicants."

Rodgers, et al., studied bioaccumulation via *Lemna perpusilla Torrey* in an ash settling basin. The Duckweed was the only abundantly occurring macrophyte. Duckweed was chosen for observation "because its morphology or growth form made possible the collection of specimens subject to and reflective of the aquatic habitat and chemical conditions at each sampling site. The plant significantly concentrated the halogens, Cl and Br, and appeared to be an efficient concentrator for 20 of the 22 elements measured: Fe, Al, Ti, Cu, Zn, Sn, Cr, Mn, Co, Ca, Mg, Ba, Sr, Na, Cs, Cl, Br, Cd, Se, As and Hg.

This project has focused on naturally assisted restoration via *Lemna minor* accumulation, of "non-spec" waters associated with Air Force facilities.

METHODS AND PROCEDURES

A. Materials

The Duckweed as used in this study was *Lemna minor*[Sculthrope]:

Division	*Spermiatophyta*	Seed Plants
Class	*Angiosperma*	Flowering Plants
Subclass	*Monocotyledonese*	Monocots
Order	*Arales*	Calla Order
Family	*Lemnaceae*	Duckweed family
Genus	*Lemna*	Duckweed
Species	*minor*	Small Duckweed

The genus is nearly cosmopolitan with approximately forty species. Duckweed are tiny aquatic herbs, having no woody structures, which are restricted to fresh or slightly brackish water. The leaf structure is very simple; cells having large lacunae allow the plants to remain at or on the water surface and the leaves are covered with a waxy cuticle which prevent wetting. TRA's *Lemna* isolate has one unbranched root per thallus or leaf.

Duckweed (*Lemna minor*) was acquired from 3 sites on the east bank of the Jordan River, approximately 7800 South and 1095 West, (next to the Gardner Historical Village) Salt Lake City, Utah, and from 4 sites on the Two Ring Levee in the Jean Lafayette National Park, Baratoria Division, 18 miles south of New Orleans, Louisiana, across the Mississippi River.

The Jordan River water is known to be highly polluted and contains cations such as uranium (U), lead (Pb), arsenic (As), aluminum (Al), molybdenum (Mo), antimony (Sb), tin (Sn), selenium (Se), gallium (Ga), germanium (Ge), silicon (Si), calcium (Ca), sodium (Na), magnesium (Mg), zinc (Zn), copper (Cu), nickel (Ni), chlorine (Cl), bromium (Br), iron (Fe), rare earths, etc. and anions such as phosphate (PO_4), sulfate (SO_4), carbonate (Co_3), nitrate/nitrite (NO_3/NO_2). Contributors to this water are Geneva and Sheran Steel Mills (metal oxides, metalloids alkaline earths), Kennecott Copper Mine (metals and processing residuals), National Semiconductor (rare metals and rare earths), a soap company, multiple restaurants, two waste water treatment plants (free Cl) and a coal processing plant. Farm land (fertilizers, pesticides) and road run-offs (grease and oil) are also present.

The Utah Jordan River Duckweed were gathered from under an overpass; the swift water was 11.8°C with a pH of 8.12. The Duckweed were on the surface of the water, attached to long-leaved plants which were attached to the bank river mud. All duckweed plants were 0.3 cm and had shiny dark green coloration and 1.0 to 2.0 cm roots. A great deal of animal life was evident, e.g. spiders and small worms.

The Louisiana Duckweed were gathered from both sides of a levee in separated ponds. Temperature ranged from 20° to 30°C and pH was from 7.0 to 8.8. Site 1-3 plants were 0.2 cm and had shiny dark green coloration and 1.0 cm roots. Site one plants were gathered from a heathy site just inside the levee. Site two plants were gathered from a healthy site on the opposite side of the site one levee. Site three plants were gathered from the opposite side of the levee; water was stagnant and contaminated with oil. The number four site Duckweed were extremely small (<0.1 cm) with very long roots (>4 cm). Site four plants were gathered from under a bridge and were interspersed with large-leafed, large-root plants.

B. Apparatus/Instrumentation

All experiments were carried out in sterile 50 ml capped, plastic sample bottles and 250 ml Erlenmeyer flasks. A Nuair down-draft laminar flow hood was used for preparation. Tests were conducted on TRA designed and constructed room temperature and temperature controlled (24-flask) orbital shakers as well as a test tube shaker.

A Barnstead Nanopure* water purifier system delivered 18 megohm-cm water (H_2O). Napco model 8000-DSE and Hirayama model HA-240M/1300M autoclaves were used for sterilization. Sartorius E 1200 S and Mettler A20 analytical balances were used to measure nutrient additions as well as initial weight and weight loss. Eppendorf/Brinkmann and Rainin/Gilson digital pipettes (2-10 microliter (μL), 10-100 μL, 100-1000 μL were used for serial dilutions.

Corning PC 101 stir/hot plates were used for mixing. Orion SA 250 meters were used to measure and equilibrate pH and temperature. A Yellow Springs Instrument (YSI) model 58 oxygen meter with a YSI O_2 probe measured dissolved oxygen.

A BH-2 Olympus biological microscope with DIC and Phase and Olympus C-35AD-4 was used with a Leica Wild stereo-microscope for analysis.

C. Methodology

This first years's work defined the ability of *Lemna minor* to accumulate contaminants from waste streams as a function of: 1) nutrient specifications; 2) pH, temperature, salinity, light and conductivity restrictions and 3) oxygen requirements. Second year work would define 1) flow rates and turbidity; 2) reservoir profiles and 3) interaction with other macrophytes or algae. Third year work would produce a pilot field design.

To begin the project, an Air Force waste stream was identified. It contained polyurethane and epoxy paints, substituted acrylic acids, metallics and inorganics, organic solvents and phospho-organic insecticides. **Figures 10 through 13**, in the **Appendix**, depict the range of selected materials over 900 days. These data were used a baseline.

Lemna minor cultures were first established on modified Jacob's medium which is a four part medium prepared as follows:

Stock A: 15.0 grams Calcium Nitrate--$Ca(NO_3)_2*4H_2O$--weighed and dissolved into 200 ml-18 megohm-cm H_2O. The volume was then corrected to 250 ml.

Stock B:
1. 12.5 grams Magnesium Sulfate--$MgSO_4*7H_2O$
2. 25.0 grams Potassium Monobasic Phosphate--KH_2PO_4
3. 3.5 gm Potassium Nitrate--KNO_3.

Dissolved all three Stock B into 100 ml-18 megohm-cm H_2O and corrected to 250 ml.

Stock C:
1. 0.070 grams Molybdic Acid
2. 0.200 grams Zinc Sulfate--$ZnSO_4$
3. 3.0 grams Boric Acid
4. Manganese chloride ($MnCl_2$)--dissolved 1 gm metal into 5.0 ml HCL and diluted to 10 ml.

Numbers 1,2,3, Stock C, were added to 200 ml tap water and dissolved. The volume was then corrected to 500 ml. $MnCl_2$ was then added as a 2.0 ml aliquot and the volume was corrected to 1 liter. Precipitation occurred if the $MnCl_2$ was added prior to this. The tap water was added to provide traces of iron (replacing iron ethylene diamine di-ortho hydroxy phenol acetic acid). To 1 liter of culture solution, 10 ml each of A, B and C was added.

Stock cultures were kept at low light intensities and temperatures to obviate the need for frequent transfers. Five initial fronds were used in the accumulation tests. By counting the number of fronds daily, the growth was plotted linearly. For all values depending on frond number, all fronds were counted, no matter how small, in order to avoid subjective decisions as to frond maturity. *Lemna minor* frond and root color, shape and condition data, average number of fronds per colony, growth and death rates were also compared after accumulation of values (uptake) in each Task.

Table I below displays the test materials for the accumulation tests. Each test material was run with and without full light, at 10° and 30°C, at pH 7.5, 8.0 and 8.5, with and without shaking, and with and without attachment. Organic tests were run with concentrations of 0.1M, 0.3M, 0.5M and 1M and inorganic tests with concentrations of 10 ppm and 100 ppm. Both single and continuous feeding tests were run.

TABLE 1
Accumulation Materials

Chemicals	Metals
Ethanol	Aluminum
Mannitol	Gallium
Glycerol	Germanium
Lactose	Strontium
Sucrose	Gold
Ribose	Silver
Arabinose	Cadmium
Maltose	Copper
Sodium Phosphate	Chromium
Sodium Succinate	Lead
Sodium Tartrate	Arsenic
Sodium Acetate	Zirconia
Ammonium Chloride	Titanium
Potassium Chloride	Sulfur
Starch	Silicon
	Potassium
	Sodium
	Iron

RESULTS AND DISCUSSION

Each test material was run with and without full light, at 10° and 30°C, at pH 7.5, 8.0 and 8.5, with and without shaking, and with and without attachment. Full light was moderately tolerated only under continuous shaking near 100 RPM (movement of liquid and O_2/CO_2 required), lower temperatures and daily feeding of nutrients. TRA's *Lemna minor* preferred temperatures between 10° and 15°C. Higher temperatures de-aerated the water, and decreased the life-span of each individual frond and the rate of daughter-frond production. pH between 8.0 and 8.3 was also preferred. Lower pH caused acidosis and death; higher pH decreased accumulation and, again, the rate of daughter-frond production. Tests also showed that TRA's *Lemna minor* preferred attachment, whether on algae, long-leaved plants or on the side of the vessel. Continuous feeding allowed the plants to adapt and accumulate at a faster rate; accumulation was also more complete.

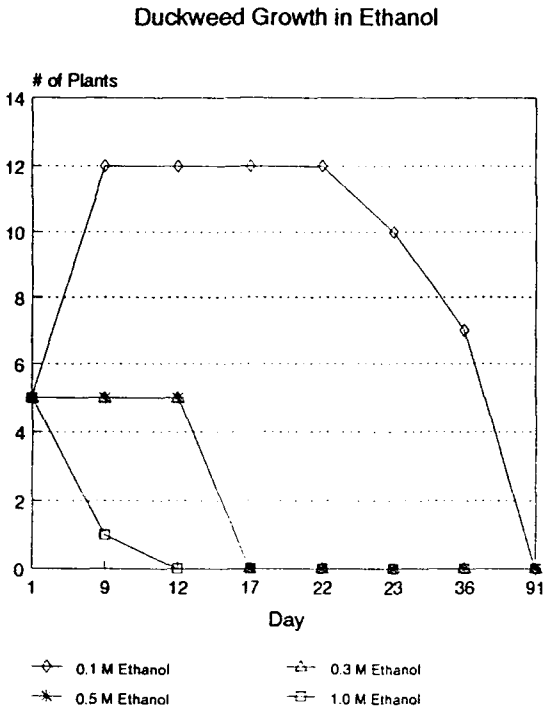
Chemical Tests

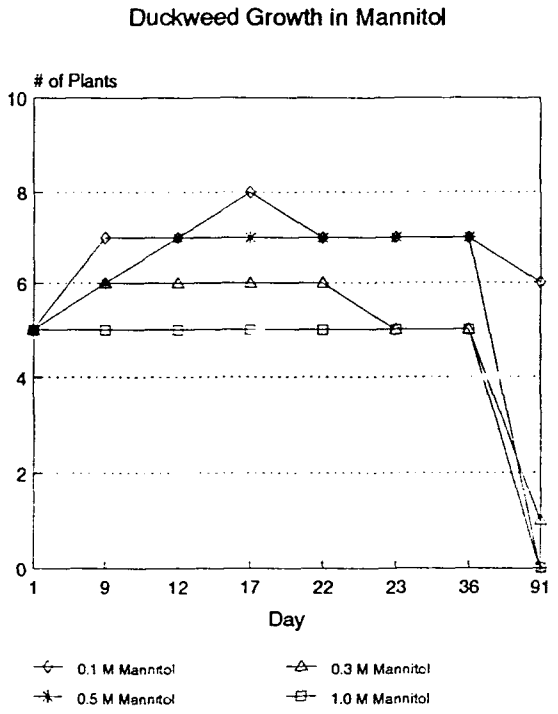
Once optimum conditions were established (10°-15°C, pH 8.0-8.3, moderate shaking of 100 RPM (yielding 5.6 to 6.6 mg/L O_2--saturation in Salt Lake City is 7.2), continuous feeding, low light), the following results were shown over a period of 91 days, as represented in **Figures 1 through 4**. Each test began with five healthy dark green plants with roots of 2 cm.

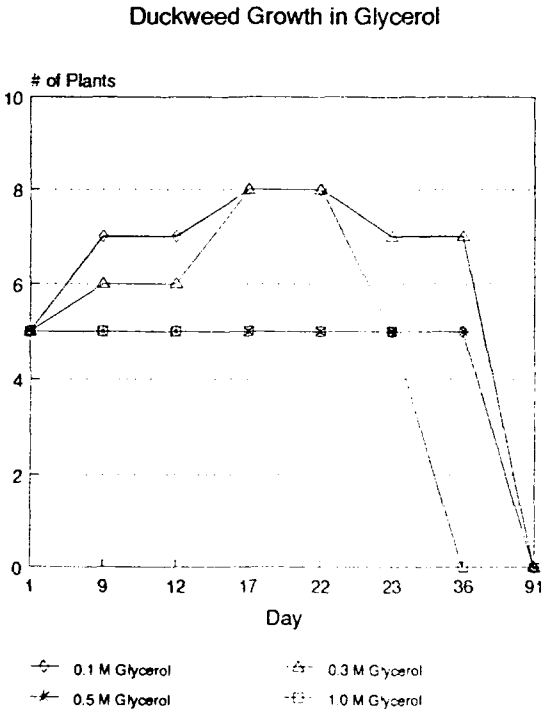
1. Ethanol-C_2H_5OH. Plants grew exceedingly well in 0.1 M ethanol; healthy dark green plants duplicated to 12 but white roots were short (<1 cm). Two plants were producing daughter fronds, upon termination of the test. Plants did not fare well in 0.3 M, 0.5 M or 1.0 M; no duplication was observed, white roots were very long (≥4 cm) and die-off occurred within the first two weeks.

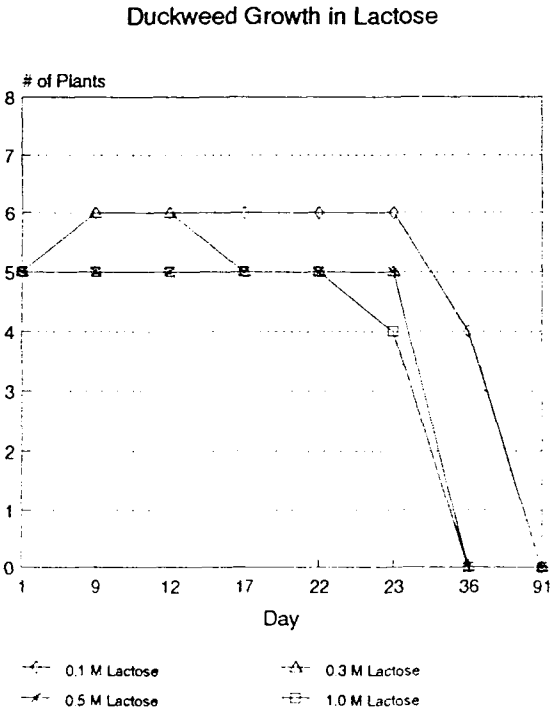
2. Mannitol-$C_6H_{14}O_6$. Plants were healthy dark green in all tests but did not duplicate significantly, although all were producing daughter fronds upon termination of the test. The 0.1 M plants duplicated to 8 and had very long green roots (≥4 cm). The 0.5 M plants duplicated to 6 while the 0.5 M plants duplicated to 7. The 1.0 M plants did not duplicate. All three groups had long white roots (≥4 cm). The 0.5 M and 1.0 M plants were pale green and were eventually covered with black *Aspergillus niger* fungus.

FIGURE 1


Duckweed Growth in Ethanol
# of Plants
14
12
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Ethanol
0.3 M Ethanol
0.5 M Ethanol
1.0 M Ethanol


Duckweed Growth in Mannitol
# of Plants
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Mannitol
0.3 M Mannitol
0.5 M Mannitol
1.0 M Mannitol


Duckweed Growth in Glycerol
# of Plants
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Glycerol
0.3 M Glycerol
0.5 M Glycerol
1.0 M Glycerol


Duckweed Growth in Lactose
# of Plants
8
7
6
5
4
3
2
1
0
1
9
12
17
22
23
36
91
Day
0.1 M Lactose
0.3 M Lactose
0.5 M Lactose
1.0 M Lactose

3. Glycerol-$C_3H_8O_3$. The 0.1 M and 0.3 M plants duplicated to 8, after 2 weeks, but were soon engulfed with an unknown white fungus. The 0.1 M plants died; only one of the 0.3 M plants died but the plants were pale green. Both groups had very short white roots (<1 cm). Neither the 0.5 M plants or the 1.0 healthy dark green plants duplicated but grew very long white roots (≥4 cm). Both groups were fungus-free through the test period.

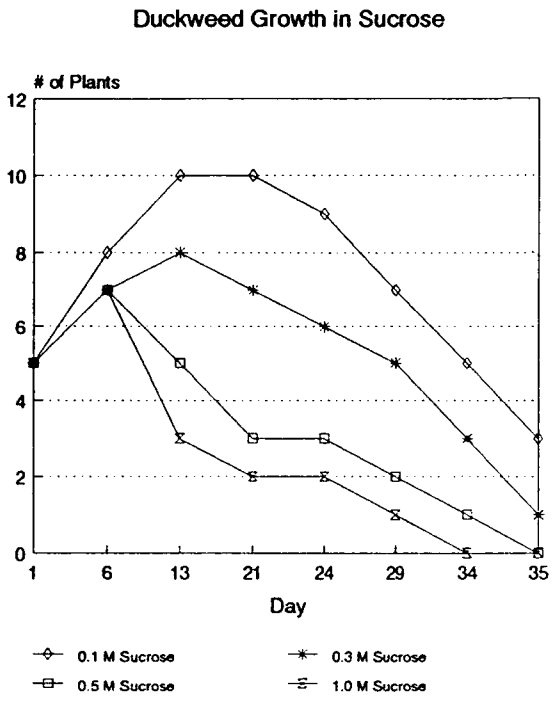
4. Lactose-$C_{12}H_{22}O_{11}$. The 0.1 M plants duplicated to 6, were healthy green and had very long green roots (≥4 cm). This group was fungus-free throughout the test period and was producing daughter fronds upon completion of the test. The 0.3 M, 0.5 M and 1.0 M plants did not duplicate, were pale green with very short white roots (<1 cm) and were quickly covered with an unknown white fungus.

5. Sucrose-$C_{12}H_{22}O_{11}$. The 0.1 M pale green plants duplicated to 10; the 0.3 M plants to 8; the 0.5 M to 7 and the 1.0 M to 7. The 0.1 M, 0.3 M and 1.0 M groups were quickly engulfed with black *Aspergillus niger* fungus, causing complete die-off. The 0.5 M groups was covered with the unknown white fungus. All roots were very short (<1 cm).

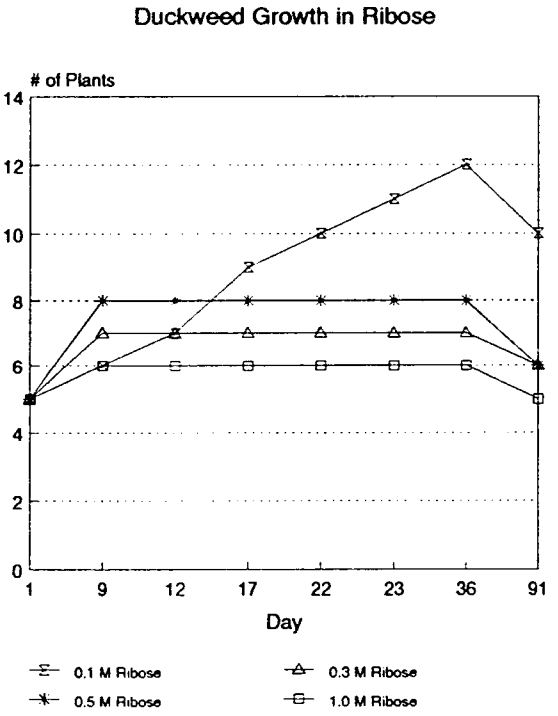
6. Ribose-$C_5H_{10}O_5$. The 0.1 M plants duplicated to 12, after a linear increase. The plants were very healthy and dark green with long green roots. All were producing daughter fronds upon completion of the test. The 0.3 M plants duplicated to 7; the 0.5 M to 8 and 1.0 M to 6. These plants remained healthy, with moderately long white roots, but were eventually covered with the white fungus.

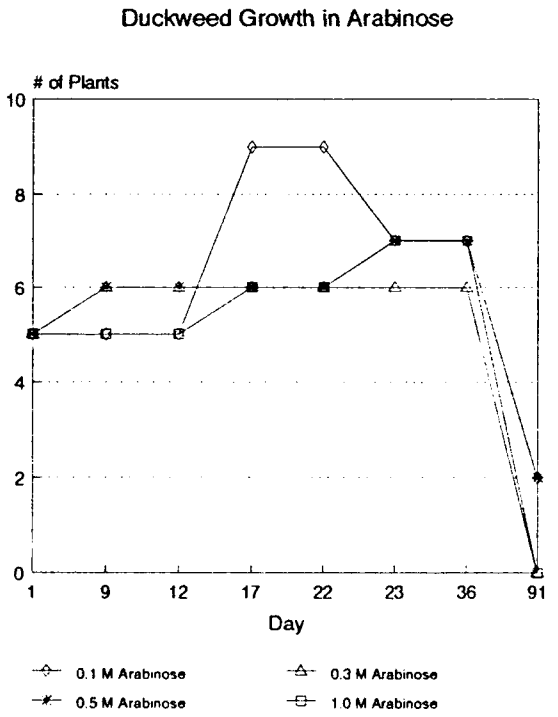
7. Arabinose-$C_5H_{10}O_5$. The 0.1 M plants duplicated to 9, were moderately green and had very short white roots (<1 cm). Two plants were producing daughter fronds upon completion of the test. The 0.3 M plants duplicated to 6; the 0.5 M duplicated to 7 and the 1.0 M duplicated to 7. All these plants were producing daughter fronds upon completion of the test. Plants were moderately green. The 0.3 M plants had very short white roots (<1 cm) and the 0.5M and 1.0 M plants had very long white roots (≥4 cm). All plants were eventually emersed in the white fungus.

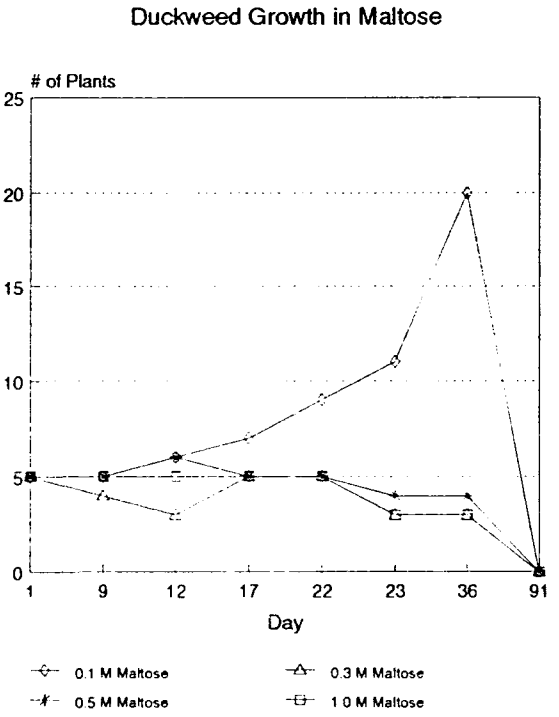
Figure 2



Bacterial and Fungal Encapsulation Interferred with Growth.







8. Maltose-$C_{12}H_{22}O_{11} * H_2O$. The 0.1 M plants duplicated to 20 after a linear increase. The plants were healthy green with long green roots (≥4 cm); all were producing daughter fronds upon completion of the test. The 0.3 M plants died to 3 and grew to 5 healthy green plants; the 0.5 M plants duplicated to 6 and then died to 4 pale green plants and the 1.0 M plants did not duplicate and were pale green. All these plants were producing daughter fronds upon completion of the test while they were completely encapsulated by the white fungus.

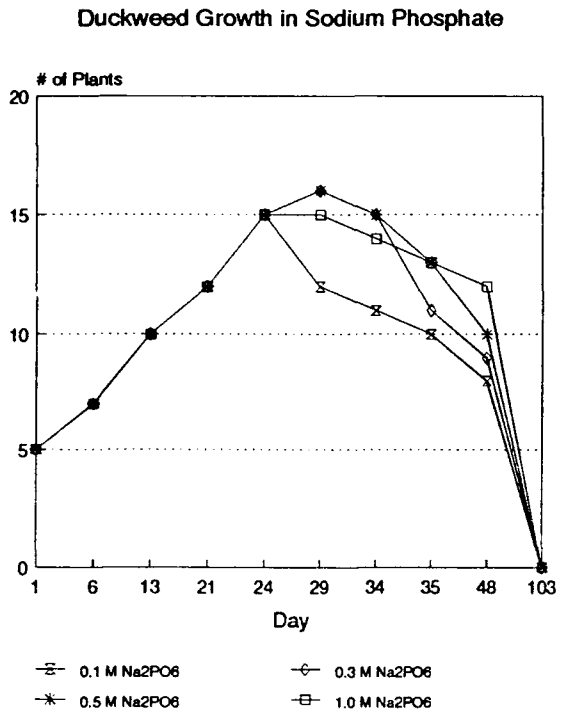
9. Sodium Phosphate-Na_2PO_6. All groups duplicated to 16 after a linear increase. Plants were pale green with very white short roots (<1 cm). Some die-off did occur after two months but all plants were producing daughter fronds upon completion of the test.

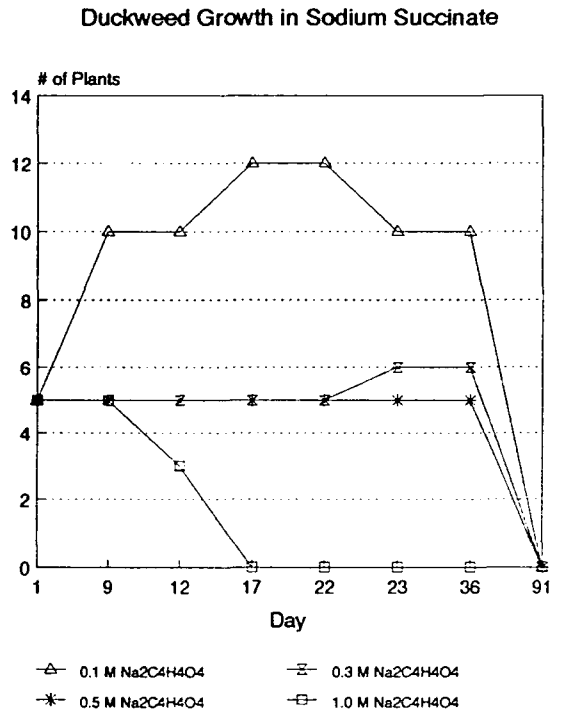
10. Sodium Succinate-$Na_2C_4H_4O_4 * 6H_2O$. The 0.1 M plants duplicated to 12 while the 0.3 M plants duplicated to 6. The 0.5 M plants did not duplicate and the 1.0 M plants died within 2 weeks. All live plants were pale green with long white roots (≥4 cm). At the end of the test period, 2 of the 0.1 M plants, and all of the 0.3 M and 0.5 M plants, were producing daughter fronds although each was individually wrapped in the white fungus.

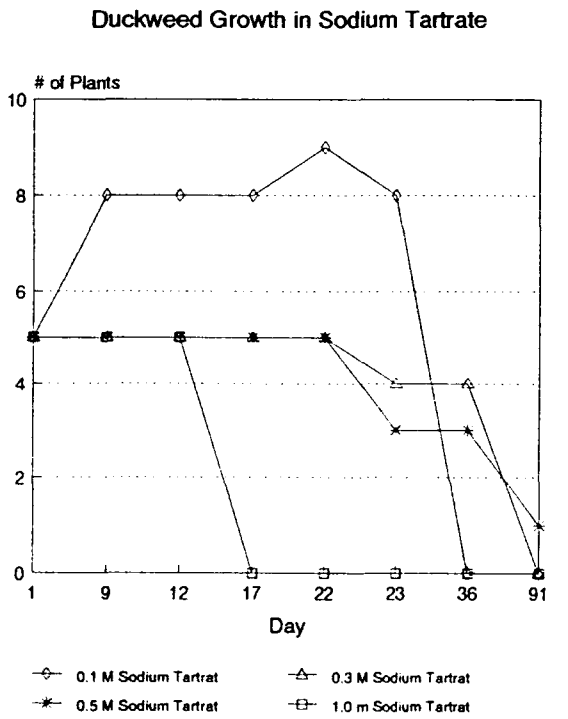
11. Sodium Tartrate-$Na_2C_4H_4O_6 * 2H_2O$. The 0.1 M plants duplicated to 9 within 20 days and were dead by 36 days. None of the other groups duplicated. The 1.0 M group was dead by the second week (encapsulated by the white fungus) while the 0.3 M and 0.5 M plants were steady for a month. All were pale green and had very short white roots (<1 cm). All were producing daughter fronds upon completion of the test.

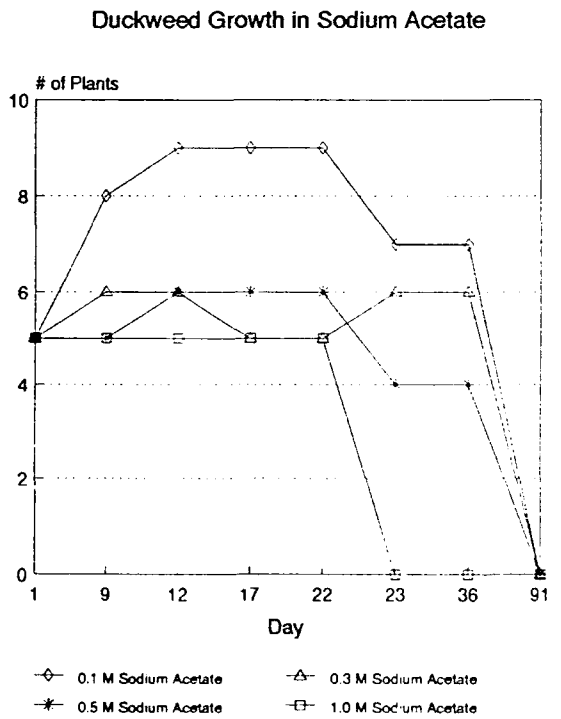
12. Sodium Acetate-$CH_3COONa * 3H_2O$. The 0.1 M plants duplicated to 9 and the 0.3 M and 0.5 M plants to 6. All plants were pale green with long white roots (>4 cm). Upon completion of the test, 2 of the 0.1 M plants were producing daughter fronds while all plants in the 0.3 M and 0.5 M groups were producing daughter fronds. They were also engulfed in fungus. The 1.0 M plants did not duplicate and died within 23 days.

FIGURE 3


Duckweed Growth in Sodium Phosphate
# of Plants
20
15
10
5
0
1
6
13
21
24
29
34
35
48
103
Day
0.1 M Na2PO6
0.3 M Na2PO6
0.5 M Na2PO6
1.0 M Na2PO6


Duckweed Growth in Sodium Succinate
# of Plants
14
12
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Na2C4H4O4
0.3 M Na2C4H4O4
0.5 M Na2C4H4O4
1.0 M Na2C4H4O4


Duckweed Growth in Sodium Tartrate
# of Plants
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Sodium Tartrat
0.3 M Sodium Tartrat
0.5 M Sodium Tartrat
1.0 m Sodium Tartrat


Duckweed Growth in Sodium Acetate
# of Plants
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Sodium Acetate
0.3 M Sodium Acetate
0.5 M Sodium Acetate
1.0 M Sodium Acetate

13. Ammonium Chloride-NH_4Cl. All groups fared poorly in this test material. The 0.1 M plants died to 3 by the second week and to 2 by the 48th day. The 0.3 M plants died to 3 by the second week and to 2 by the 36th day. The 0.5 M plants died to 2 by the second week and to 0 by the 36th day. The 0.1 M plants died to 0 by the second week. Roots were white and very short (<1 cm).

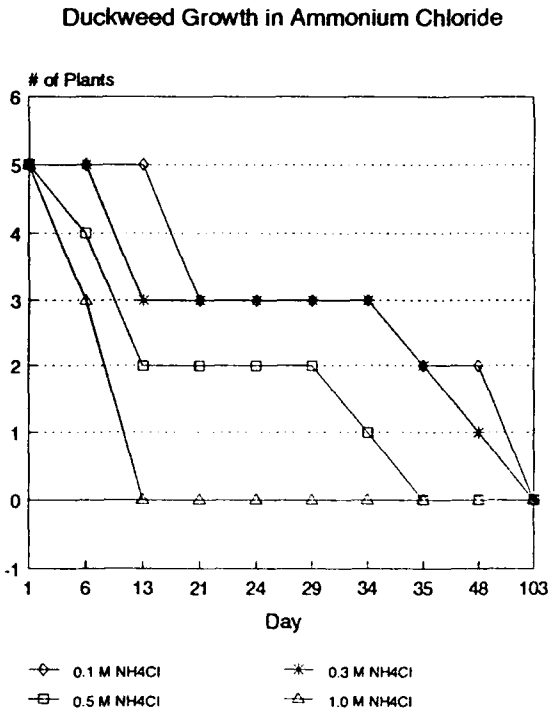
14. Potassium Chloride-KCl. The 0.1 M plants duplicated to 20 by the 3rd week and then died to 15 by the 4th week. By the completion of the test, all 15 were healthy green, had very short (<1 cm) green roots and were producing daughter fronds. The 0.3 M plants duplicated to 16 by the 3rd week and died to 13 by the 4th week. The plants were moderately green with very short white roots (<1 cm).

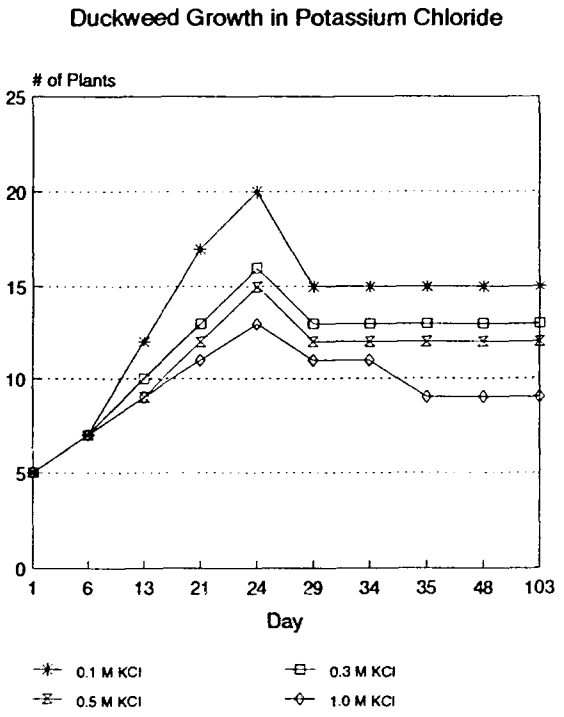
15. Starch-$(C_6H_{10}O_5)_n$. The 0.1 M plants duplicated to 11 plants by the 3rd week but soon died back to 10. The final plants were healthy green with moderately long green roots (3 cm); all were producing daughter fronds. The 0.3 M plants duplicated to 8 and were healthy green with moderately long green roots (3 cm); all were producing daughter fronds upon completion of the test. The 0.5 M plants duplicated to 11 after a steady linear increase. All plants were healthy green with moderately long green roots (3 cm) and were producing daughter fronds upon completion of the test. This group, however, was encapsulated in black, yellow and white fungus. The 1.0 M plants duplicated to 10 after a steady linear increase. All plants were healthy green with moderately long green roots (3 cm) and were producing daughter fronds upon completion of the test.

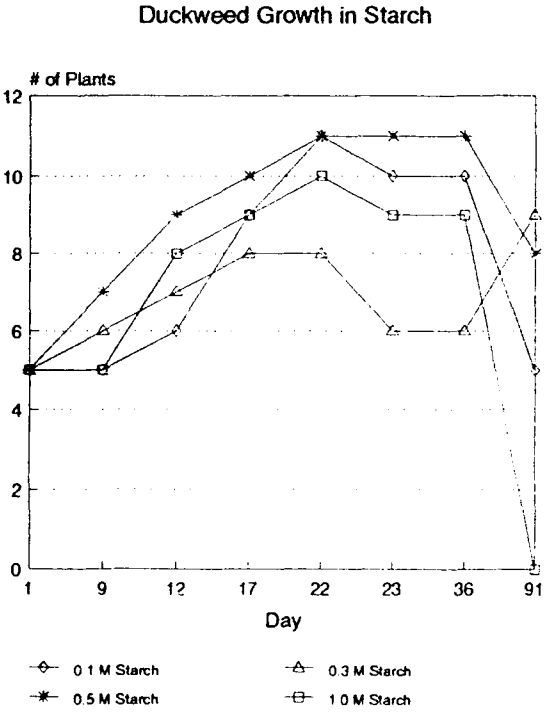
Metal Tests

Once optimum conditions were established (10°-15°C, pH 8.0-8.3, moderate shaking of 100 RPM (yielding 5.6 to 6.6 mg/L O_2--saturation in Salt Lake City is 7.2), continuous feeding, low light), the following results were shown over a period of 50 days, as represented in **Figures 5 through 9**. Each test began with five healthy dark green plants with roots of 2 cm. Concentrations were 10 and 100 ppm, unless otherwise noted.

FIGURE 4


Duckweed Growth in Ammonium Chloride
# of Plants
6
5
4
3
2
1
0
-1
1
6
13
21
24
29
34
35
48
103
Day
0.1 M NH4Cl
0.3 M NH4Cl
0.5 M NH4Cl
1.0 M NH4Cl


Duckweed Growth in Potassium Chloride
# of Plants
25
20
15
10
5
0
1
6
13
21
24
29
34
35
48
103
Day
0.1 M KCl
0.3 M KCl
0.5 M KCl
1.0 M KCl


Duckweed Growth in Starch
# of Plants
12
10
8
6
4
2
0
1
9
12
17
22
23
36
91
Day
0.1 M Starch
0.3 M Starch
0.5 M Starch
1.0 M Starch

1. Aluminum-Al. The 10 ppm plants duplicated to 11 plants after one month, after a steady linear increase. There were nine light brown plants upon completion of the test with moderate white roots (3 cm). Two plants were producing daughter fronds. The 100 ppm plants duplicated to 7 by the 2nd week but died back to 5 for the duration of the test. Plants were light green with moderate white roots (3 cm). Three plants were producing daughter fronds.

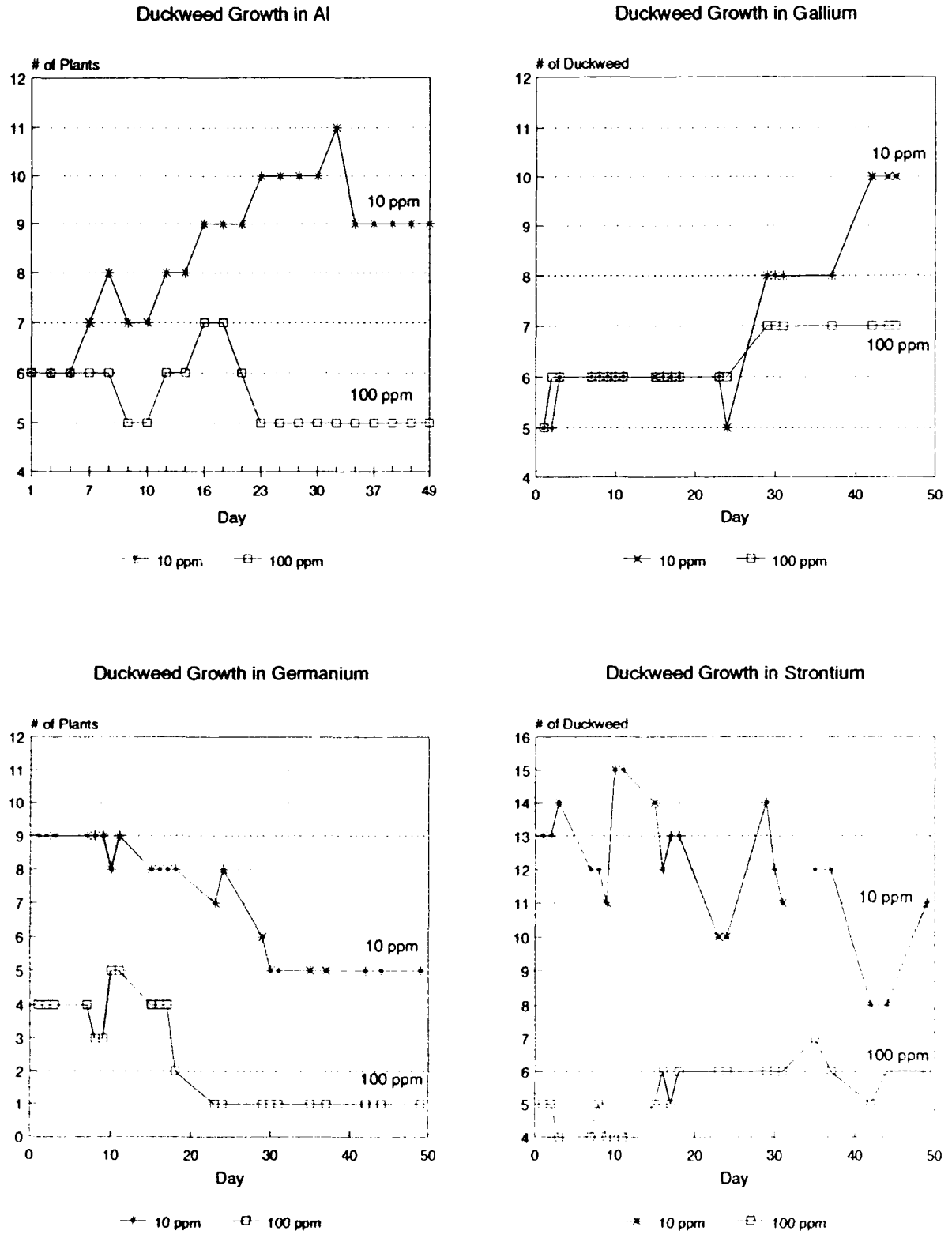
2. Gallium-Ga. The 10 ppm plants duplicated to 10 plants after 40 days, after a steady linear increase. Each final light green plant had moderate white roots (3 cm). No plants were producing daughter fronds. The 100 ppm plants duplicated to 7 in 30 days. Final plants were light brown with moderate white roots (3 cm). Four plants were producing daughter fronds.

3. Germanium-Ge. The 10 ppm plants duplicated to 9 plants immediately, then died back to 5 for the duration of the test. Each final white plants had moderate white roots (3 cm). One plant was producing a daughter frond. The 100 ppm plants died to 4, duplicated to 5 and then died to 1, in 20 days. Final plants were white with moderate white roots (3 cm). One plant was producing a daughter frond.

4. Strontium-Sr. The 10 ppm plants duplicated to 13 plants immediately, then died back to 11 and duplicated to 15 by the 10th day; duplication and death were erratic for the duration of the test. Each final light brown plant had moderate white roots (3 cm). Two plants were producing daughter fronds. The 100 ppm plants died to 4, duplicated to 7 and then died to 6. Final plants were light green with moderate white roots (3 cm). One plant was producing a daughter frond.

5. Gold-Au. The 10 ppm plants duplicated to 6 plants immediately, then died back to 5 and duplicated to 6 by the 10th day; duplication and death were erratic for the duration of the test. Each final cherry red plant had moderate white roots (3 cm). Three plants were producing daughter fronds. The 100 ppm plants died to 4, duplicated to 5 and died to 4, in 20 days. Stabilization at 5 plants occurred in 25 days. Final plants were cherry red with moderate white roots (3 cm). No plants were producing daughter fronds.

FIGURE 5


Duckweed Growth in Al
# of Plants
10 ppm
100 ppm
Day
10 ppm
100 ppm
Duckweed Growth in Gallium
# of Duckweed
10 ppm
100 ppm
Day
10 ppm
100 ppm
Duckweed Growth in Germanium
# of Plants
10 ppm
100 ppm
Day
10 ppm
100 ppm
Duckweed Growth in Strontium
# of Duckweed
10 ppm
100 ppm
Day
10 ppm
100 ppm

6. Silver-Ag. The 10 ppm plants duplicated to 6 plants immediately, then died back to 5 and duplicated to 6 by the 10th day; duplication and death were erratic for the duration of the test. Each final black plant had moderate white roots (3 cm). One plant was producing a daughter frond. The 100 ppm plants peaked to 9 immediately, died to 6 and duplicated to 10; duplication and death were erratic for the duration of the test. Final plants were black with moderate white roots (3 cm). Three plants were producing daughter fronds. Black Precipitation was also evident.

7. Cadmium-Cd. The 10 ppm plants duplicated to 12 plants immediately, then 14 by the 20th day. Each small final light green plant had moderate white roots (3 cm). No plants were producing daughter fronds. The 100 ppm plants did not peak and did not die-off. Final plants were light green with moderate white roots (3 cm). Four plants were producing daughter fronds.

The following tests were run only with 10 ppm metal:

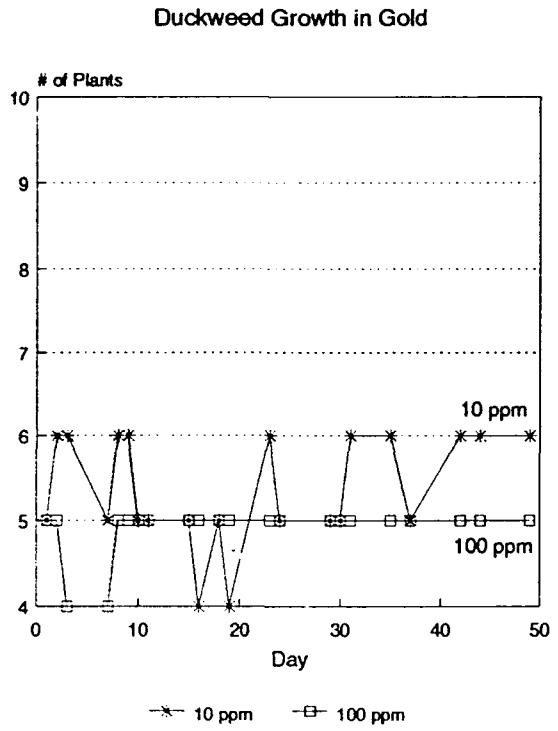
8. Copper-Cu. Plants duplicated to 7 plants, then died back to 5, then duplicated to 6 and then stabilized to 5 plants for the duration of the test. Each final plant was light brown with moderate white roots (3 cm) and was overwhelmed with the unknown white fungus.

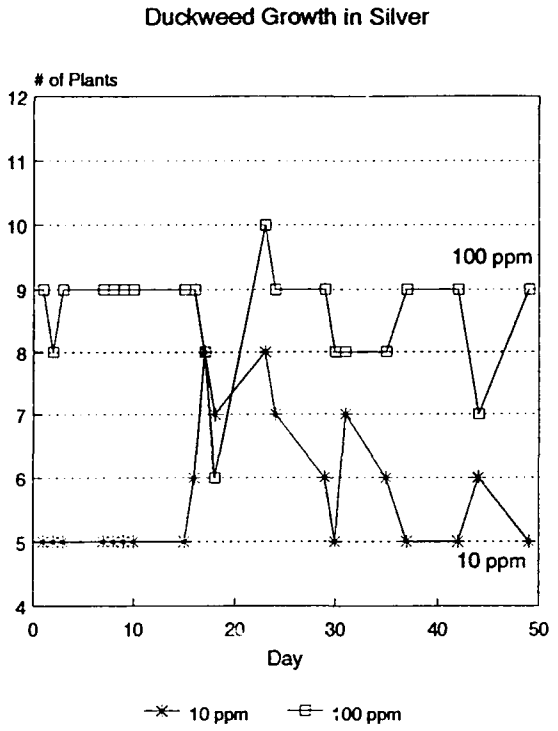
9. Chromium-Cr. Plants duplicated to 7 plants; duplication and death were erratic for the duration of the test. Each final plant was light green with moderate white roots (3 cm). One plant was producing a daughter frond.

10. Lead-Pb. Plants duplicated to 6 plants, then died to 5 and finally stabilized at 6. Each final plant was light brown with moderate white roots (3 cm) and was overwhelmed with bacteria.

11. Arsenic-As. Plants duplicated to 9 plants, then died back to five. Each final plant was light green with moderate white roots (3 cm). No plants were producing daughter fronds.

FIGURE 6


Duckweed Growth in Gold
# of Plants
10
9
8
7
6
5
4
10 ppm
100 ppm
0
10
20
30
40
50
Day
10 ppm
100 ppm


Duckweed Growth in Silver
# of Plants
12
11
10
9
8
7
6
5
4
100 ppm
10 ppm
0
10
20
30
40
50
Day
10 ppm
100 ppm

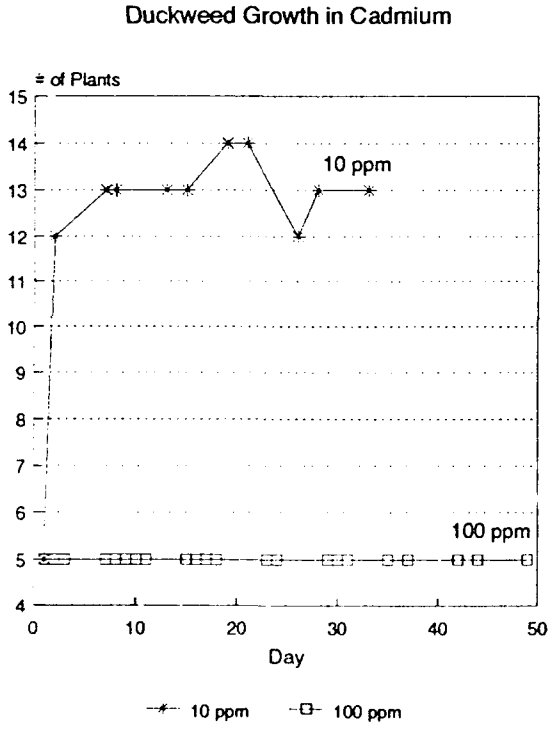
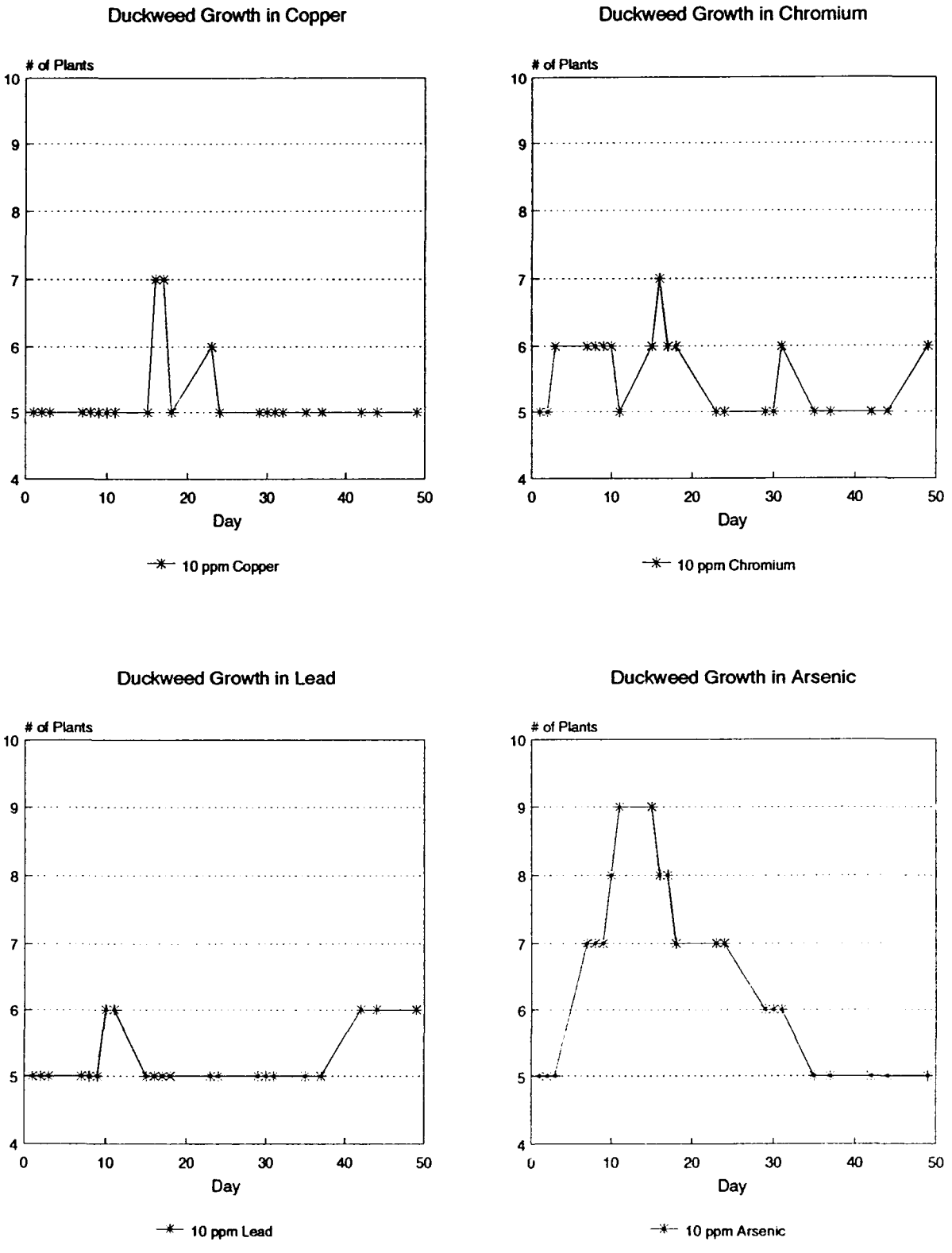

Duckweed Growth in Cadmium
# of Plants
15
14
13
12
11
10
9
8
7
6
5
4
10 ppm
100 ppm
0
10
20
30
40
50
Day
10 ppm
100 ppm

FIGURE 7


Duckweed Growth in Copper
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Copper
Duckweed Growth in Chromium
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Chromium
Duckweed Growth in Lead
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Lead
Duckweed Growth in Arsenic
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Arsenic

12. Zirconia-Zr. Plants duplicated to 9 plants after three weeks; duplication and death were then a little erratic for the duration of the test. Each final plant was dark green with moderate white roots (3 cm). Six plants were producing daughter fronds.

13. Titanium-Ti. There was no duplication during this test, although three plants were producing daughter fronds upon completion of the test. Each final plant was light brown with moderate white roots (3 cm).

14. Sulfur-S. Plants duplicated to 9 plants immediately; duplication and death were then a little erratic for the duration of the test. Each final plant was dark green with moderate white roots (3 cm). Four plants were producing daughter fronds.

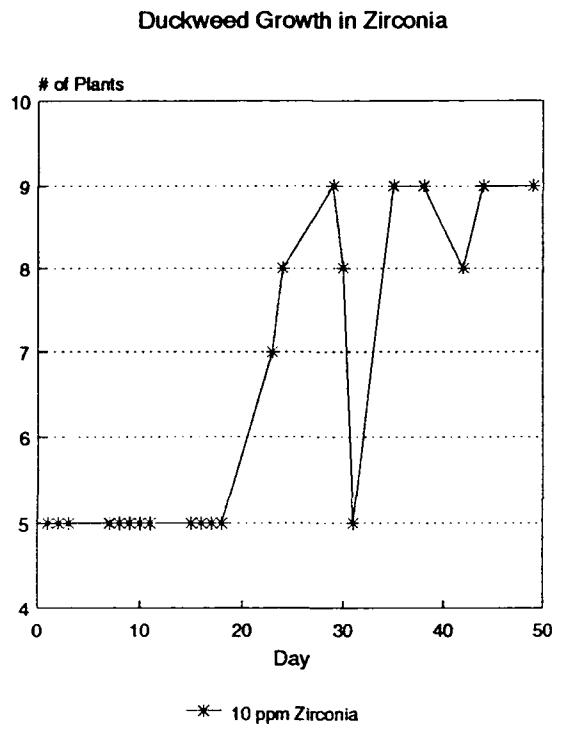
15. Silicon-Si. Plants duplicated to 6 plants after 3 weeks, then died back to 5. Each final plant was light brown with moderate white roots (3 cm). No plants were producing daughter fronds.

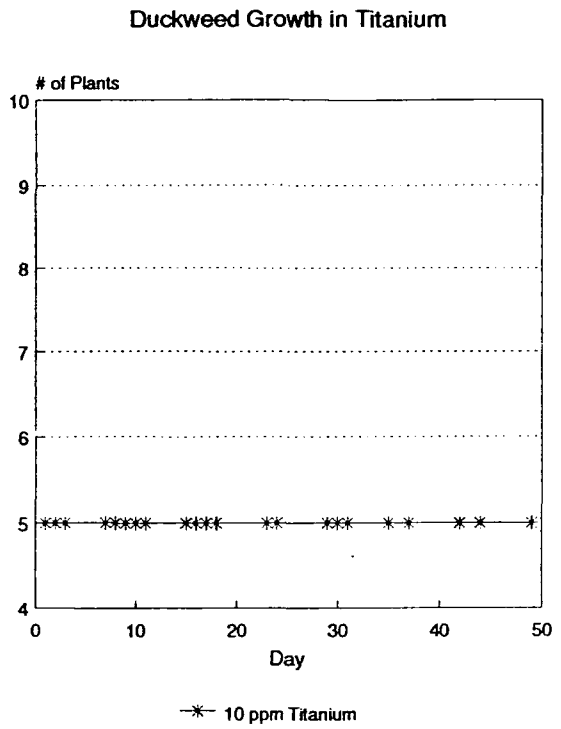
16. Potassium-K. Plants duplicated to 9 plants immediately; duplication and death were then erratic for the duration of the test. Each final plant was dark brown with moderate white roots (3 cm). Two plants were producing daughter fronds, although bacteria surrounded the plants.

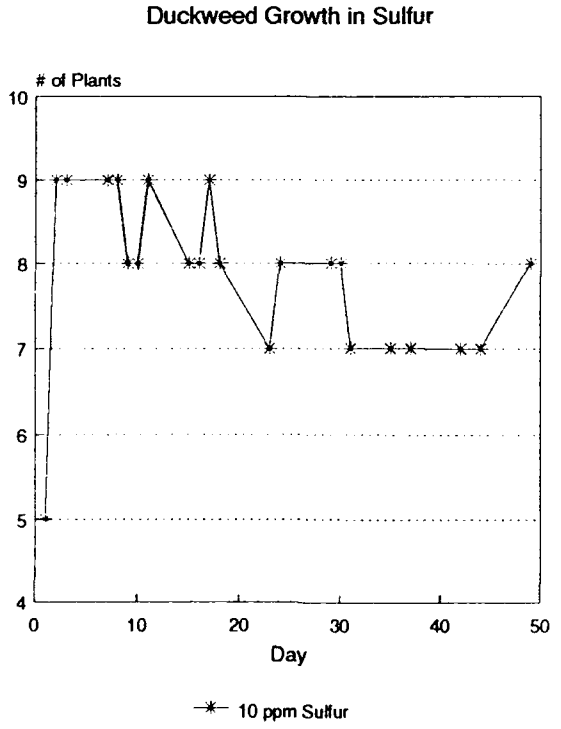
17. Sodium-Na. Plants duplicated to 10 plants after 8 day; duplication and death were then erratic for the duration of the test. Each final plant was dark brown with moderate white roots (3 cm). No plants were producing daughter fronds, although bacteria surrounded the plants.

18. Iron-Fe. Plants duplicated to 9 plants immediately; duplication and death were then erratic for the duration of the test. Each final plant was dark brown with moderate white roots (3 cm). Two plants were producing daughter fronds, although fungi surrounded the plants.

FIGURE 8


Duckweed Growth in Zirconia
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Zirconia


Duckweed Growth in Titanium
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Titanium


Duckweed Growth in Sulfur
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Sulfur

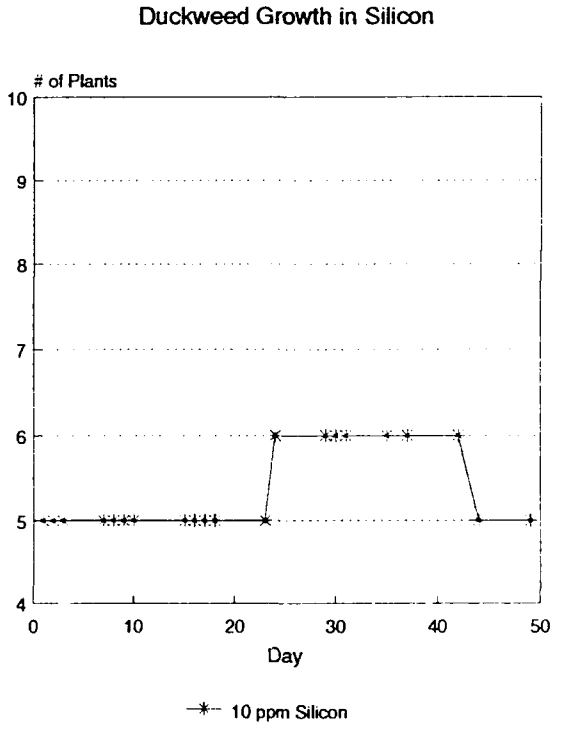
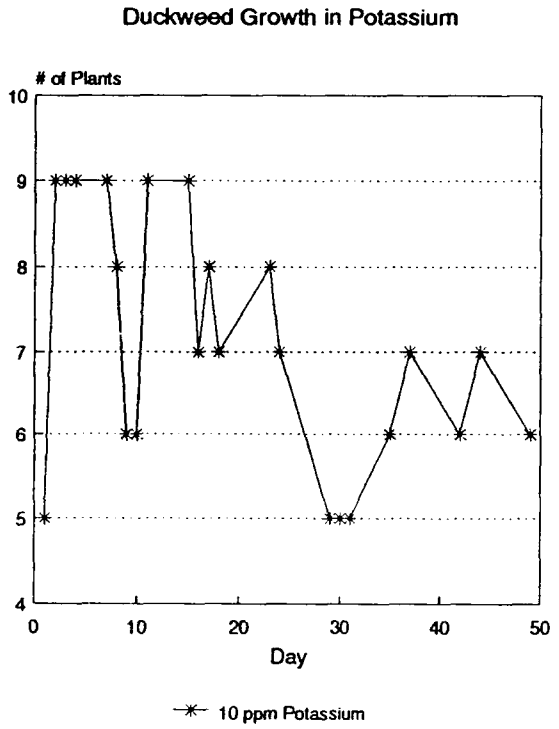
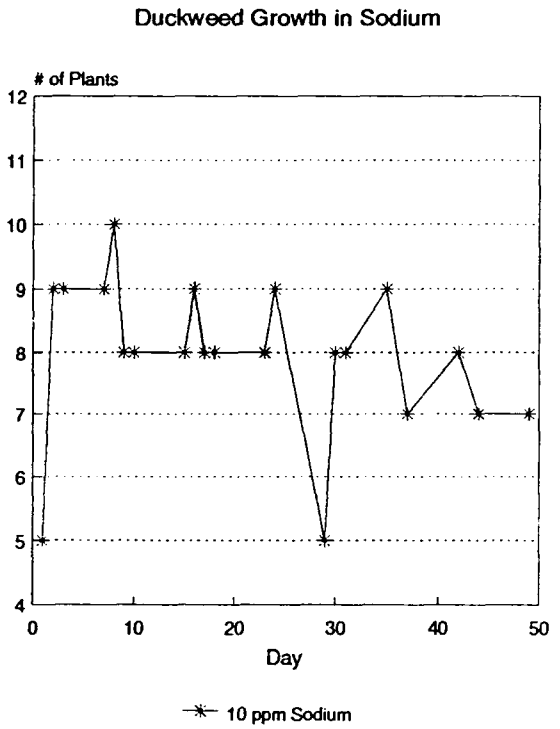
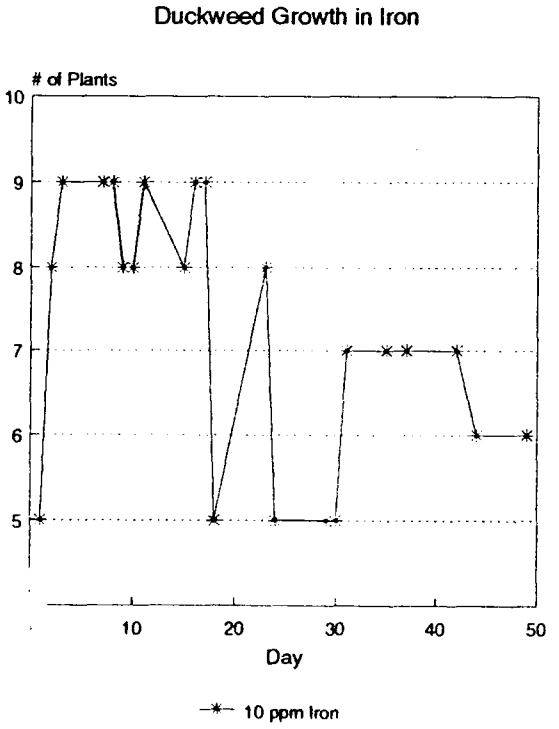

Duckweed Growth in Silicon
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Silicon

FIGURE 9


Duckweed Growth in Potassium
# of Plants
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Potassium


Duckweed Growth in Sodium
# of Plants
12
11
10
9
8
7
6
5
4
0
10
20
30
40
50
Day
10 ppm Sodium


Duckweed Growth in Iron
# of Plants
10
9
8
7
6
5
10
20
30
40
50
Day
10 ppm Iron

CONCLUSIONS

After acclimation, TRA's *Lemna minor* not only survived but thrived. Experiments showed that:

1. pH below 8.0 was detrimental to growth in this variety of *Lemna* (reported literature pH has been 3-4) and at or below the prescribed pH, the plants were not resistant to fungus, bacteria, or algal attacks in culture. pH between 8.0 and 8.3 was preferred.

2. Temperatures above 15°C were also detrimental to growth in this variety of *Lemna* (reported literature temperature has been 20° to 30°C). Higher temperatures de-aerated the water, and decreased the life-span of each individual frond and the rate of daughter-frond production.

3. Metals were slowly absorbed in non-acclimated plants but accumulation accelerated, as plants became induced. Metals were also absorbed more rapidly by plants which previously absorbed near-toxic levels. The erratic accumulation behavior shown in several tests displayed this adaptation/accumulation performance.

4. Continuous flow and feeding allowed the plants to adapt and accumulate at a faster rate; accumulation was also more complete. In addition, bacterial and fungal encapsulation does not occur.

5. Tests also showed that TRA's *Lemna minor* preferred attachment, whether on algae, long-leaved plants or on the side of the vessel.

6. TRA's *Lemna* isolate was capable of accumulating both organic and inorganic substrates. As indicated by the gold and silver tests, it appears that the duckweed can interact with the substrate and waters upon accumulation: Au^0 -> $AuCl_3$; Ag^0 -> Ag_2S.

7. Plants overwintered successfully with small roots, which is important in northern areas. As warmth and nutrient availability increase, the plant dimensions and root length increase 10-fold. Where severe freezing of the water is common, many plants sink to the bottom and lie pressed to the mud while other plants are frozen into the ice. Survival of both is normal, even though the plants may be frozen solid. More plants are revived if thawing is slow (simulating natural conditions) but recolonization is always rapid.

Planned Publications

1. Bowers-Irons, G.L.A., Nelson, R.J. and R.J. Pryor, " The Minimization of Organic and Metallic Industrial Waste Via *Lemna minor* Concentration," to be submitted to Environmental Science and Technology.

2. Bowers-Irons, G.L.A., Pease, J., Nelson, R.J., Pryor, R.J. and F. Hedberg, "Gallium Absorption in *Lemna minor*," to be submitted to The Journal of Experimental Botany.

3. Pease, J. and G.L.A. Bowers-Irons, "Copper and EDTA Toxicity in *Lemna minor*," to be submitted to Botanical Gazette.

4. Bowers-Irons, G.L.A., Pease, J., Nelson, R.J. and R.J. Pryor, "Seasonal Changes and Adaptation in Thallus shape and Size of *Lemna minor*," to be submitted to Plant Science.

5. Bowers-Irons, G. L.A., Pease, J., Nelson, R. J. and R.J. Pryor, "Iron, Copper and Zinc Toxicity in *Lemna minor*," to be submitted to Botanical Gazette.

6. Pease, J. and G.L.A. Bowers-Irons, "Varying Ecotypes of *Lemna minor* clones", to be submitted to Botanical Gazette.

7. Pease, J. and G.L.A. Bowers-Irons, "Gallium Deposition in Lacunae of *Lemna minor*," to be submitted to Journal of Experimental Botany.

Next Work

TRA received a letter from Dr. William Berry, Director of Life and Environmental Sciences, June 15, 1992, indicating that further funds were not available from the Defense Environmental Restoration Account (DERA). DERA no longer supports research. Should the funding be reinstated, Year Two's experiments will concentrate on contaminant uptake as a function of geometry. Task I will focus on uptake vs. reservoir profiles and Task II will study uptake vs. flow rates and turbidity. Task III will focus on uptake vs. *Potamogeton L.* (river weed)/*Lemna* interaction. Task IV, in Year Three, will focus on uptake vs. *Elodea canadensis/Lemna* interaction and Task V will investigate uptake vs. red algae/*Lemna* interaction. Finally, Task VI will analyze and correlate the project data and produce a pilot field design thirty-six months from the start of the project.

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GLOSSARY

absorb: to take up or in by chemical or molecular action, as gases, heat, liquid, light, etc.

acclimate: to adapt to a new temperature, pH or environment.

accumulator: one that accumulates or amasses or collects materials from liquid.

adaptation: modification of an organism or of its parts fitting more perfectly for existence under the conditions of its environment and resulting from the action of natural selection upon variation.

anion: the electronegative ion of an electrolyte, wnich moves toward the anode during electrolysis; opposite to the cation.

aquatic: living or growing near or in water.

autoclave: an airtight chamber that can be filled with steam under pressure or surrounded by another chamber for the steam that is used for sterilizing, cooking or other purposes that require moist or dry temperatures above 212°F without boiling. To sterilize or cook above 212° without boiling.

bacteria: any of a large group of unicellular microorganisms having round, rodlike, spiral, or filamentous single-celled or non-cellular bodies that are aggregated into colonies, are enclosed by a cell wall or membrane, living in soil, water, organic matter or living bodies of plants or animals.

bacterial: belonging to, consisting of, resulting from, or caused by bacteria.

centrifugation: the process of rapidly whirling fluids to separate substances of different densities.

calla: a plant of the arum family, native to South Africa, but cultivated in the United States, and having a large milk-white spathe that resembles a flower.

cation: the electropositive ion of an electrolyte, that moves toward

the cathode in electrolysis; opposed to anion.

concentration: the amount of a substance per unit volume.

cuticle: the transparent film covering the surface of a plant, derived from the layers of epidermal cells.

frond: a leaf-like extension in which the functions of stem and leaf are not fully differentiated.

fungal: consisting of fungi.

fungus: any of numerous plants that lack chlorophyll, that can be used for fermentation or degradation.

incineration: a procedure of heating organic substances with free access to air until only ash remains.

incubator: the process of maintaining under prescribed and controlled conditions such as temperature and moisture favorable for development and/or growth of bacteria or fungi. An apparatus that maintains the prescribed and controlled conditions for the cultivation of microorganisms.

indicator: a substance that by color, change, or in some other visible way, shows the condition of or some change in a system.

inorganic: being or composed of material other than of plant or animal origin, such as minerals or chemical salts.

isolate: to separate from other substances, such as other microorganisms in a mixed culture.

lacunae: an intercellular space or passage in plant tissue.

macrophyte: a member of the macroscopic (as opposed to microscopic) plant life especially of a body of water.

media: pl. of medium. any nutrient system for the artificial cultivation of microorganisms that is sometimes a simple substance but more often a complex combination of several organic and inorganic materials in a fluid or solid base. also called nutrient media.

minimization: to reduce to the smallest possible amount or degree.

monitor: something that indicates a system change.

monocots: any of a great subclass of seed plants, including palms, lilies, etc.

non-spec: not meeting military or industrial specifications.

nutrient: additive to a medium that gives nourishment or promotes development.

organic: of, pertaining to, or of the nature of compounds containing carbon.

oxidized: to convert an element into its oxide; combine with oxygen. to increase the valence of an atom or group of atoms by the loss of electrons.

precipitate: to separate in solid form out of solution by reagents or mechanical means. The separated solid.

restoration: the rehabilitation or renewal to former condition or state.

spectroscopy: the study and analysis of the phenomena observed with an optical instrument which analyses spectrum emitted by bodies or substances.

stereomicroscopy: a microscope that three dimensionally blends two images into two from different viewpoints.

thallus: a plant body without true root, stem or leaf.

toxicant: that which is poisonous.

TRA: Technical Research Associates, Inc.

APPENDIX

FIGURE 10

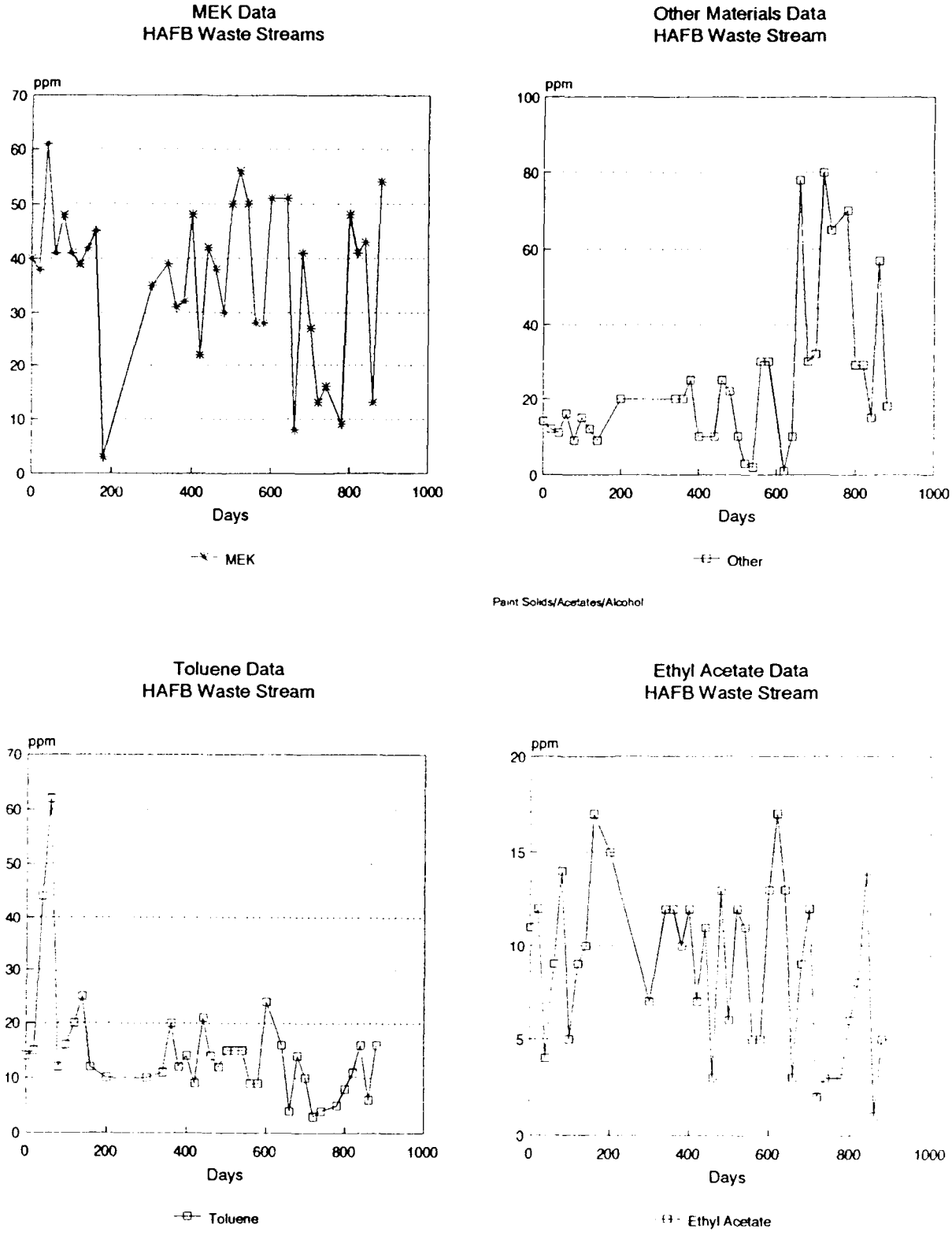
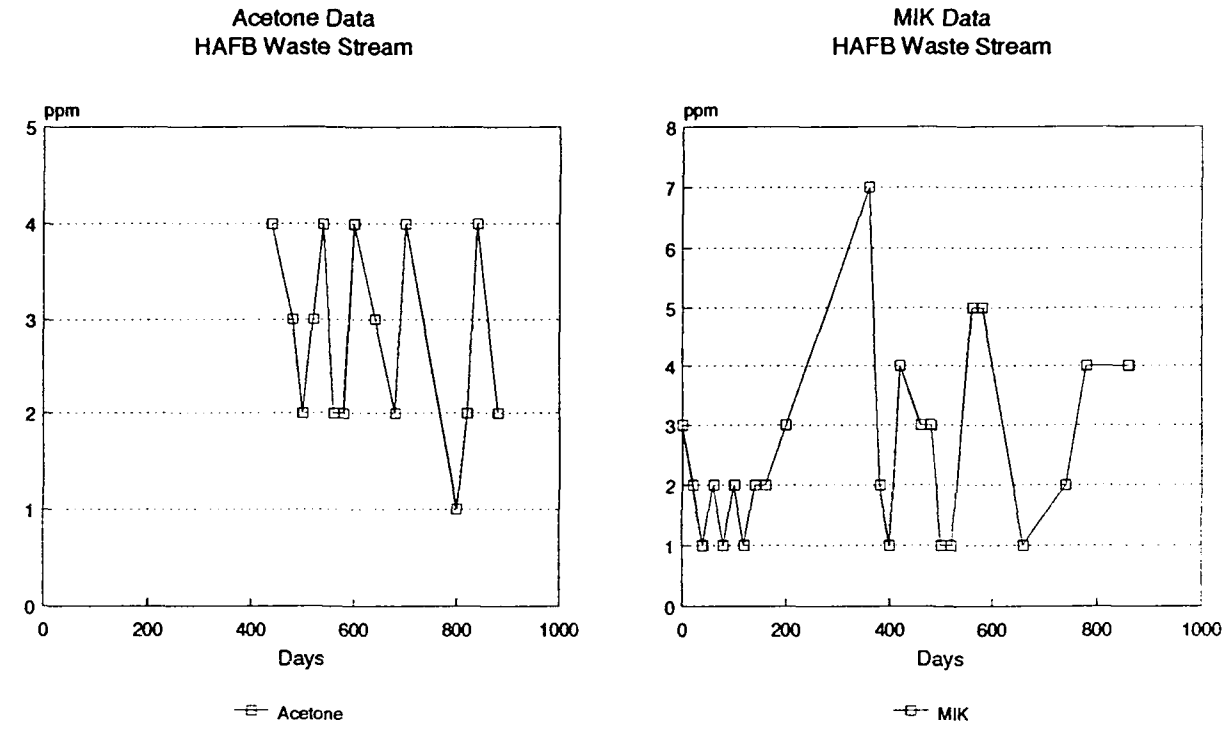

MEK Data
HAFB Waste Streams
ppm
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
Days
MEK
Other Materials Data
HAFB Waste Stream
ppm
100
80
60
40
20
0
0
200
400
600
800
1000
Days
Other
Paint Solids/Acetates/Alcohol
Toluene Data
HAFB Waste Stream
ppm
70
60
50
40
30
20
10
0
0
200
400
600
800
1000
Days
Toluene
Ethyl Acetate Data
HAFB Waste Stream
ppm
20
15
10
5
0
0
200
400
600
800
1000
Days
Ethyl Acetate

FIGURE 11



November 12, 1991

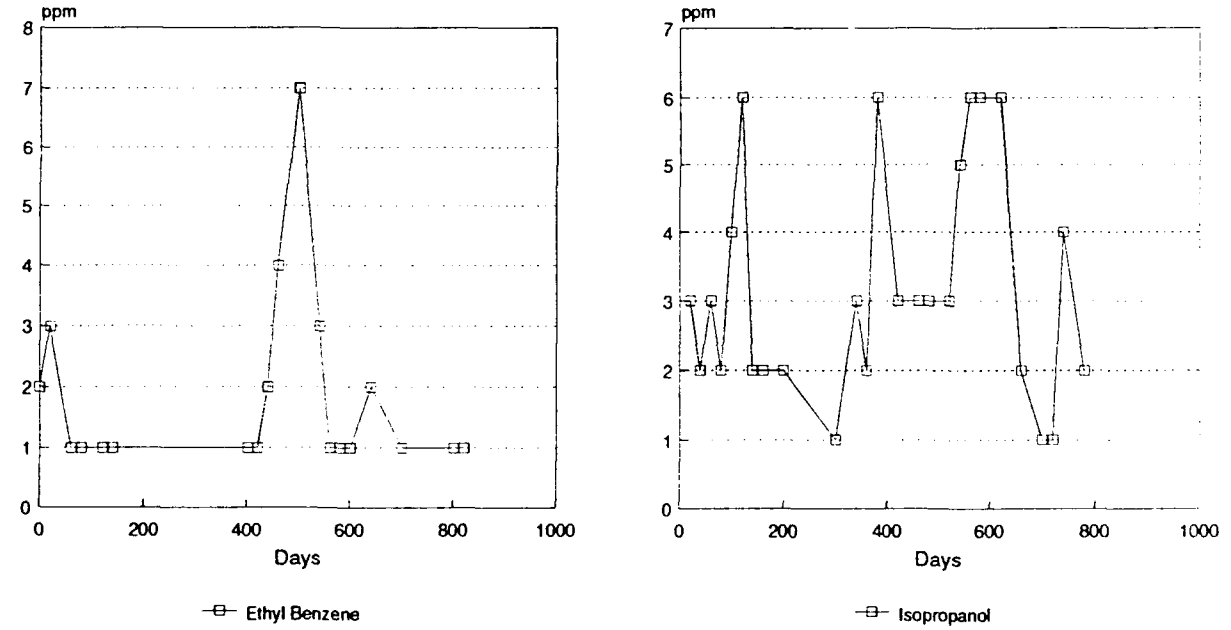
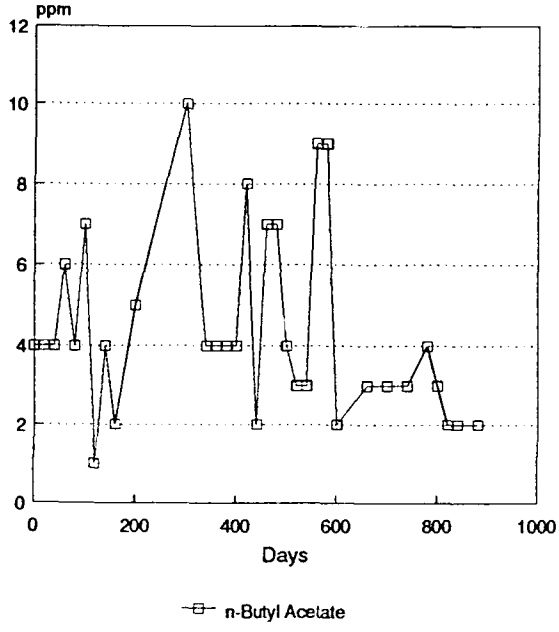
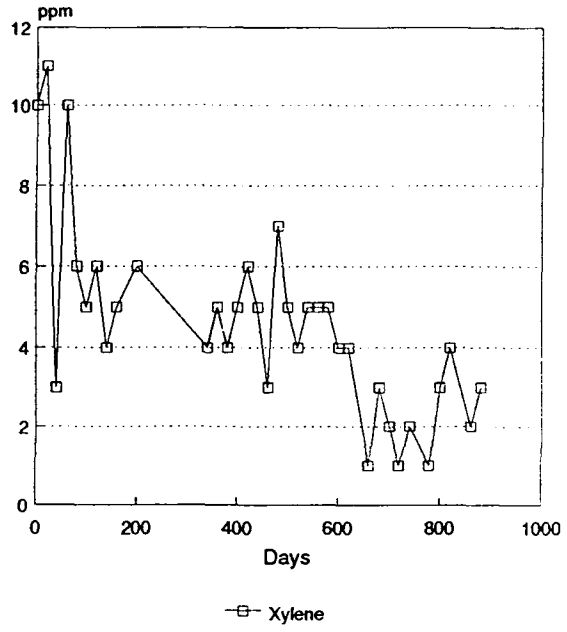


FIGURE 12

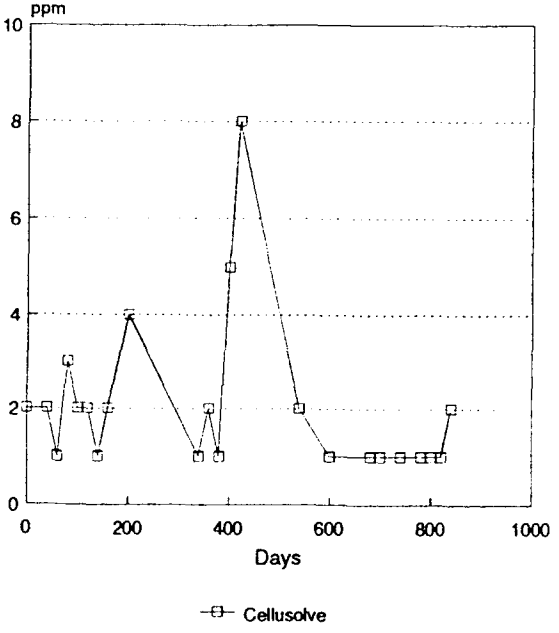
n-Butyl Acetate Data
HAFB Waste Stream



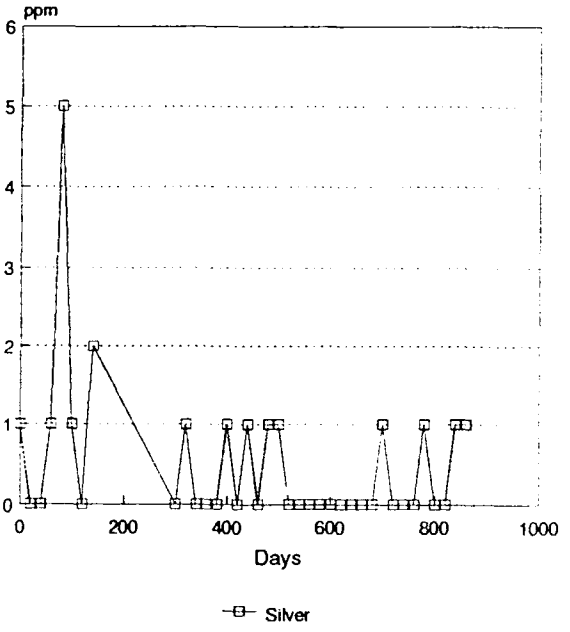
Xylene Data
HAFB Waste Stream



Cellusolve Data
HAFB Waste Stream



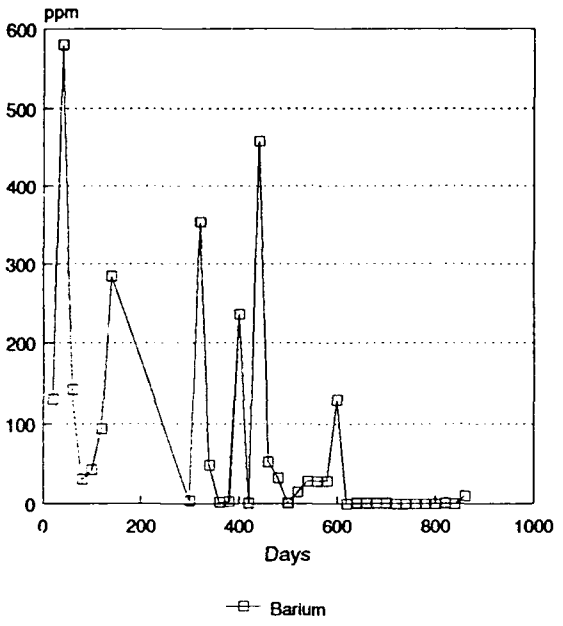
Silver Data
HAFB Waste Steam



0 = <1.0

FIGURE 13

Barium Data
HAFB Waste Stream



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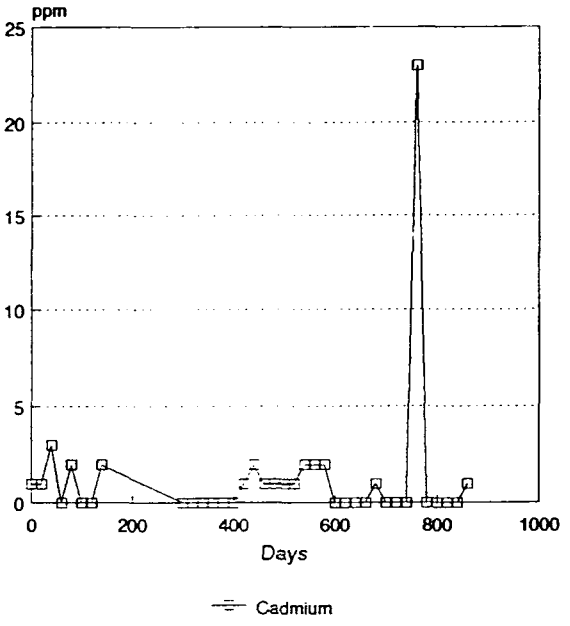
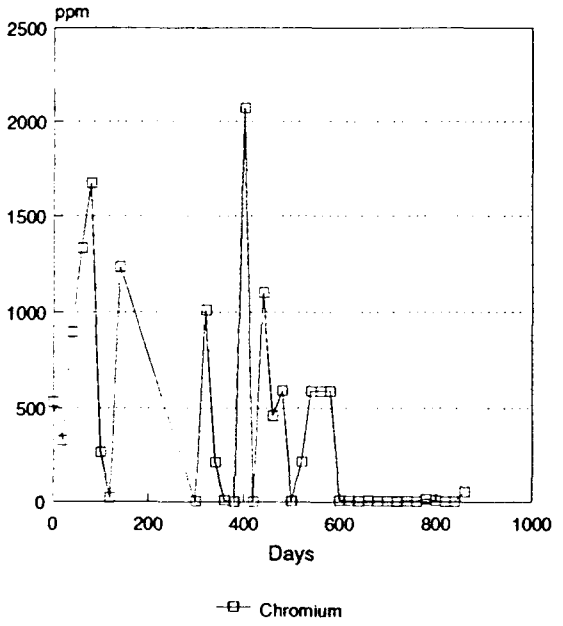
Cadmium Data
HAFB Waste Stream



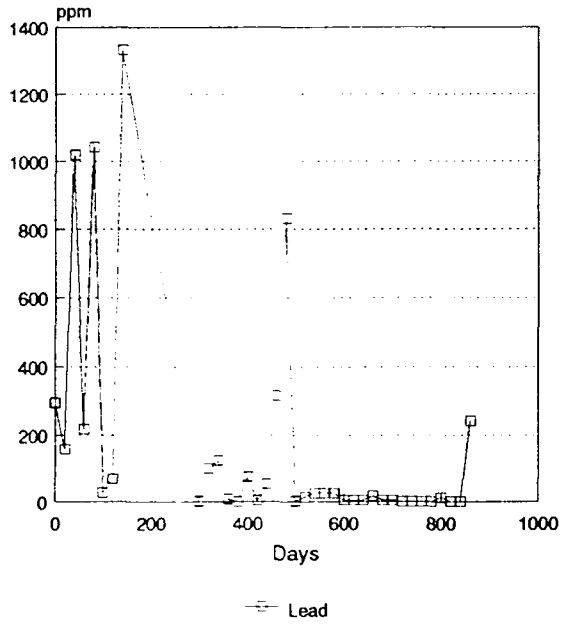
0 = <1.0

Chromium Data
HAFB Waste Stream



0 = <1.0

Lead Data
HAFB Waste Stream



0 = <1.0