EFFECTS OF HEAVY METALS ON WATERHYACINTHS AND ALLIGATORWEED AND TWO PHYTOPHAGOUS BIOCONTROL INSECTS

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1980
DEDICATED

TO

I proudly dedicate this dissertation to the memory of my father and mother, John Bellamy and Martha Haman Kay.
ACKNOWLEDGEMENTS

I wish to thank my major professor, Dr. William T. Haller, and the members of my committee, Drs. Leon A. Garrard, George Bowes, Donald A. Graetz, and Dale H. Habeck for their helpful comments and consultation.

I am grateful to Drs. C. C. Hortenstine and D. L. Myhre of the Department of Soil Science for the use of their muffle furnaces, to the Department of Entomology and Nematology for the use of the outdoor cages at the apiculture facility, and to the Department of Agronomy for the use of the leaf area meter.

Greater appreciation is expressed to Dr. P. C. Quimby, Jr., of the Southern Weed Science Laboratory, USDA, Stoneville, Mississippi, for providing the alligatorweed used in this study and for his personal encouragement throughout the course of my graduate studies.

Appreciation is expressed to Jim Fealy and Amy Lichtman for their assistance in portions of the laboratory and field work.

I am grateful to Mrs. Helen Huseman for her excellent graphics works and to Mrs. Mary Hunnicutt for the expert typing of the manuscript.

Finally, I wish to express my most sincere appreciation to my wife, Theresa, for her patience and understanding as well as for her assistance with portions of my experiments.
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A study was conducted to determine the effects of lead, cadmium, and copper in solution on waterhyacinths *Eichhornia crassipes* (Mart.) Solms] and alligatorweed *Alternanthera philoxeroides* (Mart.) Griseb. and to ascertain the effects of plant-absorbed lead, cadmium, and copper upon the waterhyacinth weevil *Neochetina* sp.) and the alligatorweed flea beetle *Agasicles hygrophila* Selman and Vogt. These studies were conducted to gain information on growth responses of waterhyacinths and alligatorweed to potentially toxic heavy metals. This information may have application in the development of these plants as self-perpetuating biological filters for wastewater treatment and in ascertaining the impact of heavy metal accumulation upon the efficacy of insects for biological control of waterhyacinths and alligatorweed.

There was no appreciable effect of lead upon either waterhyacinths or alligatorweed, but both cadmium and copper were highly toxic and...
caused a substantial reduction in the growth of both plants. Threshold toxicity of cadmium was < 0.5 ppm for waterhyacinths and between 0.5 and 1.0 ppm for alligatorweed, whereas that for copper was between 1.0 and 2.0 ppm for both waterhyacinths and alligatorweed. Toxic levels of cadmium and copper caused the reduction of root development and function in both plants. Beyond threshold toxicity, growth of the root system was negligible. Roots were short, brittle, and few lateral roots were present. Severe leaf chlorosis and reduction in overall plant growth appeared to be the results of nutrient imbalances and/or deficiencies, but direct interference of the metals in metabolism could not be ruled out. Relative growth rates of plants exposed to toxic levels of cadmium and copper were about ten percent of either the controls or plants exposed to lead.

Uptake and partitioning of metals depended upon the plant species, mobility of the metal, and level of exposure. The order of increasing mobility was lead < cadmium < copper. Metal concentrations within a plant tissue and total plant accumulation of metals increased linearly as exposure was increased. All three metals were transported to stem and leaf tissues in waterhyacinths, whereas only cadmium and copper were detected in stems and leaves of alligatorweed.

Results of these studies indicate that caution should be exercised when extrapolating from plant growth rates under optimum conditions and from short-term laboratory studies to the abilities of aquatic plants to remove toxic substances from wastewater when plant growth is inhibited.

There were no effects of lead, cadmium, or copper upon the feeding of adult waterhyacinth weevils or alligatorweed flea beetles.
levels of exposure encountered in these experiments. Lack of any effect of lead upon the flea beetles may be attributed directly to the lack of any detectable lead in the leaf tissues. Feeding of alligatorweed leaf tissue from plants that had been exposed to 0.5, 1.0, or 2.0 ppm cadmium in the nutrient solution significantly reduced the fecundity of the flea beetles as measured by egg counts. These experiments suggest that cadmium in the natural environment may be toxic to the alligatorweed flea beetle and may interfere with biocontrol efforts under some circumstances. Lead, cadmium, and copper are unlikely to have any impact upon biocontrol efforts involving the waterhyacinth weevils.
INTRODUCTION

The development of technology in an unending quest for improvement upon the quality of life has led inadvertently to the discharge of the waste materials and by-products of human society into the environment. As a result of human ignorance and, all too often, human apathy, environmental contamination has proceeded, essentially unchecked, to the present day.

Among the important, but often over-looked, environmental contaminants are the trace metals. Cook (1977) defined trace elements as those which are present in the environment in concentrations of > 400 parts per million (ppm). Many of the trace elements are essential nutrients and may be required biochemically as components of a wide range of enzymes. In natural minerals these trace metals are usually present as insoluble compounds that are harmless to living organisms, whereas the soluble forms are frequently very toxic. The metals which form poisonous compounds are frequently called heavy metals. According to Cook (1977), heavy metals are defined as metals having a density greater than 5. This strict definition would exclude beryllium (density 1.83), the oxide of which is very toxic and prevents the healing of wounds, but would include iron, which is generally regarded as non-toxic. Consequently, the more useful definition would appear to be the general definition that includes those metals which form toxic compounds.
Heavy metals are widely dispersed throughout the environment and are usually present in concentrations so small as to cause no concern. In recent years, however, human activities have enhanced levels of toxic metallic compounds in the biosphere. Toxic compounds of mercury, cadmium, and lead have caused the greatest environmental concern, primarily because of public health implications. Compounds of arsenic, zinc, chromium, copper, nickel, vanadium, zinc and many other metals have also been implicated at one time or another in air, soil and water pollution. The ultimate sink for such potentially toxic substances is the aquatic environment. Pollution of our most precious natural resource, water, has been increasing at an alarming rate over the past twenty or so years.

The pollution of our natural waters has not been limited to toxic substances, however. Discharges from domestic sewage facilities and runoff from agricultural lands have added large quantities of nutrients to the aquatic system. In recent years, especially during the past decade, increasing environmental awareness of the general public has led to the recognition of the consequences of environmental pollution, including the potential health hazards of toxic compounds as well as the problems of aquatic weed growth in response to cultural eutrophication. The impact of pressures generated by informed public opinion has prompted environmental scientists to search for solutions to these problems. Among the potential environmentally acceptable solutions to the problems of pollution and aquatic weeds, respectively, are the use of aquatic plants in the removal of water pollutants and the employment of biological agents for the suppression of undesirable aquatic plant populations.
The use of aquatic plants for the removal of organic and inorganic pollutants from domestic and industrial sewage effluents has become the objective of intensive investigations by environmental engineers and scientists. Among several plants with apparently great potential for the removal of pollutants from the water are alligatorweed and waterhyacinths (Wolverton et al. 1975b). Studies on the utilization of these and other aquatic plants for pollution control have centered solely upon the quantity of pollutants removed over a relatively short period of time. Little consideration has been given to the effects of toxic pollutants, especially the heavy metals, upon the growth of the plants employed in the pollution control or to the possible seasonal differences in plant uptake and response. In order to have a self-perpetuating biological filter for the removal of toxic compounds from the water, the plants must be able to grow and reproduce adequately under the prevailing conditions. Furthermore, the ability to absorb pollutants may possibly vary with season. To evaluate adequately the potential of an aquatic plant for use in pollution abatement, information is desperately needed concerning the toxicity of pollutants to the plants, the growth and reproduction of the plants when exposed to toxic pollutants, and seasonal responses in plant growth and development in the presence of pollutants.

The second aspect of the environmental problem related to aquatic plants is the rampant growth of certain aquatic weeds. Weedy infestations of aquatic plants may cause problems through interference with transportation, irrigation, city water supplies, fishing, recreation, and by harboring the vectors of human disease (e.g., mosquitos). The classical approach to aquatic weed control since the advent of 2,4-D
in the early 1940's has been treatment with phytotoxic chemicals. Since the outset, however, chemical control has been beset with certain difficulties. Although waterhyacinths were quite susceptible to 2,4-D, alligatorweed was quite resistant, and many areas once occupied by waterhyacinths were soon infested with alligatorweed. Waterhyacinths also grow prolifically in backwater areas quite inaccessible to chemical control operations. Consequently, there is a constant source of reinfestations of waterways by the waterhyacinth, and continual maintenance programs were necessary to maintain waterhyacinth populations at an environmentally acceptable level.

Difficulties associated with the control of alligatorweed and waterhyacinths led a search of their native habitat (South America) for natural enemies. From 1960 to 1962 a joint USDA-U.S. Army Corps of Engineers effort to find biological suppressants of these weeds was conducted. The results of these studies ultimately led to the introduction of insects for the control of alligatorweed and waterhyacinths. Three host-specific insects, the alligatorweed flea beetle, *Agasicles hygrophila* (Coleoptera: Chrysomelidae), the alligatorweed stem borer, *Vogtia malloi* (Lepidoptera: Pyralidae), and the alligatorweed thrips, *Amynothrips andersoni* (Thysanoptera: Phlaeothripidae), were introduced over a period from 1965 through the early 1970's. The details of the search, release of the insects, and their subsequent spread and impact upon alligatorweed have been reviewed extensively by Coulson (1977), Spencer and Coulson (1976), Maddox et al. (1971), Brown and Spencer (1973), and Hawkes et al. (1967). Two weevils (specific for the Pontederiaceae, but preferring waterhyacinths) *Neochetina eichhorniae* and *N. bruchi* (Coleoptera: Curculionidae) were introduced during the
mid-1970's for the biological suppression of waterhyacinths. Background information and details of the search for, host-specificity of, and introduction of these insects may be found in papers by Bennett and Zwolfer (1968), Perkins (1972), and Perkins and Maddox (1976).

Although the biological control of alligatorweed has been extremely successful, initial efforts on waterhyacinths have had variable results. There have also been indications that alligatorweed has made a resurgence in certain areas, particularly in the Carolinas (Gary Buckingham, USDA, Gainesville, Florida, personal communication). In other areas alligatorweed control has never been attained. In some cases explanations such as, that the alligatorweed flea beetle does not overwinter well in the more northerly range of alligatorweed, are inadequate to explain the lack of control. Two recent papers have suggested that environmental contamination may be a potential factor in determining the success of biological control of weeds with insects. Quimby et al. (1979) indicated that the alligatorweed flea beetle appeared to be sensitive to plant accumulation of cadmium. Kreasky et al. (1979) have shown that the alligatorweed flea beetle is very sensitive to toxaphene and methyl parathion, two insecticides that are employed extensively in the lower Mississippi Valley for insect control on cotton. These studies suggest that further investigation of the influence of environmental contaminants upon phytophagous insects is warranted.

The objectives of this study are: 1) to investigate the effects of the heavy metals lead, cadmium, and copper upon the growth of waterhyacinths and alligatorweed; 2) to ascertain whether or not there are seasonal differences in the growth of waterhyacinths when exposed
to potentially toxic metals; 3) to determine whether or not there are seasonal differences in the abilities of the waterhyacinths to absorb toxic metals; and 4) to ascertain the influences of plant accumulation of the heavy metals lead, cadmium, and copper upon the alligatorweed flea beetles, *Agasicles hygrophila*, and the waterhyacinth weevils, *Neochetina* sp.
LITERATURE REVIEW

Lead, Cadmium, and Copper in the Physical Environment

Lead

Lead is a natural constituent of the environment with the greatest quantity occurring naturally in the soil. Using the assumption of an average abundance of 16 ppm, Nriagu (1978a) has estimated that the earth's crust and soil contain $3.8 \times 10^{20}$ g and $4.8 \times 10^{15}$ g of lead, respectively. The main source of the environmental lead problem comes not from naturally occurring levels of lead in the earth's crust, but from that of anthropogenic origins.

Lead in the air and deposition. Nriagu (1978c) has estimated the worldwide emission of lead into the atmosphere from natural sources to be approximately $1.9 \times 10^{10}$ g, about 85 percent of which is contributed by wind-blown dusts; these natural emissions comprised only about 4 percent of the total lead emissions, the rest being attributable to technologic (i.e., anthropogenic) sources. For the period 1974-75, of the $44 \times 10^{10}$ g of lead released into the atmosphere, 61 percent came from the combustion of leaded gasoline, 23 percent from the production of steel and base metals, 8 percent from the mining and smelting of lead, and 5 percent from non-automotive combustion of fossil fuels (Nriagu 1978c). The majority of these atmospheric lead contaminants will eventually be removed by precipitation and dry
deposition. Nriagu (1978c) states that about 10 percent of the airborne lead is deposited in the southern hemisphere, and the average annual lead deposition in the northern and southern hemispheres are approximately 0.8 mg \cdot m^{-2} and 0.4 mg \cdot m^{-2}, respectively. The annual lead deposition in the United States varies from about 1.0 mg \cdot m^{-2} in isolated mountainous areas of California (Hirao and Patterson 1974) to > 400 mg \cdot m^{-2} in urban areas (Chow and Earl 1970).

Lead in the soil. With the exception of the immediate vicinity of streets and highways, the major source of elevated lead levels in soils is deteriorating lead-base paint. In a study in Detroit, Ter Haar (1975) found that within 2 feet of houses soil lead averaged just over 2000 ppm, while at a distance of only 10 feet from houses, lead averaged about 400 ppm. Nriagu (1978b) compiled a table of soil lead concentrations of different soil types and different regions. By weighting the data according to the land area of the countries or regions involved, he arrived at a mean value of 17 ppm for lead in uncontaminated soils. Nriagu (1978b) also emphasized the heterogeneity of lead distribution and pointed out that natural soils may contain from < 1.0 ppm lead in uncontaminated areas to well over ten percent in ordinary deposits.

The major cause of environmental lead pollution has been shown to be the use of tetraethyl lead as an antiknock compound in fuels, and hundreds of reports are available in the literature. The major findings are summarized in reviews by Smith (1976), Laxen and Harrison (1977), Nriagu (1978b), and Purves (1977) as follows: a) Lead concentrations in roadside soils are related to traffic density and mode of operation of vehicles. Greater lead build up occurs along highways than along city streets and arterial roadways. This is apparently
the result of lower emissions per unit of fuel at lower driving speeds than at high speeds. b) Automotive lead pollution of roadside soils depends upon local meteorological conditions, particularly wind speed and direction and rainfall. c) Surface topography and vegetation may affect lead concentration in roadside soil. Soils in wooded areas adjacent to roads contain less lead than soils in open fields, primarily due to trapping of lead deposits on vegetation. d) There is a curvilinear decrease in soil and plant lead contamination with distance from the roadway, with greatest levels between 0 and 10 m from the roadway and decreasing sharply thereafter to essentially background levels beyond 100 m distance. e) An exponential decrease in lead concentration in soil occurs with depth of soil, with most lead being deposited in the upper 2 to 4 cm of the soil. Significant elevation of lead above background level was uncommon below 10 cm depth. f) Lead is transferred to the soil primarily by sedimentation and precipitation. g) Lead is exhausted to the atmosphere primarily as relatively soluble particulate inorganic lead salts, particularly chlorobromides, and about 80 mg of lead are released by vehicles per km. These halides are apparently oxidized rapidly to sulfates and relatively insoluble oxides and carbonates. h) Lead is apparently bound tightly by the soil. This immobilized lead, consequently, contributes little to water pollution. Durham and Haffty (1961) reported a median lead concentration of 4 ppb with a range of 0-55 ppb; their data indicated lead to be more pronounced along the Atlantic Coast than elsewhere in the United States. Values reported for sea water were also in the range of 3 to 4 ppb. These values are somewhat higher than those tabulated by Bowen (1966) for lead in sea water (0.00003 ppm), and may be assumed to be the result of lead pollution along the Atlantic Coast.
Lead in water. The pollution of natural waters by lead has been studied extensively. The average lead concentration in sea water is reported by Purves (1977) to be 0.03 ppb. Purves (1977) reports that the lead concentration in rivers and estuaries may reach 100 to 1000 ppb. These enhanced levels were associated with industrialization. Durham and Haffty (1961) found the following lead levels in several major U.S. rivers: Colorado, 11 ppb; Columbia, 3.2 ppb; Hudson, 7.5 ppb; Mississippi, 6.3 ppb; Sacramento, 3.3 ppb; and Susquehanna, 3.5 ppb. Durham and Haffty (1963) also reported that major rivers in North America contained lead concentrations from undetected to 55 ppb, with a mean value of 4 ppb, and that elevated lead levels were more common along the Atlantic Coast than elsewhere. Elzerman et al. (1979) emphasized that trace elements in particulate matter in the surface microlayer is especially important in trace-element enrichment of natural waters. Mean surface enrichments for lead were 0.15 and 2.6 µg · m⁻² for the dissolved and particulate fractions, respectively. These data suggest strongly that the majority of lead entering natural waters would be associated with the particulate fraction, and very little would enter the free aqueous phase. This is in precise agreement with the data of other researchers (Chow and Patterson 1962; Nriagu 1978a; Wixon 1978; Baier and Healy 1977; C. P. Huang et al. 1977).

The presence of lead in Florida waters has been reported (USGS 1979). The range of lead found in surface and ground waters in Florida varies greatly, depending upon both the area of the state and the proximity to urbanized areas. Suspended, dissolved and total lead were 0 to 570, 0 to 230 and 0 to 600 ppb, respectively, in ground waters. The highest
levels found in surface waters were generally found in polluted canals and rivers in close proximity to large urban areas, whereas, high levels in ground waters were usually associated with urban landfill sites.

**Lead in sediments.** Much as soils are in terrestrial ecosystems, benthic sediments are the primary sinks for lead in aquatic ecosystems (Nriagu 1978b; Ter Haar 1975) and are frequently employed in assessing lead pollution in the aquatic environment (Pasternak 1974; Hamilton-Taylor 1979). The concentration of lead in pelagic sediments is extremely variable and depends greatly upon the source of sediments, water chemistry, proximity to a polluting source, and a myriad of other factors. Nriagu (1978b) has provided an excellent review of this subject and has provided tables of mean values compiled from the literature. Pelagic sediments in the major oceans ranged from 47 to 61 ppm, 12.6 to 17 ppm and 784 to 1250 ppm lead in clays, carbonate oozes, and nodules, respectively. Lead concentrations in sediments of the other seas generally approximated the mean crustal value of 16 ppm. Lead concentrations in brackish and coastal sediments have been greatly influenced by cultural activities and clearly reflect the degree of urbanization and industrialization of a given area. Brackish and coastal sediment lead concentrations ranged from 1 ppm at Card Sound, Florida, to over 11000 ppm at Sorfjord, Norway, which is polluted by heavy industrial discharges. Harbor sediments are usually contaminated with lead. In recent years the contamination of freshwater lakes with lead has caused concern. Lead profiles in lake sediments generally show increased loading in recent deposits.
due to increased cultural use of lead and lead products. Nriagu (1978b) has compiled from the literature a table showing lead levels in surface layers and deep sediments. Surface layer values range from 3 ppm in Lake Mary, N. Wisconsin, to 3500 ppm in Lake Coeur D'Alene, Idaho; lead concentration from the deep layers in the same lakes were < 0.1 ppm and 25 ppm, respectively. These elevated surface levels were, again, due to cultural enrichment.

Lead has also been reported in sediments in a few inland bodies of water in Florida. Water resources data (USGS 1979) show that lead levels varied from < 10 to as high as 600 μg · g⁻¹. The high levels, as was true for waters in Florida, were found in areas close to large urban areas. In Alachua County, lead levels measured on unreplicated mid-lake samples from a single sampling date were 25 and 21.8 ppm, respectively, for Lochloosa and Orange Lakes (author, unpublished data).

Lead cycling and the fate of lead in the aquatic environment. The forms, cycling and ultimate fate of lead in the aquatic environment have been the subjects of numerous studies and have often produced conflicting conclusions. A number of generalizations, however, may be made concerning lead cycling and the fate of lead in the aquatic environment.

Lead is generally considered to enter the aquatic system predominantly through atmospheric precipitation and, to a lesser extent, through runoff from the land (Rickard and Nriagu 1978). Lead enters the water primarily in association with particulate matter, organic or inorganic, and, to a lesser extent, as free dissolved lead ions
($\text{Pb}^{2+}$) and compounds and as dissolved compounds chelated with water and organic materials (Chow and Patterson 1962). The physicochemical form of lead is important, as it determines the distribution of lead in the aquatic environment as well as the availability of lead to, and consequently the impact upon, the aquatic ecosystem. Lead in the particulate form may be found bound in organic solids, such as algae and zooplankton, absorbed to inorganic solids, such as clays and oxides of iron and manganese, or be present as precipitates or coprecipitates, such as $\text{PbCO}_3$, $\text{PbS}$, and $\text{Pb}_5(\text{PO}_4)_3\text{Cl}$ (Laxen and Harrison 1977).

Numerous interactions and changes may occur once the lead has entered the aquatic system. Some direct exchange may occur between the dissolved and particulate fractions. In general, the particulate lead pool is incorporated into the sediments. Lead released from the sediments by organic decomposition is, for the greatest part, quickly adsorbed by the hydrous oxides or immobilized under anaerobic conditions through reaction with sulfide ions to form $\text{PbS}$ (Rickard and Nriagu 1978; Huang et al. 1977). Adsorption by materials such as the clay particulates depends greatly upon water pH, cation exchange capacity of the particulate fraction and competing cations (Laxen and Harrison 1977). Free $\text{Pb}^{2+}$ ions are largely the result of the dissolution of lead carbonates. Since lead carbonates are rather insoluble, however, very little $\text{Pb}^{2+}$ exists free in the water under most conditions. Soluble lead can be absorbed by living organisms, notably algae (Malanchuk and Gruendling 1973; Wollery and Lewin 1976), and concentrated biologically as it passes up the food chain. The majority of the lead incorporated into organic material is deposited in the sediments upon death of the organisms; a portion may be released,
however, as the result of biological decomposition and thus be recycled into the aquatic system.

**Cadmium**

In nature cadmium is present in extremely low concentrations as the product of the weathering of rocks and is not normally of environmental concern. Human activities, however, have led to the concentration of cadmium and cadmium compounds and their ultimate release into the environment. Cadmium is considered to be among the most toxic of naturally-occurring substances. Bioaccumulation of cadmium and bioconcentration in the food chain are well known and have caused serious concern, especially in the areas of environmental health and toxicology (Friberg *et al.* 1971; Cook 1977). Excellent reviews of the biomedical aspects of cadmium in the environment are provided by Flick *et al.* (1971) and Friberg *et al.* (1971).

The contamination of the environment with cadmium has been largely the result of activities associated with mining and refining of metals, particularly lead, copper, and zinc (Leland *et al.* 1974, 1975; Gale *et al.* 1973). Cadmium is found in association with zinc in calamine and zinc blende (Cook 1977). During the process of zinc extraction, the more volatile cadmium may be lost as metal oxide fumes from the smelters (Buchauer 1973).

Industries employing cadmium compounds in alloys, plastics and paints may contribute cadmium via their sewage effluents. Cadmium sulfides and selenides also are used as pigments in paints. Consequently, effluents from industries producing cadmium-containing paint pigments may contribute to cadmium loading of the environment (Cook...
Other sources of environmental cadmium contamination include fossil fuel power plants (Klein and Russell 1973), phosphate fertilizers (Williams and David 1976; Schroeder and Balassa 1963), sewage sludge soil amendments (Linnman et al. 1973), and motor vehicles (Burton and John 1977; Lagerwerff and Specht 1970).

**Cadmium in the air and deposition.** Data for cadmium concentrations in the air are quite incomplete, and results from one area to another, or from one time to another, show considerable variation. Many locations in the U.S. were reported to have zero cadmium in the air, probably as the result of insensitive analytical techniques (Friberg et al. 1971). Kneip et al. (1970) reported that lower Manhattan had a yearly mean of 0.023 μg • m⁻³, compared to 0.003 μg • m⁻³ in a non-urban site. Friberg et al. (1971) states that weekly means of 0.3 μg • m⁻³ were found 500 m from a factory in Sweden employing cadmium alloys, and at 100 m distance a monthly mean of 0.6 μg • m⁻³ was found; in the center of Stockholm weekly means were 0.005 μg • m⁻³, as opposed to 0.0009 μg • m⁻³ rural areas isolated from cadmium-emitting factories.

The Public Health Service (1966) reported minimum values of over 0.3 μg • m⁻³ at El Paso, Texas, over the period of 1960-64. Most of the cadmium in the air is associated with particulate matter. Deposition of cadmium is therefore associated particularly with precipitation and dry deposition. Buchauer (1973) pointed out that metal oxide fumes escaping from zinc smelters were responsible for heavy metal contamination of soils and vegetation up to 1 km distant and that contamination entered the environment as aerosals. Aerial deposition of cadmium at
Gary, Indiana, varied with season, rainfall, and location and ranged from < 0.020 to 0.297 mg · m⁻² mo⁻¹ (Peyton et al. 1976). It becomes immediately apparent that the level of cadmium in the air, as well as its rate of deposition, is directly a function of the distance from the cadmium-emitting source.

**Cadmium in the soil.** Cadmium enters the soil primarily through dry deposition of particulates and from cleansing of the air by precipitation. Both routes of cadmium entry into the soil are dependent upon the extent of industrialization, automotive traffic, and proximity to the polluting source. In unpolluted areas, cadmium levels in the soil are generally < 1 ppm (Schroeder and Balassa 1963). Burton and John (1977) reported cadmium levels of approximately 0.24 ppm in uncontaminated rural areas and 5 ppm in urban areas of the Rhondda Fawr, South Wales; depth profiles indicated that most of the soil cadmium was located in the top 5 cm of the soil profile. Buchauer (1973) reports that cadmium levels in the soil within 1 km of two zinc smelters in Palmerston, PA, were as high as 1750 ppm, and, at about 20 km distance from the smelters, levels were 5-7 ppm, and were considered normal background levels. Lagerwerff and Specht (1970) reported cadmium levels in roadside soils to be in the range of 0.90 to 1.82, 0.40 to 1.51, and 0.22 to 1.02 ppm for the upper 5 cm of soil at 8, 16, and 32 meters distant from the road, respectively. They attributed the elevated soil cadmium levels to motor oils, diesel oils, and automobile tires, which contained from 0.20 to 0.26 ppm, 0.07 to 0.10 ppm, and 20 to 90 ppm, respectively. An important source of soil cadmium contamination in agricultural areas has come from the use of inorganic phosphate fertilizers.
Williams and David (1976) stated that present-day fertilizers contain 0.00042 units of cadmium per unit of phosphorus and that about 80 percent of this is retained by the cultivated layer. Several Australian soils that were investigated contained 0.055, 0.024, 0.030, 0.114, 0.033, and 0.016 ppm cadmium in unfertilized areas and 0.085, 0.303, 0.270, 0.342, and 0.076 ppm cadmium in adjacent fertilized fields, respectively.

Cadmium in water. Cadmium in unpolluted natural waters is normally present in only extremely small quantities, and values of less than 1 ppb have been reported (Friberg et al. 1971). For sea water, Purves (1977) reported a mean value of 0.1 ppb. In a study of 136 Norwegian lakes Henriksen and Wright (1978) reported values of 0.1 to 0.5 ppm. Untreated Canadian drinking water supplies contained from < 0.01 to 1.13 ppb, with most values in the range of 0.01 to 0.02 ppb (Meranger et al. 1979). Taylor (1963) noted cadmium levels as high as 10 ppb for U.S. drinking water supplies. Data compiled for 1962-63 by the U.S. Public Health Service showed cadmium concentrations in U.S. drinking water supplies averaging 0.12 ppb in New England (maximum reported was 0.3 ppb), 1 to 10 ppb in New York City, 11.2 ppb in the South Central States, 6 to 20 ppb in Los Angeles, 0.8 and 8 ppb from two water plants in Birmingham, and a high of 30 ppb recorded for ground water at Galveston, Texas. In general, ground waters used for drinking water supplies contained the highest levels of cadmium. Many of these values are considerably in excess of the 10 ppb established by the World Health Organization and by the Department of Health, Education and Welfare as the maximum safe
level allowable for cadmium in drinking water (U.S. Dept. of Health, Education and Welfare 1962). The major sources of elevated cadmium levels have been discussed previously, but an additional source should be mentioned for cadmium in water supplies. Friberg et al. (1971) pointed out that, in addition to other sources, metal and plastic pipes used to deliver water to the consumer may contribute significantly to cadmium in drinking water.

Although a large portion of the cadmium entering surface waters is associated with particulate matter (Elzerman et al. 1979; Florence 1977; Gardiner 1974a,b), a substantial portion of the cadmium in water is present as the free (i.e., uncomplexed) dissolved Cd\(^{2+}\) ion (Florence 1977; Gardiner 1974a). The proportion of free Cd\(^{2+}\) ion increases with decreased pH. Humic substances, followed by carbonates, account for most of the complexation in natural waters. In relatively pure ground water, most cadmium is present as free ion, which would explain why, unlike copper, cadmium toxicity to living organisms is relatively independent of water hardness (Gardiner 1974a). The ultimate destiny of cadmium in water is to become bound by inorganic and organic matter and, consequently, to be precipitated in benthic sediments. Under anaerobic (i.e., reducing) conditions, however, a substantial proportion of the bound cadmium may become chemically available due to low pH (Henriksen and Wright 1978) and be absorbed by organisms, macrophytes in particular. This biologically-absorbed cadmium could then be recycled into the water upon the death of these organisms (McIntosh et al. 1978; Mayes et al. 1977). Cadmium has been reported in Florida waters by the U.S. Geological Survey (USGS 1979). As for lead, the range for cadmium varies greatly from
area to area and with proximity to urban areas. The ranges reported for suspended, dissolved and total cadmium were 0 to 32, 0 to 20 and 0 to 38 ppb, respectively, for surface waters, and were 0 to 10, 0 to 23 and 0 to 57 ppb, respectively, for ground waters. A similar relationship existed as for lead, with respect to the association of high levels in surface waters and ground waters with urban areas and urban landfill, respectively.

Cadmium in sediments. Levels of cadmium in benthic sediments vary widely from area to area, depending upon background levels in minerals and soils in the watershed and input from anthropogenic sources. Mayes et al. (1977) reported values ranging from 0.48 ppm to 3.76 ppm in an uncontaminated lake and 88.40 to 125.3 ppm in a contaminated lake, both located in Indiana. Similar values have been reported by McIntosh et al. (1978) for the eutrophic Palestine Lake, Indiana, where the upper 5 cm had up to 2.54 and 805 ppm, respectively, for uncontaminated and contaminated areas of the lake. At a depth of 30 cm in the sediment profile, this contamination was still evident: the average cadmium concentration was 165 ppm, as opposed to an average of only 0.49 in the uncontaminated site. Enhanced cadmium levels in the sediments have been clearly shown to be the result of enrichment from anthropogenic sources both in freshwaters (Iskandar and Keeney 1974; Mathis and Kevern 1975; Walters et al. 1974) and in marine waters (Moyer and Budinger 1974, cited by Leland et al. 1975).

Some disagreement exists in the literature as to the form in which cadmium is present in sediments. McIntosh et al. (1978)
maintain that about 80 percent of the cadmium is present as carbonates, either as CdCO₃ or coprecipitated with CaCO₃, and that the precipitation of CdCO₃ and coprecipitation with CaCO₃ may determine the solubility of cadmium in the system. They further state that about 10 percent of the cadmium is present as sulfates and that < 5 percent of cadmium in the sediments is organically bound. On the other hand, organic material has been suggested as being responsible for the adsorption of cadmium (Gardiner 1974b). As indicated by Florence (1977) and Gardiner (1974a), a large portion of the cadmium can be expected to be available as free Cd²⁺ ion, especially under conditions of low pH (Henriksen and Wright 1978; C. P. Huang et al. 1977), which would be expected frequently in benthic sediments. Consequently, it seems illogical that 80 percent of the cadmium in sediments would be bound as carbonates. The apparent discrepancy is likely the result of differing local conditions under which the studies have been made and the use of different analytical techniques. Very likely the form in which cadmium occurs in benthic sediments (and, hence, its availability for recycling in the system) eventually will be shown to depend upon a myriad of local conditions, including total cadmium input into the system, water hardness, presence of other cations and anions, soil type of the watershed area, organic content of the sediments, biotic influences, and many other factors.

Data for cadmium in sediments in inland waters of Florida have been reported (USGS 1979). Few sediment analyses were done, and, at all stations where sediment cadmium was measured, values were reported as < 10 μg · g⁻¹. All sediment analyses which included cadmium were from the large urban areas in South and Southeast Florida.
Copper

Naturally occurring copper in the environment is produced largely as a by-product of the weathering of shales and igneous rocks (Bowen 1966) and is of special concern in the environment because of its essentiality in plant and animal nutrition and also because of its potential toxicity to living organisms when present in greater than trace amounts. Bowen estimates the mean copper concentration in the earth's crust to be about 50 ppm. Beneficial effects of copper may be found in texts on nutrition (Epstein 1973) or biochemistry (Lehninger 1975; Bonner and Varner 1976). Toxic levels of copper in the environment are largely the result of enrichment from anthropogenic sources. Major sources of elevated environmental levels of copper include effluents from industrial complexes, mining operations, and sewage treatment plants, and airborne emissions from metal smelters (Buchauer 1973), fossil fuel power plants (Klein and Russell 1973), and industries utilizing metals in their products (Peyton et al. 1976; Cook 1977). Copper has been used extensively in fungicides and copper compounds have been widely used for herbicidal control of algae and aquatic macrophytes (Wagemann and Barica 1979).

Copper in the air and deposition. Data on heavy metal contamination of the air contain little information concerning the relative contribution of copper to air pollution. In general, the sources of copper in the air are the same as those discussed previously for lead and cadmium. Jacobs (1960) states that maximal urban air loadings
of copper are about 4.68 μg · m⁻³; analyses indicated further that copper levels in the air of large cities (over 2 million) were in the range of 0.13 to 1.34 μg · m⁻³, 0.01 to 0.60 μg · m⁻³ for cities of 500,000 to 2 million, 0.04 to 0.31 μg · m⁻³ for small cities, and < 0.01 to 0.28 μg · m⁻³ in non-urban areas. Klein et al. (1975) reported that the inlet fly ash at a coal-fired power plant contained up to 140 ppm copper, but he made no statement concerning the downwind air levels of copper contributed by the plan. Studies of surface contamination of mosses from areas in which the only known source of heavy metals was aerial deposition have pointed out that aerial deposition can contribute substantially to elevated copper levels in plants (Pakarinen and Tolonen 1976).

Copper in the soil. Copper occurs as a natural component of the soil as a product of the weathering of copper-containing minerals and varies considerably with the type of parent material. The ferromagnesium minerals such as pyroxene and biotite generally have copper concentrations averaging 140 ppm; sand stones, shales, and marine black shales may contain 10 to 40 ppm, 30 to 150 ppm, and 20 to 300 ppm, respectively (NAS 1977). Uncontaminated soils contain an average of about 20 ppm copper, and agriculturally productive soils may contain from 1 to 50 ppm (NAS 1977).

In the soil, copper exists in a number of forms. Bowen (1966) indicated that copper is strongly adsorbed by humic materials and that a substantial portion of the soil copper would be bound in the organic fraction. C. P. Huang et al. (1977) stated that adsorption on soil minerals is the most important process in regulating the
concentration of copper in a soil-water environment. Humic materials are colloidal in nature and are bound strongly to clays and hydrous oxides. Copper is found in the soil-soil solution association primarily in the forms of hydroxides, carbonates, sulfates, and sulfides. The availability of copper and the particular copper species present is greatly dependent upon soil pH and the presence of anions (C. P. Huang et al. 1977).

Anthropogenic copper enrichment is responsible for the vast majority of high and/or potentially toxic levels of copper in the soil and is related primarily to the aerial deposition of particulate copper aerosols from power plants, metal smelters, and industries employing metals. Klein and Russell (1975) noted that soils around a power plant had 4.6 ppm copper, compared with 2.8 for unenriched soils. Burton and John (1977) found that mean levels of copper were 10 to 20 times higher in urban than rural soils and indicated that aerial deposition, possibly from coal as a heating source, was the primary source. Copper levels in the upper 5 cm of the soil profile ranged from approximately 150 to 375 ppm in the contaminated urban area, whereas, in an uncontaminated rural area, the upper 5 cm contained a maximum copper concentration of about 8 ppm (within the range of background levels of 5 to 15 ppm estimated from deep cores). Some correlation was found between soil copper levels and proximity to roads, but the relationship was not as clear as for lead and cadmium. Buchauer (1973) reported that soil copper levels in the vicinity of a zinc smelter was dependent upon distance from the smelter. The A_1 horizon as far as 1 km from the smelter contained 600 to 1200 ppm copper, and the O_2 horizon in one case contained
2000 ppm. Background levels in the O₂ horizon ranged from 27.5 to 32.8 ppm and were attained only at distances of 12 to 19 km from the smelters. The above data clearly show that the contribution of copper from anthropogenic sources is the primary cause of elevated copper levels in the soil.

Other sources of elevated copper in the environment are the use of sewage sludge as a nutritive soil amendment and the employment of copper-containing fungicides in agriculture. Bowen (1966) states that about $7 \times 10^7$ kg of copper is applied for fungicidal purposes each year. Total copper content of a sludge investigated by Neuhauser and Hartenstein (1980) was 973 ppm, which could contribute significantly to soil copper content.

**Copper in water.** Copper is present in natural waters only in extremely low concentrations. Background levels for unpolluted fresh waters are estimated to be in the vicinity of $\leq 10.0$ ppb (Bowen 1966). Henriksen and Wright (1978) reported values of 0 to 2.0 ppb as background levels for uncontaminated Norwegian Lakes. In a survey of major rivers and streams in North America, median copper content was reported to be 5.3 ppb with a range of 0.83 to as high as 105 ppb (Durhum and Haffty 1963). Values of 1 to 9 ppb were found for marsh waters in Lake St. Clair, Ontario (Mudroch and Capobianco 1978). A recent study of Canadian drinking water supplies reported a median copper level of 20 ppb, with a range of 5.0 to 620 ppb (Meranger *et al.* 1979). Ocean waters reportedly contain an average copper level of 0.003 ppm (Bowen 1966; Purves 1977) with a normal range of 0.001 to 0.01 ppm (Durhum and Haffty 1963). Montgomery and Price
(1979) stated that about 2.1 ppb Cu/l is particulate-associated, whereas, approximately 1.9 ppb is soluble copper.

Copper contamination of surface waters has been shown to originate almost exclusively from anthropogenic sources (Leland et al. 1974, 1975). The major sources of enhanced copper levels in surface water are aerial input of particulate matter from urban, industrialized areas (Elzerman et al. 1979), municipal and industrial waste effluents (Namminga and Wilhm 1977), mining and smelting activities (Besch and Roberts-Pichette 1970; Pasternak 1974; Morishima and Oka 1977), and, more recently, the use of copper compounds for the treatment of algae and aquatic weeds (Chancellor et al. 1958; Toth and Riemer 1968).

In general, analyses of polluted waters have demonstrated that dissolved copper concentrations are often in the ranges described previously for supposedly uncontaminated waters. Ernst and Marquenie-van der Werff (1978) reported copper levels for polluted ditches in the Netherlands, ranging from 12.7 to 31.8 ppb at the source to 6.4 ppb at 1 km distance from the source. Data reported by Besch and Roberts-Pichette (1970) for a copper and zinc mining area in northern New Brunswick were 0.33 to 12.1 ppm at a station adjacent to the mining area and decreased downstream to < 0.0001 to 0.034 ppm. These data represented minimum and maximum values for copper from samples collected at irregular intervals. Part of the problem might be explained by the tendency for water-borne copper to be associated with suspended particulate matter (Pasternak 1974), much of which would very likely be deposited near the contaminated effluent. Tuschall (1978) noted that a substantial portion of copper in the aquatic environment was complexed with proteinaceous matter. Sylva (1976) and Wagemann and
Barica (1979) point out that, even when high levels of total copper are present, the processes of precipitation, adsorption hydrolysis, and chemical complexation would often be sufficient to reduce the free dissolved copper to low levels. Sylva (1976) noted further that the concentration of free copper as $\text{Cu}^{2+}$ was almost negligible above pH 7.5 and that the precipitation of copper was a function of the pH and the amount of bicarbonate present in solution. The precipitation of $\text{Cu}_2(\text{OH})_2\text{CO}_3$, $\text{Cu}_3(\text{OH})_2(\text{CO}_3)_2$, and $\text{Cu}(\text{OH})_2$ from a solution containing 2 ppm total copper began at pH 6, 6.2, and 6.8, respectively. Wagemann and Barica (1979) further pointed out that in hardwater lakes less than 0.5 percent of the total dissolved copper would be present as free cupric ion, $\text{Cu}^{2+}$. Consequently, water analyses, without concomitant studies of sediments and effluent loading, would appear to be of limited value in assessing the extent of copper contamination in a given body of water (Pasternak 1974).

Copper levels in Florida surface and ground waters have been recorded by the U.S. Geological Survey (USGS 1979). Values reported for suspended, dissolved and total copper ranged from 0 to 8, 0 to 285, and 0 to 55 ppb, respectively, for surface waters, and from 0 to 4, 0 to 39, and 0 to 130 ppb, respectively, for ground water. The apparent discrepancy for total copper in surface water was the result of no available data for total copper from the station at which dissolved copper was reported to be 285 ppb. The association of high levels of copper in surface and ground waters with urban areas and urban landfills, respectively, was similar to that reported for lead and cadmium.

Copper in sediments. Benthic sediments, the ultimate repository for copper in the aquatic system (McIntosh 1975), contain varying levels
of copper depending upon background levels in rock and soil from the
drainage basin, groundwaters, and the extent of anthropogenic enrich-
ment via polluted waters, erosion of contaminated soils, and the
aerial deposition of dusts, smoke particles, and aerosols into the
water. Background copper levels approaching a mean value of 25 ppm
have been estimated from sediments in unpolluted waters and from
deep-core samples in culturally enriched waters. Pasternak (1974)
found sediment copper concentrations varying from 4.0 to 26.5 ppm
in the relatively uncontaminated portions of the River Biala Przemsza
and tributaries in Poland. Values of 10 and 19 ppm have been
reported for the Mississippi and Illinois (Mathis and
Cummings 1971) Rivers, respectively, in the United States, and 24
and 28 ppm for the Rideau and Ottawa Rivers, respectively, in
Canada (Oliver 1973). A very low value of 2 ppm was reported for
Skeleton Creek, Oklahoma (Namminga and Wilhm 1977). Similar values
were reported for uncontaminated lake sediments. In a survey of
ponds in several areas of Poland, Oporowska (1976) reported copper
concentrations of 8 to 25 ppm in uncontaminated sediments. McIntosh
(1975) cited a mean value of 27.68 ppm for two ponds in Michigan.
In a study of Lake St. Clair, Ontario, Mudroch and Capobianco (1978)
found copper levels varying from 7.0 to 43.7 ppm, with only a single
station containing > 21.0 ppm. Hamilton-Taylor (1979) found 22 to
25 ppm copper in the 35 to 84 cm levels of cores taken from Windermere.

Copper in sediments from culturally enriched water was generally
much higher than these "background levels." Pasternak (1974) reported
copper levels of 56 and 128 ppm for two contaminated areas in the River
Biala Przemsza system in Poland. Morishima and Oka (1977) found that
soils in rice fields irrigated with water polluted by drainage from a copper mine had an average copper concentration of 218.3 ppm (range, 150 to 310 ppm) at the irrigation inlet and 63.0 ppm (range 47 to 106 ppm) at the outlet. Oporowska (1976) found contaminated sediments in a number of ponds in Poland to contain 175 to 535 ppm copper, depending upon promixity to a source of copper pollution. Hamilton-Taylor (1979) found that the surface two centimeters contained from 63 to 140 ppm copper and that the upper 20 cm varied from about 45 to 140 ppm copper in sediments from Windermere. He stated further that biological uptake appeared to be important in the depositional process. A certain amount of recycling of sediment copper appears to occur. C. P. Huang et al. (1977) noted that the action of microorganisms can modify the redox potential of the sediments and, thus, influence substantially the solubility of metals in the sediments. Uptake of copper from sediments by aquatic macrophytes and subsequent release from the plants may also contribute somewhat to the recycling of sediment-bound copper (McIntosh 1975).

Sediment analyses also demonstrated the presence of copper, primarily in streams and canals in the southern parts of Florida. Sediment concentrations of copper ranged from < 7 to 40 μg · g⁻¹ (USGS 1979). The higher levels reported were from the canals and might have been partially related to the use of copper compounds for aquatic weed control and copper-containing fungicides employed on agricultural crops, as well as to other sources, such as domestic and industrial sewage effluents and possible leaching from copper-containing parent materials in recently excavated canals.
Physiological Aspects of Lead, Cadmium, and Copper Contamination on Aquatic Plants

Physiological studies of the effects of lead, cadmium, copper, and other heavy metals on vegetation have been concerned largely with plants of economic importance, particularly agronomic and vegetable crops. The contamination of crops with sewage sludge used as a source of nutrients and from the employment of municipal sewage effluents in irrigation have been well documented. With increasingly higher levels of environmental pollution, concern has been expressed over the levels of toxic substances in the environment and the potential human health hazards from exposure through direct contact and dietary intake as the result of biological concentration of these substances in the food chain.

In the aquatic environment, the majority of plant studies conducted have involved algae. As a primary link in the aquatic food chain, algae have been studied with special regard for their ability to concentrate toxic substances and, thereby, to pass them on to higher trophic levels. Their potential use in the purification of sewage and as indicators of pollution is also of great interest. In recent years, however, more attention has been paid to the uptake of metals by aquatic vascular plants, especially from the standpoint of their use as biological filters in the purification of tertiary sewage effluents. Copper has been of special interest because of its toxicity to plants and, therefore, its herbicidal properties. Consequently, the vast majority of the information concerning copper in aquatic plants has dealt with the use of copper as an aquatic herbicide, either alone, or in conjunction with other herbicidal compounds.
The anatomy, physiology, and biochemistry of aquatic vascular plants, with some exceptions among the submersed species, are generally considered to be essentially the same as for terrestrial plants. Perhaps the major difference between the aquatic species and their terrestrial counterparts is that aquatic species are rarely subjected to the water stresses of the terrestrial environment. Furthermore, a large part of our knowledge of the physiology of terrestrial plants has been derived from studies of algae. Consequently, any treatment on the subject of the physiological effects of heavy metals upon aquatic vascular plants must, out of necessity, include numerous references to the algae and to terrestrial plants. No attempt will be made to review the literature on algae and terrestrial plants, and citations will be limited to those of apparent value in relation to studies of higher aquatic plants. Studies involving ion absorption and heavy metals other than lead, cadmium, or copper will be discussed wherever necessary, but primary attention will be focused upon the literature dealing specifically with these three metals in aquatic vascular plants.

Uptake, Transport, and Partitioning of Lead, Cadmium, and Copper

The apparent effects of heavy metal pollution on aquatic flora were reported as early as 1924. In a study of pollution of the River Rheidol, Cardiganshire, by drainage from lead mines, Carpenter (1924) noted that the macroscopic vegetation was limited to only a few bryophytes and the red algae *Batrachospermum* and *Lemanea*. Later studies (Jones 1958) indicated that effects on the algal flora were still visible long after the closing of the mines. Whitton (1970 noted, however, that from these earlier studies it was difficult to conclude
whether the effects were due to heavy metal contamination or sedimentation. Studies of micro- and macroalgae have shown that the uptake of heavy metals by algae is largely a passive phenomenon depending greatly upon the metal concentration in the environment (Trollope and Evans 1976; Hart and Scaife 1977; Briand et al. 1978). Manganese absorption, however, appears to be at least partially regulated (Morris and Bale 1975). Bowen (1966) reported that algae could concentrate heavy metals by factors of many thousands from solutions containing relatively low concentrations of metals. Reiniger (1977) also reported a concentration in algae. The relative sensitivity of algae to metal contamination follows no apparent taxonomic grouping, and sensitive and resistant strains may be found in all major taxa. Whitton (1973) demonstrated that planktonic gas-vacuolate bluegreen algae are the only known ecological grouping of algae that are sensitive as a whole to heavy metals. For further information on the toxicity of heavy metals to algae the review by Whitton (1970) should be consulted.

Bryophytes. The uptake and concentration of heavy metals by bryophytes has been studied by a number of researchers. McClean and Jones (1975) measured the accumulation of zinc, lead, and copper by bryophytes growing in the polluted Rivers Clarach and Ystwyth in Cardiganshire. Metal uptake was high despite low levels in the water at many locations. Among several species studied, the highest lead levels were found in *Scapania undulata*, *Fontinalis squamosa*, *Eurynchium riparioides*, and *Brachytecium rivulare*, which had maximum accumulations of 14,825, 10,800, 9650, and 8206 ppm, respectively. These same species also contained the highest copper levels. *Scapania*,
generally had the highest metal levels observed, *Fontinalis* was able to concentrate to significantly greater levels from lower metal concentrations in the water. Dissolved nutrients also directly affected the competitiveness of *Scapania* and *Fontinalis*. Under conditions of higher nutrients and lower metals, *Fontinalis* was more competitive than *Scapania*, whereas at higher metal levels and lower nutrients the reverse was true. A regional survey of *Sphagnum* peat mosses (Pakarinen and Tolonen 1976) reported high lead levels from bogs in southern Finland, where *S. balticum*, *S. fuscum*, *S. angustifolium*, and *S. magellanicum* contained 40.5, 38.8, 20.9, and 16.9 ppm lead, respectively. In northern Finland, lead concentrations in *S. fuscum* ranged from 2.5 to 9.1 ppm; and in Canada *S. fuscum* and *S. angustifolium* 0.7 to 7.3 ppm and 1.1 ppm, respectively. Ranges for cadmium and copper were 2.8 to 22.2 ppm and 0.17 to 0.63 ppm, respectively, and were totally independent of either species or regional distribution. In other studies, Ruhling and Tyler (1970, cited by Pakarinen and Tolonen 1976) reported 1 ppm cadmium and a range of 54 to 116 ppm lead in *S. magellanicum* in Swedish bogs. Gorham and Tilton (1972) found mean lead levels of about 23 ppm in *S. fuscum* in Minnesota and Wisconsin. Ruhling and Tyler (1973) reported mean lead and cadmium levels in *Hylocomium* were 11.0 and 0.18 ppm, respectively, in northern Finland. In southern Finland, these values were reported to be 32.0 and 0.49 ppm, respectively. The available data suggested that elevated metal levels in peat mosses were closely associated with the degree of urbanization of the respective regions and that peat mosses might serve as indicators of the extent of metal pollution on a regional and on a world-wide scale (Pakarinen and Tolonen 1976).
Vascular plants. Most of the research related to the uptake of heavy metals by vascular aquatic plants has been done in the last decade. A large number of the publications deal with emergent and free-floating plants, especially waterhyacinths, and are concerned largely with their potential for use in the purification of polluted waters and sewage effluents. The ability of vascular plants to remove nutrients and trace pollutants from water has been well documented (Sheffield 1967; Steward 1970; Boyd 1970, 1976; Rogers and Davis 1972; Wolverton 1975a,b,c; Wolverton and McDonald 1976, unpublished manuscript; Wolverton et al. 1975a,b; Dinges 1979). As studies of nutrient and pesticide removal are beyond the scope of this review, they will not be treated further except when specifically related to heavy metal uptake by aquatic plants.

The uptake of copper by young waterhyacinth plants was studied by Sutton and Blackburn (1971a). Plants were cultured in 900 ml nutrient solution containing from 0 to 16 ppmw copper. After two weeks, plants grown in solutions containing 0, 0.5, 1.0, 2.5, and 4.0 ppm copper had copper accumulations of 16, 26, 39, 114, and 247 ppm, respectively, in the petioles, and 15, 14, 15, 33, and 69 ppm, respectively, in the leaves. Over a 24 h time period Wolverton et al. (1975a) found that waterhyacinths were able to remove 0.176 mg lead and 0.67 mg cadmium per g dry weight and alligatorweed was able to remove 0.10 mg lead per g dry weight from polluted water. No data were given concerning initial or final water levels of lead and cadmium or volume of water filtered. In related studies waterhyacinths were found to remove as much as 0.67 mg cadmium per g dry weight from river water containing 1.9 ppm cadmium (Wolverton 1975b). Waterhyacinth and alligatorweed
were able to remove 0.192 to 0.216 and 0.087 to 0.101 mg cadmium per g dry weight, respectively from 800 ml river water containing 1 ppm cadmium (Wolverton and McDonald 1975a). The most rapid removal was evident during the first 3 h of the experimental period. Dinges (1978) noted an accumulation of 6.5 mg lead and 8.3 mg copper per kg dry weight by waterhyacinths grown in a wastewater treatment stabilization pond.

The absorption, transport, and partitioning of lead, cadmium, and copper in waterhyacinths was studied by Tatsuyama et al. (1977). Lead was absorbed in the highest amounts, followed by copper and cadmium. Lead content of plants grown for 4 weeks in 1 ppm lead solution was greatest in roots, followed by stems and leaves, with tissue levels of about 60 to 100 ppm, 10 to 20 ppm, and 10 to 15 ppm, respectively. Lead content in roots reached a maximum level after a 1-wk exposure and remained more or less constant thereafter. No data were given for tissue levels of cadmium and copper. Tatsuyama et al. (1979) showed further that absorption of lead, cadmium, and copper by waterhyacinth increased as the concentration of metal in solution was increased. Wolverton and McDonald (unpublished manuscript) noted an accumulation of 6500 ppm lead and 667 ppm cadmium in roots and 92 ppm lead and 60 ppm cadmium in tops of waterhyacinths cultured for 96 h in 10 ppm solutions of the respective metals. Reiniger (1977) reported a similar tendency for cadmium to concentrate in the roots of rice and Montia rivularis. Cadmium accumulation in rice roots was about 40 to 100 times as great as in leaves, whereas, for Montia, the roots contained about 3.5 times more cadmium than did the combined leaves and stems. In rice, the root:leaf cadmium
ratio increased sharply as soil cadmium level was increased, whereas ratios remained fairly constant in Montia. Johnson and Sheenan (1978) found that copper levels in the roots of waterhyacinths increased as copper levels in solution were increased. After 7 weeks in solutions containing 1.0, 2.5, 5, and 10 ppm copper, roots contained 23.2, 30.0, 43.8, and 52.6 μg · g⁻¹ dry weight. Thus, copper accumulation shows a curvilinear response with respect to plant uptake. The response of leaves was quite variable, with no apparent relationship to solution concentration. Thakurta and Mitra (1978) also noted that copper accumulations by waterhyacinths increased with increased rate of application. A study of marshes in Lake St. Clair, Ontario, showed that metal concentrations in Typha latifolia, Lythrum salicaria, and Pontederia cordata followed similar trends; the roots contained about twice as much copper and lead as the above-ground biomass. Dabin et al. (1978) noted similar concentration of cadmium in roots of rice. Root uptake of cadmium was very rapid whereas the translocation to the shoot portion was very slow. Analysis of cellular fractions after ultracentrifugation indicated a large portion of the root cadmium was associated with the non-cytoplasmic fraction, suggesting binding by cell walls and, consequently, with very little entrance of cadmium into metabolic tissues. Montgomery and Price (1979) have indicated a net uptake of lead, copper, mercury, and zinc from sludge by red mangrove roots. Walsh et al. (1979) were unable to demonstrate any significant translocation of lead from the roots of red mangrove seedlings and suggested that the lead might be largely adsorbed to the root surface. Cadmium, however, was mobile. Cadmium levels in stems were approximately the same as for the roots, with considerably
lower levels in the leaves. The uptake of cadmium from solution, with some transport and accumulation in the leaves, has also been noted in alligatorweed (Quimby et al. 1979). Laboratory studies of cadmium accumulation by waterhyacinths further support the generalization that the order of metal concentration by aquatic plant tissue is roots > stems > leaves. Chigbo et al. (1979) reported that waterhyacinths contained slightly greater concentrations of lead and cadmium in the leaves than in stems.

Studies of metal uptake and transport by terrestrial plants have shown that lead tends to be immobilized, at least partially, in the roots and that tissue concentrations are usually in the order of roots > stems > leaves (Jones et al. 1973; Kannan and Keppel 1976; Patel et al. 1977). Similar observations have been made for copper (Wallace and Romney 1977) and cadmium uptake in terrestrial plants (Cutler and Rains 1974; Root et al. 1975; Jarvis et al. 1976), but cadmium has occasionally been reported to concentrate more in the plant tops than in roots (Haghiri 1973). The effect of aerially deposited heavy metals apparently has not been studied with respect to uptake by emergent and floating aquatic plants. The work of Krause and Kaiser (1977) has shown that lead, cadmium, copper, and manganese are absorbed from metal-containing dust on the leaves of terrestrial plants. Lead, cadmium, and manganese were translocated to the roots by radish, whereas copper was immobilized in the leaves.
Although the absorption of nutrients and trace elements by terrestrial plants and emergent and floating aquatic plants is well known, the mechanisms of uptake, translocation, and partitioning are essentially unknown in submersed plants. Sutcliffe (1962) reported that foliar absorption was the predominant route of uptake, while Sculthorpe (1967) contended that the roots were primarily responsible. Denny (1972) concluded that rooted aquatic macrophytes could absorb nutrients from either the water or the substrate, or both. Studies of phosphorus absorption (McRoy and Barsdate 1970; Bristow and Whitcombe 1971; McRoy et al. 1972; Demarte and Hartman 1974) all demonstrated root absorption. McRoy and Barsdate (1970) and McRoy et al. (1972) have demonstrated the absorption of phosphorus by the roots of eelgrass and subsequent transport and release from the leaves. Nitrogen has also been shown to be absorbed and translocated by aquatic plants (Toetz 1974; Nicholas and Keeney 1976). The absorption of iron by hydrilla (Hydrilla verticillata) and subsequent translocation from the roots has been reported by Basiouny et al. (1977). With respect to copper and non-essential heavy metals, however, very little information is available.

Bartley (1969) noted copper concentrations as high as 4820 ppmw in some aquatic plants after applications of copper sulfate in an irrigation canal. Sutton et al. (1970) reported copper residues of 1325 and 2996 ppmw in southern naiad (Najas guadalupensis) and hydrilla, respectively, 3 days after treatment with 1 ppm copper as copper sulfate pentahydrate (CSP). Uptake of copper by hydrilla and southern naiad was most rapid in the first 3 days after treatment and declined thereafter. Uptake of copper by egeria (Egeria densa) continued to be a linear function of time during the 12 days after treatment.
Copper levels of egeria 3, 6, 9, and 12 days after treatment were approximately 2800, 3200, 3600, and 4000 ppmw, respectively. Sutton et al. (1971a) found 84 ppmw copper in the roots and 15 ppmw in the shoots of emersed parrotfeather (Myriophyllum brasiliense) exposed for 1 week to a root application of 1 ppm copper as CSP; and hydrilla exposed to a nutrient solution containing 1 ppm copper accumulated 1680 and 1700 ppm copper after 1 and 2 weeks exposure, respectively. Similar results have been reported in other studies on hydrilla (Sutton et al. 1971b; Sutton and Blackburn 1971a). In parrotfeather (Sutton and Blackburn 1971b) the accumulation of copper in roots was an almost linear function of the solution content during the first week of solution concentrations of 2 to 16 ppm copper. Very little of the copper was translocated to the shoots, however. After 2 weeks, a 4 ppm copper treatment contained 812 ppmw in roots and only 25.5 ppmw in the shoots, compared to a control which contained 14 ppmw in the roots and 14.5 ppmw in shoots. Relatively equal distributions of copper in leaves and petioles has been reported by Riemer and Toth (1969) for spaddddock (Nuphar advena), fragrant water lily (Nymphaea odorata), and white water lily (Nymphaea tuberosa). The accumulation of heavy metals by aquatic plants can vary greatly within a given species and among species (Mudroch and Capobioanco 1978; Ernst and Marquenie-van der Werff 1978). McIntosh (1975) noted that the accumulation of copper depended upon plant species and the growth conditions of the species, and that the plants with the most rapid growth are likely to accumulate the most copper. The influence of pollution upon copper content of submersed plants has also been documented. Ernst and Marquenie-van der Werff (1978) found that elodea (Elodea nuttallii) and coontail (Ceratophyllum
demersum) contained 439.4 and 33.0 ppmw copper, respectively, at the source of pollution, again demonstrating the difference in uptake by different species. Studies on copper and lead uptake in Potamogeton by Peter et al. (1979) showed that copper was translocated readily from roots to shoots, but very little was translocated downward. On the other hand, lead appeared to be readily absorbed by either roots or shoots and immobilized within the tissue, so that little or no transport of lead occurred. The absorption of lead and copper were reported for leaves of turtlegrass (Thalassia testudinum) by Montgomery and Price (1979). Studies on absorption of copper and lead by leaves of Alisma plantago-aquatica indicated significant absorption of both metals by the plant and also demonstrated that a large part of the accumulated metal was actually associated with the bacterial epiphytes (Patrick and Loutit 1977). Mayes et al. (1977) demonstrated that Elodea canadensis could accumulate significant levels of both lead and cadmium from the substrate and that the accumulation of metal was a function of the sediment concentration. Levels in the shoots were attributed to translocation from the roots. With the open box system used in the study there was contact between water and sediments so that dissolution and subsequent uptake from the water may have occurred. Consequently, the contribution of translocation of lead and cadmium from roots to shoots can not be adequately assessed. But, as Peter et al. (1979) have pointed out, very small levels in the water, as the result of dissolution from the sediment-water interface, can be accumulated by the shoots. The uptake of cadmium has also been demonstrated for southern naiad (Cearley and Coleman 1973) and for Nitella flexilis and Elodea canadensis (Kinkade
and Erdman 1975). Nakada et al. (1979) studied the uptake and partitioning of metals by Elodea nuttallii collected from the Sagami River in Japan. Lead was absorbed in greatest quantities by the roots, followed by copper and cadmium. The levels of lead, cadmium, and copper in the leaves were about 4, 7, and 46 percent, respectively, of the levels found in the roots. Although considerably less copper than lead was absorbed by the roots, copper was many times more mobile.

Subcellular localization of heavy metals has been demonstrated in a few studies. Neish (1939) noted that more copper was localized in the chloroplast fraction than in the non-chloroplast fraction of leaves of Elodea canadensis. A study of Elodea nuttallii indicated that, after a 2-h exposure to a 50 μM (3.18 ppm) copper solution, about 55 percent was associated with the cytoplasm, vacuome, and microbodies, 38.1 percent with the cell walls and nuclei, and about 6 percent with the chloroplasts. Electron microscope studies on Potamogeton pectinatus (Sharpe and Denny 1976) have indicated that lead is readily absorbed through the thin cuticle of submersed leaves and is deposited largely in the cell walls as fine granules along the middle lamella and outer wall and as coarse granules in the inner cell wall towards the plasma-lemma. No sign of any transport of lead to the roots was observed after one week. After eight days in a toxic (100 ppm) lead treatment, lead deposits were found in the cytoplasm and along the grana of the chloroplasts.

Relatively few studies of sorption rates of heavy metals have been conducted on aquatic vascular plants, and most of the information available is concerned with waterhyacinths. Wolverton and McDonald (unpublished manuscript) indicated that the rate of removal of lead,
cadmium, and mercury was greatest during the first hour of exposure and decreased rapidly thereafter. After 1 h, lead in solution was reduced from 10 to about 3 ppm; and, after 6, 12, and 96 h of exposure, lead concentrations remaining in solution were about 2, 1.5, and 0.5 ppm, respectively. For cadmium and mercury, after 1 h, solution levels were reduced to about 0.42 ppm and 0.47 ppm, respectively. After 6, 12, and 96 h, metal concentrations were about 0.32, 0.28, and 0.08 ppm, respectively, for cadmium, and 0.26, 0.24, and 0.0 respectively for mercury. Losses from the control solutions were negligible for cadmium, negligible for lead until after 24 h, but substantial for mercury. Lead loss was about 1.5 ppm from 24 to 96 h. About 30 percent of the mercury was lost from the control solutions after 1 h, and, by 96 h, the loss was almost 60 percent. Consequently, any study of uptake of heavy metals of necessity should include reference to loss other than by plant uptake. A similar study on waterhyacinths by Tatsuyama et al. (1977) demonstrated that sorption of lead, cadmium, and copper from 10 ppm solutions was most rapid during the first five minutes of exposure and decreased rapidly thereafter. After a 5-minute exposure, waterhyacinths had removed about 60 percent of the lead, 25 percent of the cadmium, and 45 percent of the copper from solution. After 10 minutes, removal of lead, cadmium, and copper from solution was about 80, 40, and 65 percent, respectively. After 15 minutes waterhyacinths had removed about 85, 45, and 70 percent of the lead, cadmium, and copper, respectively. Solutions were changed at 15 minutes and again at 30 minutes. After each solution change there was a similar, initial rapid uptake during the first five minutes, followed by a slowing in the rate of absorption. The total amount absorbed decreased
progressively each time the solutions were changed. This was consistent with a rapid uptake into root free space (free water and Donnan free space) until saturated, followed by a slower, more constant uptake thereafter as the metals are absorbed into root cytoplasm and transported to other tissues. This has been observed by Kannan and Keppel (1976) for lead absorption by pea seedlings, Cutler and Rains (1974) for cadmium absorption by excised barley roots, and Veltrup (1976) for uptake of copper in excised barley roots. A similar rapid absorption period during the first few hours has also been noted for the absorption of cadmium by *Elodea canadensis* and the macro-alga *Nitella flexilis* (Kinkade and Erdman 1975), copper uptake by *Elodea nuttallii* (Ernst and Marquenie-van der Werff 1978; Marquenie-van der Werff and Ernst 1979) and zinc absorption by *Elodea nuttallii* (Marquenie-van der Werff and Ernst 1979).

Uptake of zinc and copper by leaves of *E. nuttallii* from 50 µM solution (3.18 ppm) occurred in three distinct phases, the first ending at about 25 min, the second beginning at 25 min and ending at 60 min, and the third beginning at 60 min and lasting to 120 min. This initial phase corresponds with uptake into root free space, as has been determined by exchange with Ca²⁺. After the first 25 min, further uptake is non-exchangeable, and uptake occurs in two steps. By use of differential centrifugation, the authors showed that the phase from 25 to 60 min represented accumulation by the cytoplasm and cell organelles, particularly the chloroplasts, and that, after 60 min, further accumulation in tissues, is greater for roots than for leaves. The multiphasic uptake was considered to be indicative of an active, rather than passive, uptake mechanism. Root uptake characteristics were also cited as an indication that the roots of *Elodea* function in ion uptake and that they
are not simply attachment structures. The pattern of initial rapid absorption of heavy metals into plant tissue followed by a slower, more constant rate, thereafter, and the species specificity and concentration dependence observed for terrestrial and vascular aquatic plants has also been shown in both unicellular and macroalgae (Gutknecht 1963; Hart and Scaife 1977; Conway and Williams 1979).

The uptake of heavy metals has been shown to be influenced by a considerable number of other factors besides external metal concentration and time of exposure. Sutton et al. (1971a) reported that 1 ppm copper as an organic copper complex, Cutrine (copper sulfate triethanolamine), more than doubled the uptake of copper by hydrilla 1 or 2 weeks after treatment compared to 1 ppm copper as CSP. The addition of metal chelating agents used in plant nutrition has also been shown to affect uptake and transport of heavy metals. The use of DTPA (diethylene triamine pentaacetic acid) significantly increased the translocation of lead to barley shoots and bush bean leaves at a wide range of environmental lead concentrations, pH, and lime conditions (Patel et al. 1977). The use of the chelators NTA (nitriloacetic acid) and DPTA approximately doubled the leaf cadmium concentration, and EDTA (ethylene diamine tetraacetic acid) increased leaf cadmium by a factor of almost 7 in bush beans supplied with 150 μg cadmium per g soil.

The effect of pH upon the uptake of copper by aquatic angiosperms has been examined for Elodea nuttallii by Ernst and Marquenie-van der Werff (1978). In the pH range of 5.5 to 8.5 maximum uptake occurred at pH 5.5, with a rapid decrease in uptake as pH was increased to 7.0. From pH 7.0 to 8.5 copper uptake decreased only slightly. Tatsuyama et al. (1977) noted that very low pH or very high pH inhibited the
rate of absorption of lead, cadmium, and copper. Maximum inhibition occurred at pH 9, probably due to the limited solubility (hence, limited availability) of these metals at high pH. At pH 3 an approximately 50 percent inhibition occurred after 5 min, and no further absorption had occurred after 20 min. Maximum availability and uptake of cadmium occurred in the range of pH 5 to 7. The removal of cadmium from solution by Chlorella has been shown to be substantially greater at pH 7 than at pH 8 (Hart and Scaife 1977). Uptake of zinc by the benthic marine macro-algae Porphyra, Ulva, and Laminaria, however, increased as the pH was increased from 7.3 to 8.6. Studies on barley and bush beans have indicated pH dependent uptake of lead from soil (Patel et al. 1977). In the absence of chelators, lead uptake increased slightly as pH decreased from 7.8 to 2.3. The presence of DTPA completely reversed the pH effect on lead accumulation. In the presence of DTPA, accumulation in leaves was 25 and 36 fold higher for barley and bush beans, respectively, at pH 8.5 than at pH 4.0. Closely related to the effect of pH is the effect of anaerobiosis upon metal uptake. Bingham et al. (1976) noted that the availability of soil-applied cadmium to rice was less under flooded than non-flooded culture conditions. The authors suggested that, under the conditions in flooded soil, sulfate ion was reduced, resulting in the precipitation of CdS. Also closely related to pH are the effects of water hardness components. Kinkade and Erdman (1975) found that the uptake of cadmium by Elodea and Nitella was considerably greater in soft water than in hard water, although the initial rate of uptake was somewhat more rapid in hard water than in soft water. No mention was made of the possibility that precipitation of cadmium as carbonate
or coprecipitation with magnesium and calcium carbonates may have rendered the cadmium unavailable.

Most of the studies of heavy metal uptake have been conducted in the laboratory under static water conditions. Tatsuyama et al. (1979) investigated the effects of stirring and bubbling of the culture solution upon the sorption of lead by waterhyacinths. The sorption rate of lead during the first 30 min was directly proportional to the rate of bubbling or stirring, and sorption rate increased as the rate of bubbling or stirring was increased. This suggests that, in nature, the rate of removal of metals might be enhanced under flowing water conditions.

Another variable that apparently has not been compared in studies of aquatic plants is the effect of solution culture vs soil-culture upon the uptake of heavy metals by a given plant species. The binding capacity of soils, sediments, and organic components for heavy metals is greater than that of a water solution and markedly influences the amount of available metal for absorption by aquatic plants. Studies on terrestrial plants grown in solution culture and in soils amended with heavy metals have demonstrated quite clearly that high total metal concentration in the soil may not be reflected in the concentrations of metal in plant tissues. Plants grown in solution culture, however, tend to show metal accumulation that is more dependent upon solution level (Patel et al. 1977; Wallace et al. 1977b; Wallace and Romney 1977).

The influence of temperature on the rate of sorption of heavy metals has been studied by Tatsuyama et al. (1977). Increasing temperature from 10 to 20 C slightly increased the initial absorption rate of
cadmium and copper by waterhyacinth but had no effect on the absorption of lead until after the first five min of exposure. A temperature of 60 C greatly increased lead and cadmium absorption and slightly increased the absorption of copper. After 10 min, however, there was very little difference in the amounts of lead absorbed at either 20 or 60 C. At 60 C, cadmium absorption was greatly enhanced, whereas copper absorption was considerably depressed. Absorption of cadmium by Chlorella was more than 20-fold greater at 27 C than at 4 C (Hart and Scaife 1977). Of obvious importance, particularly for submersed plants, are the effects of temperature on desorption of heavy metals from the plant. This has been observed for marine macro-algae by Gutknecht (1963) and should be mentioned with regard to the potential recycling of heavy metals by aquatic plants. The rate of loss of Zn\textsuperscript{65} from Porphyra was enhanced by increasing temperature from 1 C to 11 and 25 C. Most rapid loss occurred at 25 C during the first 12 h, and, after 12 h, the rates at all temperatures were approximately the same. Studies on terrestrial plants have provided conflicting results. Kannan and Keppel (1976) found no significant difference in the absorption of lead and subsequent transport to the shoots of pea seedlings at either 15 or 25 C and, consequently, concluded that the uptake of lead must be largely nonmetabolic. Arvik and Zimdahl (1974), however, noted that lead uptake by beans, corn, and soybean was about 50 percent greater at 24 C than at 4 C. Cutler and Rains (1974) have shown temperature-dependent uptake of cadmium by excised barley roots. Root tissue was exposed for 30 min to a 10 ppm cadmium solution, followed by a 30 min desorption period. Absorption of cadmium by fresh roots or air-dried roots increased linearly as
temperatures were increased from 3 to 50 C, suggesting that cadmium sorption by barley is nonmetabolic in nature.

The effect of light upon heavy metal uptake has been studied in algae. Gutknecht (1963) noted that light enhanced the uptake of Zn\textsuperscript{65} by Porphyra at pH 7.3 and 8.6. Light had only a slight effect upon desorption of Zn\textsuperscript{65} from Fucus, Porphyra, or Ulva. Hart and Scaife (1977) found no accumulation of cadmium in the dark by Chlorella, and, therefore, suggested that cadmium uptake in the light was an active process requiring the expenditure of energy. Conway and Williams (1979) reported light enhancements of cadmium uptake in the diatom Asterionella formosa but no enhancement in a closely related (phylogenetically and ecologically) species, Fragilaria crotonensis.

The interactions among heavy metals and other ions in solution affecting metal uptake by aquatic plants have not been studied extensively. Tatsuyama et al. (1977) stated that sorption of lead, cadmium, or copper by waterhyacinths was inhibited by the presence of one or more of the other metals in solution. Wolverton and McDonald (unpublished manuscript) claimed, however, that there was no significant difference in the absorption of lead, mercury, or cadmium by waterhyacinth when in combination. Tatsuyama et al. (1979) reported that the presence of phosphate depressed the sorption of lead by waterhyacinths, but attributed the decrease in lead uptake to a decrease in available lead solution. The presence of phosphate or nitrogen had no significant effect upon the sorption of cadmium or copper. Hart and Scaife (1977) demonstrated that the alga, Chlorella pyrenoidosa, was unable to accumulate cadmium in the presence of 0.2 ppm manganese, but calcium, magnesium, molybdenum, zinc, or copper in the culture medium had no effect upon cadmium
uptake. The presence of iron at 120 μM also blocked cadmium absorption by Chlorella. The presence of zinc in the culture medium significantly reduced cadmium uptake by Euglena gracilis (Nakano et al. 1978). In terrestrial plants the absorption of lead by pea seedlings was significantly reduced by increasing the concentrations of Ca$^{2+}$, Mg$^{2+}$, or K$^+$ in the medium (Kannan and Keppel 1976). The presence of lead in the soil enhanced the uptake of cadmium by corn shoots, but cadmium in the soil reduced lead uptake (Miller et al. 1977). The presence of CaCO$_3$ in the soil greatly reduced the uptake of cadmium by corn (Wallace et al. 1977), probably through a pH effect on the availability of cadmium in the soil. The presence of zinc and/or copper decreased the absorption of cadmium by bush bean in solution culture (Wallace and Romney 1977).

**Effects upon Some Basic Metabolic Functions in Higher Aquatic Plants**

The effects of lead, cadmium, and copper contamination upon the physiological and biochemical processes of higher aquatic plants are largely unknown. Consequently, the following discussion will refer frequently to information gained from studies of algae and terrestrial plants that may be applicable to higher aquatic plants with respect to nutrient relations, transpiration and water relations, photosynthesis, and respiration.

**Nutrients.** Sutton and Blackburn (1971b) noted that phosphorus in both shoots and roots of parrotfeather decreased after treatments with 2 and 4 ppm copper as CSP. Treatment with 1 ppm copper as CSP and 0.5 or 1 ppm copper as Cutrine caused a significant reduction in phosphorus content of hydrilla (Sutton et al. 1971a). Johnson and
Sheenan (1977) found no apparent effects of 1 to 10 ppm copper upon foliar nitrogen, potassium, magnesium, or calcium content and variable effects upon phosphorus, zinc, and manganese levels after 5 weeks growth. Dabin et al. (1978) noted that cadmium increased the level of zinc in leafy shoots of rice but found no consistent effect upon other tissues. Data presented by Nakano et al. (1978) showed a decrease in zinc in Euglena gracilis grown with 20 ppm cadmium. The diatom, Asterionella formosa, exposed to > 10 μg cadmium · l⁻¹ showed an enhanced phosphate utilization initially, but after 20 to 30 h exposure microelement utilization ceased (Conway 1978). In another study Conway and Williams (1979) reported that utilization of phosphorus was enhanced by low cadmium concentrations of (0.05 to 0.4 μg · l⁻¹) and depressed by higher cadmium concentrations (1.8 to 8.5 μg · l⁻¹). The confusion as to the effects of cadmium on nutrient uptake also extends to the terrestrial plant literature. Turner (1973) indicated that cadmium enhanced this uptake of zinc by plant tops of several vegetables and suggested that this could be a result of root damage in the presence of cadmium. Root et al. (1975) reported a decrease in zinc concentration and an increase in iron in corn exposed to cadmium. The supposed cadmium-induced iron-deficiency chlorosis of the corn plants were shown not to be the result of iron deficiency in the tissues, and an increase in the Fe:Zn ratio was proposed as a possible causal factor of cadmium toxicity in corn. The uptake of potassium by oats was depressed by a 1 μM CdSO₄ solution (Keck 1978). Keck also found inhibition of a membrane-bound, Mg²⁺-dependent, K⁺-stimulated, acid ATPase in the present of 1 μM CdSO₄, and proposed that one of the first sites of cadmium action in oat roots is K⁺ carrier (ATPase).
Studies on pea seedlings (Kannan and Keppel 1976) have indicated that lead was inhibitory to the absorption of iron, manganese, and zinc, and that this inhibition appeared to be physical in nature, possibly the blocking of entry or the binding of ions to the ion carriers. The general confused state of the literature leaves the impression that the effects of lead, cadmium, and copper (and, probably, other heavy metals) upon nutrient uptake depends greatly upon the plant species, ambient metal concentration, form of the metal (inorganic or organic complex), and metal concentration, as well as the particular nutrients involved and their respective concentrations.

Transpiration. The effects of high levels of copper uptake on transpiration has been studied in parrotfeather (Sutton and Blackburn 1971b). The transpiration of parrotfeather was significantly reduced by CSP concentrations where copper > 2 ppm. Sutton and Blackburn (1971c) also found that 4.0 ppm copper (as CSP) significantly reduced transpiration in waterhyacinths. In terrestrial plants, high levels of lead reduced transpiration of corn (Bazzaz et al. 1974b; Carlson et al. 1975), soybean (Bazzaz et al. 1974b), sunflower (Carlson et al. 1975; Bazzaz et al. 1974c), loblolly pine and autumn olive (Rolfe and Bazzaz 1975), and pea seedlings (Kannan and Keppel 1976). The reduction in transpiration by cadmium has been reported for corn (Bazzaz et al. 1974a; Carlson et al. 1975), sunflower (Bazzaz et al. 1974a,c; Carlson et al. 1975), chrysanthemum (Kirkham 1978), and silver maple (Lamoreaux and Chaney 1978). These reductions in transpiration by lead or cadmium have been attributed to increased stomatal resistance (Bazzaz et al. 1974a,b,c; Lamoreaux and Chaney 1978; Kirkham 1978).
Kirkham (1978) noted further that increasing cadmium concentrations caused a reduction in turgor pressure in leaves of chrysanthemum. Gas exchange in sunflower was reduced in the presence of lead and cadmium as the result of stomatal closure (Bazzaz et al. 1974c). Although yet to be proven, transpiration effects of lead, cadmium, and copper on emergent and floating aquatic plants would very likely be due largely to stomatal closure and impairment of stomatal function.

**Photosynthesis.** The effects of lead, cadmium, and copper on photosynthesis have been studied extensively with algae and terrestrial plants, but no information is available concerning the effects upon aquatic vascular plants. The closest approximation to the effect of a heavy metal on photosynthesis of aquatic vascular plants is the study of the effects of arsenic upon a microcosm dominated by *Elodea* (Giddings and Eddlemon 1978). The ratio of net photosynthesis:night-time respiration was estimated from the changes in dissolved oxygen concentrations from day to night in the *Elodea*-dominated community. Under arsenic levels of 11.5 and 143 ppm both photosynthesis and respiration (as measured by dissolved oxygen concentrations) declined and P/R ratio fell to zero. This study was a measure of community metabolism as discussed by Odum (1956) and does not adequately demonstrate specific effects of arsenic upon *Elodea*.

Of particular importance to photosynthesis is the effect upon the light-absorbing pigments in the chloroplasts. Lead concentrations as low as 1 ppm have been shown to reduce the chlorophyll a content of *Chlorella vulgaris* by 17 percent (Rosko and Rachlin 1977). Conflicting results have been published for the effects of cadmium on chlorophyll
content. The presence of cadmium decreased the chlorophyll content of Chlorella pyrenoidosa (Hart and Scaife 1977) and Asterionella formosa (Conway 1978) but increased chlorophyll slightly in Chlorella vulgaris (Rosko and Rachlin 1977) and 4-fold in Euglena gracilis (Nakano et al. 1978). Similar conflicting results have been reported for copper.

Gross et al. (1970) stated that total pigments of Chlorella were reduced by copper concentrations of < 80 μM (≈ 9 ppm), whereas Rosko and Rachlin (1977) reported an increase in chlorophyll at copper concentrations of 0.042 to 0.32 ppm. A decrease in chlorophyll content occurred in Chlorella sorokiniana exposed to 100 μM CuSO₄ under anaerobic conditions, but there was little effect under aerobic conditions (Cedeno-Maldonado and Swader 1974). The increased cell level of chlorophyll in all cases is related to a decrease in total cell number as the result of retarded cell growth and division. Gross et al. (1970) noted a blue shift in the chlorophyll a absorption spectrum concomitant with the decrease in absorption in Chlorella cells exposed to copper. A similar shift in absorption spectrum and decrease in peak height was found by Puckett (1976) in the lichen, Umbilicaria muhlenbergii, exposed to 2 x 10⁻² M CuCl₂ for 15 h, but no shift was found for either lead or cadmium.

Conway (1978) also noticed a significant decrease in carotene in Asterionella formosa.

Measurements of carbon uptake and fixation by algae have indicated a species-specific response. Lead (20 ppm) decreased CO₂ fixation in Anabaena, Chlamydomonas, Cosmarium, and Navilcula but increased CO₂ fixation in Ochromonas (Malanchuk and Gruendling 1973). A reduction of CO₂ fixation in the presence of cadmium was reported for Chlorella pyrenoidosa by Hart and Scaife (1977). Cadmium concentrations as high
as 4.1 μg · l⁻¹ (ppb) did not affect carbon uptake by Asterionella formosa; however, a 45-46 percent reduction in carbon fixation occurred at a cadmium concentration of 8.8 μg · l⁻¹, respectively, for A. formosa and Fragilaria crotonensis (Conway and Williams 1979). Lead has also been shown to significantly decrease photosynthetic carbon fixation in soybean (C-Y Huang et al. 1974; Bazzaz et al. 1974b), corn (Bazzaz et al. 1974b, 1975), loblolly pine and autumn olive ( Rolfe and Bazzaz 1975). Corn and sunflower grown hydroponically showed no response of carbon fixation to lead (Carlson et al. 1975). This lack of response was attributed to poor translocation of lead under conditions of hydroponic culture. The lichen, Umbilicaria muhlenbergii, showed enhanced carbon fixation when exposed to lead for 1 h but had greatly reduced carbon fixation after long-term exposure to lead. Inhibition of carbon fixation by cadmium has been shown in soybean (C-Y Huang et al. 1974), corn (Carlson et al. 1975; Bazzaz et al. 1974b), sunflower (Bazzaz et al. 1974a,c; Carlson et al. 1975) and excised silver maple leaves (Lamoreanx and Chaney 1978). Puckett (1976) has also demonstrated decreased carbon fixation by the lichen U. muhlenbergii. The influence of lead upon enzymes of the reductive pentose phosphate pathway has been studied by Hampp et al. (1973). The activities of ribulose-1, 5-diphosphate carboxylase and ribulose-5-phosphate kinase were inhibited by 5 μM (≈1 ppm lead) Pb(NO₃)₂, while lactate dehydrogenase was less sensitive. Pyruvate kinase activity increased as Pb(NO₃)₃ concentrations were increased up to 100 μM (≈20 ppm). Concentrations of 100 to 500 μM and 100 to 1100 μM activated the NAD⁺- and NADP⁺-dependent glyceraldehyde-3-phosphate dehydrogenase, respectively; whereas, higher concentrations were inhibitory. Thus, on the basis of this study, the
inhibitory effects of lead (and, perhaps, cadmium or copper) discussed previously may be due primarily to inhibition of the primary carbon-fixing enzyme of the C₃ pathway, ribulose-1,5-biophosphate.

Oxygen evolution as a measurement of photosynthesis has also been studied. Wollery and Lewin (1976) found that 1 to 10 mg Pb · l⁻¹ significantly depressed photosynthetic oxygen evolution in the diatom (Phaeodactylum tricornutum) after a 24 h exposure; after 72 h, as little as 0.1 mg Pb · l⁻¹ caused a 25 percent reduction in oxygen evolution. A similar effect of lead was found for Chlamydomonas reinhardii (Overnell 1975). Cadmium has been shown to similarly depress oxygen evolution in whole cells of C. reinhardii (Overnell 1975), but no effect was found on either the Hill reaction (transfer of electrons from water to dichloroindophenol) or the modified Mehler reaction (transfer of electrons from dichloroindophenol to methylviologen). Lead and copper, however, did greatly depress both Hill and modified Mehler reactions. Overnell (1976) reported reduction of oxygen evolution by very low levels of copper (approximately 0.6 ppm) for Attheya decora, Isochrysis galbana, and Phaeodactylum tricornutum; the latter two species were relatively insensitive to 10 μM cadmium (~200 ppm). Oxygen evolution has also been shown to be inhibited in Chlorella by cadmium (Hart and Scaife 1977) and copper (Cedeno-Maldonado and Swader 1974). These results are in agreement with the data for inhibition of oxygen evolution in spinach chloroplasts by cadmium (Li and Miles 1975) and copper (Cedeno-Maldonado et al. 1972).

The effect of copper on photosynthetic electron transport has been reported by Cedeno-Maldonado and Swader (1974) for intact cells of Chlorella. Copper depressed photosystem I (PS I) and combined activity of PS I and II. No measurement of isolated PS II activity could be
made, as the authors were unable to prepare a chloroplast suspension for *Chlorella* with intact PS II activity. Earlier work on spinach by Cedeno-Maldonado *et al.* (1972) showed that PS I was more resistant to copper inhibition than PS II, and that the site most sensitive to copper inhibition was the oxidizing side of PS II. The amount of inhibition was also shown to depend not only on copper concentration but also upon the ratio of Cu²⁺ to chloroplast number. The greater the amount of chlorophyll, the higher the concentration of copper required to cause the same degree of inhibition, suggested that Cu²⁺ ions are bound by the chloroplast membranes. In the dark, copper was apparently bound by non-inhibitory sites in the chloroplast; therefore, a period of dark incubation reduced the amount of inhibition. The inhibition of electron transport by lead has been shown to operate similarly. Little inhibition was noted for PS I activity in spinach chloroplasts even at lead concentrations as great as 2.4 μM (≈497 ppm) when PS II was bypassed and ascorbate-reduced DCIP was the electron donor, but significant inhibition was observed with water as the electron donor (Miles *et al.* 1972). The Hill reaction was also greatly inhibited by lead. Proton transport also decreased. Fluorescence induction curves for tomato leaves indicated the primary site of inhibition was the oxidizing side of PS II, and hydroxylamine restoration of normal fluorescence after lead inhibition demonstrated that inhibition occurred between the primary electron donor of PS II and the site of water oxidation. Wong and Govindjee (1976) found that the addition of lead ions to corn chloroplast suspensions resulted in an inactivation of P700 photooxidation and an alteration of the kinetics of the re-reduction of P700⁺. In studies of the
effect of cadmium on isolated maize chloroplasts, Bazzaz and Govindjee (1974) found no inhibition of PS I but substantial inhibition of PS II and indicated that this inhibition was at the oxygen-evolving site in PS II. Li and Miles (1975), working with spinach chloroplasts, also noted PS II inhibition and lack of PS I inhibition when an electron donor was provided, but the results of studies of PS II activity measured by dye reduction, oxygen evolution, and chlorophyll fluorescence indicated that cadmium acted directly upon the PS II photoreaction rather than upon the electron transport chain near the oxygen-evolving site. Lucero et al. (1976) have shown cadmium inhibition of both cyclic and non-cyclic photophosphorylation and electron transport. Chloroplast ATP synthesis was completely inhibited by cadmium. Electron transport uncoupled by arsenate was also inhibited by cadmium, and, therefore, the authors suggested that the effect of cadmium must be near the phosphorylating site. At cadmium concentrations (25 to 500 \( \text{\mu M} \)) which inhibited phosphorylation, no effect was found on either the light-induced pH rise of the chloroplast suspensions or trypsin-activated Ca-ATPase, but inhibition of the Ca-ATPase occurred at cadmium concentrations fifty times higher.

Associated with the effects of lead and cadmium on photosynthesis are ultrastructural changes. Rebechini and Hanzley (1974) reported that exposure of Ceratophyllum demersum to lead resulted in the disruption of the lamellar system of the chloroplasts. Granal stacking was greatly reduced, as well as the amount of stroma in relation to the lamellar system. Starch granules in the chloroplasts were either absent or greatly reduced in size. Plastoglobuli were smaller, more numerous, and exhibited a total loss of electron density. Such loss
of lamellar integrity and the reduction in granal stacking would limit chloroplast function, particularly the integrated functioning of PS I and II, which requires thylacoid stacking (Arntzen et al. 1972). A similar poor development of thylocoid stacking and numerous plasto-globulii have been noted for chloroplasts of Sphagnum exposed to lead (Simola 1977). Poor starch synthesis and some degeneration of the chloroplasts were also found. Cadmium, however, did not have any apparent effect on the structure of Sphagnum chloroplasts other than slight increase in starch content.

Respiration. The effects of lead, cadmium, and copper upon respiration are essentially unknown for aquatic vascular plants. Studies on algae and terrestrial plants have indicated pronounced effects upon dark respiration. Gross et al. (1970) demonstrated a reduction of respiration rate after 1 h for Chlorella exposed to 0.1 μM CuSO₄. Cedeno-Maldonado and Swader (1974) noted a similar reduction in respiration in Chlorella exposed to copper. The inhibition of respiration was considerably less affected than photosynthesis, and only at a high copper concentration (1000 μM) was respiration inhibited 50 percent, whereas 200 μM completely inhibited photosynthesis. The effect was also more rapid upon photosynthesis than upon respiration. Wollery and Lewin (1976) have also shown a similar pattern for the effects of lead upon respiration in the diatom Phaeodactylum tricornutum. Cadmium-induced ultrastructural changes in mitochondria have been reported for freshwater green algae by Silverberg (1976). Mitochondria in the algae exposed to cadmium showed swelling, vacuolation, and the presence of prominent dense granules, which were interpreted to contain
Cadmium. Cristae were not as abundant in mitochondria of cells exposed to cadmium, and degeneration of mitochondria was noted. The changes were suggested to be extensive enough to disrupt cell metabolism. Consequently, Silverberg (1976) considered the mitochondria to be the primary target for cadmium-associated toxicity. No apparent ultrastructural changes were found for other cellular organelles. Studies of terrestrial plants show increased respiration in the presence of cadmium in soybeans (Lee et al. 1976b) and silver maple leaves (Lamoreaux and Chaney 1978), but decreased respiration in roots of oats (Keck 1978). Lead increased respiration in soybean leaves (Lee et al. 1976a).

**Effects of Lead, Cadmium, and Copper on Growth of Aquatic Plants**

*Higher plants.* In addition to effects on plant metabolism, toxic effects of lead, cadmium, and copper are usually manifested as a reduction in plant growth. Sutton and Blackburn (1971c) found that 9 ppmw copper significantly reduced shoot growth of 1-week-old waterhyacinths after 1 week root exposure to CSP and that after 2 weeks significant reduction in overall growth occurred at copper levels of 3.5 ppmw. Root length and root and shoot biomass of *Typha latifolia* were suppressed by soil with high heavy metals (zinc, cadmium, and lead) content (McNaughton et al. 1974). The growth of *Sphagnum nemoreum* (Simola 1974a) and *Sphagnum fimbriatum* (Simola 1977b) were significantly inhibited by 1 μM lead (207 ppm) and 0.1 μM cadmium (11.2 ppm), and 1 μM cadmium was lethal to *S. fimbriatum*. The grain yield of rice was depressed significantly by soil cadmium levels of 20 ppm and 320 ppm under non-flooded and flooded culture, respectively; 80 ppm completely inhibited grain
production under non-flooded conditions (Bingham et al. 1976). Exposure to alligatorweed for 3 weeks to 1 ppm cadmium under hydroponic culture in a controlled environment chamber caused a 63 percent reduction in overall growth and 17 percent reduction in the specific leaf weight (Quimby et al. 1979). Several reports are also available for submersed species. Sutton et al. (1970) reported no effect of 1 ppm copper on hydrilla 8 days after treatment with CSP. The organo-copper herbicide, cutrine, at 1 ppm copper, caused a decrease in dry weight of hydrilla compared to both CSP and CuCl₂, which had no effect at 1 ppm copper (Sutton and Blackburn 1971a). Synergistic effects upon hydrilla growth have been shown for CSP (1 ppm copper) in combination with the herbicide ametryne (1 ppm) Sutton et al. 1971b) and the combination of 5 ppm endothal + CSP at 1 ppm copper (Sutton et al. 1971a). Treatment of parrotfeather with CSP at 1 ppm copper had no effects upon either shoot length or plant dry weights (Sutton et al. 1971a; Sutton and Blackburn 1971b) after 1 week exposure. Sutton and Blackburn (1971b) noted that, after 1 week, shoot lengths were significantly reduced by 4 ppm copper and shoot dry weights by 8 ppm copper, and that, after 2 weeks, a 1 ppm copper level significantly reduced shoot dry weights; but no effects were found on root dry weights.

Algae. The growth of various species of algae has been shown to be reduced by lead (Stewart 1977; Christensen et al. 1979), cadmium (Rosko and Rachlin 1977; Hart and Scaife 1977; Nakano et al. 1978; Conway 1978; Conway and Williams 1979), and copper (Christensen et al. 1979; Rosko and Rachlin 1977). That the effects of algal growth depends upon both the metal involved and algal species have also been
demonstrated (Rosko and Rachlin 1977). Similar effects of these metals have also been noted for terrestrial plants (Krause and Kaiser 1977; Malone et al. 1978; Lepp and Roberts 1977; Wallace et al. 1977a; Page et al. 1972; Miller et al. 1977; Hassett et al. 1976).

Effects of Heavy Metals on Insects Feeding upon Contaminated Plants

Although both aquatic and terrestrial habitats are frequently subject to contamination by toxic heavy metals, very little is known about the influence of heavy metals absorbed by plant tissues upon the phytophagous insect fauna feeding upon these plants. As early as 1936 Hurd-Karrer and Poos (1936) reported that selenium was toxic to aphids. Byers and Zeiders (1976) indicated that the feeding of the frit fly, Osinella frit, and the cereal leaf beetle, Dulema melanopus, was less upon reed canarygrass which was spray-irrigated with sewage effluent than upon controls grown without spray-irrigation. Quimby et al. (1979) have shown that alligatorweed flea beetles, Agasicles hygrophila, feeding upon alligatorweed exposed to 1.0 ppm cadmium in hydroponic culture, were sensitive to leaf accumulations of 8.7 ppm cadmium, whereas nutsedge moths, Bactra verutana, feeding either upon diet containing from 0 to 18.0 ppm cadmium or upon purple nutsedge containing 6.5 ppm cadmium were not affected adversely.
EFFECTS OF WATERHYACINTHS

Materials and Methods

Plant Materials

Waterhyacinths for this study were collected in the field from Lake Oklawaha and the Oklawaha River, Florida, and held in outdoor pools filled with lake water until the beginning of the experimental period.

Experimental Setup and Design

All experiments were established outdoors in approximately 70-liter barrels lined with polyethylene bags. Barrels were filled with tap water to within 5 cm of the top. To each barrel were added 10 ml of a commercially prepared 15-7-7 liquid nutrient, which would provide a calculated 20 to 21 ppm N (approximately equivalent to 0.1 strength Hoagland's solution), and 70 ml of Sequestrene 330 iron chelate (1 ppm iron in final solution) and microelements. The microelements were equivalent to those in full-strength Hoagland's No. 1 solution. Heavy metals were provided as Cd(NO₃)₂, Pb(NO₃)₂, or CuSO₄. Stock solutions of 1000 ppm cadmium, lead, or copper were slowly added to the barrels while stirring to provide calculated initial solution concentrations of 0, 0.5, 1.0, 2.5, or 5.0 ppm. Water samples were taken at the outset within 30 minutes to determine initial loss of metals due to precipitation from solution and again at each harvest. Initial water analysis data are present in Table 1. Experiments were set up in a completely random design with three replications and harvests at 3 and
Table 1. Metal concentrations in initial solutions of waterhyacinth experiments as measured by atomic absorption spectrophotometry.

<table>
<thead>
<tr>
<th>Calculated Initial Metal Concentrations (ppm)</th>
<th>0.00</th>
<th>0.50</th>
<th>1.00</th>
<th>2.50</th>
<th>5.00</th>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>Pb</td>
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<tr>
<td>Cu</td>
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<tr>
<td>Experiment 2</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Pb</td>
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<td>1.03</td>
<td>2.35</td>
<td>4.84</td>
</tr>
</tbody>
</table>
6 weeks. The first experiments were conducted during the spring of 1979 and were repeated in late summer. Cadmium experiments were begun in the first week, followed by lead and copper experiments during the second and third weeks, respectively, in order to allow sufficient time for harvesting as well as to conduct the experiments as concurrently as possible for comparative purposes. Neither nutrients nor metals were renewed during the 6-week course of each experiment. Tap water was added as needed to maintain solution level. Mature waterhyacinth plants were selected from the pool, and all dead material and floral spikes were removed. Plants were sponged with paper towels to remove excess water, and initial fresh weights were recorded. Five plants were placed into each experimental unit (i.e., barrel) and an additional three replications of five plants each were taken for determination of fresh weight:dry weight ratios at the outset of each experiment.

**Growth Observations and Measurements**

Experiments were observed closely each week for appearance of any symptoms of toxicity, such as chlorosis and/or browning of the tissues. Plants were harvested at three and six-week intervals. Plant material was separated into leaf lamina, stems (petioles + rhizomes + stolons), flowers, and roots and was placed in a forced-air drying oven at 65 C for 72 h. Dry weights for each plant part were recorded. Growth measurements included dry weights of leaves, stems, roots, and dead plant tissues, number of flowers, and flower weights. Flower weights were included with stem weights for the overall growth analysis. From the dry weight:fresh weight ratios of the plants, an estimated initial dry weight (hereinafter called original weight) was calculated for
plant material in each experimental unit. Total plant growth after three or six weeks was expressed as the difference between biomass and original weight.

**Tissue Analyses**

Leaves, stems, roots, and dead tissues were ground in a Wiley mill to pass a 40-mesh screen; and the ground plant material was redried at 65 C for 24 h. Approximately 0.5 g samples were placed into 50 ml beakers and ashed for 5 h at 450 C in a muffle furnace. Spiked samples were included for the determination of percent metal recovery. The ash was weighed and then digested with 2 ml of 2 N HCl, filtered, and brought to a total volume of 50 ml with deionized water. Samples were sent to the University of Florida Soils Analytical Laboratory for metal determination by atomic absorption spectrophotometry. Deionized water and HCl blanks were included with the samples to assess contamination during the digestion process. All data were adjusted for both contamination and percent metal recovery. The concentrations of lead, cadmium, or copper were calculated as ppm metal in leaves, stems (including floral portions), roots, and dead tissues. Total uptake of each metal was calculated for each tissue as well as on a whole-plant basis.

**Statistical Analysis**

All data were analyzed by linear regression. Log 10 transformations were used for all regressions involving growth variables. Variation in initial plant weights necessitated the use of original weight as a covariate in the overall analyses. Actual initial solution concentrations were used in all regressions of variables against solution levels to
allow for the variation in metal concentrations and some loss of metals due to precipitation from solution.

Results and Discussion

Effects of Lead

Appearance of plants

At the outset of the experimental period all plants were healthy, uniformly green, and uniform with respect to infestation with water-hyacinth weevils, Neochetina spp. Plants used in the spring experiment had a considerably lower level of infestation with weevils than those used in the late summer experiment. There were also some signs of disease on plants used in the summer experiment, whereas plants used in the spring experiment were visibly disease free. During the course of both experiments there were no visible differences in plants subjected to different lead levels. After six weeks all plants were showing some signs of nutrient stress, particularly a slight chlorosis. Plants were densely packed in all treatments with many new ramets actually hanging out of the barrels and no longer in contact with the solution. Nutrient stress was more prevalent in the second experiment than in the first. Plants in the second experiment were severely diseased and had marked Neochetina infestations; damage resulting from Neochetina spp. larvae was extensive.

Growth measurements

Flowering and flower weights. The effects of lead upon flowering was not significant at either harvest date in either experiment. There
were no flowers at all after three weeks in the first experiment (spring), whereas some flowering had occurred in all treatments after three weeks in the second experiment (late summer). The difference in flowering due to season may be attributed to the fact that the initial plants used in the second experiment were more mature physiologically and were in flower prior to the beginning of the experimental period. Plants used in the first experiment, however, were not flowering. After six weeks, however, all treatments were flowering in both experiments. The mean number of flowers was slightly greater in the second experiment than in the first and may be the result of seasonal influences. The mean number of flowers, mean dry weights, and mean weight per flower increased very slightly with increased solution concentration of lead, but differences were not significant.

**Dry weights of leaves.** During the spring there was very little effect of lead upon partitioning of dry weight into leaf tissue (Fig. 1). After three weeks no significant differences were noted. After six weeks there was a slight, but significant, decrease in leaf dry weights in plants exposed to lead. An overall regression, however, indicated that there were no differences between the leaf dry weights for the two harvest dates. The second experiment, however, showed a different trend. After three weeks, dry matter partitioning into leaves showed a slight, but significant increase with exposure to lead. Leaf weights were 20 to 30 percent greater (highly significant) after six weeks than after three weeks. The first experiment, however, showed no differences in leaf weights. An overall regression indicated that response of leaf dry weights to level of exposure to lead was significantly affected by season.
Dry weights of stems. No differences were observed in stem dry weights regardless of exposure level during the spring experiment (Fig. 2). There was a highly significant increase in growth of stems in all treatments between the three and six-week harvests. After three weeks, a significant increase in stem growth was observed in the second experiment as the level of exposure to lead was increased, but, after six weeks, there were no significant differences. Increases in dry stem weights from Harvest 1 to Harvest 2 were markedly influenced by time but were not influenced greatly by initial lead concentration. Seasonal differences had a highly significant interactive effect upon stem dry weight.

Dry weights of roots. Figure 3 shows that root dry weights were not affected by exposure to lead during the spring of 1979. The increase in root growth from three to six weeks was also independent of lead concentration. In the second experiment, however, there was a highly significant increase in root weight with increased level of exposure to lead after either three or six weeks. Significant differences in the slopes of the two regression lines indicated that the effects of metal were interacting with length of exposure time. Regression analysis further indicated significant differences in the seasonal response of stem dry weights to increasing lead levels.

Dry weights of dead tissue. The only observable significant differences in dead plant material were the slight increase in the dry weights with increasing lead exposure in the three-week harvest of Experiment 2 (Fig. 4). All differences in dry weights between harvest dates were solely due to the longer growing period. The seasonal
Figure 1. Dry leaf weights of waterhyacinths exposed to lead:
(A) Experiment 1 - Harvest 1, \( \log Y = 1.41 + 0.01 \log (X + 1) \);
(B) Experiment 1 - Harvest 2, \( \log Y = 1.43 - 0.12 \log (X + 1) \);
(C) Experiment 2 - Harvest 1, \( \log Y = 1.14 + 0.13 \log (X + 1) \);
(D) Experiment 2 - Harvest 2, \( \log Y = 1.34 - 0.10 \log (X + 1) \).
Figure 2. Dry stem weights of waterhyacinths exposed to lead:
(A) Experiment 1 - Harvest 1, $\log Y = 1.57 + 0.03 \log (X + 1)$;
(B) Experiment 1 - Harvest 2; $\log Y = 1.81 - 0.08 \log (X + 1)$;
(C) Experiment 2 - Harvest 1, $\log Y = 1.15 + 0.16 \log (X + 1)$;
(D) Experiment 2 - Harvest 2, $\log Y = 1.58 + 0.01 \log (X + 1)$. 
Figure 3. Dry root weights of waterhyacinths exposed to lead:
(A) Experiment 1 - Harvest 1, log $Y = 1.24 + 0.09$
    $\log (X + 1)$; 
(B) Experiment 1 - Harvest 2, log $Y = 1.37 + 0.06$
    $\log (X + 1)$; 
(C) Experiment 2 - Harvest 1, log $Y = 0.91 + 0.29$
    $\log (X + 1)$; 
(D) Experiment 2 - Harvest 2, log $Y = 1.17 + 0.28$
    $\log (X + 1)$. 
Figure 4. Dry dead-tissue weights of waterhyacinths exposed to lead:
(A) Experiment 1 - Harvest 1, log \( Y = 0.39 - 0.09 \log (X + 1) \);
(B) Experiment 1 - Harvest 2, log \( Y = 1.31 - 0.07 \log (X + 1) \);
(C) Experiment 2 - Harvest 1, log \( Y = -0.05 + 0.35 \log (X + 1) \);
(D) Experiment 2 - Harvest 2, log \( Y = 1.04 - 0.02 \log (X + 1) \).
The graph shows the relationship between dry weight (g x 10^-1) and initial Pb concentration (ppm) for different conditions.

- **A**: \( r^2 = 0.0216 \)
- **B**: \( r^2 = 0.0271 \)
- **C**: \( r^2 = 0.3462 \)
- **D**: \( r^2 = 0.0011 \)

The graph includes data points and trend lines for each condition, indicating the variability and correlation between dry weight and Pb concentration.
effect was significant only for Harvest 1. The covariate, original weight, was marginally significant (P > F = .0750). If this alpha level is accepted as a significant covariate effect, then the seasonal effect may be accepted as significant for the second harvest also.

**Total growth of plants.** Perhaps the most consistent and acceptable measurement of plant response to a potentially toxic substance is the combined effect upon whole plant growth, expressed as the difference between the total dry plant weight at harvest (i.e., biomass) and original plant weight (estimated original dry weight). Such measurements tend to offset artifactual differences in such variables as leaf weight that may be influenced by uncontrolled factors, such as partial leaf defoliation by insects. Figure 5 shows that there were no significant differences in growth after either three or six weeks in the first experiment, and the increase in growth between three and six weeks was a simple function of time. The second experiment showed a highly significant increase in growth with increased lead level after three weeks, but no significant differences were apparent after six weeks. Whether or not differences would have been significant had the solutions been renewed after three weeks can be left only to conjecture. It would appear that the seasonal effect was more important to overall growth than the level of exposure to lead. Growth was significantly greater during the spring than during the late summer and early fall. See Fig. 5 for data.

**Leaves.** During the spring experiment lead was not found in leaf tissues until solution concentrations reached approximately 1.8 and 0.8 ppm for the three and six-weeks harvests, respectively; in the
Figure 5. Total growth of waterhyacinths exposed to lead:
(A) Experiment 1 - Harvest 1, $\log Y = 1.80 + 0.04 \log (X + 1)$;
(B) Experiment 1 - Harvest 2, $\log Y = 2.09 - 0.06 \log (X + 1)$;
(C) Experiment 2 - Harvest 1, $\log Y = 1.33 + 0.30 \log (X + 1)$;
(D) Experiment 2 - Harvest 2, $\log Y = 1.88 + 0.07 \log (X + 1)$.
second experiment these threshold levels were about 2.3 and 0.8 ppm, respectively (Fig. 6). Above these threshold values the concentration of lead in leaf tissue was an approximately linear function of the lead level in solution. In the first experiment a comparison of regression lines indicated significantly greater levels of lead in leaf tissue with time, but this trend did not hold true for the second experiment. Time of year had no significant influence upon lead concentrations for either harvest. With a single exception, maximum lead concentrations after six weeks exposure were 39.9 ppm (initial solution level = 3.8 ppm) and 39.8 ppm (initial solution level = 4.8 ppm) for the first and second experiments, respectively.

**Stems.** Lead uptake occurred in all treatments except controls in both experiments, and, as in the case of leaves, the concentration of lead in stem tissues was essentially a linear function of the initial solution concentration (Fig. 7). The increase in lead concentration was highly significant for both harvests of each experiment. Differences between harvests, however, were not significant, and the only significant seasonal effect was for the second harvest. Leaf lead concentrations ranged from 0 in the control plants to a high of 1393 ppm (initial solution level = 5.0 ppm) in Harvest 2 of the second experiment.

**Roots.** Highly significant differences in root lead concentrations were also linearly related to the initial solution lead concentration (Fig. 8). No significant differences in root lead concentrations were found between harvests, but a significant seasonal effect was noted. For the second harvest, the range in tissue concentration was from 0 (controls) to 4933 ppm (initial solution level = 3.8 ppm) and 9.58
Figure 6. Concentration of lead in water hyacinth leaves:
(A) Experiment 1 - Harvest 1, \( Y = -2.35 + 6.2X \);
(B) Experiment 1 - Harvest 2, \( Y = -1.57 + 11.37X \);
(C) Experiment 2 - Harvest 1, \( Y = 0.31 + 7.28X \);
(D) Experiment 2 - Harvest 2, \( Y = 0.008 + 8.83X \).
Figure 7. Concentration of lead in waterhyacinth stems:
(A) Experiment 1 - Harvest 1, \( Y = 7.85 + 108X \);
(B) Experiment 1 - Harvest 2, \( Y = 20.6 + 178.7X \);
(C) Experiment 2 - Harvest 1, \( Y = 14.77 + 73.56X \);
(D) Experiment 2 - Harvest 2, \( Y = 72.8 + 243.6X \).
(control) to 6987 ppm (initial solution level = 5.0 ppm) for the first and second experiments, respectively.

**Metal uptake and partitioning**

**Leaves.** Translocation of lead to leaf tissue was minimal. Total accumulation of lead rarely exceeded 1.0 mg and represented only about 0.5 percent of the total lead uptake. No lead was detected in leaves after three weeks until solution concentrations exceeded 1.9 ppm. After six weeks lead appeared in leaves at an exposure level of 0.8 ppm.

**Stems.** Figures 9 and 10 indicate that lead partitioning into stem tissue was proportional to the total lead uptake in the plant, with some apparent seasonal differences during the first three weeks of the experiments. In Experiment 1, stem tissue contained between 10 and 15 percent of the total plant lead. After six weeks stem lead content was about 20 to 35 percent of total plant lead in both experiments. Maximum lead accumulation in stems after three weeks was 21.8 and 7.3 mg for Experiments 1 and 2, respectively. Maximum accumulation after six weeks was 48 and 57 mg for Experiments 1 and 2, respectively.

**Roots.** Root tissue contained by far the major portion of the total plant accumulation of lead. Figures 9 and 10 show clearly that the proportion of total plant lead partitioned into roots is a function of time. After three weeks exposure, root lead represented about 70 to 90 percent of total plant uptake. After six weeks, however, a substantial proportion of total plant lead had been translocated to stem tissue, and roots contained about 50 to 65 percent of the total lead accumulation. Maximum root accumulations of lead for the three and six-weeks
Figure 8. Concentration of lead in waterhyacinth roots:
(A) Experiment 1 - Harvest 1, $Y = 136.7 + 1177X$;
(B) Experiment 1 - Harvest 2, $Y = -85.3 + 1283X$;
(C) Experiment 2 - Harvest 1, $Y = 344.9 + 1262X$;
(D) Experiment 2 - Harvest 2, $Y = 235 + 1333X$. 
Figure 9. Lead accumulation by waterhyacinths in Experiment 1: (A) Harvest 1; (B) Harvest 2.
Initial Pb Concentration (ppm)

Pb Accumulation (mg x 10^-1)

- --- Total
- --- Roots
- --- Stems
- --- Dead

Initial Pb Concentration (ppm)
Figure 10. Lead accumulation by waterhyacinths in Experiment 2: 
(A) Harvest 1; 
(B) Harvest 2.
harvests were 95 and 145 mg, respectively, for Experiment 1, and 94 and 164 mg, respectively, for Experiment 2.

**Dead tissues.** The amount of lead bound by dead tissues is important only with respect to the inavailability of this lead to the live plant portions. The total lead in dead tissues is highly dependent upon the concentration of lead in the various plant parts as well as the proportion of these plant parts in the dead biomass and their respective degree of decomposition. The approximate proportion of dead biomass in both experiments after three and six weeks were 5 and 15 percent, respectively. The relative tissue composition of dead plant parts could not be assessed adequately, but the majority of dead tissues after six weeks were roots. In the second experiment, a larger proportion of total dead tissues were leaves and stems. Maximum lead bound in dead plant parts was 43 and 32 mg for Harvest 2 of Experiments 1 and 2, respectively.

**Total uptake and mean plant concentration.** Figures 9 and 10 show that total lead uptake was an almost linear function of exposure level and time, but seasonal influences upon total lead uptake were not apparent. Maximum lead accumulations on a whole plant basis for Experiments 1 and 2, respectively, were 107 and 102 mg for Harvest 1 and 228 and 234 mg for Harvest 2. Upon examination of the mean lead concentration (ppm) in whole plants, however, the seasonal influences were quite clear. Figure 11 shows the average lead concentrations in whole plants. A comparison of the slopes of the regression lines indicated highly significant interactive effect upon plant absorption of lead due to season as well as harvest time. Maximum total lead accumulation by whole plants for Harvests 1 and 2 were 107 and 228 mg, respectively,
Figure 11. Concentration of lead in whole waterhyacinths:
(A) Experiment 1 - Harvest 1, \( Y = 12.14 + 340.6X \);
(B) Experiment 1 - Harvest 2, \( Y = -34.69 + 421.4X \);
(C) Experiment 2 - Harvest 1, \( Y = 51 + 383.3X \);
(D) Experiment 2 - Harvest 2, \( Y = 85.6 + 471.7X \).
A $r^2 = 0.9362$

B $r^2 = 0.9873$

C $r^2 = 0.9395$

D $r^2 = 0.9888$
in Experiment 1, and 102 and 224 mg, respectively, in Experiment 2. Mean lead concentrations in whole plants corresponding to the above values for uptake for Harvests 1 and 2 were 1243 and 1621 ppm, respectively, in Experiment 1, and 2030 and 2332 ppm, respectively, in Experiment 2.

Discussion of lead effects

The overall effects of lead upon waterhyacinths at the exposure levels used in these experiments were essentially negligible. Plants exposed to even the highest initial concentrations of lead grew well in both experiments, with no apparent signs of toxicity. It was obvious that the threshold of lead toxicity in waterhyacinths lay beyond the maximum level of exposure used in these experiments.

The uptake of lead increased linearly with level of exposure, and the greatest portion of total plant lead was partitioned into root tissues. Translocation of lead to the stems occurred very slowly in both experiments, and very little reached the leaf tissues, even at the highest levels of exposure. These results are in agreement with the observations of other researchers (Tatsuyama et al. 1977, 1979; Peter et al. 1979; Walsh et al. 1979; Nakada et al. 1979). The lack of toxicity of lead to waterhyacinths suggests largely apoplastic accumulation, with little or no entrance of lead into the living cells. This hypothesis is supported by the observations of Sharpe and Denny (1976) on Potamogeton, in which they demonstrated the deposition of lead in the cell walls of submersed leaves. There were no apparent interactive seasonal influences upon total lead accumulation by whole plants in the present study, but this apparent lack of seasonal influences is highly misleading. Upon closer examination, it was noted
that the mean lead concentrations in whole plants exhibited a significant seasonal interactive effect. Plants in the late-summer experiment grew significantly less but contained significantly greater concentrations of lead than did plants in the spring experiment. The seasonal influence would appear to be a simple dilution effect resulting from differences in growth rates. Tatsuyama et al. (1977) reported that high temperatures (60 C) significantly increased the absorption rate of lead by waterhyacinths. The lack of any significant effect of increasing the temperature from 10 to 20 C, however, suggests that seasonal differences in temperature would most likely have little effect upon the rate of absorption of lead by waterhyacinths.

Effects of Cadmium

Appearance of plants

At the outset of the experiment all plants were uniform with respect to condition and level of insect infestation. After ten days all plants exposed to more than 1 ppm cadmium were showing signs of insipient chlorosis, and by 14 days, all plants except controls and those exposed to less than 0.5 ppm cadmium were chlorotic. By the time of the first harvest (three weeks) there was very little green color apparent, and a substantial portion of the tissues were dead or dying in plants exposed to > 0.5 ppm cadmium. After six weeks the toxicity of cadmium was even more pronounced. All plants exposed to > 0.5 ppm cadmium were obviously dying. Symptoms of insipient nutrient stress, including very slight chlorosis, were evident even in the control plants at the time of the six-week harvest. Production of new ramets in controls and at cadmium exposure < 0.5 ppm appeared
to be normal, whereas, in all plants showing symptoms of toxicity, few new ramets had been produced. Root tissue was severely damaged by > 0.5 ppm cadmium. Most of the original root tissue was dead and decomposing, and roots forming on the few new ramets were very small, stunted, blackened, and had poor lateral root development. Roots of controls and cadmium treatments < 0.5 ppm appeared normal. In the first experiment (spring 1979) flowering did not occur until after the three week harvest date, and only a few flowers were produced in any treatments after six weeks. In the second experiment (late summer 1979) original plant materials were flowering prior to the initiation of the cadmium tests. By six weeks the controls were flowering prolifically, but few flowers appeared in any of the cadmium treatments.

Growth measurements

Flowering and flower weights. There were no differences in flowering during the entire first experiment, probably due to lack of physiological maturity of the plants. In the second experiment, however, at the six-weeks harvest the effects of cadmium were apparent. Control plants averaged 36.6 ± 10.4 flowers per experimental unit, with a mean dry weight of 7.9 ± 2.5 g and an average flower weight of 0.2 ± 0.01 g. The maximum number of flowers in any cadmium treatment was 6 at 4.7 ppm cadmium, with a corresponding flower weight of 0.3 g and average flower weight of 0.05 g.

Dry weights of leaves. In both harvests of each experiment the differences in leaf dry weights were highly significant. Figure 12
Figure 12. Dry leaf weights of waterhyacinths exposed to cadmium:

(A) Experiment 1 - Harvest 1, $\log Y = 1.12 - 0.74 \log (X + 1)$;
(B) Experiment 1 - Harvest 2, $\log Y = 1.35 - 1.42 \log (X + 1)$;
(C) Experiment 2 - Harvest 1, $\log Y = 1.06 - 1.01 \log (X + 1)$;
(D) Experiment 2 - Harvest 2, $\log Y = 1.02 - 1.44 \log (X + 1)$. 
$r^2 = 0.8459$

$A$

$B$

$C$

$D$

$Q_i \cdot V$

$Q_i = 0.7595$

Initial Cd Concentration (ppm)
shows that a decline in dry leaf weights occurred with increasing cadmium exposure up to about 1.0 ppm, after which little further change occurred. Seasonal influences on plant growth affected leaf dry weights equally over all treatments, and the overall leaf response to cadmium did not change with season.

Dry weights of stems. Figure 13 shows that the influence of cadmium on dry stem weights was somewhat less pronounced than the effects of leaf weights, but the trend was similar. Regression analysis indicated that increased cadmium concentrations caused a highly significant reduction in stem growth. At concentrations > 1 ppm cadmium very little new stem tissue was produced between the first and second harvests. There was no seasonal effect upon the response of stem growth to increasing cadmium levels.

Dry weights of roots. Root dry weights also decreased with increasing cadmium exposure (Fig. 14). After three weeks exposure, there was a significant decrease in root dry weight in the first experiment but no significant differences in the second experiment. Increased length of exposure resulted in more pronounced differences in root growth with increasing cadmium exposure. A comparison of regression lines indicated that there was a seasonal effect upon root dry weights after three weeks growth but not after six weeks growth.

Dry weights of dead tissue. During the first three weeks of each experiment there was a significant increase in the amount of dead tissues with increasing exposure to cadmium (Fig. 15). After six weeks the trend had reversed. In the spring experiment there were no significant
Figure 13. Dry stem weights of waterhyacinths exposed to cadmium:
(A) Experiment 1 - Harvest 1, log $Y = 1.24 - 0.25$
    $\log (X + 1)$;
(B) Experiment 1 - Harvest 2, log $Y = 1.53 - 0.84$
    $\log (X + 1)$;
(C) Experiment 2 - Harvest 1, log $Y = 1.18 - 0.42$
    $\log (X + 1)$;
(D) Experiment 2 - Harvest 2, log $Y = 1.31 - 0.93$
    $\log (X + 1)$. 
Initial Cd Concentration (ppm)

A \( r^2 = 0.4514 \)

B \( r^2 = 0.8693 \)

C \( r^2 = 0.6384 \)

D \( r^2 = 0.5823 \)
Figure 14. Dry root weights of waterhyacinths exposed to cadmium:
(A) Experiment 1 - Harvest 1, \( \log Y = 1.06 - 0.47 \log (X + 1) \);
(B) Experiment 1 - Harvest 2, \( \log Y = 1.30 - 0.91 \log (X + 1) \);
(C) Experiment 2 - Harvest 1, \( \log Y = 1.04 - 0.15 \log (X + 1) \);
(D) Experiment 2 - Harvest 2, \( \log Y = 0.93 - 1.09 (X + 1) \).
Figure 15. Dry dead-tissue weights of waterhyacinths exposed to cadmium;

(A) Experiment 1 - Harvest 1, log Y = 0.02 + 0.52 log (X + 1);
(B) Experiment 1 - Harvest 2, log Y = 1.04 - 0.19 log (X + 1);
(C) Experiment 2 - Harvest 2, log Y = -0.25 + 1.03 log (X + 1);
(D) Experiment 2 - Harvest 2, log Y = 1.24 - 0.38 log (X + 1).
differences in dead tissues after six weeks. In the second experiment, however, there was a significant decrease in dead tissue with increasing cadmium levels. As would be expected, there were significantly more dead tissues at all levels of exposure after six weeks than after three weeks. Seasonal effects were significant only during the first three weeks of exposure to cadmium.

**Total growth of plants.** The overall growth of waterhyacinths was significantly reduced by exposure to cadmium in both harvests of the two experiments (Fig. 16). The growth of control plants approximately tripled between harvest dates. At concentrations of approximately 0.5 ppm cadmium, the growth after six weeks was almost twice that after three weeks. At concentrations > 1 ppm, there was essentially no further growth after three weeks. Season of the year had no interactive influence upon the growth response of waterhyacinths exposed to cadmium.

**Metal concentrations in tissues**

**Leaves.** The concentration (ppm) of cadmium in waterhyacinth leaves was a linear function of level of the concentration of cadmium in the initial solution (Fig. 17). The presence of cadmium in leaf tissue even at very low exposure levels indicates that cadmium is translocated relatively well. In the spring, there was a significant interactive effect between harvest date and exposure level. The concentration of cadmium in leaf tissue at solution concentrations of > 1.0 ppm cadmium was about threefold greater after six weeks than after three weeks. There were also no influences of season upon leaf cadmium concentrations. Maximum leaf concentrations of cadmium after three and six weeks,
Figure 16. Total growth of waterhyacinths exposed to cadmium:
(A) Experiment 1 - Harvest 1, log $Y = 1.43 - 0.79 \log (X + 1)$;
(B) Experiment 1 - Harvest 2, log $Y = 1.86 - 1.35 \log (X + 1)$;
(C) Experiment 2 - Harvest 1, log $Y = 1.32 - 0.95 \log (X + 1)$;
(D) Experiment 2 - Harvest 2, log $Y = 1.67 - 1.80 \log (X + 1)$. 
Figure 17. Concentration of cadmium in waterhyacinth leaves:
(A) Experiment 1 - Harvest 1, \( Y = 4.14 + 43.96X \);
(B) Experiment 1 - Harvest 2, \( Y = -7.28 + 134.8X \);
(C) Experiment 2 - Harvest 1, \( Y = 4.85 + 55.98X \);
(D) Experiment 2 - Harvest 2, \( Y = 12.02 + 66.71X \).
respectively, were 219 and 634 ppm for Experiment 1 and 428 and 459 ppm for Experiment 2. Very low levels in controls (< 2 ppm) indicated possible cadmium contamination either in the nutrients or during analysis.

Stems. Concentrations of cadmium in stems followed the same trend as leaf tissue (Fig. 18), and tissue concentration was a linear function of cadmium exposure level. A interactive effect between harvest and exposure level was significant in both experiments, and a significant seasonal effect was demonstrated for the second harvest date. In the first experiment, a doubling of the cadmium concentration in the initial solution resulted in almost twice the tissue cadmium concentration, whereas, in the second experiment, the effect of doubling was nearly a 60 percent increase in tissue levels. Maximum cadmium concentrations in stems after three and six weeks exposure, respectively, were 793 and 1395 ppm for Experiment 1, and 856 and 1398 ppm for Experiment 2.

Roots. Concentration of cadmium in root tissues showed a highly significant increase with increasing exposure level (Fig. 19). No significant differences were observed in root cadmium concentrations either between harvest dates or on a seasonal basis. The response was very linear, and a doubling of cadmium exposure level resulted in about a two-fold increase in root cadmium concentration after either three or six weeks, regardless of season. Maximum root cadmium concentrations for Harvests 1 and 2, respectively, were 6998 and 5584 ppm for Experiment 1, and 8975 and 7284 ppm for Experiment 2.

Metal uptake and partitioning

Leaves. With a single exception, the accumulation of cadmium by waterhyacinth leaves represented only a small proportion of the total
Figure 18. Concentration of cadmium in waterhyacinth stems:
(A) Experiment 1 - Harvest 1, $Y = 36.5 + 182.2X$
(B) Experiment 1 - Harvest 2, $Y = 34.1 + 329X$
(C) Experiment 2 - Harvest 1, $Y = 43 + 162X$
(D) Experiment 2 - Harvest 2, $Y = 101.6 + 219X$. 
Figure 19. Concentration of cadmium in waterhyacinth roots:
(A) Experiment 1 - Harvest 1, $Y = -218 + 1542X$;
(B) Experiment 1 - Harvest 2, $Y = -217 + 1327X$;
(C) Experiment 2 - Harvest 1, $Y = 21.4 + 1403X$;
(D) Experiment 2 - Harvest 2, $Y = 231 + 1228X$. 
plant accumulation of cadmium. In Experiment 1, a small amount of cadmium appeared in the leaves of control plants. At undetectable initial cadmium solution levels these controls contained a total of about 0.01 mg cadmium, or about 25 to 30 percent of the total plant cadmium for Harvest 1 and about 10 percent for Harvest 2. In the second experiment, no cadmium was found in leaf tissue for control plants. The total leaf accumulation in any cadmium treatment never exceeded 1 mg and represented a maximum of about 6 percent of the total plant accumulation. In Experiment 1, the percent of total plant cadmium partitioned into leaf tissue decreased with increasing exposure level, but this trend was not apparent in Experiment 2. In both harvests of the two experiments there was a linear increase in total cadmium accumulated in leaf tissue with increasing level of exposure. An apparent seasonal effect in response was noticed between harvests. In the first experiment more cadmium was translocated to leaf tissue with increased exposure time, whereas, in the second experiment, the reverse was true. This suggests that, during the spring (Experiment 1), the increase in leaf cadmium with time was the result of increased translocation with increasing air temperatures, and, during the late summer (Experiment 2), the decrease may have been due to decreased translocation as a result of decreasing air temperatures. This apparent seasonal response may, in fact, not be real, however. A comparison of Figs. 20 and 21 show that the total cadmium uptake after six weeks by whole plants was about 15 percent greater during the second experiment than in the first, and that a considerable proportion of this was bound into dead plant material. During the late summer and early fall, a substantial proportion of total leaf tissue was in the dead fraction and, consequently, did not
Figure 20. Cadmium accumulation by waterhyacinths in Experiment 1:
(A) Harvest 1;
(B) Harvest 2.
Figure 21. Cadmium accumulation by waterhyacinths in Experiment 2:
(A) Harvest 1;
(B) Harvest 2.
Cd Accumulation (mg x 10^-1) vs. Initial Cd Concentration (ppm)

A: Total
B: Roots
- Stems
- Dead
contribute to the measured total leaf cadmium. If this is true, then the apparent seasonal effect may be an artifact created by the separation of dead from living tissues.

**Stems.** Figures 20 and 21 indicate that the partitioning of cadmium into stem tissues is an approximately linear function of exposure level during both experiments. A large portion of the stem tissue in Harvest 2 of the second experiment was in the dead plant fraction. Consequently, the apparent seasonal effects in total stem cadmium accumulation may be, as was true for leaf tissue, an artifact of separation of plant parts into living and dead tissues. In Experiment 1, the accumulation of cadmium by stems represented about 20 percent of the total plant uptake. In Experiment 2, stems contained approximately 10 percent of total plant cadmium. Maximum accumulation of cadmium in stem tissue for Harvests 1 and 2, respectively, were 9.9 and 15.2 mg for Experiment 1, and 8.8 and 7.7 mg for Experiment 2.

**Roots.** In both experiments the largest single fraction of the total plant cadmium was found in the roots (Figs. 20 and 21). Root uptake of cadmium was essentially a linear function of increasing cadmium exposure, with the exception of Harvest 2 of the second experiment. In Experiment 1, root cadmium accounted for about 60 and 40 percent of the total plant cadmium for Harvests 1 and 2, respectively. In Experiment 2 root cadmium represented about 75 percent of total plant cadmium for Harvest 1, and a highly variable 2 to 60 percent for Harvest 2. This variability may be explained, for the most part, by the large amount of root tissue in the dead plant fraction. In the second harvest of Experiment 2, one experimental unit exposed to an initial
cadmium level of 5.0 ppm contained over 90 percent of the total plant cadmium in the dead fraction and only 1 percent in the root fraction. In this case, however, dead tissue represented 16 g of the total 22.3 g of biomass and was composed largely of root tissues. Maximum total cadmium in root tissues for Harvests 1 and 2, respectively, were 35.4 and 33.6 mg for Experiment 1, and 70.9 and 52.4 mg for Experiment 2.

Dead tissues. The increase in total cadmium in dead plant parts increased as a linear function of exposure level for Harvest 1 of both experiments (Figs. 20 and 21). The linear relationship between total cadmium in dead tissues and exposure level was lost, however, for the second harvest date, especially in Experiment 2, where dead tissue frequently represented more than half of the total biomass. The most significant component of the dead tissue at this time was dead roots. As a reflection of both tissue concentration and biomass, the total cadmium accumulation in dead tissues would reflect to the greatest extent the amount of cadmium accumulated in the tissue fraction that represented the largest portion of the dead biomass. Maximum accumulation of cadmium in dead tissues for Harvests 1 and 2, respectively, were 11.4 and 48.1 mg in Experiment 1, and 10.1 and 87.0 mg in Experiment 2.

Total uptake and mean plant concentration. Figures 20 and 21 indicate that the total cadmium uptake is an essentially linear function of cadmium exposure level. Considerable variation in total plant uptake did exist, particularly at the higher exposure levels. Total cadmium uptake generally followed the pattern of total root uptake during the first harvest of both experiments. This was expected, since live
root tissue contained the highest concentration of cadmium as well as made up a large portion of total plant biomass. Recognizing that total root biomass (living and dead) also made up a substantial fraction of the total plant biomass, it is reasonable to surmise that the total cadmium uptake would closely parallel cadmium accumulation in total root biomass. The mean cadmium concentration in whole plants (Fig. 22) and the root cadmium concentrations (Fig. 19) appear to support this contention. Maximum total cadmium accumulation by whole plants for Harvests 1 and 2, respectively, were 57.7 and 80.8 mg in Experiment 1, and 86.3 and 93.3 mg in Experiment 2. The mean cadmium concentrations corresponding to the above values in whole plants for Harvests 1 and 2, respectively, were 2186 and 2960 ppm for Experiment 1 and 4665 and 4185 ppm for Experiment 2. There were significant differences in the slopes of the regression lines (Fig. 22) between harvests and between seasons. Of greater importance, however, is the seasonal effect. Waterhyacinths exposed to > 1.0 ppm cadmium were able to concentrate about 35 to 55 percent more cadmium in the same weight of plant tissue during the late summer and early fall than in the spring.

Discussion of cadmium effects
The overall effect of cadmium upon waterhyacinths was a sharp curtailment of plant growth and flowering at all levels of exposure. An earlier preliminary investigation (unpublished) had suggested that the toxicity threshold for cadmium was between 0.5 and 1.0 ppm. The present studies, however, indicate that the threshold toxicity is between 0 and 0.5 ppm. After six weeks exposure to cadmium, the total growth of waterhyacinths was significantly reduced by approximately
Figure 22. Concentration of cadmium in whole water hyacinths:
(A) Experiment 1 - Harvest 1, $Y = -30.3 + 523X$;
(B) Experiment 1 - Harvest 2, $Y = -93.9 + 674X$;
(C) Experiment 2 - Harvest 1, $Y = -17.9 + 708X$;
(D) Experiment 2 - Harvest 2, $Y = -66 + 856X$. 
Initial Cd Concentration (ppm)

A
$\text{r}^2=0.8967$

B
$\text{r}^2=0.8926$

C
$\text{r}^2=0.8882$

D
$\text{r}^2=0.9854$

Cd in Whole Plants (ppm x 10^{-3})

Initial Cd Concentration (ppm)
0.5 ppm. At cadmium concentrations > 1.0 ppm, there was essentially no further growth of the plants. The stunted condition of the roots and concomitant poor development of lateral roots suggests that the toxicity of cadmium to waterhyacinths may be the result of poor root function. Poor root function may have caused a nutrient deficiency or imbalance in the photosynthetic tissues, causing the chlorotic appearance and, consequently, resulting in poor plant growth. The chemical similarity of cadmium to the essential element zinc may have also caused direct interference in plant metabolism by the substitution of cadmium for zinc in some crucial metabolic pathway. In the present studies there are no seasonal interactive effects of cadmium upon the total plant growth.

Total plant accumulation of cadmium increased linearly as the exposure to cadmium increased. The order of both increasing cadmium uptake (mg) and increasing tissue concentrations was leaves < stems < roots. These results agree with other current research reports (Wolverton and McDonald unpublished manuscript; Reiniger 1977; Dabin et al. 1978; Tatsuyama et al. 1977, 1979). Although transport of cadmium to the leaves occurred readily, even at very low levels of exposure, the total cadmium in leaf tissues never represented more than ten percent of the total plant-absorbed cadmium. Total cadmium uptake showed a significant interactive effect due to season. In the fall, the cadmium uptake was about 35 to 55 percent greater than in the spring. Differences in mean cadmium concentrations in whole plants paralleled these differences in total plant cadmium. Since there were no seasonal interactive influences upon the growth of the plants in these experiments, and since the biomass of plants after
six weeks was only slightly greater than in the fall, these differences in cadmium uptake and mean plant cadmium concentration can not be entirely explained by a "dilution effect." Very likely the higher temperatures of the late summer and early fall promoted cadmium uptake by waterhyacinths. This temperature effect has been demonstrated by Tatsuyama et al. (1977).

**Effects of Copper**

**Appearance of plants**

At the initiation of each experiment, all plants were uniform in appearance and in degree of infestation with waterhyacinth weevils (Neochetina sp.) and diseases. After approximately 10 days, plants subjected to > 2.0 ppm copper were beginning to show signs of chlorosis. After three weeks (Harvest 1), the previously chlorotic plants had grown very little, chlorosis was pronounced, and plants appeared to be dying. Control plants and plants exposed to < 2.0 ppm copper were green, healthy, and visibly indistinguishable. During the second three-week period, some symptoms of nutrient deficiency, including a slight chlorosis and come brown streaking in the leaves, were apparent in control plants and all plants exposed to < 2.0 ppm copper. In Experiment 2, the effects of disease were severe on all plants. By the six-week harvest, plants in treatments < threshold toxicity were visibly crowded, and many new ramets were out of the water and hanging out of the barrels. Considerable quantities of dead, waterlogged tissues were apparent in Harvest 2, particularly in the second experiment. Roots of all plants exposed to < 2.0 ppm copper were normal in appearance at both harvests, whereas those exposed to > 2.0 ppm were stunted, blackened, and had poor lateral root development.
Growth measurements

Flowering and flower weights. During the first experiment, no flowers had been produced after three weeks (Harvest 1) in any treatment. After six weeks, however, flowers had formed in all treatments. There were no obvious differences in the number of flowers, flower weights, or the mean weights per flower among controls and plants exposed to < 2.0 ppm copper. At copper concentrations > 2.0 ppm, there was an approximately 30 to 50 percent reduction in the number of flowers and average flower weights, and an approximately 60 to 70 percent reduction in total dry flower weights. In Experiment 2, all treatments in both harvests were flowering. There were no apparent differences in flower number, dry weights or mean flower weights after three weeks growth. After six weeks there were no differences in flowering among controls and plants exposed to < 1 ppm copper, but, at concentrations > 1.0 ppm, flowering was drastically curtailed. Between 1.0 and 2.5 ppm, flower numbers and total dry weights were about 60 percent of the controls, but there was little influence on the average weight per flower. Copper concentrations of approximately 5.0 ppm reduced flower numbers and total dry weights to about 10 percent of the controls. There was a pronounced seasonal response in flowering which can not be explained by differences in physiological maturity at the outset of the two experiments. Control plants from Harvest 2 of Experiments 1 and 2, respectively, produced an average of 33.3 and 16.7 flowers with corresponding dry weights of 9.6 and 1.9 g and mean weights per flower of 0.29 and 0.12 g.

Dry weights of leaves. There were highly significant differences in the dry weights of leaf tissues exposed to increasing levels of
copper (Fig. 23). In both harvests of the two experiments there were no differences between controls and plants exposed to < 2.0 ppm copper. At concentrations > 2.0 ppm, however, there was a sharp curtailment in leaf weights. In Experiment 1, regression analysis demonstrated no significant differences in response to copper between the two harvest dates. In the second experiment, however, there were highly significant differences in response between harvests. After six weeks, essentially no further leaf tissue had been produced by plants exposed to > 2.0 ppm copper, while plants exposed to < 2.0 ppm showed a significant increase in dry leaf weights over the three-week period. A seasonal comparison of harvests showed a significant interactive seasonal effect upon leaf weights for Harvest 2, but no seasonal effect upon Harvest 1.

**Dry weights of stems.** Figure 24 indicates that increasing the level of exposure of copper also caused a highly significant reduction in the dry weights of stem tissues throughout both experiments. The effect of length of exposure was also significant for stems. Very little growth occurred after three weeks in plants exposed to > 2.0 ppm copper. There was a significant interactive effect upon stem weights of the second harvest, but not for the first. Seasonal differences in stem dry weights for Harvest 1 are apparently simple effects of seasonal differences in growth rates of the plants.

**Dry weights of roots.** Root weights decreased significantly as exposure to copper exceeded 2.0 ppm in both harvests of Experiment 1 and in Harvest 2 of Experiment 2 (Fig. 25). There were no significant differences in root weights in Harvest 1 of the second experiment, however, regardless of level of exposure to copper. In both experiments, there
Figure 23. Dry leaf weights of waterhyacinths exposed to copper:
(A) Experiment 1 - Harvest 1, log $Y = 1.51 - 0.72$
$\log (X + 1)$;
(B) Experiment 1 - Harvest 2, log $Y = 1.51 - 0.82$
$\log (X + 1)$;
(C) Experiment 2 - Harvest 1, log $Y = 1.11 - 0.92$
$\log (X + 1)$;
(D) Experiment 2 - Harvest 2, log $Y = 1.50 - 1.83$
$\log (X + 1)$. 
Initial Cu Concentration (ppm)

- **A** $r^2 = 0.8370$
- **B** $r^2 = 0.6988$
- **C** $r^2 = 0.7750$
- **D** $r^2 = 0.7691$
Figure 24. Dry stem weights of waterhyacinths exposed to copper:

(A) Experiment 1 - Harvest 1, log Y = 1.62 - 0.46 log (X + 1);

(B) Experiment 1 - Harvest 2, log Y = 1.90 - 0.80 log (X + 1);

(C) Experiment 2 - Harvest 1, log Y = 1.12 - 0.49 log (X + 1);

(D) Experiment 2 - Harvest 2, log Y = 1.61 - 1.20 log (X + 1).
A \quad r^2 = 0.7912

B \quad r^2 = 0.7805

C \quad r^2 = 0.6624

D \quad r^2 = 0.8413

Dry Weight (g \times 10^{-1})

Initial Cu Concentration (ppm)
Figure 25. Dry root weights of waterhyacinths exposed to copper:
(A) Experiment 1 - Harvest 1, log $Y = 1.33 - 0.41$
    $\log (X + 1)$;
(B) Experiment 1 - Harvest 2, log $Y = 1.48 - 0.56$
    $\log (X + 1)$;
(C) Experiment 2 - Harvest 1, log $Y = 0.84 - 0.03$
    $\log (X + 1)$;
(D) Experiment 2 - Harvest 2, log $Y = 1.31 - 0.79$
    $\log (X + 1)$. 
were significant between-harvest differences for dry root weights, but interactive effects were significant only in Experiment 2. Root growth was greater during the spring than in the late summer. A comparison of regression lines indicated a significant interactive effect for season in the first harvest, but non for the second harvest.

**Dry weights of dead tissues.** In Experiment 1, and in Harvest 2 of Experiment 2, there were significant differences in the dry weights of dead tissues present at different levels of exposure to copper. Dead tissue increased slightly during the first half of each experiment, but this trend was reversed during the second half of the experiments (Fig. 26). Regression analysis indicated that these differences were significant interactive effects between harvest date and exposure level. After three weeks, there were no significant differences in the slopes of the regression lines for the first harvests of the two experiments. Consequently, any observed differences in weights of dead tissues were probably the result of seasonal differences in the growth rates of waterhyacinths. For Harvest 2, however, there were significant interactive influences of season upon the weights of dead tissues at different levels of exposure to copper.

**Total growth of plants.** In both harvests of each experiment there were highly significant reductions in the total dry weights of plants as copper concentrations exceeded 2.0 ppm (Fig. 27). Regression analysis indicated that there were no significant interactive effects for harvest date in Experiment 1, and that observed differences were due only to the difference in length of growing period. An interactive response was indicated for Experiment 2. From the first to the second harvest
Figure 26. Dry dead-tissue weights of waterhyacinths exposed to copper:

(A) Experiment 1 - Harvest 1, log Y = 0.20 + 0.49 log (X + 1);
(B) Experiment 1 - Harvest 2, log Y = 1.39 - 0.66 log (X + 1);
(C) Experiment 2 - Harvest 1, log Y = -0.17 + 0.55 log (X + 1);
(D) Experiment 2 - Harvest 2, log Y = 0.68 - 0.03 log (X + 1).
Figure 27. Total growth of waterhyacinths exposed to copper:
(A) Experiment 1 - Harvest 1, $\log Y = 1.90 - 0.90 \log (X + 1)$;
(B) Experiment 1 - Harvest 2, $\log Y = 2.23 - 1.14 \log (X + 1)$;
(C) Experiment 2 - Harvest 1, $\log Y = 1.38 - 1.25 \log (X + 1)$;
(D) Experiment 2 - Harvest 2, $\log Y = 2.01 - 2.04 \log (X + 1)$. 
\[ r^2 = 0.8087 \]

\[ r^2 = 0.7193 \]

\[ r^2 = 0.7583 \]

\[ r^2 = 0.7867 \]
date very little growth had occurred in any plants exposed to > 2.0 ppm copper, but the controls and plants in treatments of < 2.0 ppm had almost tripled in weight. There were no significant interactive effects of season for the first harvest date. After six weeks (Harvest 2), season had a significant interactive influence upon growth.

**Metal concentration in tissues**

**Leaves.** The concentration of copper in leaf tissue (Fig. 28) increased linearly with increasing solution concentration. In Experiment 1 there was an apparent small increase in leaf copper from the three- to six-weeks harvest, but these differences were not significant. For Experiment 2, however, there were highly significant differences in leaf copper concentrations between harvests. Regression analysis indicated that there was no interactive influence of season upon the level of copper in leaf tissues for Harvest 1. There was a highly significant seasonal interactive effect for Harvest 2, however. Plants subjected to < 2.0 ppm showed no difference in leaf copper concentrations, whereas those given > 2.0 ppm contained considerably higher levels of copper in leaf tissues in Experiment 2 than in Experiment 1. Maximum leaf concentrations after three and six weeks were 76.6 and 139 ppm, respectively, for Experiment 1, and 81.5 and 627 ppm, respectively, for Experiment 2.

**Stems.** The concentrations of copper in stems also increased linearly with increasing level of exposure to copper (Fig. 29). Regression analysis showed that, not only were there significantly higher copper levels in stems in the second harvests of both experiments than in the first harvests, but also that these differences between harvests were
Figure 28. Concentration of copper in waterhyacinth leaves:
(A) Experiment 1 - Harvest 1, \( Y = 12.52 + 10.93X \);
(B) Experiment 1 - Harvest 2, \( Y = 0.90 + 22.10X \);
(C) Experiment 2 - Harvest 1, \( Y = 26.90 + 11.20X \);
(D) Experiment 2 - Harvest 2, \( Y = -45.9 + 110.8X \).
Figure 29. Concentration of copper in waterhyacinth stems:

(A) Experiment 1 - Harvest 1, \( Y = 21.33 + 49.7X \);
(B) Experiment 1 - Harvest 2, \( Y = -12.1 + 114X \);
(C) Experiment 2 - Harvest 1, \( Y = 2.7 + 97.4X \);
(D) Experiment 2 - Harvest 2, \( Y = -34.1 + 174.8X \).
interactive in nature. Responses for both harvests were also significantly greater in the second experiment than in the first. Copper concentrations < 2.0 ppm resulted in very little differences in stem copper concentrations between the two experiments after either three or six weeks, but, at concentrations > 2.0 ppm, plants in Experiment 2 had considerably greater copper concentrations in the stems than did the plants in Experiment 1. Maximum stem copper concentrations for Harvests 1 and 2 were 316 and 657 ppm, respectively, for Experiment 1, and 553 and 985 ppm, respectively, for Experiment 2.

Roots. Highest concentrations of copper in the plants were consistently found in the roots (Fig. 30). The same significant trend of linear increase in copper concentration in roots occurred with increased copper levels in solution. Regression analysis indicated that there were no differences in response between the two harvests of either experiment. At concentrations of > 2.0 ppm copper, the concentrations of copper in roots were considerably greater in Experiment 2 than in Experiment 1. A comparison of regression lines indicated that these seasonal differences were highly significant and interactive in nature. Maximum concentrations of copper in roots for Harvests 1 and 2 were 8764 and 7659 ppm, respectively, for Experiment 1, and 10,945 and 9524 ppm, respectively, for Experiment 2.

Metal uptake and partitioning

Leaves. Total copper partitioned into leaf tissue rarely exceeded ten percent of the total plant accumulation in either harvest of either experiment, and, in most treatments, leaf accumulation was below five
Figure 30. Concentration of copper in waterhyacinth roots:
(A) Experiment 1 - Harvest 1, Y = -699 + 1511X;
(B) Experiment 1 - Harvest 2, Y = -605 + 1460X;
(C) Experiment 2 - Harvest 1, Y = -1063 + 2180X;
(D) Experiment 2 - Harvest 2, Y = -843 + 1983X.
percent of the total plant copper. In the first harvest of both experiments, however, the leaves of control plants contained from 25 to 35 percent of the total plant copper. Throughout both experiments the fraction of total plant copper partitioned into leaves declined as the copper concentration in solution was increased. Total copper in leaf tissue increased approximately linearly with increasing exposure to copper and rarely exceeded 0.5 mg. There was a decrease in total leaf copper at exposure levels of approximately 5.0 ppm. This decrease paralleled the decrease in total plant copper shown in Figs. 31 and 32, and may possibly have been the result of an increasing proportion of leaf tissue in the dead plant fraction. For Harvest 1, the total leaf copper in the first experiment generally exceeded that of the second experiment. For Harvest 2, however, this trend was reversed. The maximum accumulations of copper in leaf tissues for Harvests 1 and 2 were 0.71 and 0.6 mg, respectively, for Experiment 1, and 0.47 and 1.1 mg, respectively for Experiment 2.

**Stems.** Figures 31 and 32 indicate that the total copper partitioned into stems increased slightly with increasing exposure level. The fraction of copper in stems decreased as exposure level was increased. Control plants partitioned about 25 percent of the total plant copper into the stems, whereas plants exposed to approximately 5.0 ppm copper partitioned about 10 percent of their total copper into stem tissues. The maximum amounts of copper found in stem tissues in Harvests 1 and 2 were 4.7 and 10.8 mg, respectively, for Experiment 1, and 3.1 and 4.6 mg, respectively, for Experiment 2.
Figure 31. Copper accumulation by waterhyacinths in Experiment 1:
(A) Harvest 1;
(B) Harvest 2.
Figure 32. Copper accumulation by waterhyacinths in Experiment 2:
(A) Harvest 1;
(B) Harvest 2.
Roots. Figures 31 and 32 show that the largest portion of total plant copper was usually associated with the root fraction. The percentage of total plant copper in roots increased with increase exposure levels of copper in both harvests of each experiment. Control plants had 35 to 50 percent of their total copper in the roots, whereas at copper concentrations > 2.0 ppm, roots contained about 65 to 85 percent of the plant copper. In Harvest 2 of the first experiment, root copper represented only about 55 percent of the total plant copper at exposure > 2.0 ppm. This was apparently the result of a substantial quantity of roots in the dead tissue fraction.

Dead tissues. Figures 31 and 32 indicate that the proportion of total plant copper bound in dead tissues also increased with increasing levels of exposure to copper. In Experiment 1 this increase was only marginally linear. In Experiment 2 the apparent linear relationship was masked by sharp increases at 2.7 and 2.1 ppm for Harvests 1 and 2, respectively. These apparently aberrant increases in Experiment 2 reflect concomitant sharp decreases in total root copper and are most likely the results of large portions of root tissue in the dead fraction. Maximum accumulations of copper in dead tissues for Harvests 1 and 2 were 13.7 and 48.6 mg, respectively, in Experiment 1, and 14.9 and 26.9 mg, respectively, in Experiment 2.

Total uptake and mean plant concentration. The total plant accumulation of copper increased approximately linearly with increasing copper exposure up to approximately 4.0 ppm. At copper concentrations > 4.0 ppm, there was a decrease in total plant copper, which was most likely the result of the poor condition of the plants at high exposure levels.
A large part of the plant biomass at concentrations of copper > 4.0 ppm was dead tissue at varying stages of deterioration. Therefore some loss of copper may have occurred through leaching from the dead tissues. There was also an apparent seasonal effect upon total copper accumulation by whole plants. During the spring, when the original plants were essentially free of disease and insect damage, the total accumulation was greater than in the late-summer experiment, even though the average tissue levels (Fig. 33) were less during the spring experiment. This is clearly reflected in the average whole-plant copper concentrations and is the result of the dilution effect due to more rapid growth of the plants in the spring than in the late summer and early fall.

Regression analysis indicated that there were no significant differences in the mean whole-plant copper concentrations between Harvests 1 and 2 of either experiment, but that the seasonal effects were highly significant. Maximum total plant copper for Harvests 1 and 2 were 104 and 123 mg, respectively, in Experiment 1, and 83 and 96 mg, respectively, in Experiment 2. Mean copper concentrations in whole plants corresponding to the above uptake values for Harvests 1 and 2 were 2891 and 3586 ppm, respectively, for Experiment 1, and 4779 and 5238 ppm, respectively, for Experiment 2.

**Discussion of copper effects**

The effects of copper on the overall plant growth were essentially negligible at < 2.0 ppm exposure, but, at high concentrations, there was a sharp reduction in the plant growth and flowering. A slight reduction in growth of plants at approximately 1.0 ppm copper in the second experiment suggested that the threshold toxicity might be close
Figure 33. Concentration of copper in whole waterhyacinths:
(A) Experiment 1 - Harvest 1, \( Y = -226 + 479X \);
(B) Experiment 1 - Harvest 2, \( Y = -249 + 578X \);
(C) Experiment 2 - Harvest 1, \( Y = -467 + 981X \);
(D) Experiment 2 - Harvest 2, \( Y = -505 + 1089X \).
to 1.0 ppm. All tissues were severely affected by exposure to copper in excess of 2.0 ppm. The stunted, blackened appearance of new roots and the concomitant poor development of lateral roots suggested that for copper (as with cadmium) the primary influence of high exposure to copper was upon root function, with the secondary effects resulting from induced nutrient imbalances, as well as through direct interference in plant metabolism.

Uptake and concentration of copper by all plant tissues increased essentially linearly as the solution concentration was increased. The order of increasing concentrations of copper in the plant tissues was leaves < stems < roots. These observations agree with previous studies (Sutton and Blackburn 1971c; Wolverton and McDonald unpublished manuscript; Tatsuyama et al. 1977; Johnson and Sheenan 1978; Thakurta and Mitra 1978). Although transport of copper from the roots to the shoots was good, even in control plants (approximately 0.04 ppm copper in the nutrient solution), the proportion of total copper transported acropetally decreased as the exposure to copper was increased. At high levels of exposure, the total copper content of either stems or leaves rarely exceeded 10 percent of the total plant copper. A seasonal interactive effect was demonstrated for total plant accumulation. Total copper accumulation was greater during the spring than during the fall, even though the tissue concentrations were substantially less than in the fall. More rapid growth during the spring and the concomitant dilution effect would apparently account for the observed differences in seasonal accumulation of copper. The higher temperatures occurring in the late summer very likely would have enhanced the rate of copper absorption and, therefore, could have promoted the absorption of a larger quantity of copper by a smaller biomass.
Comparison of Metal Effects

Effects on plant growth

The effects of lead upon waterhyacinths at the levels of exposure used in these experiments can be considered minimal in comparison with the effects of either cadmium or copper. Although regression analyses did indicate significant trends in the response to lead, there were essentially no visible effects upon either the physical condition or the biomass of plants at any level of exposure. In comparison, the response to either cadmium or copper was obvious. Plants exposed to > 0.5 ppm cadmium were visibly stunted and were showing signs of chlorosis after only ten days; after three weeks, all plants exposed to > 0.5 ppm cadmium were chlorotic and appeared to be dying. Plants exposed to > 2.0 ppm copper were also becoming chlorotic after ten days and, after three weeks were visibly indistinguishable from the cadmium-stressed plants. All control plants and plants exposed to < 0.5 ppm cadmium or < 2.0 ppm copper were physically indistinguishable from any of the plants in the lead experiment. Among the most obvious signs of toxicity were the effects upon the roots. All plants adversely affected by either cadmium or copper had very poor root development, particularly on the new ramets. Roots were stunted, blackened, very brittle, and possessed very few lateral roots. Roots in such condition were probably non-functional. The production of new ramets was severely curtailed in all plants exposed to > 0.5 ppm cadmium or > 0.2 ppm copper, but there were no visible differences among controls, plants exposed to < 0.5 ppm cadmium or < 2.0 ppm copper, or any plants in the lead experiments.
The overall growth of waterhyacinths was obviously not greatly affected by exposure to lead. Exposure to approximately 0.5 ppm cadmium or 2.0 ppm copper, however, resulted in a sharp decline in plant growth. Figures 34, 35, and 36 show the relative growth rates (RGR) of plants exposed to lead, cadmium, and copper, respectively, for the six-weeks harvest dates. In Experiments 1 and 2, the RGR for plants exposed to approximately 5.0 ppm lead were about 89 and 110 percent of the controls, respectively, whereas RGR's for plants exposed to approximately the same concentrations of cadmium and copper were about 10 and 3 percent of the controls, respectively, for cadmium, and 17 and 5 percent of the controls, respectively, for copper. Exposure to lead, therefore, had very little effect upon RGR, but cadmium or copper exposure resulted in a rapid reduction in the RGR. Plants in all experiments generally grew more rapidly during the spring than in the fall, and these seasonal differences in growth rates were reflected in the RGR's as well as in the total plant growth. In any case, the gross responses to toxic doses of metals were similar. The reductions in growth and RGR at approximately 0.5 ppm cadmium or 2.0 ppm copper indicated that threshold toxicities are between 0 and 0.5 ppm for cadmium and 1.0 and 2.0 ppm for copper. Threshold toxicity of lead could not be evaluated from these studies.

Effects of metal accumulation

The adsorption, transport, and tissue concentrations of lead, cadmium, and copper depend upon length of exposure, individual metal and season of the year. The concentration and partitioning of total metal into the various plant tissues followed the pattern of leaves < stems < roots and increased in an approximately linear manner with
Figure 34. Relative growth rates of waterhyacinths exposed to lead for six weeks:
   (A) Experiment 1, log $Y = 0.1407 - 0.0024 \log (X + 1)$;
   (B) Experiment 2, log $Y = -0.9020 + 0.0459 \log (X + 1)$.

Figure 35. Relative growth rates of waterhyacinths exposed to cadmium for six weeks:
   (A) Experiment 1, log $Y = -0.9887 - 1.4116 \log (X + 1)$;
   (B) Experiment 2, log $Y = -1.1787 - 1.8257 \log (X + 1)$. 
The graphs depict the relationship between initial metal concentration (Pb or Cd) and RGR (g/g/day) for two different treatment conditions (A and B). The coefficient of determination ($r^2$) for each condition is indicated:

- For Pb conditions:
  - A: $r^2 = 0.0836$
  - B: $r^2 = 0.0438$

- For Cd conditions:
  - A: $r^2 = 0.9382$
  - B: $r^2 = 0.7480$
Figure 36. Relative growth rates of waterhyacinths exposed to copper for six weeks:

(A) Experiment 1, \( \log Y = -0.8432 - 1.0702 \log (X + 1) \);
(B) Experiment 2, \( \log Y = -0.7323 - 2.0575 \log (X + 1) \).
increasing level of exposure as well as with length of exposure for all three metals. There were no obvious differences in the total plant accumulation of lead between the spring and late summer. Average plant concentrations of lead were significantly greater in the fall than in the spring. Plants in the late-summer experiment not only contained significantly higher concentrations of cadmium, but also had a significantly higher total plant cadmium accumulation than the plants in the spring experiment. The total plant accumulation of copper was greater in the spring than in the late summer and early fall, but tissue concentrations were higher in the fall than in the spring. The general responses of waterhyacinths to lead, cadmium, and copper are summarized in Table 2, and the seasonal effects on RGR metal uptake, and tissue concentrations are shown in Table 3.

Summary and Conclusions

The effects of lead upon the growth of waterhyacinths were essentially negligible at the levels of exposure in this study. Exposure to $> 0.5$ ppm cadmium or $> 2.0$ ppm copper resulted in a pronounced chlorosis and a sharp reduction in root, stem, and leaf development, flowering, and production of new ramets.

Relative growth rates (RGR) were greatly reduced by exposure to cadmium or copper but were not substantially affected by lead. The growth of all plants was greater during the spring than during the late summer experiment and may be attributed to both increasing ambient temperatures and healthier plants in the spring, as compared with the decreasing temperatures and higher levels of diseases and insect infestations in the late summer and early fall. In the lead experiments the maximum
Table 2. General responses of waterhyacinths to exposure to lead, cadmium, and copper in comparison with control plants. Responses are for the level representing the threshold toxicity, if any, and are consistent up to the highest level tested.

<table>
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<tr>
<th>Response Variable</th>
<th>Metal</th>
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<td>Lead</td>
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<tr>
<td>Leaf Color</td>
<td>Green</td>
</tr>
<tr>
<td>New Ramets</td>
<td>Abundant</td>
</tr>
<tr>
<td>Flowers</td>
<td>Abundant</td>
</tr>
<tr>
<td>Total Plant Growth</td>
<td>Good</td>
</tr>
</tbody>
</table>
Table 3. Maximum relative growth rates, maximum total plant accumulation of metals, and maximum concentrations of metals in whole waterhyacinths for the six-weeks harvest dates.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Maximum relative growth rate, g/day</th>
<th>Maximum metal accumulation in whole plants, mg</th>
<th>Maximum metal concentration in whole plants, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls 5.0 ppm*</td>
<td>Controls 5.0 ppm*</td>
<td>Controls 5.0 ppm*</td>
</tr>
<tr>
<td>Lead</td>
<td>0.158</td>
<td>0.141</td>
<td>0.133</td>
</tr>
<tr>
<td>Fall</td>
<td>0.288</td>
<td>0.234</td>
<td>0.2462</td>
</tr>
<tr>
<td>Spring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.139</td>
<td>0.014</td>
<td>-0.001</td>
</tr>
<tr>
<td>Fall</td>
<td>0.159</td>
<td>0.365</td>
<td>0.933</td>
</tr>
<tr>
<td>Spring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>0.127</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>Fall</td>
<td>0.126</td>
<td>0.012</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*The use of 5.0 ppm as the maximum exposure level was necessary because there was some indication from water analyses that some redissolution of precipitated metals had occurred in some experimental units. Therefore the use of the level 5.0 ppm should be construed as meaning the highest level of exposure up to and including 5.0 ppm.
RGR's for controls and highest level of exposure were 0.158 and 0.141 g \cdot g^{-1} \cdot day^{-1}, respectively, in Experiment 1, and 0.133 and 0.144 g \cdot g^{-1} \cdot day^{-1}, respectively, in Experiment 2. Corresponding values for cadmium were 0.139 and 0.014 g \cdot g^{-1} \cdot day^{-1}, respectively, in Experiment 1, and 0.118 and -0.001 g \cdot g^{-1} \cdot day^{-1}, respectively, in Experiment 2. The respective RGR's for the copper study were 0.127 and 0.008 g \cdot g^{-1} \cdot day^{-1}, respectively for Experiment 1, and 0.126 and 0.004 g \cdot g^{-1} \cdot day^{-1}, respectively for Experiment 2.

Threshold toxicities to waterhyacinths were between 0 and 0.5 ppm for cadmium and between 1.0 and 2.0 ppm for copper. Threshold toxicity of lead could not be evaluated from this study.

The primary effect of cadmium and copper upon waterhyacinths appeared to be upon root growth and function, especially on the new ramets, where there was little root development. The development of chlorosis and concomitant reduction in growth probably were the results of induced nutrient imbalances and/or deficiencies, but possible direct interference with metabolic functions can not be discounted.

The concentrations and partitioning of metals in waterhyacinths increased approximately linearly with increased exposure and followed the pattern leaves < stems < roots. The uptake and partitioning of metals depended greatly upon the individual metal, the level of exposure, and season of the year. The plant concentrations of lead, cadmium, and copper were higher in the fall than in the spring. Total plant accumulations of lead were similar in both seasons, whereas cadmium accumulation was greater in the fall, and copper was greater in the spring.

All three metals were at least partly mobile and were transported to the stems and leaves in proportion to their mobility within the plant. The order of increasing mobility was lead < cadmium < copper.
The results of this study indicate that caution should be exercised when extrapolating from short-term laboratory studies to field situations in which waterhyacinths may be used for the removal of toxic substances from wastewater effluents. The use of data from short-term laboratory studies in conjunction with field measurements of the growth of waterhyacinths under optimum conditions in the prediction of the ability of these plants to accumulate toxic metals from wastewater may be unreliable.
EFFECTS ON ALLIGATOWEED

Materials and Methods

Plant Materials

Initial stem cuttings of alligatorweed were obtained from a clone of a "broad-stemmed" alligatorweed biotype growing under hydroponic culture in a greenhouse at the U.S.D.A., Southern Weed Science Laboratory in Stoneville, Mississippi. The clone was originally started from alligatorweed collected from Blue Lake near Itta Bena, Mississippi. Stem cuttings from the cloned plants materials were sprouted in bottles containing full-strength Hoagland's nutrient solution and were allowed to grow until sufficient plant material was available to being the experiments.

Experimental Setup and Design

Two-node stem cuttings were sprouted indoors in tap water under artificial lighting and were allowed to grow for one week. Plants were then selected for uniformity of shoot and root initiation. Two two-node stems were weighed and then placed into 375-ml amber bottles containing full-strength Hoagland's nutrient solution with 1 ppm iron provided by Sequestrene 330 iron chelate. Metals were provided as Cd(NO₃)₂, Pb(NO₃)₂, or CuSO₄ from stock solutions containing 375 ppm cadmium, lead, or copper. Metal treatments were 0.10, 0.5, 1.0, 2.0, 3.0, 4.0, or 5.0 as cadmium, lead, or copper. The experiments were conducted in a completely randomized design with 19 treatments and three replications per treatment.
The 0.0 ppm treatment served as the control for all three metals. An additional three replications were taken for dry weight:fresh weight ratios at the beginning of the experiments. All plants were placed in outdoor screened enclosures for the duration of the experiments and were watered as needed with deionized water. The two experiments were conducted from July 29 to August 26 and from September 2 to September 30, 1979. Water samples were taken for metal analysis by atomic absorption spectrophotometry at the outset and at the end of the four-week experimental period. Water analysis data are presented in Table 4.

Growth Observations and Measurements

Plants were observed closely for the appearance of any symptoms of toxicity during the course of the experimental period. After a four-week experimental period, plants were harvested, washed with tap water to remove surface accumulations of metals and/or algae, rinsed with deionized water, and separated into leaves, new stems, original stem cuttings, and roots. Plant tissues were dried at 65°C for 48 h and weighed. Growth measurements included the dry weights of leaves, new stems, original stem cuttings, and roots. From the dry weight:fresh weight ratios, an estimated original dry weight (hereinafter called original weight) was calculated for each experimental unit. Growth was estimated as the differences between biomass and the original weight. Relative growth rates (RGR) were calculated.

Tissue Analyses

Dry leaves, new stems, original stem cuttings, and roots were ground in a Wiley mill to pass a 40-mesh screen. Ground plant material was then re-dried at 65°C for 24 h. Approximately 0.2 g samples were weighed
Table 4. Metal concentrations in initial solutions of the alligatorweed experiments as measured by atomic absorption spectrophotometry.

<table>
<thead>
<tr>
<th></th>
<th>Calculated Initial Metal Concentrations (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.00</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.00</td>
</tr>
<tr>
<td>Copper</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>0.00</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.00</td>
</tr>
<tr>
<td>Copper</td>
<td>0.00</td>
</tr>
</tbody>
</table>
into 50-ml beakers and ashed at 450 °C for 5 h in a muffle furnace. Tissue samples that were too small to grind were ashed whole. The ash was weighed and then digested with 1 ml of 6 N HCl. Because of high residual carbon, all digested samples were evaporated to dryness on a hotplate, re-ashed at 525 °C for an additional 4 h, cooled to room temperature and re-digested with an additional 1 ml of 6 N HCl. After the second digestion, all samples were filtered and brought to a final volume of 50 ml with de-ionized water. Metal content was determined by atomic absorption spectrophotometry in the same manner as for the waterhyacinth experiments. Metal concentrations (ppmw) and total accumulations (mg) were calculated for each plant tissue and for whole plants.

**Statistical Analysis**

Data were subjected to linear regression analysis. Log 10 transformations were employed for all regressions involving growth variables. Initial solution concentrations of lead, cadmium, or copper determined by atomic absorption spectrophotometry were used in all regressions of variables upon solution concentrations to allow for loss of metals through precipitation from the solutions.

**Results and Discussion**

**Effects of Lead**

**Appearance of plants**

Initially the plants were all uniformly green, healthy, and apparently free of insects and disease. There were no visible differences among plants at any level of exposure to lead. All plants remained green and apparently healthy and were still indistinguishable from the controls.
at the time of harvest (four weeks). Roots were white, well-developed, and had a profuse development of lateral roots.

At the initiation of the first experiment, additional plants had been setup for a planned second harvest at eight weeks. At the time of the four-week harvest some insect activity was noticed on controls and on all plants exposed to lead. By six weeks into the experiment it was obvious that the second harvest was going to be impossible to conduct because of extensive damage to the controls and all lead treatments. After eight weeks essentially no viable leaf or stem tissue remained on either the control plants or the plants exposed to lead. At this time, the second experiment was in progress and some insect activity was noticeable. Three insects were identified as jointly responsible for the damage. The first, a thrips, was identified as the host-specific alligatorweed thrips, *Amynothrips andersoni* (Thysanoptera: Phlaeothripidae), which had been imported from South America in the early 1970's for the biological suppression of alligatorweed. This insect was found to be feeding upon the terminal buds and new leaves of all stems of the controls and lead treatments. Extensive damage from this insect resulted in the wilting and abscission of most of the plant terminals. The second insect was a lepidopteran, *Spodoptera latifascia* (Lepidoptera: Noctuidae), a polyphagous feeder of the armyworm group. The larvae were responsible for an almost complete defoliation of the controls and lead treatments. The third insect, a cockroach (Orthoptera: Blattidae) was seen emerging from large holes in the original stem cutting and from openings of the bottles, but could not be captured and was, therefore, not specifically identified. The cockroach was presumably responsible
for the holes in the original stem cuttings, concomitant waterlogging of these original stems, and for several cases in which the original stem was completely destroyed, severing the new plant portions from the root system and causing their death through desiccation. The second experiment, conducted in an adjacent cage, was harvested before insect damage was sufficiently extensive to adversely affect the four-weeks harvest.

Growth measurements

Flowering. Few flowers were observed at any time during the course of either experiment. It is assumed that the new growth was not sufficiently mature physiologically to begin flowering, as alliatorweed in nature was in full flower at this time.

Dry weights of leaves. There were no significant differences in the dry weights of leaf tissue at any level of exposure to lead in either experiment (Fig. 37). On the average, the growth of leaf tissue was significantly greater in the first than in the second experiment. The differences were not interactive in nature, however, and may be presumed to be the result of a more rapid growth rate at the higher temperatures obtained in the first experiment.

Dry weights of new stems. Again there were no significant differences in growth response to lead in either experiment (Fig. 38). As for leaves, there was significantly greater growth during the first than during the second experiment. These differences possibly may be the result of seasonal differences in ambient temperature.

Dry weights of roots. Again there were no significant differences in growth response to lead in either experiment (Fig. 39). The reduction in root growth with the cooler ambient temperatures during the second
Figure 37. Dry leaf weights of alligatorweed exposed to lead:
(A) Experiment 1, \( \log Y = -0.06 - 0.03 \log (X + 1) \);
(B) Experiment 2, \( \log Y = -0.19 - 0.012 \log (X + 1) \).
Figure 38. Dry stem weights of alligatorweed exposed to lead:
(A) Experiment 1, \( \log Y = -0.019 - 0.147 \log (X + 1) \);
(B) Experiment 2, \( \log Y = -0.115 - 0.123 \log (X + 1) \).
Figure 39. Dry root weights of alligatorweed exposed to lead:

(A) Experiment 1, log $Y = -0.845 + 0.223 \log (X + 1)$;
(B) Experiment 2, log $Y = -1.276 + 0.160 \log (X + 1)$. 
experiment was of the same magnitude as the reductions in leaf and stem weights.

**Total growth of plants.** Figure 40 shows the total growth of alligatorweed at all levels of exposure to lead. The growth of alligatorweed in Experiment 2 was about 70 percent of that in the earlier experiment. This may possibly be a seasonal effect resulting from temperature differences and can not in any way be construed as the result of exposure to lead, as differences were not interactive in nature, but were of equal magnitude for all levels of exposure.

**Metal concentrations in tissues**

**Leaves, new stems, and original cuttings.** At no time in either experiment was lead detected in either leaf or new stem tissues. In Experiment 1, lead was not detected in original stem cuttings. In Experiment 2, however, very low levels were found in the original stem cuttings. At measured original lead exposure levels of 1.1, 1.7, 1.9, and 2.2 ppm (corresponding to the calculated initial levels of 2, 3, 4, and 5 ppm, respectively) the mean concentrations of lead in original stem cuttings were 53.14 ± 61.69, 9.82 ± 17.00, 8.32 ± 14.41, and 55.04 ± 10.56 ppm, respectively. The wide range of variation and the lack of any apparent relationship to the concentration of lead in the nutrient solution suggest that these apparent tissue concentrations may have been the result of surface contamination (algae, bacteria) that were not removed in the rinsing procedure.

**Roots.** The concentration of lead in root tissues increased with increased exposure level (Fig. 41). In Experiment 1, the increase was significant and relatively linear as exposure level increased. In the second experiment, the lead concentrations in roots were substantially
Figure 40. Total growth of alligatorweed exposed to lead:

(A) Experiment 1, \( \log Y = 0.373 - 0.056 \log (X + 1) \);

(B) Experiment 2, \( \log Y = 0.263 - 0.103 \log (X + 1) \).
Figure 41. Concentration of lead in alligatorweed roots:

(A) Experiment 1, \( y = -6.67 + 109.56x \);
(B) Experiment 2, \( y = 392 + 1374x \).
higher than those in Experiment 1, but the linear relationship of increasing root lead with increasing exposure level was not readily observable, due to wide variation within a given treatment. Two outliers were observed in the data, one at 1.1 ppm lead (19,785 ppm in roots), and the other at 2.2 ppm lead (8778 ppm in roots). Regression analysis including these outliers indicated that there were no significant differences due to level of exposure to lead but indicated significant differences when the two outliers were deleted. The two outliers appear to be the result of either accidental contamination or analytical error. The regression line shown in Fig. 41-B is based upon data with these two outliers deleted. All data points except the 19,785 ppm outlier are plotted, however. Differences in the slopes of the regression lines are not significantly different. The apparent interactive differences between the two experiments were most likely the product of wide variation with treatments. With the exceptions of the two outliers, maximum lead concentration in roots was 5974 ppm at an exposure level of 2.2 ppm lead.

**Metal uptake and partitioning**

*Leaves, new stems, and original stem cuttings.* There was no detectable contribution of either leaves or new stems to the total plant lead accumulation (Fig. 42). Original stem cuttings contained from 0 to 75 percent of the total plant lead accumulation. There was no detectable lead in original stems during the first experiment, while the level of lead in the second experiment varied greatly and showed no obvious trend. Extreme variation in the fraction of lead in the original stems seems to somewhat support the hypothesis of external contamination,
Figure 42. Lead accumulation by alligatorweed:
(A) Experiment 1;
(B) Experiment 2.
particularly when the variation within a given treatment went from 0 to 75 percent of the total plant accumulation. Maximum accumulation associated with original stem cuttings was 71 mg at 1.1 ppm lead. This incidentally, did not correspond to the outlier (19,785 ppm) mentioned previously for lead concentration in roots.

Roots. The total lead accumulation in root tissues represented the total plant lead uptake for Experiment 1 and closely paralleled the curve for total plant lead in Experiment 2 (Fig. 42). The maximum lead found in root tissue during the first experiment was 71 µg to 1.0 ppm lead. In Experiment 2, the maximum lead accumulation was 363 µg, which represented the 19,785 ppm outlier at initial lead level of 1.1 ppm. A second high value was 362 µg at 2.2 ppm.

Total uptake and mean plant concentrations. In Experiment 1, the total plant accumulation of lead was bound in the root tissue (Fig. 42). In Experiment 2, the total plant lead accumulation was the same as that for roots up to the 0.5 ppm exposure level. Total plant lead fluctuated widely within a treatment, but the general trend was toward higher accumulation at higher exposure levels. The wide fluctuations in lead content within a single treatment were apparently not related to root biomass. A reasonable portion of this fluctuation may have been the result of absorption of lead by epiphytic algae and/or bacteria. A visual assessment of algal level in the solutions at harvest, however, did not support an hypothesis of algal absorption of lead as a factor affecting lead content of the plants. The maximum accumulations of lead in whole plants were 71 µg (at 1.1 ppm lead) and 389 µg (at 2.2 ppm lead) in Experiments 1 and 2, respectively. Metal concentrations
in whole plants were about ten times greater at all levels for Experiment 2 than for Experiment 1 (Fig. 43). A significant interactive difference was noted when the two regression lines were compared. This apparent interaction is at least partly due to the two outliers (at 1.1 and 2.2 ppm) in Experiment 2 discussed previously in the section on concentrations throughout the entire second experiment. A portion of the overall differences in whole-plant lead concentrations (but not the apparent interactive effect) may also be attributed to the dilution effect due to greater growth of plants during Experiment 1. The whole-plant lead concentrations corresponding to the maximum levels of accumulation for Experiments 1 and 2 were 22.7 and 191 ppm, respectively.

**Discussion of lead effects**

There were no significant differences in the growth response of alligatorweed to lead at the exposure levels obtained during these experiments. The lack of any observable differences in growth or any symptoms of toxicity may be attributed to any of several factors. First, lead was not readily transported from the roots to the growing shoots, and lead was not detected in either leaves or new stems. Lead was not found in the original stems in the first experiment and was found only occasionally during the second experiment at the higher levels of exposure. This suggests that lead enters the plant entirely, or largely, through the root system. The lack of any toxicity symptoms and presence of normal root development further suggest that the plant-accumulated lead was bound apoplastically in the root tissues with little or no absorption into the symplast. A second possibility is
Figure 43. Concentration of lead in whole alligatorweed:
(A) Experiment 1, $Y = -0.23 + 7.27X$;
(B) Experiment 2, $Y = 13.16 + 57.28X$. 
A

$r^2 = 0.3198$

B

$r^2 = 0.4023$

Pb in Whole Plants (ppm x 10)

Initial Pb Concentration (ppm)
that lead measured in root tissues is largely extraneous and associated
with the periphytic assemblage on the root surfaces. This has been
noted by Patrick and Loutit (1977). Procedures have not yet been
developed to adequately remove adhering algal and bacterial periphyton
from plant roots without destroying the roots, themselves. Consequently,
there was no way to adequately assess the contribution of the periphytic
assemblage to root lead accumulation. The error in measurement may
very likely be substantial and may have been responsible for a large
part of the wide variation of root and total plant lead observed in
these experiments. Another factor that very likely may be important
is the possibility that lead may not be very toxic to the plants.
Substantial precipitation of initial metal from solution occurred
(e.g., one lead solution intended to have a lead concentration of 5.0
ppm was found to be only 0.8 ppm upon analysis), and may have also
resulted in the reduction of the solution lead level below the
threshold for toxicity. Any, or all, of the above factors, and
possibly many others, may have acted to render the lead non-toxic to
alligatorweed at the levels of exposure obtaining in these experiments.

The uptake and concentrations of lead by alligatorweed observed
in these experiments tended to increase, albeit erratically, with in-
creasing level of exposure. The maximum accumulations of plant metal
appear to be reasonably consistent with values reported previously
for alligatorweed (Wolverton and McDonald 1975a). A significantly
greater plant concentration of lead, as well as total plant accumula-
tion, was found in Experiment 2 than in Experiment 1. This must be
at least partially attributed to the dilution effect from greater
growth that occurred during the first experiment. The dilution effect
is inadequate to explain completely the difference in magnitude of lead uptake, as total plant growth in the second experiment was about 70 percent that of the first experiment.

Effects of Cadmium

Appearance of plants

At the initiation of the experiments all plants were uniformly green and healthy. After about two weeks, the plants exposed to > 2 ppm cadmium were heavily chlorotic and had grown very little. Control plants and all cadmium treatments \( \leq 2 \) ppm were green, healthy, and were growing well. By the harvest (4 weeks) the plants exposed to 1 and 2 ppm were also slightly chlorotic and appeared to have less growth than the controls. Plants exposed to 0.5 ppm cadmium were indistinguishable from the controls. The roots were reduced in the higher level cadmium treatments more or less in proportion to exposure level. At cadmium exposure of 4 to 5 ppm, there were few roots present, and those present were greatly reduced, both in length and in the extent of development of lateral roots. At the harvest in Experiment 1, some insect activity was noticed on the plants exposed to \( \leq 2 \) ppm cadmium. The same insects were present as were noted for the controls and lead experiment. At the time of the second harvest of Experiment 1 (8 weeks), all cadmium treatments \( \leq 2.0 \) ppm had been destroyed by the insects, so that the second harvest had to be aborted. No insect feeding was found on any plants exposed to > 2 ppm cadmium, however. Apparently the insects had either totally avoided the plants exposed to high cadmium levels or had been killed by the cadmium in the plant tissues before any visible damage had been done. Since the
alligatorweed thrips was among the insects feeding upon controls and cadmium treatments $\leq 2.0$ ppm, these observations suggest that cadmium may be a potential factor that could have deleterious effects on the biological control of alligatorweed.

Growth measurements

**Flowering.** As in the case of the lead experiments, very few flowers were found in the cadmium experiments, and the only adequate conclusion is the lack of physiological maturity.

**Dry weights of leaves.** Dry leaf weights decreased sharply in response to cadmium concentrations $> 0.5$ ppm, and, at concentrations $> 1.0$ ppm, there was little further effect on the leaves (Fig. 44). Regression analysis indicated that the response to cadmium was highly significant. There were no significant difference in response of dry leaf weights to cadmium between the two experiments.

**Dry weights of new stems.** The responses of the stems to increasing cadmium concentrations (Fig. 45) were similar to the leaf responses. Beyond $1.0$ ppm exposure very little new stem tissue was produced in either experiment, and the reduction in dry stem weights was shown to be highly significant. The dry stem weights in Experiment 2 were significantly greater than in Experiment 1. A comparison of regression lines showed no significant differences in slopes for the two experiments. Therefore, the observed differences were not interactive in nature.
Figure 44. Dry leaf weights of alligatorweed exposed to cadmium:
(A) Experiment 1, log Y = -0.186 - 1.116 log (X + 1);
(B) Experiment 2, log Y = -0.276 - 0.849 log (X + 1).
Figure 45. Dry stem weights of alligatorweed exposed to cadmium:
(A) Experiment 1, \( \log Y = -0.257 - 1.29 \log (X + 1) \);
(B) Experiment 2, \( \log Y = -0.206 - 0.915 \log (X + 1) \).
Dry weights of roots. A sharp reduction in root growth occurred at cadmium exposure of > 1.0 ppm (Fig. 46). There was little new root tissue produced by alligatorweed at cadmium concentrations > 2.0 ppm in either experiment. A comparison of regression lines indicated no significant differences in the response of alligatorweed root growth between the two experiments.

Total growth of plants. The growth of alligatorweed exposed to approximately 0.5 ppm cadmium was not different than the control plants (Fig. 47). A decrease in growth occurred between 0.5 and 1.0 ppm exposure, and beyond 1.0 ppm there was very little growth of the plants. Regression analysis indicated that the decrease in growth was not significant in Experiment 1 (P > F = 0.0595). Differences were significant, however, for Experiment 2. It must be noted, however, that this apparent lack of significant differences for growth in Experiment 1 may be an artifact of the inclusion of original stems in the calculation of growth (Growth = Biomass - Estimated Original Dry Weight). The attack of the insects upon the controls and cadmium treatments had caused some damage to the original stem cuttings. Loss of tissue in this manner may have affected the overall growth response sufficiently to cause differences to be non-significant. It also must be pointed out that alpha = 0.0595 is marginally significant, if one accepts 94 percent as an acceptable level of significance, rather than the customary 95 percent level. For all practical purposes, considering the uncontrollable source of variation (insect feeding), there was a significant effect of cadmium on alligatorweed in the first experiment. A comparison of the slopes of the two regression lines also indicated no significant
Figure 46. Dry root weights of alligatorweed exposed to cadmium:
(A) Experiment 1, log $Y = -0.849 - 1.998 \log (X + 1)$;
(B) Experiment 2, log $Y = -1.155 - 0.854 \log (X + 1)$. 
Figure 47. Total growth of alligatorweed exposed to cadmium:
(A) Experiment 1, log $Y = 0.025 - 0.944 \log (X + 1)$;
(B) Experiment 2, log $Y = 0.160 - 0.872 \log (X + 1)$. 
differences between the two experiments, further supporting the contention that differences observed in Experiment 1 are real and should be considered significant.

Metal concentrations in tissues

**Leaves.** Cadmium concentration in alligatorweed leaves increased with increasing cadmium exposure in both experiments (Fig. 48). Although there was rather wide variation among treatments, regression analysis indicated that the increases in leaf cadmium concentrations were highly significant. There were not significant differences between the two experiments, however. Maximum leaf cadmium concentrations for Experiments 1 and 2 were 18.1 and 36.0 ppm, respectively.

**New stems.** The cadmium concentrations in new stems (Fig. 49) were somewhat less variable at lower levels of exposure than was true for leaves. As exposure level increased, the variation within a treatment also increased, and both experiments had one widely divergent measurement between 415 and 510 ppm. Difficulties in measurement on very small tissue samples in treatments > 2.0 ppm resulted in some zero or near-zero ppm readings at 2.8, 3.7, and 4.7 ppm cadmium exposure in Experiment 2. Regression analysis indicated, despite the variation, that cadmium concentrations in new stems increased significantly with increasing level of exposure, but differences observed between the two experiments were not significant. Maximum cadmium concentration in new stems for Experiments 1 and 2 were 199 and 142 ppm, respectively.
Figure 48. Concentration of cadmium in alligatorweed leaves:
(A) Experiment 1, $Y = 3.70 + 2.71X$;
(B) Experiment 2, $Y = 3.93 + 3.24X$.

Figure 49. Concentration of cadmium in alligatorweed stems:
(A) Experiment 1, $Y = 3.62 + 14.99X$;
(B) Experiment 2, $Y = 5.69 + 8.24X$. 
Original stem cuttings. The concentrations of cadmium in original stems increased linearly up to approximately 2.0 ppm exposure, after which, the linearity was destroyed. In many cases, at exposures > 2.0 ppm, the cadmium concentration in the original stems decreased. Cadmium was very toxic at high exposure levels, and the stems in contact with the solutions were often partially, or totally, waterlogged and in various stages of decomposition. One result would be a dilution of the cadmium in these tissues. In Experiment 1, the maximum cadmium concentration in original stem cuttings was 996 ppm at 4.6 ppm cadmium exposure. This value was considerably above the 164 and 177 ppm measured in two other experimental units also at 4.6 ppm. The two lower values, however, were associated with more than twice as much dry weight as the 996 ppm outlier. Hence, the 996 ppm outlier may possibly be attributed to an analytical error. Maximum cadmium found in Experiment 2 was 215 ppm at 4.7 ppm exposure. Meaningful regression analyses could not be performed on data from original stem cuttings, because of the inability to adequately quantitate either the degree of waterlogging or the state of decomposition of these tissues.

Roots. With the exception of a single outlier in Experiment 2 (3.7 ppm level), the concentration of cadmium in alligatorweed roots increased linearly with increasing exposure level (Fig. 50). Some slight deviation from linearity may have been the result of inaccurate measurements at the higher exposures because of small tissue samples. The observed increases were shown to be highly significant in both experiments. The root cadmium concentrations were also significantly greater in the second experiment than in the first. Regression
Figure 50. Concentration of cadmium in alligatorweed roots:

(A) Experiment 1, \( Y = 412 + 342X \);
(B) Experiment 2, \( Y = 795 + 708X \).
analysis indicated that the differences were only in magnitude and were not interactive in nature. Maximum levels of cadmium observed in roots for Experiments 1 and 2 were 1702 and 7267 ppm, respectively.

**Metal uptake and partitioning**

**Leaves.** Total cadmium in leaves was essentially constant at all levels of exposure in both experiments (Fig. 51). At any level of exposure total leaf cadmium never exceeded 5 percent of the total plant cadmium accumulation. Lack of increase in total leaf cadmium with increasing exposure was apparently the result of greatly reduced leaf weights as exposure levels were increased beyond approximately 1.0 ppm. Maximum accumulation of cadmium in leaf tissues for Experiments 1 and 2 were 0.01 mg at 0.45 and ppm and 0.008 mg at 2.8 ppm, respectively.

**New stems.** Total accumulation of cadmium in new stems (Fig. 51) was also essentially constant at all levels of exposure and quite closely paralleled that of the leaves, but at a slightly higher level. The greatest accumulation of cadmium occurred at a concentration of 0.46 ppm, where total cadmium in new stems was 0.017 and 0.024 mg for Experiments 1 and 2, respectively. These maximum values represented 13 and 19 percent of the total plant cadmium for these two experiments, respectively. Total cadmium in new stems generally represented 10 percent of the total plant accumulation. The apparent lack of increase in total cadmium accumulation in the new stems may be attributed to the reduction in stem growth with increasing cadmium exposure.
Figure 51. Cadmium accumulation by alligatorweed:
(A) Experiment 1;
(B) Experiment 2.
Initial Cd Concentration (ppm)
Original stem cuttings. The original stems must be treated separately as they were in direct contact with the cadmium-containing solution and are, therefore, subject to possible accumulation of cadmium on the surface of the stems, as well as through actual absorption into the tissues. In both experiments, the cadmium accumulation in original stems increased linearly with increasing exposure level (Fig. 51). In Experiment 1, cadmium in original stems represented about 65 to 75 percent of the total plant cadmium at exposures > beyond 2.0 ppm. This increase paralleled a reduction in growth of other tissues, particularly roots, as cadmium exposure increased. In the second experiment, however, the proportion of total plant cadmium bound in original stems did not reach 50 percent until the level of exposure was > 3.0 ppm. The apparent anomaly may be the result of an enhanced rate of export from the roots at slightly higher temperatures in the first experimental period and/or a possibly greater surface contamination from cadmium absorbed by periphyton that was not removed in the rinsing process. Possible temperature-enhanced absorption directly through the stem epiderm's and callous tissues in the first experiment can not be ruled out, however. Maximum accumulations of cadmium in Experiments 1 and 2 were 0.181 mg at 4.6 ppm and 0.096 mg at 4.7 ppm, respectively.

Roots. Total cadmium uptake by roots (Fig. 51) was closely related to both the root biomass and exposure level. In both experiments, there were sharp peaks of accumulation at approximately 0.5 ppm exposure, and additional major peaks occurred at 1.8 ppm (Experiment 1) and 2.8 ppm (Experiment 2). In each case, the peaks were associated with substantially
greater root biomass than for exposure levels immediately above or below the levels at which the peaks were observed. In Experiment 1, with the exception of the peak at 1.8 ppm, roots contained the majority of total plant cadmium only at levels of exposure < 1.9 ppm, and uptake of cadmium by roots increased somewhat as exposure increased. In Experiment 2, the total cadmium bound by roots represented the majority of total plant cadmium until exposure exceeded 4.0 ppm. The higher root cadmium accumulation in the second experiment appears to be a function of a somewhat higher root biomass in Experiment 2 than in Experiment 1. Maximum root cadmium accumulations were 0.084 mg at 0.45 ppm and 0.525 mg at 0.46 ppm in Experiments 1 and 2, respectively. It should be noted that the value shown in Fig. 51-B represents a mean for 0.46 ppm, and, without the outlier of 0.525 mg, mean root cadmium would have been approximately 0.44 mg. The high level for the outlier was related to considerably greater root biomass than either of the other two replications at 0.46 ppm.

Total uptake and mean plant concentrations. Total plant cadmium closely paralleled root cadmium up to 1.8 ppm exposure in Experiment 1 and throughout the entire second experiment (Fig. 51). Fluctuations in total plant cadmium reflected the variation in root biomass discussed previously. Maximum total plant accumulations for Experiments 1 and 2 were 0.185 mg at 4.6 ppm and 0.537 mg at 0.46 ppm, respectively. Whole-plant cadmium concentrations were greater for Experiment 1 than for Experiment 2 (Fig. 52). Noting that growth (Fig. 47) was greater in Experiment 2, the differences observed in whole-plant concentrations of cadmium can be explained as an effect of differences in plant biomass.
Figure 52. Concentration of cadmium in whole alligatorweed:

(A) Experiment 1, \( Y = -2.74 + 62.9X \);
(B) Experiment 2, \( Y = 79.87 + 16.94 \).
(i.e., the dilution effect). Maximum whole-plant cadmium concentrations for Experiments 1 and 2 were 869 ppm at 4.6 ppm cadmium exposure and 418 ppm at 0.46 ppm cadmium exposure, respectively. If the outlier is excluded for 0.46 ppm cadmium in Experiment 2, maximum concentration was 192 ppm at 4.7 ppm.

Discussion of cadmium effects

A major effect of cadmium upon alligatorweed was the reduction in the development of the root system with increasing levels of exposure to cadmium in solution. Concomitant with the poor root growth was a sharp reduction in overall plant growth and a pronounced chlorosis of the leaves. This suggests that a major effect of cadmium is interference in root function with resultant nutrient imbalances and/or deficiencies. Substantial reduction in growth of alligatorweed in solution culture with 1.0 ppm cadmium has been demonstrated previously (Quimby et al. 1979). The effects of iron deficiency on alligatorweed have also been studied previously (Kay and Quimby 1977), and the results of the iron deficiency studies were similar to the effects of cadmium observed in the current experiments. The possible metabolic interference of cadmium by substitution for zinc can not be ruled out, however. The threshold for cadmium toxicity to alligatorweed appears to lie between 0.5 and 1.0 ppm exposure.

The uptake, partitioning, and concentrations of cadmium within the plant are apparently greatly affected not only by the level of exposure to cadmium, but also by the plant biomass. This suggests that under conditions where growth is not inhibited, alligatorweed can absorb large amounts of cadmium from solutions containing relatively
low levels of cadmium ($< 1.0$ ppm). The present study has also shown that cadmium is slightly mobile within the plant. The amount transported to leaf tissue, however, was somewhat lower than that reported in growth-chamber study by Quimby et al. (1979) at the same solution concentration (1.0 ppm). These differences may be artifacts of the greater variation in environmental factors experienced in an out-of-doors study, as opposed to the rigidly controlled conditions of the controlled-environment chamber.

Effects of Copper

Appearance of plants

At the beginning of the experimental period, all plants were uniformly green and healthy. After two weeks, the controls and plants exposed to $\geq 2.0$ ppm copper were chlorotic and appeared to have grown little. After four weeks the plants in the 2.0 ppm treatments were also chlorotic and had less growth than the controls or plants exposed to $> 2.0$ ppm copper. The development of roots, particularly lateral roots, was also severely curtailed, and at concentrations $> 2.0$ ppm copper there was little or no root development. As in the cases of lead and cadmium, there was some insect activity noticed by the time of the harvest. In the intended second harvest of the first copper experiment the plants exposed to $\leq 2.0$ ppm copper were totally destroyed by the same three insects that destroyed the lead and cadmium experiments. At copper exposure of $> 2.0$ ppm, no insect activity was observed with the sole exception of one small lepidopterous feeding lesion on a single leaf of a 3.0 ppm treatment.
Growth measurements

Flowering. The plants in both copper experiments appeared to be physiologically immature, as no flowering was observed in any of the copper treatments.

Dry weights of leaves. Among the controls and plants exposed to \( \leq 1.0 \) ppm copper there was wide variation and very little overall difference in dry leaf weights. A decrease in leaf weights occurred at approximately 1.8 ppm exposure, and, beyond 2.0 ppm, there were essentially no further copper effects in either experiment (Fig. 53). Regression analysis indicated that the response to increasing copper levels was highly significant and that the responses in both experiments were similar.

Dry weights of new stems. The responses of new stem growth to copper was similar to that of the leaves. There were no effects of copper at exposure levels \( \leq 1.0 \) ppm (Fig. 54). Stem growth was sharply curtailed in both experiments at approximately 1.8 ppm exposure, with a further reduction at approximately 2.7 ppm for Experiment 1. Beyond 2.7 ppm in Experiment 1 and 1.8 ppm in Experiment 2 no further effects of copper were observed. Regression analysis indicated that the reduction in the dry weights of new stems with increasing level of exposure to copper were highly significant. There were no differences in response between the two experiments.

Dry weights of roots. In both experiments there was a significant reduction in root growth as copper exposure was increased above 1.0 ppm (Fig. 55). A comparison of regression lines indicated that there were
Figure 53. Dry leaf weights of alligatoweed exposed to copper:
(A) Experiment 1, log Y = 0.155 - 1.714 log (X + 1);
(B) Experiment 2, log Y = -0.075 - 1.503 log (X + 1).
Figure 54. Dry stem weights of alligatorweed exposed to copper:
(A) Experiment 1, $\log Y = 0.223 - 1.937 \log (X + 1)$;
(B) Experiment 2, $\log Y = -0.021 - 1.468 \log (X + 1)$.
Dry Weight (g x 10) vs. Initial Cu Concentration (ppm)

A: $r^2 = 0.8232$
B: $r^2 = 0.7335$
Figure 55. Dry root weights of alligatorweed exposed to copper:
(A) Experiment 1, $\log Y = 0.060 - 5.658 \log (X + 1)$;
(B) Experiment 2, $\log Y = -1.195 - 1.230 \log (X + 1)$. 
significant differences between the two experiments and that these differences were interactive in nature. Upon examination of the graphs, (Fig. 55), it becomes apparent that this interactive influence has manifest itself as approximately two- to three-fold greater growth in the controls and copper treatments of < 1.0 ppm in the first experiment, whereas there was essentially no between-experiment differences in root weights at copper exposures of > 2.0 ppm. The higher ambient temperatures in the first experimental period may have enhanced the toxicity of copper at high levels of exposure. This, combined with a greater root growth rate of controls and copper treatments of < 1.8 ppm at the warmer temperatures, may have caused the observed interactive differences.

Total growth of plants. There was little difference between growth of controls and treatments of < 1.0 ppm copper in either experiment (Fig. 56). A sharp decline in growth occurred between 1.0 and 2.0 ppm exposure and beyond 2.0 ppm there was little further change with increasing copper concentration. Regression analysis demonstrated that the decreases in total plant growth in both experiments were highly significant and that there were no differences between the responses observed in the two experiments.

Metal concentrations in tissues

Leaves. The concentrations of copper in leaves increased linearly with increasing level of exposure to copper (Fig. 57). The increases observed were highly significant. A comparison of the two experiments showed that there were no significant differences. The apparent difference in the slopes of the regression lines is the result of greater leaf
Figure 56. Total growth of alligatorweed exposed to copper:
(A) Experiment 1, log Y = 0.684 - 2.18 log (X + 1);
(B) Experiment 2, log Y = 0.368 - 1.358 log (X + 1).
A

\[ r^2 = 0.7443 \]

B

\[ r^2 = 0.5356 \]
biomass (hence, a dilution effect) in the controls and approximately 0.5 ppm treatments in Experiment 1 as compared to Experiment 2. The outlier at the 2.7 ppm exposure level in the first experiment also may have influenced the slope of the regression line sufficiently to cause this apparent interaction. Copper concentrations in leaves of both experiments were similar within the range of about 0.9 to 3.7 ppm exposure. With the exception of the outlier in the first experiment, maximum leaf copper concentrations were 145 ppm at 4.6 ppm and 113 ppm at 4.4 ppm, respectively, for Experiments 1 and 2. The outlier in the first experiment at 2.7 ppm exposure was 161 ppm copper in the leaves.

**New stems.** A highly significant linear increase was noted in the concentration of copper in new stems (Fig. 58). The levels of copper accumulation in Experiment 2 were slightly, but significantly, higher than in Experiment 1. A comparison of regression lines indicated that these differences were not interactive in nature. The differences observed for copper concentrations in new stems can not be attributed to a dilution effect in the first experiment, as no significant differences between experiments were noted for dry weights of new stems (Fig. 54). Maximum concentrations of copper for Experiments 1 and 2 were 120 ppm at 4.6 ppm copper and 126 ppm at 3.7 ppm copper, respectively.

**Original stem cuttings.** There were essentially no differences in the concentrations of copper in the original stems until exposure level reached 1.8 ppm copper, after which the concentrations of copper in the original stem cuttings increased greatly. In Experiment 1, the maximum concentration reached was 866 ppm at 2.7 ppm exposure. In Experiment 2,
Figure 57. Concentration of copper in alligatorweed leaves:
(A) Experiment 1, \( Y = 4.63 + 26.68X \);
(B) Experiment 2, \( Y = 17.21 + 15.96X \).

Figure 58. Concentration of copper in alligatorweed stems:
(A) Experiment 1, \( Y = 11.24 + 17.51X \);
(B) Experiment 2, \( Y = 28.67 + 15.44X \).
the tissue concentrations of copper increased in a linear manner from 1.8 to 4.4 ppm exposure, and the maximum copper concentration in the original stems was 326 ppm at 4.4 ppm copper exposure. The differences between experiments are probably the result of more extensive waterlogging and tissue deterioration at the highest level of exposure in Experiment 1 and consequent dilution of copper accumulated in the original stems. Very likely the warm temperatures of August promoted more rapid stem deterioration than did the somewhat cooler temperatures in September.

**Roots.** Copper concentrations in root tissues increased significantly in a linear manner as exposure to copper increased (Fig. 59). A comparison of regression lines demonstrated no significant differences in response to copper between the two experiments. There was a rapid curtailment of root growth as exposure level was increased above 2.0 ppm. No root growth occurred at copper concentrations > 4.0 ppm. Maximum copper concentrations in roots occurred at 3.7 ppm exposure and were 4323 and 2308 ppm, respectively, for Experiments 1 and 2.

**Metal uptake and partitioning**

**Leaves.** The total copper partitioned into leaf tissues increased with increasing exposure to copper up to approximately 1.0 ppm and then declined rapidly (Fig. 60). At copper concentrations > 2.0 ppm, leaf copper remained fairly constant, with the exception of a small peak at 4.5 ppm copper in Experiment 1. The decline in total copper accumulation in leaves at levels of exposure > 1.0 ppm was the result of copper toxicity and concomitant reduced leaf growth. Maximum leaf copper accumulations for Experiments 1 and 2 were 0.035 mg at 0.87 ppm and 0.028 mg at 0.91 ppm, respectively.
Figure 59. Concentration of copper in alligatorweed roots:
(A) Experiment 1, Y = -42.17 + 940.7X;
(B) Experiment 2, Y = -32.82 + 673.6X.
Figure 60. Copper accumulation by alligatorweed:
   (A) Experiment 1;
   (B) Experiment 2.
New stems. Total copper partitioned into new stem tissues very closely paralleled copper in the leaves in both experiments (Fig. 60). In the first experiment, total copper in new stems exceeded that in the leaves only up to approximately 1.0 ppm exposure. In the second experiment, however, stem copper was consistently higher at all levels of exposure except 4.4 ppm. This was the result of greater concentrations of copper in the same stem biomass in the second experiment. Maximum copper accumulations in Experiments 1 and 2 were 0.035 mg at 0.87 ppm and 0.39 mg at 0.91 ppm, respectively.

Original stem cuttings. Total copper accumulation in original stems increased in a curvilinear manner as exposure to copper increased (Fig. 60). Beyond approximately 2.0 ppm in both experiments the original stems contained from 75 to 90 percent of the total plant copper accumulation. Such a large proportion of total plant copper in the original stems suggests either that there was considerable absorption through the stem surface and/or callous tissue, or that the accumulation was largely due to surface contamination that was not removed by the washing procedure. The poor root development at higher exposure levels would favor the latter hypothesis, but does not exclude the former. Maximum copper accumulations in original stems were 0.220 mg at 4.6 ppm and 0.183 mg at 4.4 ppm for Experiments 1 and 2, respectively.

Roots. Total root copper increased with increasing exposure up to approximately 1.8 ppm (Fig. 60). At concentrations > 1.8 ppm, copper was very toxic and very little root development occurred. Consequently, even with increasing root concentrations of copper, the total copper partitioned into roots declined rapidly. At the 1.8 ppm exposure level,
the roots contained about 45 percent of the total plant copper, whereas at 3.7 ppm, roots contained about 10 percent of the total plant copper. Maximum copper in roots for Experiments 1 and 2 were 0.064 and 0.028 mg, respectively, both at initial solution concentrations of 1.8 ppm copper.

**Total uptake and mean plant concentrations.** In Experiment 1, total plant copper accumulation increased rapidly up to 1.8 ppm, formed a plateau between 1.8 and 2.7 ppm, and then increased rapidly again as exposure was increased to 4.6 ppm. In Experiment 2, the total copper uptake was apparently biomodal in nature. No plateau in copper uptake occurred at 1.8 ppm. Instead, at approximately 1.9 ppm copper, a peak was reached, after which there was a sharp decline through about 2.5 ppm. Dry weights of roots (Fig. 55) show that the drop in total plant copper at about 1.8 ppm in Experiment 2 was the result of substantially less root biomass, and, hence, less root accumulation of copper than at the same exposure level in the first experiment. Both the plateau in Experiment 1 and the decline in Experiment 2 were the result of decreased root biomass. The increases observed at approximately 2.7 ppm in both experiments were the results of greater partitioning of copper into the original stems with increasing exposure levels. Total accumulation at the higher exposure levels was slightly greater in Experiment 1 than in Experiment 2. Maximum copper accumulations in whole plants were 238 mg at 4.6 ppm and 204 mg at 4.4 ppm, respectively, for Experiments 1 and 2. There were also significant differences in whole plant copper concentrations between the two experiments (Fig. 61). With the exception of an outlier at 2.7 ppm in Experiment 1, the copper concentrations showed a highly significant linear increase with
Figure 61. Concentration of copper in whole alligatorweed:
   (A) Experiment 1, Y = -9.84 + 84.9X;
   (B) Experiment 2, Y = 15.98 + 32.52X.
increased level of exposure. The between-experiment differences in whole-plant copper concentrations were not interactive in nature. Maximum whole-plant copper concentrations for Experiments 1 and 2 (excluding a 668 ppm outlier at 2.7 ppm copper in Experiment 1) were 487 and 243 ppm, respectively, and correspond to the values given previously for total plant accumulation of copper.

**Discussion of copper effects**

The major effect of copper on alligatorweed was the severe curtailment in root development as the level of exposure exceeded approximately 1.0 ppm. Restriction in root growth resulted in reduced growth of new stem and leaf tissues and a pronounced chlorosis in leaves which did develop. The toxicity of copper to root tissues very likely caused a nutrient imbalance and/or deficiency as the result of poor root function, with concomitant adverse effects upon the growth of new stems and leaves. The overall greater growth of plants not showing symptoms of copper toxicity in Experiment 1, compared with Experiment 2, would be the expected result of an increased metabolic rate at the higher ambient temperatures prevailing in August. Threshold toxicity of copper for alligatorweed appeared to be between 1.8 and 2.0 ppm.

The concentration of copper in alligatorweed tissues are shown to increase in a linear fashion with increasing exposure to copper. The total accumulation of copper in the various plant parts increased up to the point of threshold toxicity, after which there was a decline in all but the original stems. This was shown to be a function of decreased growth of new tissues. The substantially greater biomass
of original stems continued to accumulate copper in a curvilinear fashion as exposure level was increased, so that total plant copper continued to rise as exposure level increased. Total copper accumulations and whole-plant concentration of copper were slightly greater in Experiment 1 than in Experiment 2 and were attributed to a greater growth rate due to the higher ambient temperatures prevailing during the first experiment.

Comparison of Metal Effects

Effects of plant growth

The effects of lead upon alligatorweed were negligible in comparison to the effects of cadmium or copper. There were no visible differences in either biomass or the condition of plants among the controls and those exposed to all levels of lead. Plants exposed to > 2.0 ppm of cadmium or copper were chlorotic and greatly stunted. The root system of these stunted plants was essentially non-existent compared to the well-developed roots of control plants, lead treatments, and cadmium or copper treatments < 2.0 ppm. All stunted plants also had very poor development of lateral roots, whereas plants that appeared normal also had normally-developed lateral roots. Most of the original stem cuttings in cadmium and copper treatments > 2.0 ppm were waterlogged and in an advanced state of deterioration. Controls, all lead treatments, and cadmium and copper treatments of < 2.0 ppm had healthy, green, original stems at the time of harvest.

The lack of toxicity of lead in these experiments seems to be related to several factors. First, no plants were exposed to an initial lead concentration > 2.2 ppm, as a large portion of the calculated upper range of 5.0 ppm had apparently precipitated from solution
very rapidly. Second, lead was apparently less toxic than either cadmium or copper solution concentrations of approximately 2.0 ppm. Both cadmium and copper treatments were visibly stunted at this level, but no effects could be detected for lead. The lack of any adverse effects of approximately 2.0 ppm lead in solution on the root system seems to support this hypothesis. Figures 62, 63, and 64 show the relative growth rates (RGR) after four weeks for alligatorweed exposed to varying levels of lead, cadmium, and copper, respectively. In Experiments 1 and 2, the RGR for plants exposed to lead changed very little. Exposure to copper resulted in a precipitous decline in the RGR between 1.0 and 2.0 ppm. A similar but somewhat less precipitous, decline in RGR occurred in plants exposed to cadmium. A comparison of RGR's at concentrations of approximately 2.0 ppm reveals RGR's for lead, cadmium, and copper of approximately 0.198, 0.030, and 0.040 g · g\(^{-1}\) · day\(^{-1}\), respectively, in Experiment 1, and 0.150, 0.040, and 0.030 g · g\(^{-1}\) · day\(^{-1}\), respectively in Experiment 2. The RGR for control plants in Experiments 1 and 2 were approximately 0.225 and 0.150 g · g\(^{-1}\) · day\(^{-1}\), respectively. The effects of cadmium and copper upon the RGR suggest that the threshold of toxicity to alligatorweed lies in the range of 0.5 to 1.0 ppm for cadmium and 1.0 to 2.0 ppm for copper.

**Effects of metal accumulations**

The absorption, transport, and tissue concentrations of lead, cadmium, and copper were dependent both upon individual metal and upon level of exposure. The pattern of metal dispersion within the plant was generally leaves < stems < roots. Lead was only poorly transported
Figure 62. Relative growth rates of alligatorweed exposed to lead:
(A) Experiment 1, \( \log Y = -0.72 + 0.03 \log (X + 1) \);
(B) Experiment 2, \( \log Y = -0.75 - 0.17 \log (X + 1) \).
Figure 63. Relative growth rates of alligatorweed exposed to cadmium:
(A) Experiment 1, \( \log Y = -1.06 - 0.91 \log (X + 1) \);
(B) Experiment 2, \( \log Y = -0.86 - 1.11 \log (X + 1) \).
Figure 64. Relative growth rates of alligatorweed exposed to copper:
   (A) Experiment 1, \( \log Y = -0.37 - 2.21 \log (X + 1) \); 
   (B) Experiment 2, \( \log Y = -0.62 - 1.89 \log (X + 1) \).
within the plant and, in the first experiment, was not detected in tissues other than roots. In the second experiment, a small amount of lead was detected in original stems but was considered to be due to external contamination and not genuine absorption by the plant tissues. Both cadmium and copper were found to be mobile at low treatment concentrations (0 to 1.0 ppm), but the mobility decreased sharply as threshold toxicity was approached. Above the level of threshold toxicity, cadmium and copper were bound largely in the roots and original stems. As treatment levels were increased beyond 2.0 ppm, an increasing proportion of plant-absorbed cadmium and copper was associated with the original stem tissues. At solution levels exceeding 3.0 ppm, copper was almost entirely bound within original stems, but a substantial proportion of the cadmium was still present in roots. Whole-plant concentrations of lead, on the average, were considerably greater during the second experiment than during the first, whereas the reverse was true for both cadmium and copper. This appeared to be a dilution effect due to greater plant growth during the warmer seasons in the case of lead. For cadmium, the greater growth during Experiment 2 in all but the controls suggest that higher temperatures enhanced cadmium toxicity. For copper, the growth was approximately identical in both experiments, and it would appear that copper toxicity was not enhanced by higher temperatures. The general responses of alligatorweed to lead, cadmium, and copper are summarized in Table 5, and the effects upon RGR, metal uptake, and whole-plant metal concentrations are presented in Table 6 for controls and highest levels of exposure.
Table 5. General responses of alligatorweed to exposure to lead, cadmium, and copper in comparison with control plants. Responses are for the level representing threshold toxicity, if any and are consistent up to the highest level tested.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Lead</th>
<th>Cadmium</th>
<th>Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold Toxicity</td>
<td>Not observed</td>
<td>0.5 to 1.0 ppm</td>
<td>1.0 to 2.0 ppm</td>
</tr>
<tr>
<td>Root Development</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Lateral Roots</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Leaf Color</td>
<td>Green</td>
<td>Chlorotic</td>
<td>Chlorotic</td>
</tr>
<tr>
<td>New Shoot Growth</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Total Plant Growth</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Treatments Damaged by Insects</td>
<td>All</td>
<td>All \leq 2.0 ppm</td>
<td>All \leq 2.0 ppm</td>
</tr>
</tbody>
</table>
Table 6. Maximum relative growth rates, maximum total plant accumulation of metals, and maximum concentration of metals in whole alligatorweed plants after a four-week growth period.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Experiment</th>
<th>Maximum relative growth rate, g/g/day</th>
<th>Maximum metal accumulation in whole plants, mg</th>
<th>Maximum metal concentration in whole plants, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Controls 5.0 ppm*</td>
<td>Controls 5.0 ppm*</td>
<td>Controls 5.0 ppm*</td>
</tr>
<tr>
<td>Lead</td>
<td>1</td>
<td>0.286 0.282</td>
<td>0.0 0.043</td>
<td>0.0 14.1</td>
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<tr>
<td></td>
<td>2</td>
<td>0.207 0.243</td>
<td>0.0 0.389</td>
<td>0.0 191</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1</td>
<td>0.286 0.041</td>
<td>0.001 0.185</td>
<td>0.23 869</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.207 0.085</td>
<td>0.026 0.215</td>
<td>15.9 192</td>
</tr>
<tr>
<td>Copper</td>
<td>1</td>
<td>0.286 0.025</td>
<td>0.037 0.238</td>
<td>15.4 487</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.207 0.024</td>
<td>0.040 0.204</td>
<td>30.4 243</td>
</tr>
</tbody>
</table>

*The use of 5.0 ppm as the maximum exposure level was necessary because there were some indications from water analyses that some redissolution of precipitated metals had occurred in some experimental units. Therefore the use of 5.0 ppm should be construed as meaning the highest level of exposure up to and including 5.0 ppm.
The overall effects of exposure of alligatorweed to cadmium and copper suggest that caution should be exercised when making judgements based upon laboratory-accumulated data as to the potential of alligatorweed in the removal of toxic substances, especially heavy metals, from polluted effluents. The present study shows that the heavy metals, cadmium and copper, very rapidly limit plant growth.

The present study has indicated that cadmium or copper might also interfere with biological agents used to suppress the growth of alligatorweed. Cockroaches, an armyworm (Spodoptera latifascia), and the alligatorweed thrips (Amynothrips andersoni) all attacked an intended second harvest. All controls, all lead treatments, and the cadmium and copper treatments of \(< 2.0\) ppm were totally destroyed by insect feeding. Plants exposed to \(> 2.0\) ppm of either cadmium or copper were not affected by the insects, however.

**Summary and Conclusions**

There were no observable effects of lead on the growth of alligatorweed at the levels of exposure in this study. Exposure to \(> 2.0\) ppm of either cadmium or copper resulted in a pronounced chlorosis and a sharp reduction in root, stem, and leaf development.

Relative growth rates (RGR) were reduced substantially by exposure to cadmium and copper but were not affected by lead. The growth of plants was greater in the first experiment when ambient temperatures were higher. In the first experiment, the RGR for controls and for the highest levels of exposure to lead, cadmium, and copper were 0.224, 0.198, 0.030, and 0.040 g · g⁻¹ · day⁻¹, respectively. In the second experiment, the corresponding RGR's were 0.160, 0.150, 0.040, and 0.030 g · g⁻¹ · day⁻¹.
Threshold toxicities for cadmium and copper were 0.5 to 1.0 ppm and 1.0 to 2.0 ppm, respectively, but could not be determined for lead.

The primary effect of cadmium and copper upon alligatorweed appeared to be a reduction in root development and function, with secondary effects (i.e., reduced growth, chlorosis) occurring as the probable result of nutrient imbalances and/or deficiencies.

The concentrations of metals in the plants increased linearly with increasing level of exposure. Concentrations of metals in the different tissues followed the pattern of leaves < stems < roots and were dependent upon the relative mobility of the particular metal.

Partitioning of lead, cadmium, and copper within the plant depended upon both the mobility and the relative toxicity of the particular metal. Lead was bound almost entirely within the roots, and none was detected in new stems or leaves at any level of exposure. Cadmium and copper were more readily transported within the plant. The order of increasing mobility was lead < cadmium < copper. The total plant accumulation of all three metals generally increased with increased level of exposure. This trend was masked somewhat by rather wide variation in the cadmium experiment. The fraction of total plant cadmium and copper accumulation in the original stems increased as initial solution concentrations were increased.

The lack of any insect damage on plants exposed to > 2.0 ppm cadmium or copper suggests that these heavy metals (and possibly others) might in some cases interfere with biological control agents. The fact that the alligatorweed thrips (*Amynothrips andersoni*), an insect imported from South America for the control of alligatorweed, was among the
three insects which did not attack alligatorweed exposed to > 2.0 ppm of cadmium or copper seems to support this hypothesis.
EFFECTS OF PLANT ACCUMULATIONS OF LEAD, CADMIUM, AND COPPER ON THE ALLIGATORWEED FLEA BEETLE, AGASICLES HYGROPHILA, AND THE WATERHYACINTH WEEVIL, NEOCHETINA SP.

Materials and Methods

Agasicles Feeding Experiment

Alligatorweed to be used as a source of stem-cuttings for this experiment was greenhouse-grown in vermiculite in 6" (15.24 cm) plastic pots placed in dishpans containing about 10 l of tap water. Nutrients were provided by the addition of 10 ml of the 15-7-7 nutrient and 10 ml each of micronutrients and iron chelate described previously. This nutrient solution provided nitrogen approximately equivalent to the 210 ppm N in full-strength Hoagland's solution. Plants were allowed to grow for four months with monthly renewal of nutrients. Two three-node stem cuttings were then placed into 375-ml amber bottles containing full-strength Hoagland's solution. A three-week incubation period was allowed prior to the initiation of the feeding experiments inorder to insure that adequate leaf material would be available. At the end of the three-week incubation period the nutrient solutions were changed and metals were added. Treatments were controls 0.5, 1.0, and 2.0 ppm cadmium, and 2.0 ppm each lead or copper. A three-week absorption period was allowed, with complete solution changes at the end of each week. All plant tissue was maintained in the greenhouse. Insects for the feeding study were collected in February from Hume Pond on the campus of the University of Florida, Gainesville, and maintained in
indoor cages on greenhouse-grown alligatorweed until all danger of frost was past. The colony was then moved outdoors and supplied with fresh alligatorweed.

Leaves for the feeding tests were collected from treated plants, and four leaf discs were cut from each leaf with a cork borer. The initial area of eight leaf discs of a given treatment was measured with a Lambda leaf area meter. The area of each set of eight discs was measured five times, and the mean was taken as the best estimate of the actual area. The leaf discs were then placed in a circle on moist blotter paper in clear plastic boxes measuring approximately 3.5 x 7 x 9.5 cm, and two male and two female Agasicles flea beetles were placed into each box. Each treatment was replicated three times. An additional three boxes containing control leaf discs were established without insects to provide a measure of tissue shrinkage or swelling due to either loss or gain of water, respectively. All boxes were placed on a tray and sealed inside a large plastic bag to retard moisture loss. Insect boxes were placed inside a controlled-environment chamber with a 14 h photoperiod and 30/25 C day/night temperature regime. Insects were transferred daily for the first four days, and then, every other day, to a new box containing fresh tissue. An additional eight discs of each treatment were taken at each change for the determination of specific leaf weights (SLW). Feeding was estimated as the difference between the initial and final area, adjusted for tissue shrinkage or swelling and reported as feeding per insect to allow for differences due to insect mortality. Mortality by sex, number of egg clutches, and total number of eggs laid were observed at each change. Leaf tissue left after removing leaf discs was dried.
and saved for metal analyses. Dead insects were also saved, and, at
the end of the experimental period, all insects in a particular treat-
ment were dried and combined for metal analysis. An approximation of
the amount of metal ingested per insect was obtained by calculating
the total feeding per insect during the 10-day experiment period,
the specific leaf weights for each treatment, and the metal concentra-
tion in leaves from the tissue analyses. Eggs were retained until
hatched. Data were subjected to an analysis of variance, and means
were compared with a Duncan's multiple range test.

**Neochetina Feeding Experiment**

Waterhyacinths for use in the **Neochetina** feeding experiments were
propagated from plants that grew from seed in an outdoor cage during
the summer of 1979. Progeny from these plants were maintained in
hydroponic culture in 70-l barrels in a greenhouse during the winter
of 1979-80 and were transferred to outdoor cages after all danger of
frost was past. Plant material was cultured as described in the
waterhyacinth growth study except that well water was used for pre-
paring the solutions and that the plants were inside cages to prevent
natural infestation by **Neochetina** sp. After a five-week growth period,
the metal solutions and fresh nutrients were added to the barrels.
Treatments were established so as not to exceed the minimum solution
metal concentrations which were toxic to waterhyacinths in the growth
studies. Metal treatments were: controls, 0.5 and 1.0 ppm cadmium,
1.0 and 2.5 ppm copper, and 1.0 and 5.0 ppm lead. The 1.0 ppm treat-
ment was selected in order to compare the effects of similar levels
of exposure to the different metals. Plants were given a four-week
uptake period prior to the initiation of feeding studies.
Feeding studies were conducted indoors at ambient room temperature in front of a glass door with a west exposure. *Neochetina* sp. were collected in the field from Camp's Canal and the River Styx in Alachua County, Florida, and were held indoors for four to six days in 3.8 l glass jars containing waterhyacinth leaves. Ten adult *Neochetina* weevils were randomly selected from the jar for each experimental unit. One mature waterhyacinth leaf was placed into a 3.5 x 7 x 9.5 cm plastic box with ten adult weevils to constitute one replication of one treatment. Boxes were then placed inside individual zip-loc plastic bags and were sealed to prevent moisture loss. Each treatment was replicated five times. After 24 h feeding, old leaves were removed and replaced with fresh leaves. The number of feeding spots and insect mortality were recorded daily for the first ten days. Any insect mortality during the first 24 h of the experiment was assumed to be the result of old or unhealthy insects or damage due to handling. Consequently, insects which died during the first 24 h were not counted and were immediately replaced. Mortality counts were continued for an additional ten days, and the experiment was terminated at the end of 20 days. All insects remaining at the end of the experimental period were combined with the dead insects, killed by freezing, and prepared for metal analysis as described for the *Agasicles* flea beetles. All replications of a treatment were combined for a single analysis. Leaf metal analysis were performed in the same manner as described previously for the waterhyacinth growth study, except that ash weights were not recorded. Mean cumulative feeding per insect was subjected to an analysis of variance, and means were compared with a Duncan's multiple range test.
Results and Discussion

Agasicles Experiment

The experiments involving the alligatorweed flea beetles were besought with difficulties that have rendered interpretation of the data extremely difficult. A major source of variation involved the regulation of humidity and the accompanying shrinkage and swelling of the leaf discs. On several occasions one replication of the control discs for shrink-swell measurement behaved aberrantly, resulting in decreased leaf area measurement that indicated loss of leaf moisture and tissue shrinkage, whereas the other two replications absorbed moisture and, consequently, showed and increased leaf area. This also occasionally occurred in the experimental leaf discs. Previous experimental indicated that the leaf discs would swell somewhat through moisture absorption and that the swelling was quite uniform. The reason for using leaf discs in these tests was two-fold. First, the insects would all be presented the same amount of leaf tissue upon which to feed, thereby reducing a first source of variation. Second, previous experience had also indicated that whole leaf tissue would dry out rapidly and that the insects did not feed readily once the leaf began to desiccate and curl. The curled leaves were also difficult to measure in the leaf area meter, because they would not lie flat on the belts. The difficulties involved with moisture-control in this study necessitated the use of shrunken control leaf discs to allow for tissue shrinkage in experimental units which lost moisture, and swollen control leaf discs whenever the leaf discs had not obviously desiccated.
Another difficulty experienced was the complete loss of all insects in one control and in one replication each of all metal treatments except lead. The loss of these insects occurred within the first two days of the experiments, in most cases, and had to be attributed to factors other than metal exposure. The analysis of variance was greatly biased by the early loss of these insects; significant differences among the various treatments could not be demonstrated. Consequently, the data were re-analyzed after deletion of data from all experimental units in which there was total mortality early in the experiment. The following presentation of data and subsequent discussion are based upon this second data analysis.

Effects on feeding. The cumulative feeding responses of the alligatorweed flea beetles to control plants and plants that had been exposed to approximately 2.0 ppm of either lead or copper and 0.5, 1.0, and 2.0 ppm of cadmium are presented in Fig. 65. After only one day of feeding on leaves from plants exposed to approximately 1.0 or 2.0 ppm cadmium, there was an apparent reduction in feeding compared to the controls. Feeding on these treatments remained below that upon controls for the remainder of the experimental period. There were no effects of either lead or copper at 2.0 ppm or 0.5 ppm cadmium insect feeding during the first six days. After eight days, the feeding on these treatments appeared to be enhanced over the controls. Variation in data were great and the analysis of variance revealed no significant differences in ten-day cumulative feeding among any treatments.

Effects on fecundity. The effects upon fecundity of exposure of adult flea beetles to alligatorweed exposed to lead, cadmium, or copper
are summarized in Table 7. There were no significant differences in either the total number of eggs laid or in the size of the individual egg clutches in either the lead or copper treatments. All cadmium treatments had a significant reduction in total number of eggs laid, and in the 1.0 ppm treatment, a significant reduction in clutch size occurred. Oviposition was aberrant in all treatments, including controls, under the experimental conditions. Eggs were often deposited upon the plastic box or even upon the surface of the blotter paper, and eggs within a single clutch were frequently laid erratically one upon another or even spaced at wide intervals. Egg viability was assessed on the basis of 50 percent hatching of undamaged eggs. In all cases in which the eggs had not been physically damaged, eggs were found to be viable. Due to the lack of adequate time, no attempt was made to rear-out the larvae. Previous experience with cadmium effects upon Agasicles in which larvae had been reared, however, indicated that larvae of the F₁ generation were normal.

**Effects on mortality.** Under the conditions of these experiments there were no observable significant differences in mortality during the 10-day feeding test regardless of metal or level of exposure. An examination of the concentrations of metals in the insect bodies suggest that insufficient metal was accumulated from feeding to adversely affect the insect physiologically. High copper levels in control insects (127 ppm) also suggest not only a high tolerance for copper, but possibly a high nutritional requirement for this metal.
Figure 65. Cumulative feeding of alligatorweed flea beetles:
(A) Lead; (B) Cadmium; (C) Copper.
Table 7. Effects of lead, cadmium, and copper on the alligatorweed flea beetles, Agasicles hygrophila: means and standard deviations for feeding, fecundity, mortality, and plant and insect uptake.

<table>
<thead>
<tr>
<th>Metal</th>
<th>ppm</th>
<th>n</th>
<th>10-day cumulative feeding, mg/insect</th>
<th>Metal concentration in leaves ppm</th>
<th>mg metal ingested/insect</th>
<th>Metal in insects ppm</th>
<th>Total metal/insect ng</th>
<th>10-day mortality</th>
<th>Total number of eggs</th>
<th>Eggs laid/female/day</th>
<th>Clutch size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.0</td>
<td>2</td>
<td>6.97±0.29</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1±0</td>
<td>151±14.1</td>
<td>7.55±0.71</td>
<td>25.17±2.38</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>3</td>
<td>8.17±1.93</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2±1</td>
<td>98.3±17.7</td>
<td>4.92±0.88</td>
<td>20.97±1.31</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.0</td>
<td>2</td>
<td>6.97±0.29</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1±0</td>
<td>151±14.1</td>
<td>7.55±0.71</td>
<td>25.17±2.38</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2</td>
<td>8.01±1.46</td>
<td>0.0</td>
<td>0.0</td>
<td>5.24</td>
<td>10.0</td>
<td>1±1.4</td>
<td>57±4.2</td>
<td>2.85±0.21</td>
<td>23.50±4.95</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2</td>
<td>4.94±2.80</td>
<td>2.40</td>
<td>11.87±6.73</td>
<td>4.61</td>
<td>8.33</td>
<td>2±1.4</td>
<td>6.5±9.2</td>
<td>0.33±0.46</td>
<td>3.25±1.80</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2</td>
<td>5.01±2.01</td>
<td>3.46</td>
<td>17.33±6.97</td>
<td>4.39</td>
<td>8.34</td>
<td>3±0</td>
<td>22.0±3.51</td>
<td>1.10±0.14</td>
<td>22.00±2.83</td>
</tr>
<tr>
<td>Copper</td>
<td>0.0</td>
<td>2</td>
<td>6.97±0.29</td>
<td>1.86</td>
<td>12.96±0.54</td>
<td>127.30</td>
<td>257</td>
<td>1±0</td>
<td>151±14.1</td>
<td>7.55±0.71</td>
<td>25.17±2.38</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2</td>
<td>9.05±2.32</td>
<td>14.76</td>
<td>133.52±34.23</td>
<td>210.30</td>
<td>425</td>
<td>2.5±0.7</td>
<td>91±90.51</td>
<td>4.55±4.53</td>
<td>16.44±4.15</td>
</tr>
</tbody>
</table>
Discussion. The reason for the lack of any significant effect of plant-absorbed lead upon the alligatorweed flea beetles becomes quite obvious upon examination of the data in Table 7. No detectable lead had been ingested by the insects. This does not preclude possible toxicity of lead in alligatorweed to the flea beetles, however, as the present study was limited to root-absorbed metals and, obviously, was solely dependent upon transport of lead to the leaves before effects could be observed. In nature lead may very likely be deposited upon leaf surfaces where the insects would ingest it in the course of feeding. The possibility that such surface deposited lead may influence the feeding and fecundity of the flea beetles needs to be investigated.

The lack of any significant effects of copper upon the flea beetles would initially appear to be the result of the elimination of copper from the body with relatively little physiological uptake. Table 7 shows that the average copper concentration in leaves of plants exposed to 2.0 ppm copper was 14.76 ppm, (almost eight times as great as that in control plants) and that the actual amount of copper ingested was 133 ng (about ten times as great as in the controls). The concentrations of copper in insects from controls and the 2.0 ppm copper treatments were 127 and 210 ppm, respectively. Upon examination of the total copper accumulation (ng) is the insect bodies, however, it may be seen that control insects contained 257 ng copper per insect, whereas 2.0 ppm treatments contained 425 ng copper per insect (an increase of about 65 percent over the control insects). This would more than account for the difference in total copper ingested. On the basis of these observations, therefore, it must
be concluded that the copper ingested was actually absorbed by the insects and that the insects, were, in fact, resistant to copper at the levels received in these experiments.

The present experiments have demonstrated that the primary effect of cadmium upon the alligatorweed flea beetles was reduced fecundity. This is in agreement with earlier work (Quimby et al. 1979). The present study, however, did not show any significant differences in cumulative feeding or in mortality, whereas Quimby et al. (1979) had noted that flea beetles fed leaves of alligatorweed that had been exposed to 1.0 ppm cadmium in solution fed only about 25 percent as much and had about 3.4 times as great mortality as control insects. The apparent discrepancy in results is ameliorated upon examination of leaf concentrations of cadmium in the present study. The mean concentrations of cadmium in leaves in the current studies were 0.0 for controls and 0.5 ppm treatments and 2.4 and 3.46 ppm for the 1.0 and 2.0 ppm treatments, respectively. These values are considerably below those reported by Quimby et al. (1979), where leaves of controls contained 2.6 ppm and leaves of 1.0 ppm treatments contained 8.7 ppm cadmium. Although no cadmium was detected in the leaves in the 0.5 ppm treatment, approximately the same level of cadmium was detected in the insects in this treatment as was found in the higher level treatments (1.0 and 2.0 ppm cadmium). This suggests that very low (i.e., undetectable) levels were present in the leaves of the 0.5 ppm treatments, and that this undetectable amount was sufficient to adversely affect fecundity in these experiments. The lack of any apparent effects of cadmium upon mortality occurred because of inadequate exposure level, but cadmium, even at the lowest levels
tested (0.5 ppm in solution), was sufficiently toxic to reduce fecundity. These results suggest that even low environmental levels of cadmium might interfere with the reproduction of the flea beetles.

Neochetina Experiment

Effects of feeding. Figure 66 shows the cumulative feeding of the waterhyacinth weevil, Neochetina sp., when fed waterhyacinth leaves containing different levels of lead, cadmium, or copper. Plants exposed to the metals had higher levels of insect feeding (i.e., more lesions) than did the control plants, but overall analysis of variance indicated that these differences were not significant. When analyzed separately for each metal, however, there were significant differences between feeding on the controls and feeding on plants exposed to approximately 2.5 ppm copper. Cumulative feeding in either the lead or cadmium experiments was not significantly different from the controls, regardless of level of exposure.

Effects on mortality. There were no significant differences in insect mortality during the first ten days in which feeding was monitored. After 20 days, however, there were significant differences in mortality. Insects feeding on plants exposed to approximately 5.0 ppm lead, 1.0 ppm cadmium, or 2.5 ppm copper had significantly lower mortality than insects feeding upon control plants. The average leaf concentrations of metals for plants exposed to approximately 5.0 ppm lead, 1.0 ppm cadmium, and 2.5 ppm copper were 9.84, 17.20, and 44.77 ppm, respectively. Control plants contained 0.0, 0.06, and 15.35 ppm lead, cadmium, and copper, respectively. Insects feeding on leaves
Figure 66. Cumulative feeding of waterhyacinth weevils:

(A) Lead; (B) Cadmium; (C) Copper.
Table 8. Means and standard deviations for concentrations of metals in waterhyacinth leaves and waterhyacinth weevils, 10-day cumulative feeding, and 10- and 20-day mortality in the Neochetina feeding tests.

<table>
<thead>
<tr>
<th>Metal</th>
<th>ppm</th>
<th>Metal Concentration in Leaves, ppm</th>
<th>Metal Concentration in Insects, ppm</th>
<th>10-day Cumulative Feeding lesions/insects</th>
<th>10-day Mortality</th>
<th>20-day Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.0</td>
<td>0.00 ± 0.00</td>
<td>0.00</td>
<td>131.2 ± 11.8</td>
<td>0.8 ± 0.8</td>
<td>2.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>5.89 ± 5.38</td>
<td>0.00</td>
<td>158.0 ± 22.7</td>
<td>0.6 ± 0.9</td>
<td>2.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>9.84 ± 0.14</td>
<td>44.45</td>
<td>149.6 ± 14.0</td>
<td>0.0 ± 0.0</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.0</td>
<td>0.06 ± 0.05</td>
<td>6.52</td>
<td>131.2 ± 11.8</td>
<td>0.8 ± 0.8</td>
<td>2.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>8.00 ± 1.56</td>
<td>14.46</td>
<td>149.9 ± 35.6</td>
<td>0.8 ± 0.8</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>17.20 ± 3.05</td>
<td>36.67</td>
<td>162.7 ± 16.5</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Copper</td>
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<td>15.35 ± 0.82</td>
<td>30.42</td>
<td>131.2 ± 11.8</td>
<td>0.8 ± 0.8</td>
<td>2.8 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>21.62 ± 1.39</td>
<td>38.37</td>
<td>145.3 ± 19.8</td>
<td>0.2 ± 0.4</td>
<td>1.4 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>44.77 ± 1.94</td>
<td>32.77</td>
<td>174.7 ± 29.7</td>
<td>0.0 ± 0.0</td>
<td>0.8 ± 0.8</td>
</tr>
</tbody>
</table>
containing 9.84 ppm lead, 17.20 ppm cadmium, or 44.77 ppm copper were found to contain 44.45, 36.67, or 32.77 ppm, respectively, of lead, cadmium, or copper. Insects fed control leaves contained 0.0 ppm lead, 6.52 ppm cadmium, and 30.42 ppm copper. It is apparent that the concentrations of metals to which the weevils were exposed in these experiments were insufficient to cause either an acute toxicity effect or a chronic toxicity resulting from accumulation of metals in the tissues over a long period of time. Means for data are summarized in Table 8.

Discussion. Differences in cumulative feeding noted in these experiments may have been caused by several factors. An experiment in which feeding is assessed by the counting of feeding lesions is subject to considerable variation from differences in sizes of feeding spots. For the purpose of eliminating some of the variation due to lesion size, a standardized feeding spot size was estimated. Large lesions were then estimated as to how many average lesions they represented, and several unusually small lesions were counted as one. Difficulties in such a technique are apparent, especially when leaves have an extremely dense concentration of feeding lesions. Even with such inherent errors in measurement, the error in estimation would not likely cause bias in the data that so consistently favored one treatment over another. A second possible factor that may have enhanced feeding is that the presence of high plant levels of lead, cadmium, or copper may have altered the plant chemistry sufficiently to produce enhanced levels of some feeding stimulant or to possibly decrease the plant content of one or more substances essential in the insect nutrition (hence, more feeding on leaves with high levels of metal). A third possible explanation
for the enhanced feeding is that live plant tissues may have contained a greater amount of water when exposed to high levels of metals. This could have resulted in enhanced insect feeding in order to obtain the same amount of food.

The apparent increased longevity of weevils feeding upon waterhyacinth leaves containing high concentrations of metals might also be an artifact of experimental conditions. In the present study, insufficient numbers of weevils of the same species were collected to be able to conduct the experiments using insects of all of one species. Consequently, both Neochetina eichhorniae and N. bruchi were present in each treatment. This may have biased the controls in favor of one species over the other to a greater extent than in the metal treatments, with the consequence that there was on the average, both less feeding and high mortality in the controls. A second factor may have been age and/or the physiological maturity of the insects.

The general conclusions may be made. First, lead, cadmium, and copper in waterhyacinths had little or no effect upon either the feeding or mortality of adult waterhyacinth weevils. Second, at the levels of exposure in these experiments lead, cadmium, and copper apparently were not accumulated sufficiently by the insects to cause either acute or chronic toxicity. These results suggest that the waterhyacinth weevils, Neochetina sp., are not likely to be adversely affected by environmental pollution with lead, cadmium, or copper. It must be pointed out, however, that these studies were done with adult weevils. The effects upon the larvae and pupae, as well as fecundity and oviposition, have not been studied. Further experimentation is needed before any knowledgeable conclusion can be made.
concerning the impact of environmental contamination by lead, cadmium, or copper upon the efficacy of the waterhyacinth weevil as a biological suppressant of waterhyacinths.

**Summary and Conclusions**

There were no significant effects of plant accumulations of lead, cadmium, or copper upon either the feeding or mortality of adult alligatorweed flea beetles, *Agasicles hygrophila*, during 10-day feeding trials.

The feeding of leaf tissue from plants which had been exposed to 0.5, 1.0, or 2.0 ppm of cadmium in the nutrient solution caused a significant reduction in comparison to the controls in the fecundity of the flea beetles as measured by egg counts. The effects were manifested both as a reduction in the total number of eggs laid as well as in the size of individual egg clutches. The response threshold for effects of cadmium on flea beetles fecundity can be placed near 0.5 ppm plant exposure.

There were no significant effects upon feeding of the flea beetles on leaf tissue from plants which had been exposed to 2.0 ppm of either lead or copper. The lack effect of lead was due apparently to the lack of transport of lead to leaves. The lack of effect of copper appeared to be innate resistance of the insects to the levels of copper present in leaf tissue under the experimental conditions. The results of the experiments with *Agasicles* suggest that cadmium may be toxic in the natural environment, and that further research is warranted.

There were essentially no differences in either the feeding response or mortality of adult waterhyacinth weevils, *Neochetina* sp.,
when fed leaves of waterhyacinths that were exposed to lead, cadmium, or copper. The exposure levels used in these experiments were apparently insufficient to elicit either an acute or a chronic toxic effect. The results of these experiments suggest that adult waterhyacinth weevils are unlikely to be adversely affected by environmental contamination with lead, cadmium, or copper.
LITERATURE CITED


Wolverton, B. C., R. M. Barlow, and R. C. McDonald. Unpublished manuscript. Water hyacinth sorption rates of lead, mercury and cadmium.


BIOGRAPHICAL SKETCH

Stratford Haman Kay was born on May 4, 1945, in Cleveland, Ohio. He was graduated from Calhoun City High School, Calhoun City, Mississippi, in 1963. He received his B.S. and M.S. degrees in biology from the University of Mississippi in 1967 and 1974, respectively. Mr. Kay has taught school in Greenville, Mississippi, and has worked as a biological research technician in aquatic plant research at the USDA, Southern Weed Science Laboratory in Stoneville, Mississippi. Mr. Kay is married to Theresa Edwards and has a son, John Stephen. Mr. Kay is presently employed as a post-doctoral research associate at the Center for Alluvial Plain Studies, Delta State University, Cleveland, Mississippi, in a cooperative research agreement with the USDA at Stoneville, Mississippi.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

William T. Haller, Chairman
Associate Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Leon A. Garrard
Associate Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

George E. Bowes
Associate Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dale H. Habeck
Professor of Entomology and Nematology
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald A. Graetz
Associate Professor of Soil Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1980

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School