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A DISSERTATION

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JOYCE DARLEEN SHEEDY

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1977

HEAVY METAL CONTENT OF WHEAT, BERMUDA GRASS AND SOIL IRRIGATED WITH ELECTRIC PLATING EFFLUENT

APPROVED BY

Robert Y. Nelson
Larry Canter
James M. Robertson
Paul G. Risser

DISSERTATION COMMITTEE

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CHAPTER I

INTRODUCTION

Pollution is one of the many problems faced by an industrialized world. Every year industry must dispose of tons of unwanted by-products. Attention has become focused on the pollution problems facing the United States and the world. Efforts are being made to control the contamination of water, air and food by pollutants whose effects on human and animal populations have not been completely determined. It is becoming apparent in many instances that it may be necessary to pay a higher price for production to prevent irreparable harm to the environment.

In a world controlled by intricately linked cycles, an effect on one cycle eventually affects all cycles. Thus it is important that we consider the effects that our actions have on the whole system before proceeding with them. Life as we know it depends on water. Where there is an abundance of unpolluted water, there is an abundance of life. But the supply of clean water in this country has been greatly diminished over the last several decades as municipalities and industries dump liquid and solid wastes in lakes, streams and oceans. Many streams and lakes are now "dead" because of this practice. The dissolved oxygen (D.O.), B.O.D., pH, organic and temperature levels are such that aquatic life can no longer survive and thus the system of self-cleaning has been destroyed. Toxic materials such as heavy metals are often constitutens of wastewater. Even when the concentration levels are not high enough to destroy the organisms, the organisms are often capable of accumulating such toxic materials and passing them on through complex food chains. Some organisms have the ability to concentrate an effluent in the order of thousands of times above the environmental level (Kormondy, 1969). It is possible, therefore, for the final consumer to receive large amounts of the elements which are concentrated in this way. This final consumer may be a higher aquatic organism, or various species of animals. Quite often it is man.

A program has been initiated by the government to regulate the discharges into streams and bodies of water. Stringent standards are scheduled to go into effect in 1977 and the complete elimination of discharge of pollutants into navigable streams is planned for 1985 (Federal Water Pollution Control Act Amendment of 1972). This will require new methods of waste control and disposal.

Industries faced with stringent discharge standards and an ultimate no pollutant discharge edict have several methods to choose from in solving the problem of what to do with wastewater. The ultimate disposal of wastewater is to treat it to meet government standards or to store what cannot meet these standards. With shortages of raw materials that are becoming common, water reuse and by-product recovery may be more economical than cleaning and/or storing wastewater. Another partial solution is the reduction of the volume of water used in industrial processes. In the past atmopsheric, ocean and deep well disposal methods were used and are still in use in some areas, although felt by many environmentalists to be unsatisfactory.

A method of ultimate disposal of wastewater and sludge is land disposal. Recycling of sewage waste on land, if properly managed, is probably the most economical solution and has the least pollution potential of any method presently available (Sabey et al, 1973). Land disposal methods include:

1. Overland flow in which the soil surface is used as a horizontal trickling filter.
2. Infiltration-percolation which is used to recharge the ground water.
3. Irrigation of grazing and crop lands.

It is possible that a problem is being made of what is potentially a valuable asset. As the population continues to increase there is an ever increasing demand for greater crop production. In many arid and semi-arid regions the lack of water greatly limits the amount and type of crops that can be produced. While in other regions the rainfall may be adequate, it does not fall at times that are optimal for crop production. A steady supply of water for irrigation would be useful in solving water shortages in both cases. Wastewater, however, supplies more than just water - it supplies nutrients. The same nutrients that cause algal blooms and contribute to the eutrophication of lakes could be of great value when applied to land to replace those nutrients that are removed with crops. In a world where the cost of fertilizers is rising as the supply diminishes, wastewater as a source of nutrients should not be overlooked.

Wastewater effluent from municipal sewage treatment plants has been used for irrigation for years in Europe with no ill effects reported when properly managed. Many investigations have been carried out on the effects of irrigating crops with municipal and industrial effluents. (Forester, 1973; Hatcher, 1974; Seabrook, 1973; Harlin, 1973; and Lamb, 1973). Often the yields and quality of the crops have been markedly improved by the application of effluents. In Oklahoma several small municipal systems under construction utilize irrigation as a means of lessening lagoon size, thus making the sewage treatment facility less costly. In the future it may be possible for municipalities to help pay for the operation and maintenance of their sewage treatment systems by the sale of wastewater or the sale of crops grown on land irrigated by wastewater.

There are many questions still unanswered concerning the effects of effluent on the soil, groundwater, and plant and animal life. Of special interest is the ability of the soil, plants, and animals to accumulate certain materials including heavy metals. It has been generally concluded that while land disposal of effluent in the form of crop irrigation may be satisfactorily accomplished for municipal effluent and certain industrial effluents containing principally organic materials (food processing in particular), the effluent of industries such as electric plating industries would not be satisfactory for irrigation due to the accumulation of heavy metals by the soil. Such an accumulation would be expected in time to be toxic to plants and render the soil unproductive or completely unuseable for crop production, or produce a crop with a heavy metal content toxic to the animals or humans who consume the crop.

To date no study has completely answered the question of relationship of the quality of the crops harvested and the mineral content of the soil on which they grew, or the effect of these crops on the animals which consume them. It is generally accepted that the mineral quality of the soil influences the mineral quality of the foods grown on it (Goldstein, 1973a). More research in this area is vital because changes in nutrient composition of the plants could have far reaching effects on the health of the animals and the people who consume them.

The objectives of this research were to:

1. Study the feasibility of disposal of treated electroplating wastewater effluent by land irrigation
2. Study the effect of the effluent on the potential crops of wheat and bermuda grass
3. Study the accumulation of the heavy metals, chromium, copper, iron, nickel, and zinc, in the soil and plant material and assess the potential hazard to animals and man.

In order to meet these objectives a laboratory study was conducted. Winter wheat (*Triticum aestivum* var. Danne) and bermuda grass (Cynoda dactylon var. Midland) were grown in the laboratory under controlled conditions. These plants were watered with effluent obtained from the Western Electric Company located in Oklahoma City and grown on soil obtained from land belonging to that company. The plants were harvested at intervals and analyzed for five heavy metals present in the effluent (chromium, copper, iron, nickel, and zinc). The amount of effluent added to the plants was recorded and the concentrations of heavy metals in the soil prior to planting and at the completion of the growth period was measured.

Since chromium is difficult to remove by most conventional treatment processes, and since it was present in the effluent in relatively high concentrations, a special study of the cycling of chromium was undertaken using radioactive chromium-51. Winter wheat and bermuda grass were watered with deionized water containing chromium-51 and chromium-51 concentrations were determined in the plant material.

These studies demonstrated the quantities of heavy metals taken up by plants compared to the amount of heavy metals added to the soil. The results indicate the feasibility of irrigation as a method of disposal for the effluent involved.

CHAPTER II

LITERATURE SURVEY

A. Introduction

The pertiment literature on the use of industrial wastewater containing heavy metals for irrigation of croplands was surveyed with particular emphasis on the accumulation of chromium, copper, iron, nickel and zinc by soil, crops and animals and the subsequent entry into the food chain.

Municipalities and industries today are faced with the problem of disposal of effluent and sludge which often include toxic materials such as heavy metals, cyanide, and phenols. The chemical properties of industrial wastes may fluctuate widely. The pH and temperature may vary considerably depending on the industrial process. The problems are as varied as the types of industrial processes which produce the wastes. There are many methods for primary treatment of these wastes.

The ultimate disposal of wastewater effluent will be either to treat and clean it or to store or contain all that cannot be cleaned. The Federal Water Pollution Control Act Amendements of 1972 require that the discharge of pollutants into navigable waters eventually must be prevented. Atmospheric, ocean and deep well disposal methods are not looked upon favorably and in some states are illegal. Faced with zero discharge of pollutants by 1985, producers of municipal and industrial wastes must undertake the technology advances needed to bring about water reuse and by-product recovery (Forester, 1973a).

There is no one correct way to handle wastewater and sludge. Many methods are now in use with varying degrees of success. Storing the effluent in lagoons is one such method. This method has drawbacks as well as advantages. At best lagoons are only a temporary answer since they are subject to leaching into the groundwater and possible overflows during heavy rainfall. Lagoons also require rather large amounts of land area, which may not be available or economical for many industries and municipalities.

Other acceptable methods which will allow many treatment plants to meet 1977 NPDES requirements comprise what are known as tertiary treatment methods. These are usually very expensive both in capital costs and in operation and maintenance costs.

The recycling of wastewater and residuals on the land may be an acceptable method of ultimate disposal. According to Sabey et al (1973), the recycling of sewage waste on land is probably the most economical and has the least pollution potential of any method presently available, when managed properly. Of the three most commonly used land application methods, irrigation, overland flow, and groundwater recharge, irrigation is the most reliable with respect to long term use and renovation of wastewater. Current technology is sufficiently developed so that general design and operational guidelines can be prepared (Pound and Crites, 1973a). The effectiveness of irrigation for waste disposal treatment depends on several factors: the availability of suitalbe land in the vicinity; the cost of such land; and wastewater characteristics such as B.O.D., pH, toxic materials, and heavy metals.

B. Brief History of Wastewater Irrigation

The use of wastewater for irrigation is not new. The history of irrigation with wastewater is a long one. Wastewater application to land was practiced in Athens, Greece, in the B.C. period (Metcalf and Eddy, 1972) and irrigation with wastewater was recorded in Germany in the 16th century, A.D. (DeTurk, 1935). The practice of "sewage farming" spread to England in the 1700's. Crops grown were grains, grasses, root, vegetables, corn, and fruit. The yield from a sewage farm was usually at least twice that of a conventional farm in the same area. The excess wastewater was usually conveyed to nearby streams by underdrain systems with no adverse effect on the purity of the stream (Pound and Crites, 1973b). The practice of wastewater irrigation spread to South Africa, Australia and Mexico where it continues today.

Wastewater irrigation was used in the United States in the 1870's. However, only recently has the possibility of irrigating with wastewater and liquid sludge been given serious attention in the United States. This is because of the new NPDES limitations which have caused a drastic increase in the cost of "adequate" wastewater treatment.

Wastewater irrigation has proven to be reliable in terms of length of useful life. In the United States systems have been in operation at Cheyenne, Wyoming, since 1881, at Fresno, California, since

1891 and at Bakersfield, California, since 1912 (Pound and Crites, 1973a).

Application of industrial wastewater to the land has been a relatively recent development in the history of land application of wastewaters. Reports of land application operations specifically for industrial waste did not appear in the literature until the 1940's. Extensive use of land application has been restricted primarily to the food processing industry, the pulp and paper industry, and the dairy industry (Pound and Crites, 1973b).

C. Current and Recent Research

Many research projects, both laboratory and field investigations, have been conducted in an attempt to determine the effect of wastewater irrigation on soil, plants, groundwater, and human and animal consumers.

Spray irrigation has been used successfully in St. Petersburg, Florida, on a golf course and park lands with no salting out. Success with sludge and wastewater used as soil conditioners for land reclamation has been reported in Oregon on marginal desert land (Forester, 1973b). There are several studies underway on the use of effluent and sludge as soil conditioners and fertilizers for forest and crop lands (Hatcher, 1974; Seabrook, 1973; Harlin, 1973; and Lamb, 1973).

Studies on the effects of irrigation with disinfected municipal effluent on crops at Pennsylvania State University have indicated that when practiced in a cool, semi-humid climate, increased crop production can be achieved while contributing a substantial recharge to groundwater. Changes in soil chemistry were small and probably not a future problem (Harlin, 1973 and Lewicke, 1972).

Kardos et al (1974) reported on a study in Pennsylvania using chlorinated, secondary wastewater through sprinkler irrigation systems to approximately 70 acres of crop and forest land in well drained clay loam soils and sandy loam soils. Application at hydraulic loading rates of one to two inches per week resulted in a guaranteed economic level of crop production, while at the same time recharging 50 to 100 percent of the applied wastewater as potable water. Crop removal contributed substantially to the renovation of wastewater by removing the equivalent of 20 to 80 percent of the applied phosphorus and 40 to 100 percent of the applied nitrogen. The chemical composition of the harvested crops were well within the normal ranges of such crops as reported in agronomic literature. The quality of the groundwater where the recharge was taking place changed slightly. There was an increase in nitrogen and chloride in one monitoring well, but the water met the USPHS drinking water standards.

The city of Yakima, Washington, irrigates 120 acres of hay with sludge and industrial (cannery) effluent. The hay (alta-fesque grass hay) is a mixture of orchard grass, fesque grass and vernal alfalfa adapted for conditions of excessive moisture. The crop yield was generally greater than the yield from conventionally-run hay fields in the area. The saturation point where production leveled was not reached (Lamb, 1973).

Spray runoff studies were undertaken at Napoleon, Ohio, and Paris, Texas. Preliminary results over a two-year period show that the system has a capability of producing a tertiary level of treatment without producing sludge (Harlin, 1973).

Sullivan et al (1971) performed a survey of facilities using land application of wastewater. They conducted field investigation of 67 municipal and 20 industrial facilities and questionnaire investigations of 300 additional facilities. Of the 67 municipal systems studied, ten had operated prior to 1920. The earliest industrial system surveyed began operation between 1940 and 1945. The prevalent industrial types were food processing, milk processing, pulp and paper mills and organic chemical production. They found that under proper conditions land application of wastewater is a workable alternative to advanced or tertiary treatment of municipal wastes. Ninety-five percent of the industries surveyed intended to keep their irrigation systems.

Very little research has been conducted to determine the feasibility of irrigation with wastewaters that contain significant levels of heavy metals. The general consensus appears to be that such industrial waste is unsuitable for land application (Pound and Crites, 1973a and 1973b). Of primary concern is the accumulation of heavy metals in the soil itself. This could lead to a lowering of crop productivity and even to plant toxicity. Another concern is the effect such heavy metal enriched soils might have on the uptake and accumulation of these heavy metals by crops. In conjunction with this is the problem of involving possibly higher than normal levels of heavy metals in food chains.

The characteristics of industrial wastewaters vary widely not only by industry, but also by product and processing technique used. There is no typical industrial wastewater. It has been concluded that in general industrial wastewaters containing significant concentrations of heavy metals are not suitable for application to most soils. However, successful operations with such wastewater have been reported. For example, in Vicksburg, Michigan, paper processing wastewater high in lead, vanadium, and cadmium concentrations was applied to a peat-type soil. The high organic content of the soil provided a large heavy metal retention capacity. However, this capacity is not limitless and is expected to be exhausted within 20 years (Pound and Crites, 1973b). The response of the soil at a proposed site should be studied carefully prior to full-scale application of industrial wastewaters containing significant concentrations of heavy metals.

D. Factors To Be Considered in Irrigation Systems

1. Introduction

There are several factors that should be considered before land application of industrial waste by irrigation is chosen as the method for wastewater treatment: climatic conditions; amount of waste flow; industry size; population equivalent of industrial wastes; availability of open land; land use zoning; cost of land; type of crops and market needs and demands; groundwater depth and quantities and use for water supply; proximity of surface waters; nature of soil (Sullivan et al, 1971); and wastewater characteristics such as B.O.D., C.O.D., suspended solids, total fixed dissolved solids, nitrogen, pH, temperature, color, heavy metals, SAR, pesticides and other organo-compounds and salts.

2. Method of Application

Sometimes the method of application can play an important role. While spray irrigation has become the predominant method of application, the ridge and furrow methods has been used with some success in systems where the wastewater is apparently toxic to the vegetation (Pound and Crites, 1973b). Zinc, iron, and copper, usually as sulfates, are often sprayed on the leaves of plants to alleviate deficiencies. The leaf has essentially no selective capacity to preferentially absorb one nutrient over another. Thus a leaf will absorb any element contained in a spray solution (Foy et al, 1953 and Volk and McAulippe, 1954). The sensitivity of leaves to noxious elements in spray solutions has not been determined adequately and merits further research (Melsted, 1973).

3. Type of Vegetation

The type of vegetation grown at the irrigation site will also affect the success of the system. Plants vary widely in their ability to absorb nutrients from the soil and to transport nutrients or toxic substances from the roots to the vegetative sutructures above ground. Some of these differences are genetic in origin and may be associated with the physical distribution of roots and their chemical characteristics such as cation exchange capacity and pH (Melsted, 1973). The productivity of the plant on the land irrigated by industrial wastewater and the uptake and accumulation of heavy metals and other substances by the plant can vary widely from species to species and even from variety to variety within the same species.

4. Nature of Soil

The success of irrigation with wastewater depends to a great extent on the type of soil to be irrigated. Irrigation requires soils with a particular balance of two basic properites, adsorption and permeability. These two properties tend to counteract each other. A compromise must be reached between the type of soil with excellent percolation and low adsorption, which while allowing heavy hydraulic loading, would also allow movement of substances found in the wastewater into any groundwater present; and a tight clay or silt loam soil with high ion adsorption capacity and an infiltration rate too low to operate successfully (Melsted, 1973).

Many soil factors influence the uptake of heavy metals and other substances by plants. Some of the more important factors are pH, organic matter content, moisture, pore space, proportion of clay and silt, type and size of microbial life (Sauchelli, 1969), phosphate content, and cation exchange capacity (Chaney, 1973).

5. Nature of the Wastewater

A necessary preliminary step when planning a land application system is a detailed evaluation of the wastewater to be applied to the land. The following factors should be determined: total solids content, suspended solids, dissolved solids, temperature, color, odor, dissolved oxygen, dissolved organic matter, dissolved inorganic matter, nitrogen content, phosphorus content, potassium content, heavy metals content and the content of other toxic elements (Oklahoma State Department of Health 1977).

E. Heavy Metals In The Soil-Plant-Animal System

1. Introduction

In any plan for land application of water containing heavy metals it is necessary to consider the effect of the following elements on the soil-plant system: heavy metal interaction with the soil, heavy metal interaction with plants, and heavy metals in the food chain.

a. Heavy metal interactions with soil. It has been suggested that soil is the ultimate receptacle of most of our solid and liquid wastes (Korte et al, 1976). But it should be remembered that sludges and effluents containing non-essential toxic chemical compounds cannot be expected to enhance the soil for crop production, but under controlled applications they should not affect the soil to the extent that crop yield and quality are impaired (Melsted, 1973). When trace elements are added to the soil via irrigation waters they may: react with the soil, be removed from the soil through harvested plants; or move through the soil with percolating waters. The fate of any given element will depend on the chemistry of the element and on plant and soil factors. Some trace metals are not removed by leaching and can accumulate to toxic levels in the soil (Pratt, 1973).

Our knowledge of the chemistry of toxic metals in soils is very incomplete and largely speculative (Lisk, 1972). The chemistry of trace elements in soils is dominated by reactions that lead to the formation of inert and insoluble compounds or complexes (Allaway, 1968). The solubilities of soil constituents is markedly affected by several factors such as pH, oxidation processes and organic matter (Bowen, 1966).

Heavy metals are generally much more available at pH values below 6.5 to 7.0 (Chaney, 1973). The availability of cations is decreased at high pH values. In acid conditions there is an abundance of the ions of iron, zinc, and copper. As the pH increases the ionic forms are changed to hydroxides or oxides. All the hydroxides of the trace element cations are insoluble. The exact pH at which precipitation occurs varies from element to element (Buckner and Brady, 1969).

The organic matter content of the soil is important since it forms stable complexes with metal ions making them unavailable (Antonovics et al, 1971). Organic matter in soil seems to form a complex with clay minerals, thus increasing the cation exchange capacity (Reynolds and Gloyna, 1963).

The cation exchange capacity is important in binding all cations, including heavy metal cations. However, Korte et al (1976) stated that cation exchange capacity is not related to the environmental fixation of trace metals. They have proposed instead control by hydrous oxides of iron and manganese.

The divalent metal ions zinc, copper and nickel are normally not found in soils in large quantities. Therefore, their inclusion in wastewater may increase the total content of these elements in the soil significantly. Although these cations can be held as exchangeable ions most of them will precipitate out of solution. These metals are potentially toxic to plants and animals and may constitute future hazards if continually added to soils (Lindsay, 1973).

b. Heavy metal interactions with plants. There are many factors affecting plant accumulation of toxic metals: 1) Any factor that affects the plant accumulation (total amount of metal, pH, organic matter, PO_4, CEC, and reversion control the amount of excess metal available to the root); 2) Characteristics of the toxic metal (in acid soils zinc is easily translocated to plant tops, while copper and nickel are translocated in appreciable quantities only during severe injury to the plant); 3) Presence of competing ions; 4) Phosphate availability (phosphate interferes with zinc, copper, and iron metabolism of plants); 5) Rooting depth and soil distribution of metals; 6) Plant age and seasonal effects (the growth rate of some crops allows a dilution in leaf zinc as the season progresses); 7) Plant species and variety; and 8) Soil moisture, aeration and temperature (Chaney, 1973).

Agronomists have long debated to what extent the soil or the plant dictates ion uptake by roots. The nature of the plant including its species, size, growth rate, extent and depth of rooting, transpiration rate, and nutritional requirements may effect its efficiency for metal absorption from soils. The mechanism of ion uptake by roots is speculative and may involve direct ion absorption of soluble ions in soil solution as well as exudation of organic complexing anions by roots or bacteria to render fixed metals soluble for absorption. Physical contact between the root and absorbed or precipitated ions may result in direct passage of the ions into the root. Finally one ion species may interfere with root absorption of a different one in the same solution. The behavior of toxic metals in soils and the mechanisms of ion uptake by plants are largely speculative (Lisk, 1972).

Melsted (1973) however, stated that active uptake is the dominant entry pathway of cations once they are absorbed on the root exchange surface. This is an aerobic process by which the plant can accumulate cations against nutrient gradients and by which it exercises a degree of discrimination against cations present in the biosphere. According to Melsted (1973) passive entry is the major pathway of anions.

Plant species vary widely in tolerance to toxic metals and varieties within a species can vary three to tenfold. Many general farm crops (corn, small grains and soybeans) are moderately tolerant. Most grasses (fescue, lovegrass, bermuda grass, orchardgrass, and perennial ryegrass) are tolerant to high amounts of metals (Chaney, 1973).

The mechanisms whereby the living organism can grow on apparently contaminated soil may prevent the heavy metals from reaching their sites of toxic action within the plant or they may simply be external factors that prevent the metals from entering the organism. External mechanisms include: the form of metal is not directly soluble in water and/or if dissolved then rapidly diluted by surrounding water; actual amount of freely diffusable metal ions is small compared to total amount present; lack of permeability to heavy metals under specific conditions; and metal ion antagonisms. Internal mechanisms include: differential uptake of ions; removal of metal ions from metabolism by deposition in vacuole; removal of metal ions from metabolism by pumping from cell; removal of metal ions from metabolism by rendering into an innocuous form; excretory mechanisms; greater requirement of enzyme systems for metal ions; alternative metabolic pathway by-passing inhibited site;

increased concentration of metabolic that antagonize inhibitor; increased concentration of enzyme that is inhibited; decreased requirement for product of inhibited system; formation of altered enzyme with decreased affinity for inhibitor or increased relative affinity for substrate compared to the competitive inhibitor; decreased permeability of cell or subcellular units to metal ions; and alteration in protoplasm so that enzymes may function even when toxic metals replace physiological metals. All the above are possible mechanisms for metal tolerances (Antonovics et al, 1971).

c. Soil testing and plant uptake of heavy metals. There is no single approved method of determining the concentration of heavy metals in soils. Among the many methods used are tests that measure the total, exchangeable, available, and water soluble cations. Many of these measurements show no correlation with plant content of the heavy metal in question.

Antonovics et al (1971) states that most workers have resorted to measuring total metal concentrations since this has been found to reflect in a relative way on the concentration affecting the plants, and because it is easy to measure. They found no evidence that other methods reflected more accurately the amount available to plants.

In Sweden, copper extractable by strong acid digestion of a soil correlated reasonable well with the copper content of the tissues of plants grown thereon. However, at present no precise chemical method has been developed for determining the amount of copper in soil which is available to plants (Reuther and Labanauskas, 1966).

There has been no acceptable chemical test for determing iron availability approved officially in the United States (Sauchelli, 1969).

The nickel content of plants appears to be closely correlated to the exchangeable nickel of soils as determined by neutral normal ammonium acetate solution. Total nickel content is not a good measure of the availability of the element to plants (Vanselow, 1966).

d. Heavy metals in the food chain. One of the problems with heavy metals is their tendency to concentrate through both aquatic and terrestrial food chains. The more links in the chain, the more severe the bioaccumulation phenomenon (VERSAR INC., 1975).

Little information concerning accumulation in organisms, magnification in food webs and mechanism of transport throughout the environment is available for most of the heavy metals (VERSAR INC., 1975). Copper, zinc, and nickel will reduce yields and severely injure plants before they are accumulated to levels that are toxic to most animals. Thus the food chain appears to be protected (Chaney, 1973).

It may be possible to avoid potential toxicity to animals by utilizing the forage at a state when the concentration of a potentially toxic element is least (Allaway, 1968).

e. Heavy metals in the soil-plant-animal system. The soil-plant system can be viewed as a dynamic living system of competing biological, chemical and physical reactions (Lisk, 1972). Although the concentrations of trace elements in living tissue are ordinarily very low, these concentrations must often be maintained within narrow limits in order to permit optimum biological performance of plants, animals, and man. The route from soil, to plant, to animal or human remains the predominant one for many elements (Allaway, 1968).

Man, like all terrestrial animals, depends on food derived from the soil and the composition of the soil is, therefore, of vital importance to him. Having evolved against a background of food supply based on virgin soil we run the risk of creating serious biochemical difficulties for ourselves if we allow the soil to be contaminated in the long term with toxic substances which can pass freely into plants. Contamination of soils with elements such as copper, lead, and zinc appears to be largely irreversible. The trace element content of plants grown on contaminated soils can be enhanced and deleterious effects on plant growth are possible (Purves, 1972).

It is possible for feed and food plants to grow "normally" at optimum or near-optimum rates, even though they contain insufficient chromium, copper, and/or zinc to meet the dietary requirements of some animals for these elements (Allaway, 1968).

The soil plant system provides an effective barrier against toxicity from arsenic, iodine, beryllium, fluorine, nickel and zinc. Plant growth will cease or be greatly depressed before these elements will be taken up from the soil, and accumulated in concentrations that would be dangerous for animals. The soil-to-plant route is, for most trace elements, the major route of entry into the living tissues of plants, animals, and man. The soil-plant system exerts an effective buffering action on the environmental cycling of trace elements (Allaway, 1968).

2. Chromium

a. Introduction. Chromium has been determined as essential to humans and animals (National Acadamy of Sciences, 1974; Allaway, 1968; Bowen, 1966; Lisk, 1972; Underwood, 1975; and VERSAR INC., 1975) but not to plants (Allaway, 1968; Pratt, 1973; Davis, 1956 and Pratt, 1966).

Chromium is fairly abundant in the earth's crust, ranking fourth among the 29 elements of biologic importance (NAS, 1974). Chromium metal is stable and relatively non-toxic due to its insolubility in water and body fluids (VERSAR INC., 1975). However, hexavalent chromium is a strong oxidizing agent and readily reacts with organic matter in acidic solutions, leading to reduction to trivalent chromium. The toxic action of hexavalent chromium is largely due to this oxidizing reaction. Hexavalent chromium can penetrate biologic membranes easily, trivalent chromium cannot (National Acadamy of Sciences, 1974).

Chromium is one of the few essential elements for which no accumulation against a concentration gradient is evident at any point in the biological cycle from soil to plant to animal (Allaway, 1968). The published data cannot be assumed to be accurate because the analytical methods and sampling techniques have been unreliable and highly variable (National Academy of Sciences, 1974).

b. Naturally occurring chromium concentrations in soil. Chromium concentration in the soil has been reported to range from 10 to 150 ppm with an average of 40 ppm (NSA, 1974). In 19 soil samples in France chromium ranged from 1.7 to 88.4 ppm (Davis, 1956). American soils have been tested with chromium concentrations from 1 to 1500 ppm (Lisk, 1972). Bowen (1966) reported chromium concentrations ranging from 5 to 3000 ppm with a mean of 100 ppm while Norrish (1975) listed chromium in soils at 200 ppm and Pratt (1966) at 5 to 1000 ppm.

c. Chromium reactions in soil. At this time the soil chemistry of chromium is largely speculative (Lisk, 1972). The chromium in soil is relatively unavailable as insoluble oxides or in silicate lattices (NSA, 1974; Lisk, 1972; and Allaway, 1968). Lindsay (1973) reported that chromium in soils is generally oxidized or reduced to trivalent chromium and precipitated as an insoluble hydroxide.

The important chromium ions are chromates and dischromates which are easily oxidized to trivalent chromium in acid solutions and in the presence of organic matter (NSA, 1974). The unavailability of trivalent chromium may be partially due to a strong attraction to negatively charged sites on clays or in organic matter (Lisk, 1972 and NSA, 1974). Very little chromium is available to plants in soils with a pH greater than four (Davis, 1956).

Soluble chromium added to the soil probably reacts to form insoluble oxides. Even chelated chromium added to the soil may result in only a temporary increase in the soil solution (Allaway, 1968).

d. Availability of chromium to plants, its uptake and accumulation. Most of the chromium naturally occurring in the soil is insoluble and of low availability to plants (Allaway, 1968; Lisk, 1972; and Lindsay, 1973). Soluble chromium added to the soil is likely to revert to very insoluble oxides (Allaway, 1968). Thus very high amounts of trivalent chromium may be added to the soil without increasing the chromium content of crops appreciably, and constitute no hazard (Chaney, 1973).

Plants absorb only a small proportion of the chromium in soils and chromium additions to the soil usually result in very small increases in plant absorption of chromium (Lisk, 1972). Chaney (1973) reported that very high amounts of trivalent chromium added to the soil would not increase the chromium content of crops appreciably and would constitute no hazard. However, vegetables growing on soil irrigated with chromium containing wastewaters had three to ten times more chromium than control plants in some instances (VERSAR INC., 1975 and Lisk, 1972). The reason for this increase in chromium content might be due to the efficient plant absorption of continuously provided chelated chromium (Lisk, 1972).

Higher plants such as conifers, deciduous trees and shrubs usually take up less chromium than do lower plants like lichens, mosses, ferns and grasses (NSA, 1974). The roots of plants usually contain a higher concentration than the tops (Lisk, 1972 and Bowen, 1966).

Chromium concentrations of 80 ppb in potatoes, 590 ppb in hay, and 40 ppb in grains and cereals have been reported (NSA, 1974). Most plants fall between 100 to 500 micrograms/kilogram. Plants grown on silica soil with a chromium concentration of 140 ppm took up 4.9 to 7.6 ppm chromium (NSA, 1974). Allaway (1968) reported the normal concentrations of chromium in feed and food plants ranging from 0.03 to 1.0 ppm. Pratt (1973) reported chromium concentrations of 7.6 ppm in barley leaves; 4.5-6.5 ppm (low range) and 10.2-14.0 ppm (high range) in wheat leaves; and

4.0-14.0 ppm in tobacco leaves. A concentration of 18-34 ppm in tobacco leaves was toxic and 252 ppm in oat leaves was toxic.

Davis (1956) reported that vegetables from 25 botanical families contained 10-1000 micrograms of chromium per kilogram dry matter. Most of the plants fell in the 100-500 microgram/kilogram range. Bowen (1966) reported chromium in land plants at 0.23 ppm. While Melsted (1973) listed the chromium content of common agronomic crops (corn, soybeans, legumes, wheat, oats, barley and grasses) as ranging from 0.1 to 0.5 ppm with a suggested tolerance level of 2 ppm.

e. The role of chromium in plant metabolism. Chromium has not been proven as essential for plant metabolism (Allaway, 1968; Pratt 1966 and 1973; and Davis, 1956), however crop yields in Germany, Poland, France, and Russia have been improved by application of chromium (NSA, 1974).

Specific functions of chromium in plants have not been determined (Tiffin, 1972). Very little information is available on the effects of chromium on plants. Chromium appears to affect the roots of plants (NSA, 1974). Plants with induced chromium toxicity often contain the same concentration of chromium in the tops as are found in unaffected plants (Allaway, 1968).

The effects of chromium on plants vary with the species and the specific chromium compound. Other chemicals in the soil such as nickel, cobalt and magnesium may interact with chromium in its effect on vegetation (NSA, 1974).

f. Chromium toxicity in plants. The addition of large amounts of chromium to the soil has been reported to be poisonous to plants (NSA, 1974). Applications of 150 ppm to a soil was toxic to citrus seedlings and the addition of 100 and 500 ppm chromium to soils reduced the dry weight and nodulation of peas (Pratt, 1966 and 1973). Chromium has low toxicity when applied as poorly soluble compounds or in alkaline soils (Davis, 1956). Hexavalent chromium appears to be more toxic than the trivalent form (Pratt, 1966).

Excessive chromium levels in the soil cause two plant diseases: witches broom in tea plants and yellow branch in citrus plants (Davis, 1956). Phytotoxicity by chromium has been reported and inhibitation of nitrification has been observed (Lisk, 1972). Chromium has produced iron chlorosis in sugar beets grown in sand cultures (Pratt, 1966). Oat plants affected by chromium toxicity were stunted with narrow brownish red leaves containing small necrotic areas and with poorly developed roots. Toxic chromium levels in tobacco was reported as 18-34 ppm in leaves and 375-410 ppm in the roots, while the toxic level in corn leaves was 4.0-8.0 ppm and in oat leaves 252 ppm (Pratt, 1966).

g. Chromium absorption and accumulation in humans and animals. Little information is available on the degree to which the chromium contained in plants is absorbed and retained by animals. It appears that there is a relatively low absorption and retention of chromium by animals (Allaway, 1968). The availability of chromium varies with its dietary source ranging from less than 1% to 25% of an oral dose (NSA, 1974). In fact chromium intakes are insufficient in some people (Underwood, 1975). Even in its most soluble forms, the element is not readily absorbed by animals (NSA, 1974).

The chromium content has been reported for a variety of foods. Most of them contained levels in the range of 0.1 ppm. Fish and fruit contained less chromium and condiments contained as much as 2.7 ppm (Lisk, 1972). Most meats are good sources of chromium (NSA, 1974). Bowen (1966) reports that animals contain about 0.075 ppm chromium. It is apparently accumulated by RNA and insulin. A majority of the food and feed crops now produced do not contain enough chromium to meet the requirements of humans and animals (Allaway, 1968).

Institutional diets in the United States provide varying concentrations of chromium ranging from 52 to 78 micrograms/day (NSA, 1974) Lisk (1972) reported the range of chromium in institutional diets to be 0.36 to 0.89 mg per day. Chromium intake is considered marginal in the United States (NSA, 1974). Chromium intake is higher in other countries.

h. The role of chromium in human and animal metabolism. Chromium is now recognized as an essential trace element to humans and animals (NSA, 1974; Allaway, 1968; Bowen, 1966; Lisk, 1972; Underwood, 1975; and VERSAR INC., 1975). Chromium appears to play a role in glucose metabolism and the effectiveness of insulin. Chromium is an essential part of a glucose tolerance factor (NSA, 1974; Allaway, 1968; Lisk, 1972; Bowen, 1966; Underwood, 1975; VERSAR INC., 1975 and Scott, 1972). The digestive enzyme trypsin appears to contain chromium as an integram part (NSA, 1974). Chromium may play a role in preventing atherosclerosis and serve as an anti-cholesterogenic agent (Lisk, 1972 and VERSAR INC., 1975). It is also believed to be involved in carbohydrate metabolism (Lee, 1975 and Underwood, 1975) as well as in lipid and protein metabolism (Underwood, 1975).

Baetjer (1956) reported that trivalent chromium could serve as a coactivator in the activation of phosphoglucomutase and an activator in the succinic dehydrogenase cytochrome system. Nucleic acids contain chromium, however, the function of chromium in RNA is not known (NSA, 1974). Chromium may also be antagonistic to lead toxicity (Lisk, 1972). Chromate is absorbed by body organs less readily than chromite (Lisk, 1972). The overall nutritional significance and biochemical role of trivalent chromium is unknown (Scott, 1972).

i. Chromium deficiency and toxicity in humans and animals. Chromium is lost through urinary excretion at the rate of between 7 and 10 micrograms/day. Since chromium is so poorly absorbed, to compensate for this loss a daily intake of 40 to 2000 micrograms would be necessary depending on the chromium compound. Chromium deficiency is of greater concern than overexposure (NSA, 1974). A diet of 0.15 to 0.20 ppm chromium resulted in deficiency in rats (Allaway, 1968).

Impairment of the glucose tolerance is the first symptom of deficiency in experimental animals (NSA, 1974 and VERSAR INC., 1975). A lack of chromium has also been associated with arteriosclerotic heart disease, elevated cholestrol levels in the blood, and high fat content of the aorta (VERSAR INC., 1975).

Chromium, especially in the hexavalent form, is toxic at high levels to animals. However, the margin is substantial between essentiality and toxicity (Allaway, 1968). Trivalent chromium is moderately toxic (Bowen, 1966) and trivalent chromium compounds probably do not produce serious damage to body tissues. Laboratory animals ingesting fairly large doses of such compounds exhibited no sign of illness, loss of weight or tissue damage. Analysis of the ash of tissue indicated no absorption of chromium or at least no retention of chromium in the body (Baetjer, 1956).

Symptoms of excessive dietary intake of chromium is unknown in man (NSA, 1974). Internally, the chrome salts act as an irritant causing tissue corrosion in the intestinal tract. Vomiting and bloody stools are often noted. The central nervous system is often involved and dilated pupils, coma, collapse, slow respiration, shock and death sometimes occur.

The toxic dose to man is reported to be about 0.5 g $K_2Cr_2O_7$ (VERSAR INC., 1975). The toxic dose to adult mammals was shown as 200 mg/day with 2000 mg/day being lethal (Bowen, 1966).

j. Chromium limitation in irrigation water. Pratt (1973) recommended that the maximum concentration of chromium in irrigation water used for sensitive crops on soils with low capacities to retain chromium in an unavailable form be 0.1 mg/l. This concentration was recommended in view of the lack of knowledge concerning chromium accumulation and toxicity (NAS, 1974).

3. Copper

a. Introduction. Copper has been determined as essential to plant (Eyster, 1964; Boardman, 1975; Sauchelli, 1969; and Buckner and Brady, 1969) and animal nutrition (VERSAR INC., 1975 and Reuther and Labanauskas, 1966).

Copper is not normally found in soils in large quantities and its inclusion in sewage wastes may increase the total content of copper in soils significantly (Lindsay, 1973). In soil only a small amount of the copper ions will remain in the exchangeable form (Lindsay, 1973).

There are definite limits to the amounts of copper than can be applied to agricultural soils without damage to crops (Reuther and Labanauskas, 1966). Copper is highly toxic to plants at low concentrations (Bowen, 1966 and Pratt, 1973), and moderately toxic to mannals (Bowen, 1966). Copper will usually cause severe plant injury before the content is high enough to be toxic to most animals (Chaney, 1973). Therefore the chances are small of excess copper moving through the food chain from plants to animals.

b. Naturally occurring copper concentrations in soil. Various copper concentrations have been reported in soil. Bowen (1966) reported copper concentrations ranging from 2-100 ppm with a mean of 20 ppm; Sauchelli (1969) stated that a normal agricultural soil contains from 1 to over 50 ppm copper; while Reuther and Labanauskas (1966) reported mineral soils with textures ranging between loam and clay to have 10 to 200 ppm copper with a majority between 25 and 60 ppm. The total copper concentration averaged 31 ppm in 54 soil samples. In 16 Kentucky soils copper ranged from 1 to 27 ppm. In other soils copper ranged from 10 to 140 ppm (Reuther and Labanauskas, 1966). Buckner and Brady (1969) reported the normal range of copper in soils as 5-150 ppm with 50 ppm being representative.

c. Copper reactions. The divalent copper cation reacts strongly with soil colloids and organic matter (Devlin, 1967; Pratt, 1973;

Sauchelli, 1969; and Bowen, 1966). Copper added to the soil is tightly held in the surface few inches of soil and moves very slowly, if at all, with drainage water (Pratt, 1973; Sauchelli, 1969; and Reuther and Labanauskas, 1966) and is not subject to leaching out of the principal root zone (Reuther and Labanauskas, 1966). Copper retention in the soil appears to be correlated more with organic matter and soil alkalinity than with clay content of the soil (VERSAR INC., 1975). A single application of copper can produce a strong residual effect in the soil which may persist for years (Reuther and Labanauskas, 1966).

Copper can be held as an exchangeable ion, however, only small quantities will remain exchangeable as precipitation reactions lower the copper level in solution below that of the common exchangeable cations. The exact reaction products that precipitate in soils are unknown (Lindsay, 1973). A major portion of the copper is evidently held with such security that it cannot be displaced readily by other common soil cations in the ordinary range of soil acidity (Reuther and Labanauskas, 1966). Soil copper may also form very stable complexes with organic matter of soil and become nonexchangeable (Devlin, 1967). The hydroxide forms of copper are extrememly insoluble (Buckner and Brady, 1969).

The activity of copper cations decreases with an increase in soil pH (Lindsay, 1973). Copper is held to soil most securely at pH 7 and above (Buckner and Brady, 1969 and Reuther and Labanauskas, 1966). Copper is most soluble and available under acid conditions (Bucker and Brady, 1969 and Reuther and Labanauskas, 1966).

d. Availability of copper to plants, its uptake and accumulation. Factors affecting the availability of copper in soil are pH, organic matter content, proportion of sand to clay, and the presence of other chemicals (Sauchelli, 1969). Copper is less available in soils with pH values of 7 and above (Buckner and Brady, 1969) and in organic and heavy clay soils (Sauchelli, 1969). Application of zinc fertilizers to soils low in copper can induce copper deficiency in wheat (Loneragan, 1975). High rates of nitrogen fertilizer can cause copper deficiency as can phosphorus accumulation in soils (Reuther and Labanauskas, 1966).

In the soil only small amounts of the copper ions will remain in the exchangeable form (Lindsay, 1973). The amount of copper removed when a crop is harvested is infinitesimally small compared to the amount usually in the soil (Reuther and Labanauskas, 1966).

Roots contain greater amounts of copper than shoots (Bowen, 1966 and Antonovics et al, 1971). Antonovics et al (1971) found that copper uptake in the above ground parts of plants stayed low and constant when soil copper levels were low, but above some higher level of soil copper the quantity of copper in the plant tops increased abruptly. At slightly higher levels the copper in the soil was lethal. They found that species differed both in their overall copper content and in the concentration at which the sudden increase in copper uptake occurred. It appears that in some plants the roots prevented copper from reaching the upper part of the plant until the root system was swamped.

In a wide variety of plants the range of copper in the dry matter of leaves falls between 5 and 20 ppm for normal growth (Allaway, 1968 and Reuther and Labanauskas, 1966). Bowen (1966) reported the copper concentration found in land plants as 14 ppm, while Melsted (1974) found that the average concentration ranges calcualted from composition data reported in the literature for corn soybeans, legumes, wheat, oats, barley and grasses was 3 to 40 ppm with a suggested tolerance level of 150 ppm. Sauchelli (1969) reported that herbage (mixed pastures) contained 1.1 ppm copper.

Wheat (Triticum species) straw was reported to contain 9-18 ppm copper and the grain 3-4.5 ppm (Chapman, 1966). Antonovics et al (1971) found that the copper content of plants rarely varies more than 5-15 ppm. Utah wheat contained from 6 to 12 ppm when grown in soils containing from 4 to 51 ppm of total copper. From 20 to 25 ppm of total copper in organic soil and 8 to 10 ppm in mineral soils produced grass crops with a normal copper content (Reuther and Labanauskas, 1966).

e. The role of copper in plant metabolism. Copper plays an important primary role in plant metabolism (Reuther and Labanauskas, 1966). It is an essential element for plants (Eyster, 1964; Boardman, 1975; Sauchelli, 1969; and Buckner and Brady, 1969). Copper is an essential component of the electron transport chain (Boardman, 1975; Salisbury and Ross, 1969; and Buckner and Brady, 1969). Copper may function in photosynthesis (Devlin, 1967 and Salisbury and Ross, 1969) and play a catalytic role in nitrogen fixation (Salisbury and Ross, 1969). Copper is involved in respiration and the utilization of iron (Buckner and

Brady, 1969).

Copper is a component of certain enzymes such as tyrosinase (Nicholas, 1975 and Sauchelli, 1969), laccase (Nicholas, 1975 and Devlin, 1967), cytochrome oxidase (Nicholas, 1975), plastocyanin (Nicholas, 1975; Boardman, 1975; and Salisbury and Ross, 1969), ascorbic acid oxidase (Sauchelli, 1969 and Devlin, 1967), polyphenol oxidase (Salisbury and Ross, 1969), phenolases (Devlin, 1967), perphas nitrate reductase (Salisbury and Ross, 1969), and many metalloenzymes especially, those concerned with oxidation (Bowen, 1966 and Buckner and Brady, 1969).

f. Copper deficiency and toxicity in plants. In a wide variety of plants copper deficiency is characterized by levels of less than 4 ppm copper in the dry matter of leaves (Allaway, 1968 and Reuther and Labanauskas, 1966). Copper in wheat straw was reported as deficient at a level of 8.5 ppm dry weight basis (Chapman, 1966).

Copper deficiency delayed maturity, reduced straw yield and severely depressed the grain yield of 7 wheat genotypes. Genotypes differed considerably in their expression of deficiency symptoms (Nambiar, 1976).

Early stages of copper deficiency are manifested in reduced growth or yield. Deficiency symptoms of wheat (Triticum species) included paleness, lack of turgor and rolling or yellowing of terminal or new leaves; older leaves become limp and bent at the ligule. In severe cases the leaves die and dry to a bleached gray (Reuther and Labanauskas, 1966). Copper deficiency can cause a necrosis of the tip of young leaves that proceeds along the margin of the leaf giving a withered appearance (Devlin, 1967).

Copper is highly toxic at quite low concentrations in plants (Bowen, 1966 and Pratt, 1973). Amounts of 20 ppm in the dry matter of leaves should be looked on with suspicion (Allaway, 1968 and Reuther and Labanauskas, 1966). A tolerance level of 150 ppm copper was suggested by Melsted (1975).

In the early stages of copper excess growth is reduced (Reuther and Labanauskas, 1966). Excess copper reduces the root yield more than the top yield. Roots prevent much of the toxic metal from reaching the leaves (Chaney, 1973). Excess copper commonly induces iron chlorosis symptoms in plants. This is due to a depression of iron concentration in the leaves (Reuther and Labanauskas, 1966) as a result of reduced iron transport by roots (Chaney, 1973).

Copper toxicity may interfere with the uptake of certain heavy metals and phosphorus and otherwise derange the normal process of nutrient accumulation by roots (Reuther and Labanauskas, 1966).

g. Copper absorption and accumulation in humans and animals. Copper will usually cause severe plant injury before the content is high enough to be toxic to most animals (Chaney, 1973). Copper is often deficient or marginally sufficient in agronomic soils resulting in low levels of copper in crops and less availability of copper to animals (Chaney, 1973). Animals require copper levels of 1-10 ppm dependent on the molybdenum level (Allaway, 1968). Bowen (1966) reported the copper content of land animals to be 2.4 ppm. The normal amount of copper in the diet of adult mammals is 2-5 mg/day (Bowen, 1966). Sheep need between 5 and 10 mg of copper per day (Lee, 1975). The adult human requires 3 mg/day of copper and children need 2 mg/day (VERSAR INC., 1975).

h. The role of copper in human and animal metabolism. Copper is found in traces in all animal life and is essential for animal nutrition (VERSAR INC., 1975). It plays an important role in animal metabolism (Reuther and Labanauskas, 1966). The copper requirement is reported to be about 2 mg/day for children and 3 mg/day for adults (VERSAR INC., 1975). Copper is essential in the generation of hemoglobin of higher animals (Reuther and Labanauskas, 1966; VERSAR INC., 1975; and Sauchelli, 1969). Copper content is generally highest in the liver, brain, heart, and kidney (Sauchelli, 19-9 and Bowen, 1966).

There seems to be some reciporocal relationship between copper and molybedenum in animal nutrition. Molybedenum content of the diet may exert a strong effect on copper metabolism of ruminant animals (Reuther and Labanauskas, 1966) and can interfere with copper metabolism in humans (Allaway, 1968). Copper and aluminum are also reported as being antagonistic (Reuther and Labanauskas, 1966).

i. Copper deficiency and toxicity in humans and animals. Copper deficiencies often occur in animals which are fed plants with low copper concentrations (Allaway, 1968 and Chaney, 1974). Copper deficiency in lambs causes enzootic ataxia (nervous degeneration of the spinal cord). It also results in stringy wool. A falling disease in cattle characterized by sudden death of seemingly normal cattle is caused by copper deficiency (Lee, 1975). Cattle in Florida have suffered a debilitating disease associated with extreme anemia caused by a deficiency of iron and copper in the diet. A wasting disease of cattle was also shown to be caused by a diet deficient in copper (Reuther and Labanauskas, 1966).

Animals with copper deficiency show symptoms such as: blood deficient in hemoglobin, retardation of growth, failure to fatten, coarsening and depigmentation of hair, poor reproduction, diarrhea, abnormalities of bone formation, nervous disorders and general weakness (Reuther and Labanauskas, 1966).

Copper is highly toxic to invertebrates and moderately toxic to mammals. A diet of 250-500 mg/day copper is toxic to adult mammals (Bowen, 1966).

Copper resembles many other heavy metals in its systemic toxic effects which include: widespread capillary damage, kidney and liver injury, and central nervous excitation followed by depression. Hemolytic anemias are described in acute poisoning in man and chronic poisoning in sheep. Chronic feeding of copper to animals resulted in a pigmentary cirrhosis of the liver (VERSAR INC., 1975).

In a type of chronic copper poisoning in man the tissue copper levels are elevated and this accumulation has been noted to proceed the development of liver pathology which may prove fatal (VERSAR INC., 1975).

j. Copper limitation in irrigation water. The recommended maximum concentration of copper in irrigation waters used for sensitive crops on soils with low capacities to retain copper in unavailable form is 0.2 mg/l (Pratt, 1973 and VERSAR INC., 1975).

4. Iron

a. Introduction. Iron is essential to plants (Sauchelli, 1969; Buckner and Brady, 1969; Eyster, 1964; Boardman, 1975; and Salisbury and Ross, 1969) and animals (White, Handler, and Smith, 1968). It is the fourth most abundant chemical element in the earth's crust (Sauchelli, 1969). It is found in the soil in more than one valance state (Buckner and Brady, 1969). Soils are generally not deficient in iron but may be deficient in exchangeable or soluble forms of iron (Devlin, 1967).

Most iron added to the soil as simple salts is quickely rendered insoluble and is no more available to plants than the native iron (Wallihan, 1966). Iron is most available to plants in the ferrous form and that is the metabolically active form in plants (Devlin, 1967).

Iron deficiencies exist in both plants (Wallihan, 1966) and animals (White, Handler, and Smith, 1968 and Reuther and Labanauskas, 1966). However, iron toxicity has not been much in evidence in plants under natural conditions (Wallihan, 1966) and has seldom been reported in humans and animals.

Iron in irrigation waters is not apt to create a problem of plant toxicities. It is so insoluble in aerated soils at all pH values at which plants grow well that it is not toxic (Pratt, 1973).

b. Naturally occurring iron concentration in soil. Iron is the fourth most abundant chemical element in the earth's crust (Sauchelli, 1969). Bowen (1966) reported the iron concentration range of soils as 7,000 to 50,000 ppm dry soil with a mean of 38,000 ppm. Buckner and

Brady (1969) found the normal range of iron in soils to be 5,000 to 50,000 ppm with 25,000 ppm suggested as representative in surface soil.

c. Iron reactions in soil. Iron is found in the soil in more than one valance state (Buckner and Brady, 1969). In well-oxidized soils iron forms highly insoluble oxides and hydroxides. This limits the chemical activity of this metal to very low levels (Lindsay, 1973). At low pH and under reducing conditions iron can be solubilized and become mobile in the soil as the ferrous ion (Lindsay, 1973). If iron is present in a reduced form it may be toxic because it is more available (Buckner and Brady, 1969). The ferrous form of iron is soluble while the ferric form is insoluble (Bowen, 1966).

In soil the ferric ion is soluble in the pH range of 3 to 5 and is available to plants. The ferrous ion is soluble up to pH 7 and up to about pH 8, both ferric and ferrous humates are soluble (Sauchelli, 1969). If the soil reaction is held within a soil pH of 6.0 to 7.0 the toxicity of iron may be surpressed and at the same time its unavailability will be avoided unless it is lacking in the soil (Buckner and Brady, 1969). At high pH values iron is more unavailable (Sauchelli, 1969).

High levels of copper, manganese, molybdenum, vanadium,and zinc; high pH; bicarbonate ions in soil solution; substantial amounts of magnesium carbonate and calcium carbonate and excessive PO_4 restrict iron availability (Sauchelli, 1969). Most of the iron added to the soil as simple salts is quickly rendered insoluble in alkaline soils (Wallihan, 1966).

d. Availability of iron to plants, its uptake and accumulation. Iron is most available to plants in the ferrous form, but significant quantities of ferric ion may be absorbed (Devlin, 1967). Iron is most soluble and available under acid conditions (Buckner and Brady, 1969 and Devlin, 1967). Chelated iron is in an available form. There are two general sources of readily available iron in the soil: nutrients adsorbed on the colloids and salts in solution (Buckner and Brady, 1969). High levels of certain heavy metals, high pH, high levels of bicarbonate ion, magnesium carbonate, calcium carbonate and PO_4 restrict the availability of iron (Sauchelli, 1969).

The uptake of iron by plants is depressed by toxic amounts of nickel, copper, cobalt, zinc and manganese in the soil (Reuther and Labanauskas, 1966).

Scott (1973) reported the iron content of wheat standard middlings as 100 ppm, with 50 ppm in grain and 150 ppm in bran. Chapman (1966) reported the content of corn leaves as 56 to 176 ppm iron. Rice leaves had 80 ppm iron. The amount of iron in the leaves of a normal plant will generally average a few hundred ppm, the amount hardly varying (Sauchelli, 1969). Bowen (1966) reported the iron concentration of land plants as 160 ppm and Sauchelli (1969) reported 100-200 ppm iron in pasture grasses. Melsted (1973) listed the iron content of common agronomic crops as 20-300 ppm as ferrous iron with a suggested tolerance level of 750 ppm.

e. The role of iron in plant metabolism. Iron is essential to plants (Sauchelli, 1969) Buckner and Brady, 1969; Eyster, 1964; Boardman, 1975; and Salisbury and Ross, 1969). Although it may be taken up in the ferric state, it is generally felt that the ferrous state is the metabolically active form of iron in plants (Devlin, 1967). It is relatively immobile once it has arrived at the living cell via the phylem transportation system (Salisbury and Ross, 1969).

Iron is essential for the formation of chlorophyll (Sauchelli, 1969; Buckner and Brady, 1969; Devlin, 1967; and Salisbury and Ross, 1969), probably as a componenet of the catalyst involved (Sauchelli, 1969 and Salisbury and Ross, 1969). The synthesis of protein contained in the chloroplasts requires iron (Buckner and Brady, 1969). Iron is involved in photosynthesis (Nicholas, 1975 and Eyster, 1964), and electron carrier systems such as cytochromes that are important in respiration and bring about oxidation-reduction reactions (Nicholas, 1975; Buckner and Brady, 1969; Devlin, 1967; Salisbury and Ross, 1969; and Boardman, 1975). The ferredoxins which are involved in metabolic events such as N_2 fixation and hormone biosynthesis contain iron (Nicholas, 1975). Iron is necessary in certian pigment molecules (Salisbury and Ross, 1969) and a component of peroxidases, catalases (Devlin, 1967), and perhaps nitrate reductase (Salisbury and Ross, 1969).

Iron may inhibit the absorption and translocation of zinc in some plants (Brar and Sekhov, 1976b).

f. Iron deficiency and toxicity in plants. Soils generally are not deficient in iron but may be deficient in exchangeable and soluble forms of iron (Devlin, 1967). Iron deficiency results in chlorosis of the leaves, generally the younger leaves are most effected (Devlin, 1967 and Sauchelli, 1969). This is due to the relative immobility of iron in plants (Devlin, 1967). Plants with iron concentration of 10 to 80 ppm in the leaves are generally deficient in iron (Sauchelli, 1969).

A symptom of iron deficiency in green plants is chlorosis, a reduced concentration of chlorophyll. At an intermediate degree of deficiency plant leaves show a typical intervenial chlorosis. More severe deficiency results in severely chlorotic leaves, with green color absent in the finest veins, then from larger veins until in extreme cases the leaf is essentially devoid of chlorophyll. Iron deficiency also results in a reduced growth rate (Wallihan, 1966).

Iron toxicity has not been much in evidence under natural conditions (Wallihan, 1966). However, in very acid soils or under reducing conditions if a relative abundance of iron ions are present, they can be toxic to common plants (Buckner and Brady, 1969). Iron toxicity has been observed in plants which have received soluble iron salts in excessive amounts, either as sprays or as soil amendments, and it first appears in the form of necrotic spots (Wallihan, 1966). Reduced quality in tobacco leaves has been reported as the result of the spray application of 5 mg or more of soluble iron by sprinkler system (Pratt, 1973).

g. Iron absorption and accumulation in humans and animals. The various forms of iron have differing availabilities to man and animals and different absorption rates in the intestines (White, Handler, and Smith, 1968). The adult mammal requires 12-15 mg/day of ferrous or ferric iron in its diet (Bowen, 1966). Men require 10 mg of iron/day and women 15 mg/day (White, Handler, and Smith, 1968). Bowen (1966) reported the average iron content of land animals as 160 ppm.

Iron is highest in bone marrow and red blood cells, but is also high in the liver, lung and spleen in man (Bowen, 1966).

h. The role of iron in human and animal metabolism. Iron is an essential element for human and animal metabolism. It is a constituent of hemoglobin and as such is involved in respiration. Iron is also a constitutent of cytochromes and other hemoproteins. The enzyme aconitase requires ferrous iron for maximal activity. Iron is also a part of adrenodorin and several flavoproteins (White, Handler, and Smith, 1968). Iron functions as a catalyst, activates a number of oxidases and is a constitutent of many oxidizing metalloenzymes, respiratory pigments and proteins of unknown function (Bowen, 1966).

i. Iron deficiency and toxicity in humans and animals. Iron deficiency causes anemia (White, Handler, and Smith, 1968). Cattle in Florida have suffered from a debilitating disease associated with extreme anemia caused by a deficiency of iron and copper in the diet (Reuther and Labanauskas, 1966).

Iron is only slightly toxic to organisms (Bowen, 1966).

j. Iron limitation in irrigation water. Iron in irrigation water is not likely to create a problem of plant toxicities. It is so insoluble in aerated soils at all pH values at which plants grow well that it is not toxic. The disadvantages of soluble iron salts in waters are that they would contribute to soil acidification and the precipitation of iron would increase the fixation of such essential elements as phosphorous and molybdenum. Therefore 5.0 mg/l iron is recommended for continuous use on all soils (Pratt, 1973).

5. Nickel

a. Introduction. Nickel occurs in the soils of several widely scattered regions in amounts sufficient to have a deleterious effect on many plants (Vanselow, 1966). Nickel is not essential for plant and animal metabolism (Sauchelli, 1969 and Vanselow, 1966). It has been shown to be toxic to both plants and animals.

b. Naturally occurring nickel concentrations in soil. Soils normally contain from 5 to 500 ppm of nickel with an average of about 100 ppm. In continental Europe 5 to 40 ppm total nickel was found in 35 soil samples. The nickel content of 150 Scottish soils ranged from 5 to 230 ppm, while that for 77 Spanish soils was 10 to 500 ppm. In Florida nickel content of soils was found to vary from less than 1 to 100 ppm, in Southern California it ranged from 8 to 100 ppm and for 40 samples from the eastern United States nickel ranged from 2.5 to 40 ppm (Vanselow, 1966). Lisk (1972) reported nickel in soil at 40 ppm. Sauchelli (1969) reported that the total nickel in soil generally ranges between 10-40 ppm, and Bowen (1966) and Allaway (1968) found nickel in soil ranged from 10-1000 ppm with a mean of 40 ppm.

c. Nickel reactions in soil. Calcium carbonate reduces the availability of soil nickel by an increase in soil pH (Hunter and Vergnano, 1952). Nickel is mobilized in soil following flooding and aeration and is more available in poorly drained soils (Lisk, 1972).

Nickel is a divalent metal ion normally not found in soils in large quantities. Although this cation can be held as exchangeable ions, precipitation reactions lower the levels so that only small quantities will remain on the exchanger. The exact reaction products that precipitate in soils with this cation are not known. The activity of this metal cation is expected to decrease with increase in soil pH. The metal is potentially toxic to animals and plants and may constitute future hazards if continually added to soils (Lindsay, 1973).

d. Availability of nickel to plants, its uptake and accumulation. Nickel appears to be fixed and less available to plants at soil pH values above or below 6.5 to 7.0. Nickel is mobilized in soils following flooding and aeration and is more available in poorly drained soils (Lisk, 1972). Excessive acidification of ordinary soils sometimes results in a greater uptake of nickel by plants (Vanselow, 1966).

In many green plants, the degree of absorption of nickel by the roots appears to be dependent upon soil pH (VERSAR INC., 1975), being low where soil pH is high and vice versa (Hunter and Vergano, 1952). There has been little systematic work on nickel uptake. For many species, levels in the aerial parts of the plant remain low (around 100 ppm) irrespective of external concentration. This resembles the copper uptake mechanism (Atonovics et al, 1971).

For the most part, plants contain very small quantities of nickel, usually less than 1 ppm. Melsted has reported the nickel content of common agronomic crops as 0.1 to 1.0 ppm with a suggested tolerance level of 3.0 ppm. Bowen (1966) reported that land plants contain 3.0 ppm nickel. Chapman (1966) reported nickel in grasses varied from 0.2 to 3.0 ppm for tops of plants grown in fields with nickel concentrations of 9.0 to 56.0

ppm. Sauchelli, (1969) reported 0.5 to 4.0 ppm nickel in pasture herbage. Hunter and Vergano (1952) found 0.5 to 4.0 ppm nickel in pasture grasses.

In grasses, roots contain slightly more nickel than shoots and nickel is enriched in the inflorescence (Bowen, 1966). In general leaves contain more nickel than stems, young plants have more than old plants, leaf blades contain more nickel than leaf sheaths, flowers contain more nickel than peduncles and grain has more nickel than straw (Hunter and Vergano, 1952).

It is not possible to fix allowable nickel content of healthy plants because there are too few data available from experiments in which nickel excess was the only adverse factor (Vanselow, 1966).

e. *Nickel and plant metabolism.* Nickel is among the many chemical elements found in most plants, but of unproven essentiality (Vanselow, 1966 and Sauchelli, 1969). Nickel is thought to act as a catalyst in biological reactions (Sauchelli, 1969).

The root is the metal-sensitive organ in plants (Chaney, 1973). The roots of grasses will contain more nickel than do the shoots (Bowen, 1966) and excessive nickel reduces root yield more than top yield (Chaney, 1973).

Nickel interferes with plant absorption of iron (Lisk, 1972 and Hunter and Vergano, 1952) causing chlorosis due to the reduced iron transport by the roots (Chaney, 1973). The excessive absorption of nickel is thought to reduce the cation exchange capacity of roots in many plants (VERSAR INC., 1975).

f. Nickel toxicity in plants. Many workers by means of tissue culture solutions and sand culture techniques have demonstrated the high degree of nickel toxicity for many crops (Vanselow, 1966). The nickel content of plants averages between 0.5 and 5.0 ppm (Sauchelli, 1969) and is toxic at +50 ppm (Allaway, 1968).

The nickel content of the plant is usually unrelated to the major nutrient supply, though it may be reduced when this is high (Hunter and Vergano, 1952). Nickel toxicity is aggravated by low calcium, magnesium, nitrogen, and potassium, and by high phosphorus in the culture medium (Vanselow, 1966). Increasing soil pH reduces nickel toxicity (Pratt, 1973).

In early or incipient stages of nickel toxicity there are no definite symptoms, but only a dwarfing or repression of growth. Excess nickel produces a chlorosis resembling the symptoms of iron deficiency. In cases where toxicity is severe, the chlorosis is followed by white necrotic tissue (Vanselow, 1966 and Hunter and Vergano, 1952) and even death of the plant (Vanselow, 1966). High levels of copper prevent the appearance of necrotic symptoms indicating that a nickel-copper relationship may be involved in their production (Hunter and Vergano, 1952). Nickel is appreciably more toxic than copper (Reuther and Labanauskas, 1966).

g. Nickel absorption and accumulation in humans and animals. Absorption of nickel and most of its salts from the intestines is poor (VERSAR INC., 1975). The diet of adult mammals normally contains 0.3 to 0.5 mg/day of nickel (Bowen, 1966). Nickel is not found in milk or eggs

(Sauchelli, 1969). Total nickel in sea foods is 0.02 to 1.7 ppm; in cereals and grains it is 0.0 to 6.45 ppm; in fruits it is 0.0 to 0.34 ppm; in vegetables it is 0.0 to 2.59 ppm; in meats it is 0.0 to 4.5 ppm and in dairy products it is 0.0 to 0.03 ppm (Lisk, 1972). In general, land animals contain about 0.8 ppm nickel (Bowen, 1966).

h. Nickel and animal metabolism. It has not been proven that nickel is essential for the proper growth of animals (Vanselow, 1966 and Sauchelli, 1969). However, nickel deficiency in the diet of chickens has been reported to result in the production of thickened legs, enlarged hocks, and orange colored skin (Lisk, 1972 and Underwood, 1975). Nickel may be involved in the development of the integumentary color or its absence in birds and may therefore be essential in their diet (Lisk, 1972). Nickel acts as a catalyst in biological reactions (Sauchelli, 1969).

Eighty-seven to eighty-nine percent of the nickel in man occurs in the blood. Nickel ions can replace calcium ions in the generation of action potential in muscle, but the duration of the potential is increased (VERSAR INC., 1975). Nickel is apparently accumulated by RNA (Bowen, 1966). In man it appears quite frequently in the skin and intestines but does not accumulate with age (Sauchelli, 1969).

i. Nickel toxicity in humans and animals. Nickel is moderately toxic to mammals. Nickel as $Ni(CO)_4$ is highly toxic and is probably carcinogenic (Bowen, 1966). Although absorption of nickel and most of its salts from the intestines is poor, sufficient quantities are absorbed to cause systemic responses. These include capillary damage, renal injury, myocardial weakness, and central nervous system depression. Ingestion of large doses of nickel (1 to 3 mg/kg body weight), caused internal disorders, convulsions and asphyxia in dogs (VERSAR INC., 1975).

j. Nickel in irrigation waters. Based on both toxicity in nutrient solutions and on quantities that produce toxicities in soils the recommended maximum concentration of nickel in irrigation waters is 0.2 mg/l for continued use. On fine textured alkaline soils 2.0 mg/l might be used for 50 years or 5.0 mg/l might be used for 20 years without danger of toxicity to plants (Pratt, 1973).

6. Zinc

a. Introduction. Zinc is naturally occurring in soils (Buckner and Brady, 1969) and is essential to plants (Sauchelli, 1969; Buckner and Brady, 1969; and Eyster, 1964) and animals (Sauchelli, 1969; Prasad, 1966; and Stara et al, 1971). Zinc deficiency in plants is becoming more widespread and the zinc content of food and feed crops is on the average declining. Zinc deficiencies in a number of species, including man, have been observed under practical conditions (Allaway, 1968).

Zinc is moderately toxic to plants (Bowen, 1966) but is not an inherently toxic element to man (VERSAR INC., 1975 and Sauchelli, 1969). The soil-plant system provides an effective barrier against toxicity from zinc. Plant growth will cease or be greatly depressed before zinc will be accumulated in concentrations dangerous for animals (Allaway, 1968).

b. Naturally occurring zinc concentrations in soils. Zinc is a divalent metal ion not normally found in soil in large quantities (Lindsay, 1973). Bowen (1966), Viets (1966), Allaway (1968), and Chapman (1966) reported zinc levels in soils ranging from 10 to 300 ppm with a mean of 50 ppm. Buckner and Brady (1969) listed the range of zinc in soils as 10 to 250 ppm. Chapman (1966) felt that zinc was more concentrated in surface than subsurface horizons while Viets (1966) reported that zinc was fairly uniform among the various horizons of the profile.

c. Zinc reactions in soil. Zinc is a divalent metal ion not normally found in soils in large quantities. It can be held as an exchangeable ion, but only small quantities will remain exchangeable since precipitation reactions will lower the concentration. The exact reaction products that precipitate in soils with this cation are not known (Lindsay, 1973). Zinc is known to combine with soluble phosphate to form insoluble zinc phosphate. Little is known about the interactions of zinc and its compounds in soil. The zinc ion is not labile and apparently remains in the top soil layer where it may have accumulated during the years. Most zinc is not readily available to plants which can take up only water soluble or exchangeable forms of zinc (Sauchelli, 1969). Little is known about the concentration of zinc in soil solution, but it is thought to be low (Devlin, 1967). The activity of zinc cations decreases with increase in soil pH (Lindsay, 1973).

d. Availability of zinc to plants, its uptake and accumulation. Information concerning the soil factors which control availability of zinc to plants is not too precise. Zinc is more available in acid or neutral soils. Most zinc is not readily available to plants which can take up only water soluble or exchangeable forms of zinc. Phosphate can reduce the availability of soil zinc to pasture and other crops (Sauchelli, 1969).

Chelated zinc is less apt to take part in reactions with other soil constitutents and remains in solution at much higher pH values than do the inorganic ionic forms. It is apparently in an available form and assimilated fairly readily by growing plants (Buckner and Brady, 1969). The availability of zinc can be increased by acidification of the soil to pH's below 7 (Devlin, 1967 and Buckner and Brady, 1969). Phosphate fertilizers may induce zinc deficiency in some soils (Viets, 1966).

Different species vary in the degree to which they take up zinc. Plant organs accumulate varying quantities of zinc with roots and leaves generally accumulating the most and stems and inflorescences the least. The pattern of distribution depends both on the species and on the metal concentration. The quantitiy of zinc in plants changes with the growing season, increasing throughout the season. Zinc is readily taken in by plants growing on zinc-contaminated soil, and nowhere in the literature is there any evidence of these plants having an exclusion mechanism to enable them to survive on contaminated soils. The zinc content can vary from 20 to 1200 ppm (Antonovics et al, 1971). Uptake of zinc by plant roots is not linked to the metabolic process (Bowen, 1966).

Viets (1966) felt that in agriculture soils there is generally little or no relation between zinc content of the soil and the zinc status of plants grown on the soil. However, Antonovics, et al (1971) found that the quantity of zinc in plants was related, often in a clearly linear pattern, to the amount of zinc in the soil. Allaway (1968) showed that the concentration of zinc in most plants was only moderately increased by application of zinc, even though increases in crop yield were obtained.

Zinc accumulates in plants in the following descending order: roots, stem, leaves, fruits. Zinc readily accumulates in the leaves of many plants in concsiderable amounts (Sauchelli, 1969). The zinc content of common agronomic crops was reported as 15 to 150 ppm with a suggested tolerance level of 300 ppm by Melsted (1973). Bowen (1966) reported that land plants contain 100 ppm zinc. The concentration of zinc in grasses has been shown to be 15-80 ppm and wheat straw to be 11-40 ppm (Sauchelli, 1969). Allaway (1968) found that the zinc content of plants ranged from 8 to 15 ppm.

Viets (1966) listed the normal range of zinc in plants as 15 to 16 ppm of the dry weight. He found no difference in the zinc content of Coastal Bermudagrass during the season which contrasts to the findings of Antonovics et al (1971) that plants vary considerably in zinc content over the season.

e. The role of zinc in plant metabolism. Zinc is essential in enzyme systems which are necessary for important reactions in plant metabolism (Nicholas, 1975; Buckner and Brady, 1969; Devlin, 1964; Salisbury and Ross, 1969; Sauchelli, 1969; and Eyster, 1964). Zinc is involved in the biosynthesis of the plant auxin indole-3-acetic acid (IAA) by its involvement in the synthesis of tryptophan, a precursor of auxin (Devlin, 1967; Sauchelli, 1969; and Salisbury and Ross, 1969). Zinc catalyzes the process of oxidation in plant cells, is vital for the transformation of carbohydrates, regulates consumption of sugar, increases the source of energy for production of chlorophyll and promotes absorption of water. Zinc and copper may be considered a pair of coordinated catalysts in the oxidation-reduction phenomena. Zinc is not translocated from old to new tissues (Sauchelli, 1969).

Excess zinc reduces root yield more than top yield and reduces the amount of iron transported by the roots (Chaney, 1973). Zinc may depress copper absorption by wheat plants (Loneragan, 1974).

f. Zinc deficiency and toxicity in plants. Zinc deficiency in plants is becoming more widespread (Allaway, 1968). Antagonistic effects of copper, iron, and manganese on absorption and translocation of zinc may be one reason for the deficiency (Brar and Sekhon, 1976a). Deficiency exists in wheat plants or grasses with 10 ppm or less zinc (Sauchelli, 1969).

Deficiency symptoms differ among plant species (Viets, 1966). The first sign of zinc deficiency is intervenal chlorosis of older leaves starting at tips and margins (Devlin, 1967). Other symptoms include: small, curled, or mottled leaves; stunting or rosetting; intervenal chlorosis; and failure to bloom and set seed (Viets, 1966). Occurrence of zinc deficiency is not closely related to type, texture, organic matter, mineralogy or total zinc content of soils.

Zinc is moderately toxic to plants (Bowen, 1966). Excess zinc often produces iron chlorosis by injury to the plant roots. Barley and various grasses have rusty-brown flecks on the leaves followed by death when zinc is excessive (Chapman, 1966). According to Sauchelli (1969) concentrations of zinc in wheat of 150 ppm are probably toxic, while Chapman (1966) found that amounts greater than 400 ppm zinc on dry weight basis may indicate excess zinc.

g. Zinc absorption and accumulation in humans and animals. Zinc is less available to animals from diets containing plant protein than it is from diets based on animal protein (Allaway, 1968). Although zinc is present in plants such as soybeans in sufficient amounts, it may be fixed in a form such as phytate, that is nutritionally unavailable to animals (Lisk, 1972). Orten (1966) indicated only 5-10% of the dietary zinc is absorbed from the intestines while McKenney et al (1962) found 10-20% of ingested Zn-65 was absorbed from the gastrointestinal tract of rams.

The normal amount of zinc in the diet of adult mammals and the American adult is 10-15 mg/day (Bowen, 1966 and Van Reen, 1966). The daily requirement is estimated as 1-2 mg. Zinc is primarily associated with protein foods such as milk, meat, fish, eggs, nuts, whole grains and legumes (Orten, 1966).

h. The role of zinc in human and animal metabolism. Zinc is essential to animal metabolism (Sauchelli, 1969; Prasad, 1966; and Stara et al, 1971). The highest concentrations of zinc occur in the prostate, kidney, liver and muscle (Bowen, 1966).

Zinc is a constituent of certain metalloenzymes: carbonic anhydrase, pancreatic carboxypeptidase, alcohol dehydrogenase, glutamic dehydrogenase (Orten, 1966 and Luecke, 1966), lactic dehydrogenase and probably other pyridine nucleotide-dependent metallodehydrogenases. It is apparently a cofactor in a number of other enzymes and may be involved in the synthesis of RNA and hence in protein synthesis (Orten, 1966). Testicular atrophy in zinc-deficient animals has been reported. Zinc deficiency was found in patients from the Middle East who exhibited severe growth retardation and sexual hyperfunction. Evidence indicates that zinc is essential for growth and gonadal function in man (Prasad, 1966).

i. Zinc deficiency and toxicity in humans and animals. There is an increasing incidence of zinc deficiency in livestock and poultry. Zinc deficiency has also be reported in people from Egypt (Viets, 1966 and Allaway, 1968).

Zinc deficiency has been noted in cattle grazing on forage with 18-42 ppm zinc. Ram lambs showed symptoms of zinc deficiency on diets with up to 17.4 ppm zinc (Lee, 1973). A complete absence of zinc can be fatal. Deficiency symptoms include: retarded growth, bone and joint disorders, skin diseases, disorders in feathers and hair, delayed sexual maturity, sterility and even death (Sauchelli, 1969). Zinc deficiency has been accompanied by impaired protein utilization (Lee, 1975). Impaired healing of wounds in humans has also been attributed to zinc deficiency (Allaway, 1968).

Zinc is not inherently a toxic element to man (VERSAR INC., 1975) and is generally considered nontoxic to animals (Sauchelli, 1969). Zinc salts are not particularly toxic in comparison to some of the other metal salts such as copper, molybdenum and mercury. Symptoms of zinc toxicity are fever, nausea, vomiting, stomach cramps and diarrhea in 3 to 12 hours after ingestion (Van Reen, 1966). However, the danger of toxicity from excess dietary zinc is minimal (Allaway, 1968).

j. Zinc in irrigation water. On acid sandy soils the amounts required for toxicity would suggest a recommended maximum concentration of zinc of 1 mg/l for continuous use. Assuming adequate use of liming material to keep pH values relatively high (six or above) the recommended maximum concentration for continuous use on all soils is 2.0 mg/l (Pratt, 1973).

CHAPTER III

EXPERIMENTAL DESIGN AND PROCEDURES

A. Introduction

The objective of this study was to determine the feasibility of wastewater disposal by irrigation with industrial effluent containing heavy metals. The crops selected to study were winter wheat and bermuda grass. The plants were grown in pots in the laboratory. All pots were watered with either industrial effluent obtained from the Western Electric Company in Oklahoma City or deionized water. The wastewater was the effluent from the electric plating waste control system and contained trace concentrations of many heavy metals.

Three sets containing four pots each of wheat and bermuda grass plants were established. These were:

1. Wheat and bermuda grass watered with effluent;
2. Wheat and bermuda grass watered with aerated effluent; and
3. Wheat and bermuda grass watered with deionized water to serve as the control.

A fourth set consisting of 12 pots each of wheat and bermuda grass was treated with chromium-51 solution.

The plants were all grown under the same environmental conditions of controlled light and maintained temperature. The plant material was harvested apprcximately every two weeks and analyzed for chromium, copper, iron, nickel, zinc, or chromium-51.

The soil was analyzed prior to planting and after harvest for the following parameters: pH, chromium, copper, iron, nickel, zinc, or chromium-51. Soil particle size, cation exchange capacity, and organic matter content were determined prior to planting.

B. Industrial Effluent Study

1. Collection And Preparation Of Soil For Planting

Soil was obtained from a wheat field located 150 yards west of Rockwell Street and 200 yeard north of Reno Street in Oklahoma City, Oklahoma County, Oklahoma. The top six to nine inches of soil from randomly selected sites in the field were placed in polyethylene lined metal barrels for transport to the laboratory.

The soil was mixed then divided into five batches. Three kilograms of air dried soil was placed in number 10 metal cans lined with two 10 x 12 inch polyethylene bags in which 200 grams of washed gravel had been placed to provide drainage. Soil moisture content was determined on each batch of soil and the oven dried weight of the soil in each container was calculated.

The soil was prepared for seeding using the currently accepted best agricultural practices as published by Oklahoma State University (Science Serving Agriculture Nos. 2217, 2551 and 2206).

2. Industrial Effluent

a. Collection of the industrial effluent. Effluent from the Western Electric Company wastewater treatment system was collected as it discharged from the effluent meter pit (Figure 1). It was divided into two batches and one batch was aerated. Samples for analyses were collected from each batch of the wastewater at each application to the plants.

b. Wastewater treatment system. The Western Electric Company treatment plant consists of three systems: 1) Chrome, 2) Cyanide, and 3) Acid-alkali as shown in Figure 1. Each system is divided into dilute and concentrated subsystems. The average weekly flow in the total system is 1,600,000 gallons with a daily flow range of 250,000 to 340,000 gallons.

The chrome is treated at pH 2.0 with sulfur dioxide to reduce the hexavalent chromium to the trivalent form. The liquid then goes to a pH adjustment tank where it is mixed with wastewater from the other two systems.

The cyanide containing solutions are oxidized by the addition of chlorine first at pH 11 and then after the pH has been adjusted to 9. The flow goes to the pH adjustment tank to be mixed with wastewater from the other two treatments.

The concentrated acid and alkali solutions are mixed in a holding tank. The acid-alkali solution goes from the holding tank to the pH adjustment tank and is combined with the chrome and cyanide wastes.

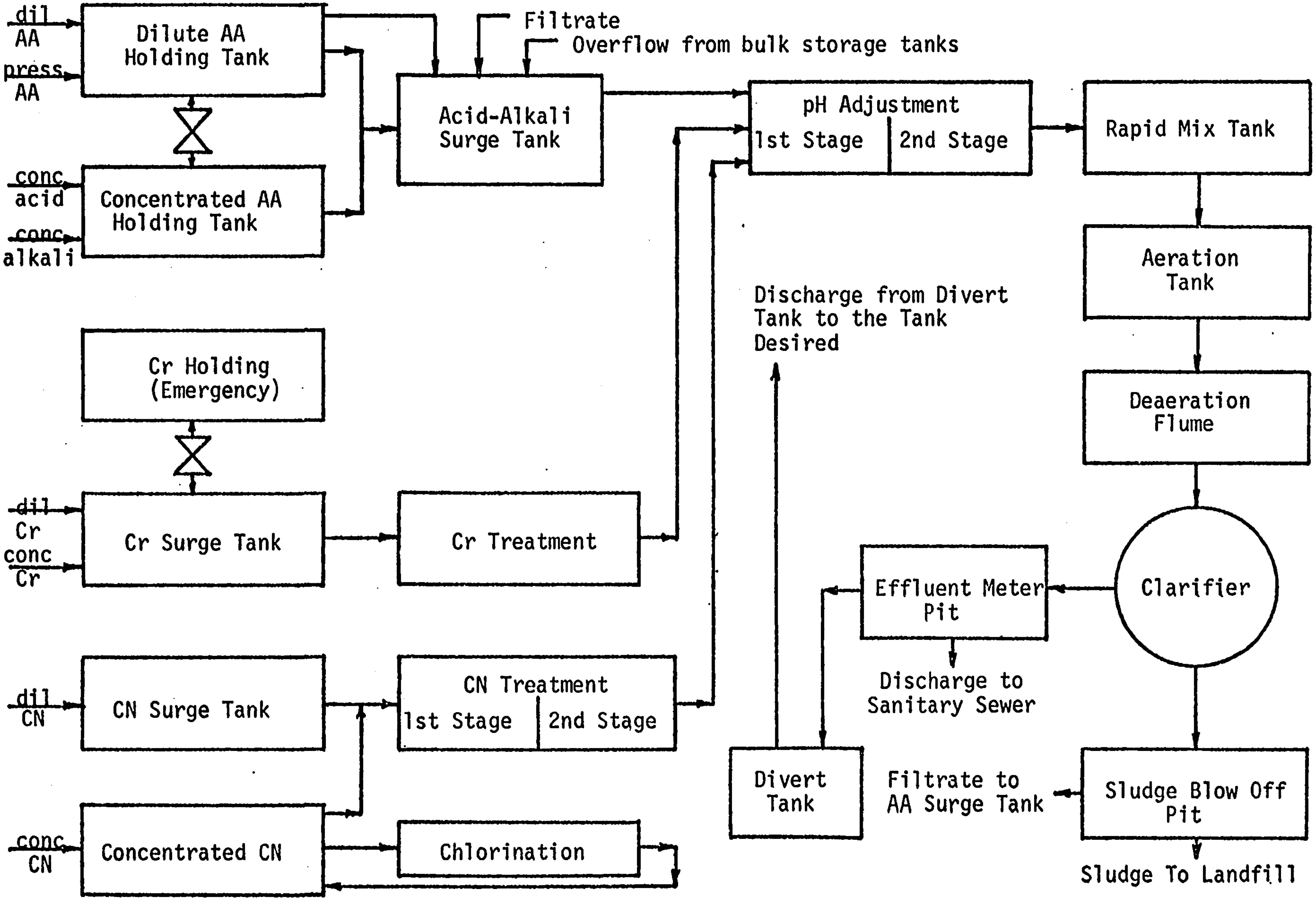

Figure 1. Flow Diagram

This combined waste is adjusted to a pH of 7.0 with NaOH or H_2SO_4. The wastewater goes to the rapid mix tank where the pH is raised to 9.0. The flow goes to the aeration tank, then to the clarifier where a coagulant is added. The floc settles and is withdrawn with a sump pump to a storage tank to await filtering. The clear water goes to the sanitary sewer through the effluent meter pit. The sludge is filtered and the water is returned to the acid-alkali surge tank. The sludge is then taken to a solid waste disposal site.

c. Toxic substances in the treated wastewater. The waste streams entering the treatment plant are very variable due to the nature of the company operation. At any time, the exact composition depends on the processes being used at the electric plating plant. Chemical analyses of the effluent from the treatment plant indicated the presence of the elements shown in Table 1.

3. Seeding and Growing Methods and Procedures

a. Wheat (Triticum aestivum var. Danne). On January 18, 1975, fifty wheat seeds were placed in each of the twenty-four containers and 200 milliliters of deionized water added. Plants were watered with deionized water until after the first harvest when the containers were divided into four groups. A different treatment was established for each group:

1. Plants watered with industrial effluent;
2. Plants watered with aerated industrial effluent;
3. Plants watered with chromium-51 solution; and
4. Plants watered with deionized water (the controls).

Table 1. Chemical Composition of the Effluent

Element	Concentration Range (ppm)
Chromium	0.11 - 0.9
Cyanide	0.00 - 0.08
Aluminum	0.01 - 0.05
Copper	0.10 - 0.3
Iron	0.08 - 0.6
Zinc	0.01 - 0.062
Cadmium	0.004
Lead	0.071
Fluorine	0.98
Nickel	0.16
Phenols	0.01

Each treatment was carried out in quadruplicate with the exception of the chromium-51 treatment which had 12 replicates.

b. Bermuda grass (Cynoda dactylon, var. Midland). On January 19, 1975, enough springs of dormant bermuda grass were planted to assure a good sod in a reasonable time period. Two hundred milliliters of deionized water were added to each container. The plants were divided into four treatment groups:

1. Plants watered with industrial effluent;
2. Plants watered with aerated industrial effluent;
3. Plants watered with chromium-51 solution; and
4. Plants watered with deionized water.

Each treatment was conducted in quadruplicate with the exception of the chromium-51 treatment which had 12 replicates. To allow the plants to establish themselves, they were watered with deionized water until after the first harvest.

c. Experimental conditions. During the experiment daytime temperature in the laboratry ranged from 23°C to 30°C with an average temperature of 28°C. Night time temperature ranged from 17°C to 25°C with an average temperature of 20°C. A 16-hour day was maintained with a light intensity of 1500 foot candles. A combination of GE Cool White F 40CW florescence and GE F 40PL plant lights were used to illuminate the plants. The plants were watered based on need.

4. Harvesting Procedures

a. Wheat. The wheat was plated January 18, 1975, and the first harvest was completed February 5, 1975. The wheat was clipped at the grazing level (2 centimeters) approximately every two weeks for the first month, then at longer intervals as the yield decreased until the final harvest May 17, 1975. Plant material was reduced to small uniform pieces by hand and oven dried at 80°C for 12 hours in preparation for analyses.

b. Bermuda grass. The bermuda grass was planted January 19, 1975, and the first harvest was February 7, 1975. The bermuda grass was harvested approximately every two weeks during the first month and then at longer intervals as the yields decreased until the final harvest May 17, 1975. Like wheat, the bermuda grass was clipped at the grazing level (2 centimeters). The plant material was prepared for analyses in the same manner as the wheat.

5. Soil Analyses

a. Preparation of soil for analyses. The soil was analyzed for pH, cation exchange capacity, organic matter content, soil particle size, and chromium, copper, iron, nickel and zinc concentrations prior to planting (Table 2). At the end of the study representative soil samples were taken at the 0-3 cm, 3-6 cm and 6-9 cm levels from each container for analyses of chromium, copper, iron, nickel and zinc concentrations. Roots were removed, the samples were air dried, ground in a mortar and pestle and passed through a two millimeter nylon seive, with the exception of those samples which were tested for soil particle size, pH, cation exchange capacity and organic matter content. All analyses were based on oven dry weight of the soil which was obtained by drying the soil at 105°C for 24 hours.

Table 2. Soil Characteristics

Parameter	Value
Texture	
Sand	35%
fine	34%
coarse	1%
Silt	50%
Clay	15%
Organic Matter	1.27%
pH	7.2
Soil Type	Loam to Silty Loam
Cation Exchange Capacity	9.6 meq/100 grams

b. Determination of chromium, copper, iron, nickel and zinc concentrations. Nitric acid digestion was performed on the soil samples and chromium, copper, iron, nickel and zinc were determined with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., 1966).

c. Determination of organic matter and cation exchange capacity. These two parameters were measured by the Soil and Water Service Laboratory, Agronomy Department, Oklahoma State University.

d. Determination of particle size. Particle size was determined by the Bouyoucos Method (Piper, 1942).

6. Plant Material Analyses

a. Preparation of plant material for analyses. After harvesting the plant material was reduced to small pieces by hand, then oven dried at 80°C for 12 hours. A weighed aliquot was placed in a kjeldahl flask and digested using a 3:4:15 nitric acid:perchloric acid:sulfuric acid mixture. After digestion the solution was made up to volume and analyzed (Perkin-Elmer Corp., 1966).

b. Analyses of plant material. Chromium, copper, iron, nickel, and zinc concentrations were determined with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., 1966).

7. Effluent Analyses

a. Preparation for analyses. Samples were taken at the time of each watering. Since pH is an important factor influencing the availability of heavy metals to plants, this parameter was measured prior to the preparation of the samples for further analyses. After the pH had been determined the samples were preserved by acidification with HNO_3 (3 mls/liter). Samples with suspended solids were digested with HNO_3 prior to analyses (National Environmental Research Center, 1974).

b. Determination of pH. The pH of the effluent was determined with an Orion Research Specific Ion Meter.

c. Determination of chromium, copper, iron, nickel, and zinc concentrations. Chromium, copper, iron, nickel and zinc were determined with a Perkin-Elmer Atomic Absorption Spectrophotometer by direct aspiration of each sample (Perkin-Elmer Corp., 1966).

C. Radioisotope Tracer Methods and Procedures

1. Radioisotope Source Preparation

Radioactive chromium-51 was received in the chemical form $CrCl_3$ in 0.5 N HCl. Over the period of the project four separate shipments of chromium-51 were necessary to maintain reasonable levels of activity. The first shipment (assay date January 6, 1975) had a specific activity of 271 mCi/mg and total solids of 0.203 mg/ml in 0.182 milliliters. The specific activity of the second shipment (assay date February 3, 1975) was 133 mCi/mg with 0.203 total solids in 0.371 milliliters. The thrid shipment (assay date March 3, 1975) had a specific activity of 98.5 mCi/mg and the total solids were less than 0.1 mg/ml in 3.5 milliliters. The final shipment (assay date March 31, 1975) had s specific activity of 104 mCi/mg with 0.33 mg/ml total soilids in 0.44 milliliters. All shipments were approximately 10 millicuries.

Upon receipt, each of the four shipments of the isotope was removed from the container, checked for contamination and opened. The total volume was made up to 5.0 milliliters by the addition of deionized water. These 5.0 milliliter volumes served as stock solutions of radiochromium. Watering solutions for the plants were prepared by diluting 2 milliliters of the stock solution to 8 liters with deionized water.

2. Counting System

A gamma counting system was set up consisting of a 3 inch sodium iodide well crystal, a photomultiplier, and an Ortec Model 420 single channel analyzer. The detection unit was fixed upright in a counting chamber constructed of lead bricks to provide low background counting rates.

A gamma spectrum of chromium-51 was run to establish the proper operating voltage, baseline and window width for the detection of the isotope. Optimum operating parameters for the counting system were: baseline setting - 320 Mev, window width - 2, coarse gain - 16, and fine gain - 8.

3. Counting Efficiency

To determine the amount of radioactivity present in a sample, the counting efficiency or machine efficiency of the counting apparatus must be known. Machine efficiency is merely the ability of the equipment to detect the radiation and is determined by counting a solution with known disintegrations per minute (dpm) and comparing the count rate of the machine to the actual count rate. In order to determine machine efficiency a 0.31 milliliter aliquot of chromium-51 was taken from the third shipment's stock solution and diluted to 2 liters with deionized water. Several 10 milliliter samples of this solution were counted and the machine efficiency determined as follows:

$$\text{Efficiency} = \text{Counts Per Minute (CPM)/dpm}$$

$$\text{dpm} = (2.22 \times 10^{9}\ \text{dpm/mCi})\ (3.08 \times 10^{-3}\text{mCl})\ (9^{-2})$$

$$= 6.15384 \times 10^{3}$$

$$\text{Efficiency} = 141{,}763/6.15384 \times 10^{3} = 23.04\%$$

where 141,763 CPM is the average count rate of the samples.

4. Plants

a. Growing conditions. Twelve pots of wheat and twelve pots of bermuda grass (planting and growing conditions are described elsewhere) were watered with a radioactive solution prepared from 1 milliliter stock solution diluted to 4 liters with deionized water.

b. Sampling. The wheat and bermuda grass were harvested by hand at the 2 centimeter level (grazing level) and placed in precounted 3 cm x 15 cm pyrex test tubes and counted. After counting, the plant material was dried in a drying oven at 60°C for 48 hours and weighed to obtain the yield based on oven dry weight.

The wheat was harvested approximately every two weeks for the first month, then at longer intervals as the yields began to decrease. During the last month the wheat was sampled weekly. The bermuda grass was harvested less often due to its slower growth. During the first two months the bermuda grass was harvested every two weeks, then at longer intervals as the yield decreased.

CHAPTER IV

ANALYSIS OF DATA

A. Introduction

This chapter consists of an analysis of the data and a summary of the findings. The raw data were converted to micrograms of heavy metal per gram of plant material or soil and to micrograms heavy metal per milliliter of effluent.

The average micrograms of heavy metal per gram plant material was calcualted for each harvest and the standard deviation determined. The same information was calculated for the soil prior to and after completion of the research. Analysis of variance and the new multiple range test were performed on the plant material data at each harvest to determine if significant differences in heavy metal concentrations existed between the three treatments (Li, 1964).

B. Compilation of Data

1. Heavy Metals

a. Plant material. Atomic absorption data from the analysis of the plant material was converted into values of micrograms per gram of dry plant material using the following formula:

$$\text{microgram/g} = \frac{\text{(g/ml sample)(mls of solution)(d.f.)}}{\text{g oven dry weight}}$$

where,

$$\text{d.f.} = \frac{\text{final diluted volume in mls}}{\text{volume in mls of aliquot taken for dilution}}$$

(Perkin-Elmer Corp., 1966).

Because the containers of plants were watered based on individual moisture needs, the containers did not always receive the same quantity of solution between harvests. Therefore, the figures obtained from the method described above were normalized according to the following formula:

$$\begin{array}{c}\text{Normalized}\\ \text{Value}\end{array} = \left(\frac{\mu\text{g h.m. in plant/harvest}}{\text{total }\mu\text{g/g h.m. added/harvest}}\right)\left(\begin{array}{c}\text{average g h.m.}\\ \text{added/harvest}\end{array}\right)$$

These results were averaged and standard deviations calculated (Tables 3 and 6). Analysis of variance and the new multiple range test were then performed on the data for each harvest to determine if significant differences existed among the three treatments (Li, 1964). The results of these statistical tests are presented in Tables 5 and 7.

b. Soil. The data from the soil analysis for the heavy metals were converted to micrograms of heavy metals per gram soil and normalized by the methods presented above for the plant material. These results were averaged and standard deviations calculated (Appendix, Table A-3).

2. Chromium-51

All samples were counted and the background substracted. Because of the relatively short half-life of chromium-51 (27.8 days), it was necessary to use three shipments of chromium-51 for preparing the watering solution. Thus during the period from one harvest to another the plants were watered with chromium-51 solutions made up from different chromium-51 shipments. Each chromium-51 solution was counted prior to watering and the amount added to each container recorded. The counts per minute added to each container were corrected for decay to the harvest date by the following formula:

$$N = N_o e^{-\lambda t/T^{\frac{1}{2}}}$$

where,

$\lambda = 0.693$

$T_{\frac{1}{2}} = 27.8$ days

The CPM for the plant material was also corrected to the harvest date. A ratio of

(CPM/100 g plant material)/(CPM/ml Cr-51 solution added)

was determined and used for comparing chromium-51 uptake of the two plant species.

C. Summary of Findings

The experiment consisted of three sets containing four pots each of wheat and bermuda grass. Three treatments were set up: 1) plants watered with effluent; 2) plants watered with aerated effluent; and 3) plants watered with deionized water to serve as controls.

1. Chromium in Wheat

The chromium content of the plant material was measured for each of the three treatments at each harvest (Table 3 and Figure 2). The

Table 3. Heavy Metals in Wheat (micrograms/gram)

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Chromium						
Control	4.50±0.16	12.31±2.36	7.84±2.34	10.44±2.77	15.57±1.42	8.40±6.10
Effluent	5.40±0.99	6.90±1.42	12.24±3.06	67.36±16.30	43.71±8.88	74.57±0.00
Aerated Ef.	6.37±0.97	4.36±0.06	20.15±2.84	80.12±2.90	23.54±1.15	67.83±0.00
Copper						
Control	13.00±1.00	12.00±2.00	5.80±0.52	11.75±0.06	11.84±0.28	9.68±0.63
Effluent	12.50±1.66	14.59±0.84	6.97±0.16	12.41±0.49	21.85±2.50	11.56±0.00
Aerated Ef.	11.00±0.00	13.75±0.24	11.41±2.96	13.17±4.85	31.89±0.00	14.54±0.00
Iron						
Control	94.20±2.49	96.00±0.00	77.17±0.19	69.48±3.82	86.82±2.78	158.83±1.24
Effluent	90.67±3.77	97.90±9.73	71.71±4.55	169.16±28.95	287.28±1.92	378.15±0.00
Aerated Ef.	86.67±0.94	125.12±3.46	83.08±1.43	138.93±6.11	301.56±53.18	213.18±0.00
Nickel						
Control	13.51±2.70	7.00±2.00	9.12±0.61	9.83±1.53	3.45±0.45	26.17±2.94
Effluent	14.43±2.40	13.83±0.16	43.11±0.29	35.65±0.31	52.97±0.06	525.20±0.00
Aerated Ef.	14.22 ±2.92	25.69±1.40	96.55±0.00	112.17±0.00	171.38±11.60	513.57±0.00
Zinc						
Control	44.47±0.66	65.87±2.12	51.26±1.34	49.18±2.13	46.58±2.11	62.13±2.34
Effluent	43.84±1.73	53.34±4.08	46.28±4.99	48.27±5.17	115.02±12.71	69.12±0.00
Aerated Ef.	43.65±0.41	55.34±4.08	43.20±2.26	69.58±2.10	91.35±10.41	62,02±0.00

All values in this table have been normalized.

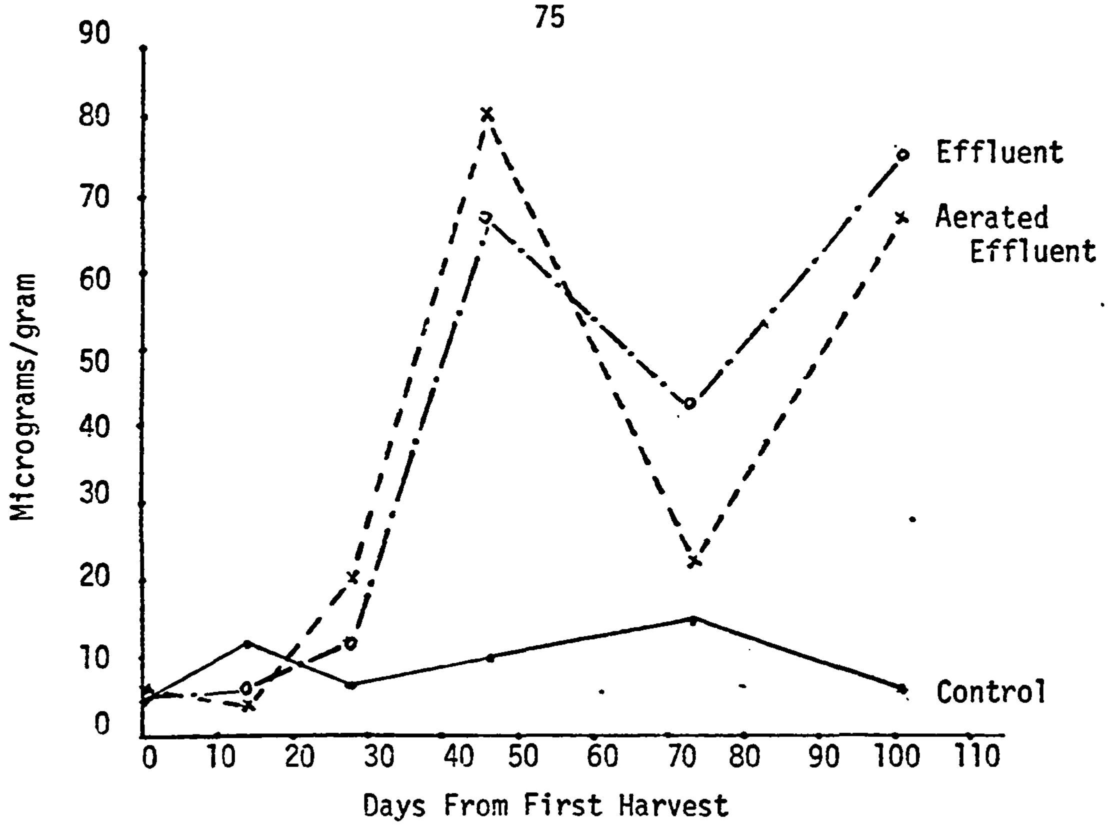

Figure 2. Chromium Concentration in Wheat

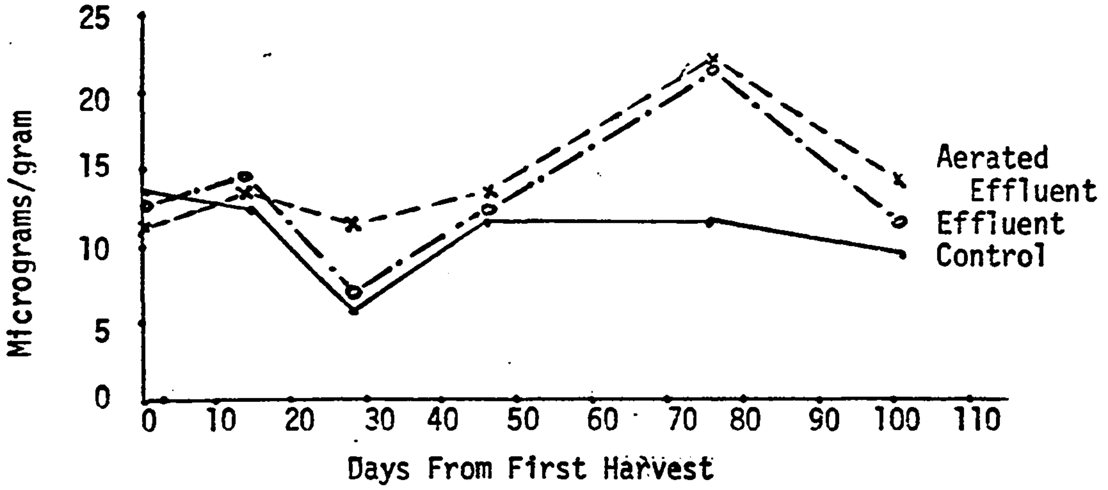

Figure 3. Copper Concentration in Wheat

wheat was planted and all three treatments were watered with deionized water until the seedlings were established. The first harvest was made on the 18th growing day. This is shown on Figure 2 as day 0. The chromium content was very similar in the three treatments ranging from 4.30 to 7.36 micrograms/gram.

The second harvest occurred 14 days after the first harvest. The control plants had been watered during this fourteen day period with deionized water. One set of plants was watered with effluent (Effluent plants) and the third set was watered with aerated effluent (Aerated Effluent plants). Differences in chromium content began to become evident at the second harvest. The chromium content was higher in the Control plants (12.30 micrograms/gram) than in the Effluent plants (6.90 micrograms/gram) or the Aerated Effluent plants (4.35 micrograms/gram), making it appear that the initial effect of the effluent was to depress the chromium uptake by the test plants.

However, by the time of the third harvest 14 days later (28 days after the first harvest) there was a reversal in chromium content. The chromium content of the Control plants (7.84 micrograms/gram) fell below that of the Effluent plants (12.24 micrograms/gram) and the Aerated Effluent plants (20.15 micrograms/gram) and remained lower for the remainder of the project.

At the fourth harvest 18 days later (46 days after the first harvest) the Control plants remained comparatively low in chromium content (10.44 micrograms/gram) while the chromium content of the other two treatments increased sharply (Effluent plants - 67.36 micrograms/gram and Aerated Effluent plants - 80.12 micrograms/gram).

The chromium content had risen slightly in the Control plants (to 15.55 micrograms/gram) by the firth harvest 27 days later (73 days after the first harvest). However, there was a decrease in the chromium content of the Effluent plants to 43.71 micrograms/gram and the Aerated Effluent plants to 23.54 micrograms/gram.

The chromium concentration of the Control plants decreased at the sixth and final harvest 28 days later (101 days after the first harvest). The chromium content of the Control plants averaged 8.40 micrograms/gram which was well below the chromium content of the Effluent plants (74.27 micrograms/gram) and the Aerated Effluent plant (67.83 micrograms/gram). The analysis of variance and the new multiple range test (Table 4) indicated no significant differences existed between the three treatments at the time of the first harvest which was to be expected since all plants had received the same treatment up to this point. At the time of the second harvest significant differences existed in the chromium level between the Control plants and both the Effluent plants and Aerated Effluent plants. There was a significant difference in chromium concentration between the Control plants and Aerated Effluent plants at the time of the third harvest. Significant differences in chromium levels between the Control plants and Effluent plants existed throughout the remainder of the experiment. The chromium concentration was significantly different between the Control plants and the Aerated Effluent plants at the time of the fourth and fifth harvests. There were no significant differences in chromium levels between the Effluent and Aerated Effluent plants throughout the experiment.

Table 4. Results From the New Multiple Range Test (Wheat)

Heavy Metal	Test	First Harvest	Second Harvest	Third Harvest	Fourth Harvest	Fifth Harvest	Sixth Harvest
Chromium	C - E	NS	S	NS	S	S	S
	C - AE	NS	S	S	S	NS	S
	E - AE	NS	NS	NS	NS	NS	NS
Copper	C - E	NS	NS	NS	NS	NS	NS
	C - AE	NS	NS	NS	NS	NS	NS
	E - AE	NS	NS	NS	NS	NS	NS
Iron	C - E	NS	S	S	S	S	S
	C - AE	NS	S	NS	S	S	S
	E - AE	NS	S	S	NS	NS	S
Nickel	C - E	NS	S	S	S	S	S
	C - AE	NS	S	S	S	S	S
	E - AE	NS	S	S	S	S	NS
Zinc	C - E	NS	S	S	NS	S	NS
	C - AE	NS	S	S	S	S	NS
	E - AE	NS	NS	NS	S	NS	NS

C = Control plants
E = Effluent plants
AE = Aerated Effluent plants
S = Significant difference
NS = No significant difference

The total accumulated chromium content in the Control plants was very close to a straight line. At the time of the second harvest the accumulated chromium content of the Aerated Effluent plants increased more sharply than the Effluent plants or the Controls, and continued to have a greater increase until the fifth harvest, when the accumulated chromium content of the Effluent plants became greater and remained so through the final harvest (Table 5 and Figure 4).

2. Copper in Wheat

The copper concentration of the plant material was measured for each treatment at each harvest (Table 3 and Figure 3). The wheat was watered for the first 18 days with deionized water and harvested on the 18th day. This is shown as day 0 on Figure 3. The copper content was practically the same in all three treatments at this point (Control plants - 13.00 micrograms/gram, Effluent plants - 12.50 microgram/gram, and Aerated Effluent plants - 11.00 micrograms/gram).

The plants were then treated with the appropriate solutions and harvested again in 14 days. Again the copper content was quite close in all three treatments (Control plants - 12.00 microgram/gram, Effluent plants -14.59 micrograms/gram, and Aerated Effluent plants - 13.75 micrograms/gram). These figures were fairly close to the copper content of the first harvest.

At the third harvest 14 days later there was a drop in copper content particularly in the Control plants (5.80 micrograms/gram) and the Effluent plants (6.97 micrograms/gram). The drop was less drastic in the Aerated Effluent plants (11.41 micrograms/gram).

Table 5. Accumulated Heavy Metals in Wheat (micrograms/gram)

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Chromium						
Control	4.30	16.61	24.45	34.89	50.46	58.86
Effluent	5.40	12.30	24.54	91.91	135.62	210.20
Aerated Eff.	6.27	10.73	30.88	110.99	134.54	202.37
Copper						
Control	13.00	25.00	30.80	42.55	54.39	64.07
Effluent	12.50	27.09	34.06	46.47	68.32	79.87
Aerated Eff.	11.00	24.75	36.16	49.33	71.23	85.76
Iron						
Control	94.20	190.20	267.37	336.86	423.68	582.51
Effluent	90.67	188.57	260.28	429.43	716.71	1094.86
Aerated Eff.	86.67	211.79	294.87	433.79	735.36	948.54
Nickel						
Control	13.50	20.50	29.62	39.44	42.98	69.06
Effluent	14.40	27.83	70.94	106.60	159.56	684.77
Aerated Eff.	14.20	39.89	136.45	248.61	420.00	933.56
Zinc						
Control	44.47	110.34	161.60	210.79	257.37	319.50
Effluent	43.80	97.14	143.42	191.70	306.71	375.83
Aerated Eff.	43.65	98.99	142.20	211.78	303.12	365.14

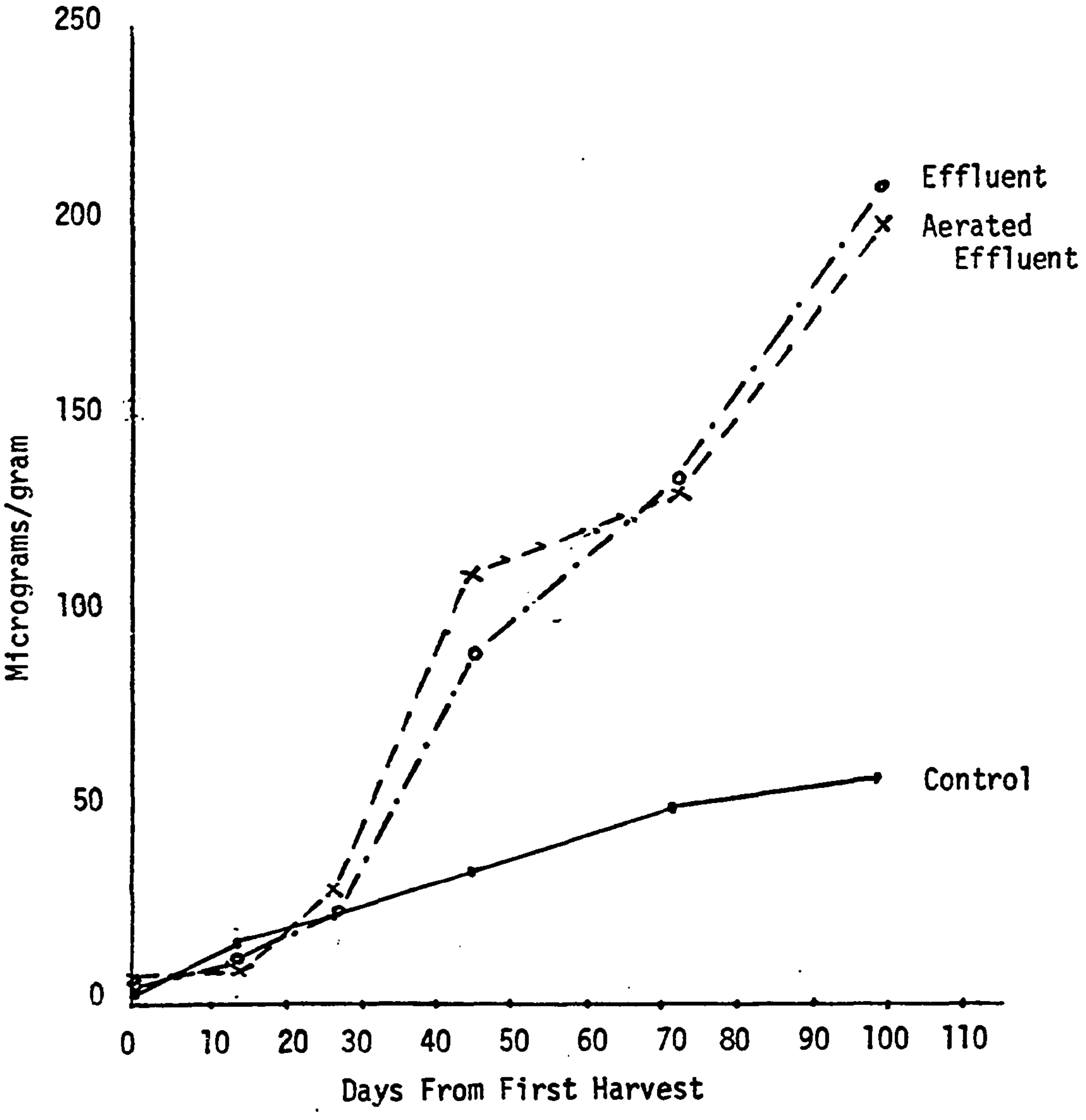

Figure 4. Accumulated Chromium in Wheat (Normalized)

There was an increase in copper content in all three treatments at the fourth harvest 18 days later. Again the copper content of all three treatments was very close (Control plants - 11.75 micrograms/gram, Effluent plants - 12.41 micrograms/gram and Aerated Effluent plants - 13.17 micrograms/gram).

The fifth harvest was made 27 days later. The copper content of the Control plants rose only slightly (11.84 micrograms/gram) while there was a much greater increase in the Effluent plants (21.85 micrograms/gram) and the Aerated Effluent plants (21.89 micrograms/gram).

The sixth and final harvest 28 days later showed a decrease in the copper content of all three treatments (Control plants - 9.68 micrograms/gram, Effluent plants - 11.56 micrograms/gram, and Aerated Effluent plants - 14.54 micrograms/gram).

The analysis of variance and the new multiple range test (Table 4) indicated there were no significant differences in the copper concentrations of the plant material in the three treatments throughout the experiment.

The total accumulated copper content in the three treatments was extremely close through the fourth harvest. After that the increase in the accumulated copper in the Effluent plants and the Aerated Effluent plants outstripped that of the Control plants. The accumulated copper content of the Effluent and Aerated Effluent plants was very close throughout the experiment (Table 5 and Figure 5).

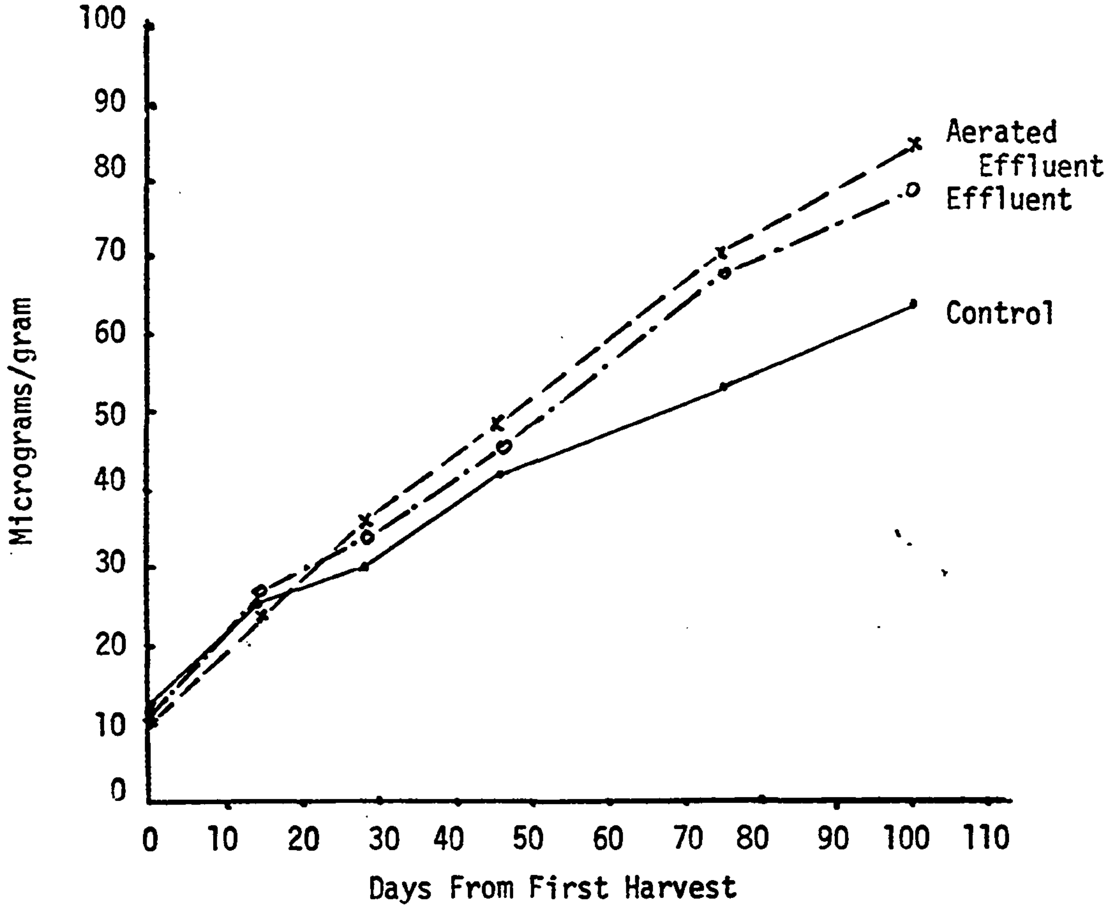

Figure 5. Accumulated Copper in Wheat

3. Iron in Wheat

The iron concentration of the plant material is presented in Table 3 and Figure 6. The first harvest was made 18 days after seeding. This is shown as day 0 in Figure 6. Up to this time plants in all three treatments had been watered with deionized water only. Even so, the iron content in the three treatments ranged from 94.2 micrograms/gram in the Control plants to 86.67 micrograms/gram in the Aerated plants. The Effluent plants were midway between the other two treatments with 90.67 micrograms/gram of iron.

At the second harvest there was an increase in the iron content of both the Effluent plants (97.90 micrograms/gram) and the Aerated Effluent plants (125.12 micrograms/gram). The iron content of the Control plants increased to 96.00 micrograms/gram.

There was a continued decrease in the iron content of the Control plants at the third and fourth harvests. The other two treatments had decreased iron contents at the third harvest, but had large increases at the fourth harvest.

At the fifth harvest there was an increase of iron content in all three treatments (Control - 86.82 micrograms/gram, Effluent plants - 287.28 micrograms/gram, and Aerated Effluent plants - 301.56 micrograms/gram).

The Effluent plants continued to increase in iron content (378.15 micrograms/gram) at the final harvest. The Control plants also increased (158.83 micrograms/gram) in iron content. However, the Aerated Effluent plants decreased from 301.56 micrograms/gram to 213.18 micrograms/gram.

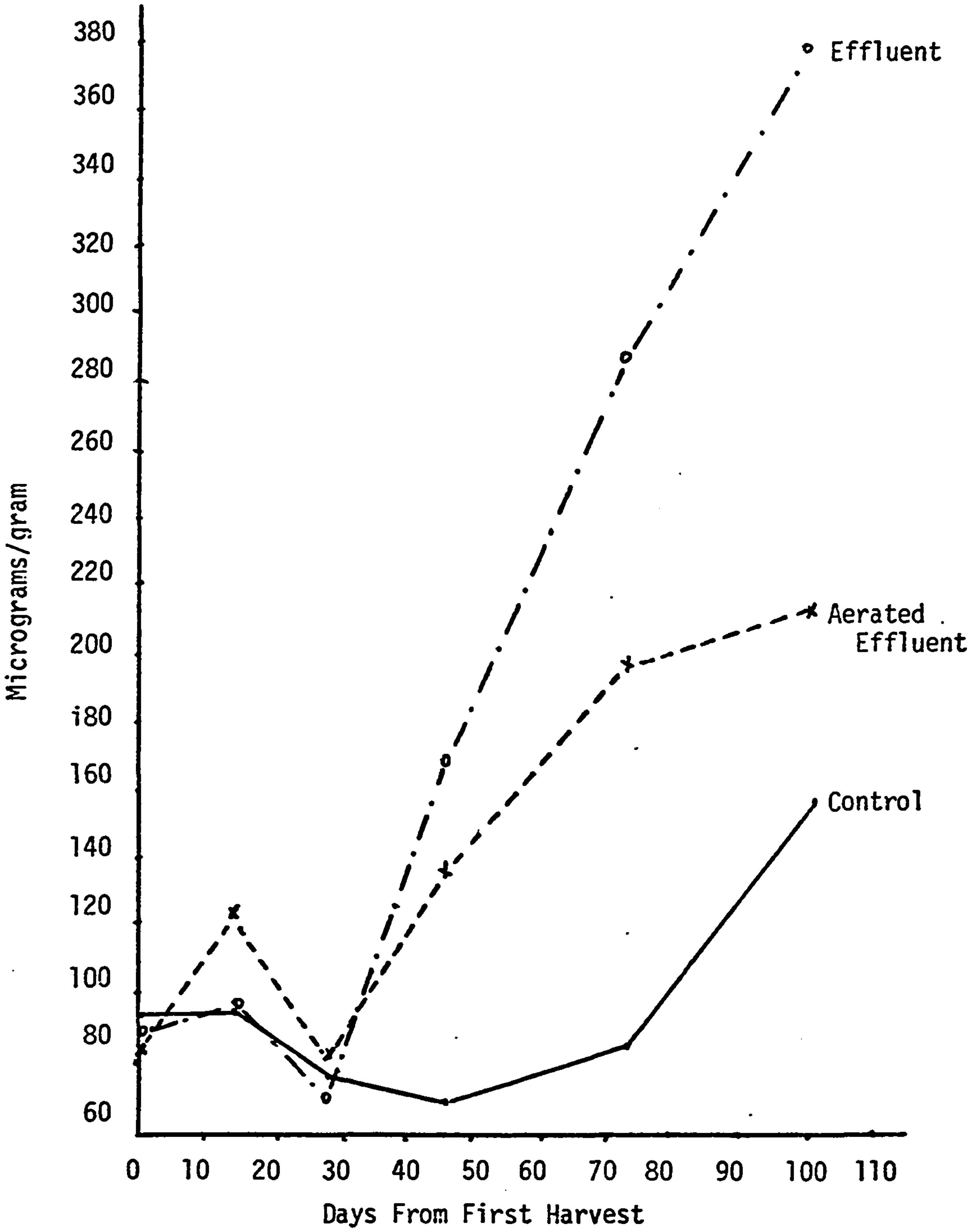

Figure 6. Iron Concentration in Wheat (Normalized)

The analysis of variance and the new multiple range test (Table 4) indicated no significant differences in the iron concentration existed in the three treatments at the time of the first harvest. However, there were significant differences in iron of all three treatments at the time of the second harvest. The iron concentrations remained significantly different between the Control plants and the Effluent plants throughout the remainder of the experiment. No significant differences in iron content was indicated between the Control plants and the Aerated Effluent plants at the time of the third harvest, but significant differences were indicated for the remainder of the experiment. Significant differences in iron content between the Effluent plants and Aerated Effluent plants were indicated at the time of the third and sixth harvests.

The accumulated iron content in the Effluent and Aerated Effluent plants were very close with a slightly greater iron content in the Aerated Effluent plants until the sixth harvest when the Effluent plants increased in accumulated iron content to 1094.86 micrograms/gram, while the accumulated iron content of the Aerated Effluent plants was 948.54 micrograms/gram. The accumulated iron content of the Control plants was very close to that of the other two treatments through the third harvest. Thereafter the accumulated iron content of the Control plants fell below that of the other two treatments. At the final harvest the accumulated iron content of the Controls was only 582.51 micrograms/gram compared to 1094.86 micrograms/gram in the Effluent plants and 948.86 micrograms/gram in the Aerated Effluent plants (Table 5 and Figure 7).

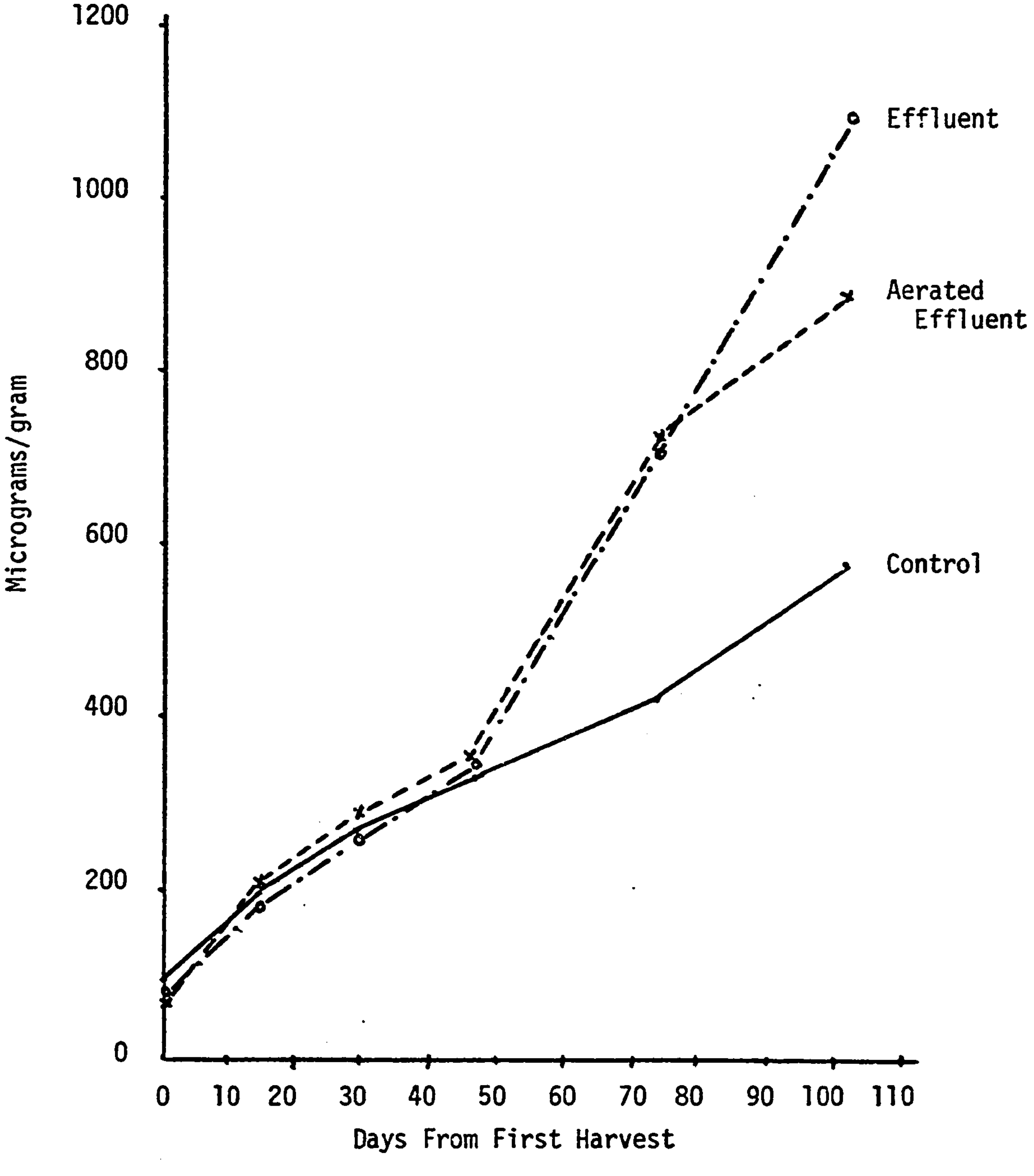

Figure 7. Accumulated Iron in Wheat

4. Nickel in Wheat

The nickel content of the wheat is presented in Table 3 and Figure 8. The nickel concentration of all three treatments was very similar at the first harvest after a period of all plants being watered with deionized water. The nickel content ranged from 13.51 micrograms/gram in the Control plants to 14.43 micrograms/gram in the Effluent plants.

The nickel content in the Control plants had decreased to 7.00 micrograms/gram by the time of the second harvest two weeks later. The Effluent plants had a decrease in nickel content from 14.43 micrograms/gram to 13.83 micrograms/gram. The Aerated Effluent plants had the greatest nickel content (25.70 micrograms/gram) at this time.

At the third harvest, the nickel content had increased slightly in the Control plants to 9.11 micrograms/gram. There were larger increases in the other two treatments (Effluent plants - 43.11 micrograms/gram and Aerated Effluent plants - 96.55 micrograms/gram).

At the fourth harvest there continued to be increases in nickel concentration in the Control plants (to 9.83 micrograms/gram) and the Aerated Effluent plants (to 112.17 micrograms/gram). There was a decrease in the nickel content of the Effluent plants (to 35.65 micrograms/gram).

There was a marked decrease in the nickel content of the Control plants (3.45 micrograms/gram) at the fifth harvest. The nickel content of the Effluent plants increased to 52.97 micrograms/gram and that of the Aerated Effluent plants increased to 171.38 micrograms/gram.

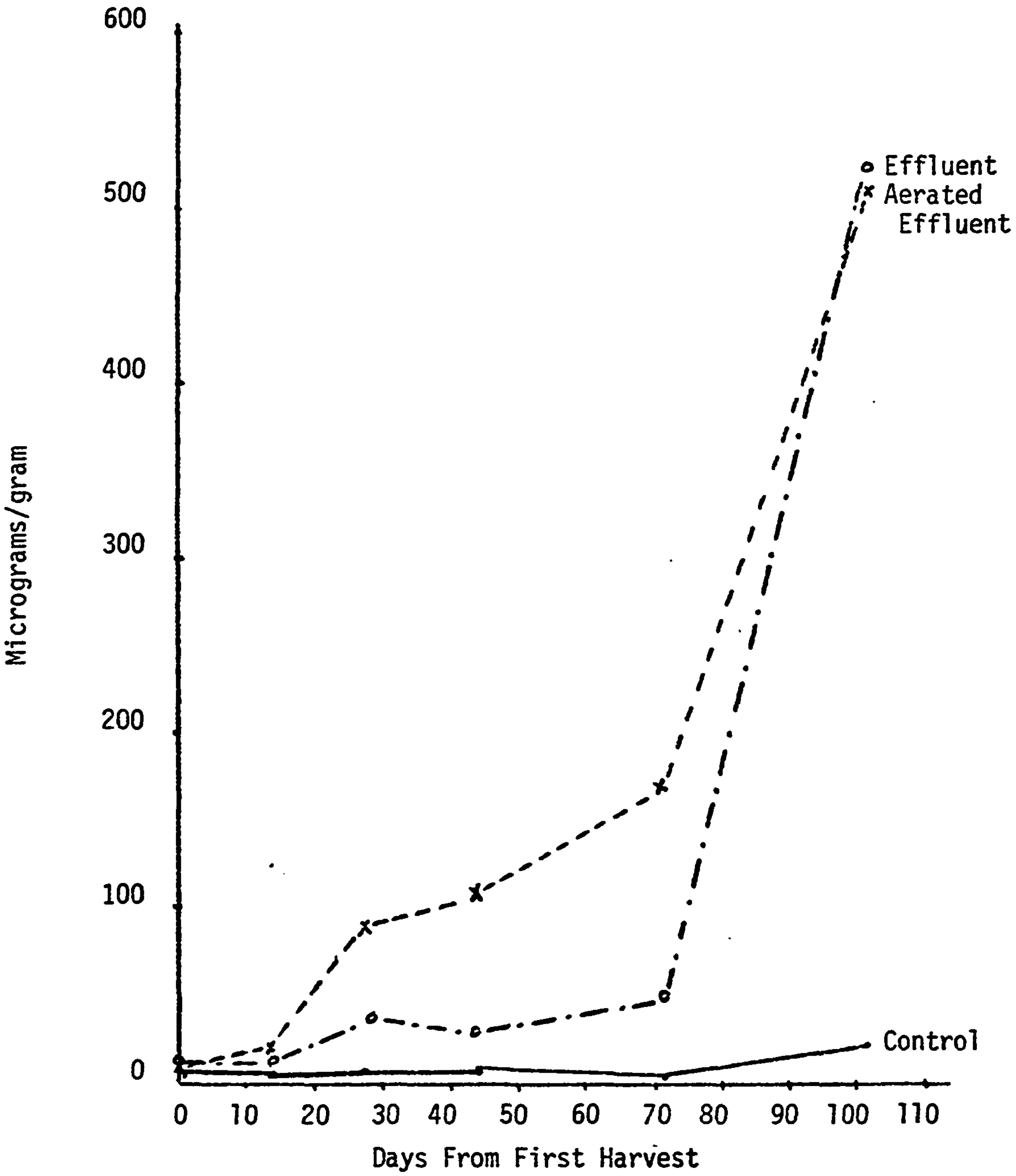

Figure 8. Nickel Concentration in Wheat (Normalized)

At the sixth harvest there was an increase in nickel content in all three treatments. The nickel content of the Control plants increased to 26.17 micrograms/gram. The nickel content of the Effluent plants and Aerated Effluent plants increased to 525.20 micrograms/gram and 513.57 micrograms/gram respectively.

The analysis of variance and the new multiple range test indicated no significant difference in the nickel content of the wheat at the time of the first harvest (Table 4). However, significant differences in nickel content were indicated between the Control plants and the Effluent plants and between the Control plants and the Aerated Effluent plants throughout the remainder of the experiment. There were also significant differences between the nickel content of the Effluent plants and the Aerated Effluent plants from the second harvest through the fifth.

From the first harvest through the final harvest the accumulated nickel content of the Aerated Effluent plants (933.56 micrograms/gram) outstripped that of the other two treatments. The Effluent plants had the second highest accumulated nickel content (684.77 micrograms/gram). The Control plants had a total nickel content of 69.06 micrograms/gram which was much less than the other two treatments (Table 5 and Figure 9).

5. Zinc in Wheat

The zinc content of all three treatments was very similar at the first harvest when the plants had been watered with deionized water. The zinc content ranged from 43.84 micrograms/gram in the Aerated Effluent plants to 44.47 micrograms/gram in the Control plants (Table 3 and Figure 10).

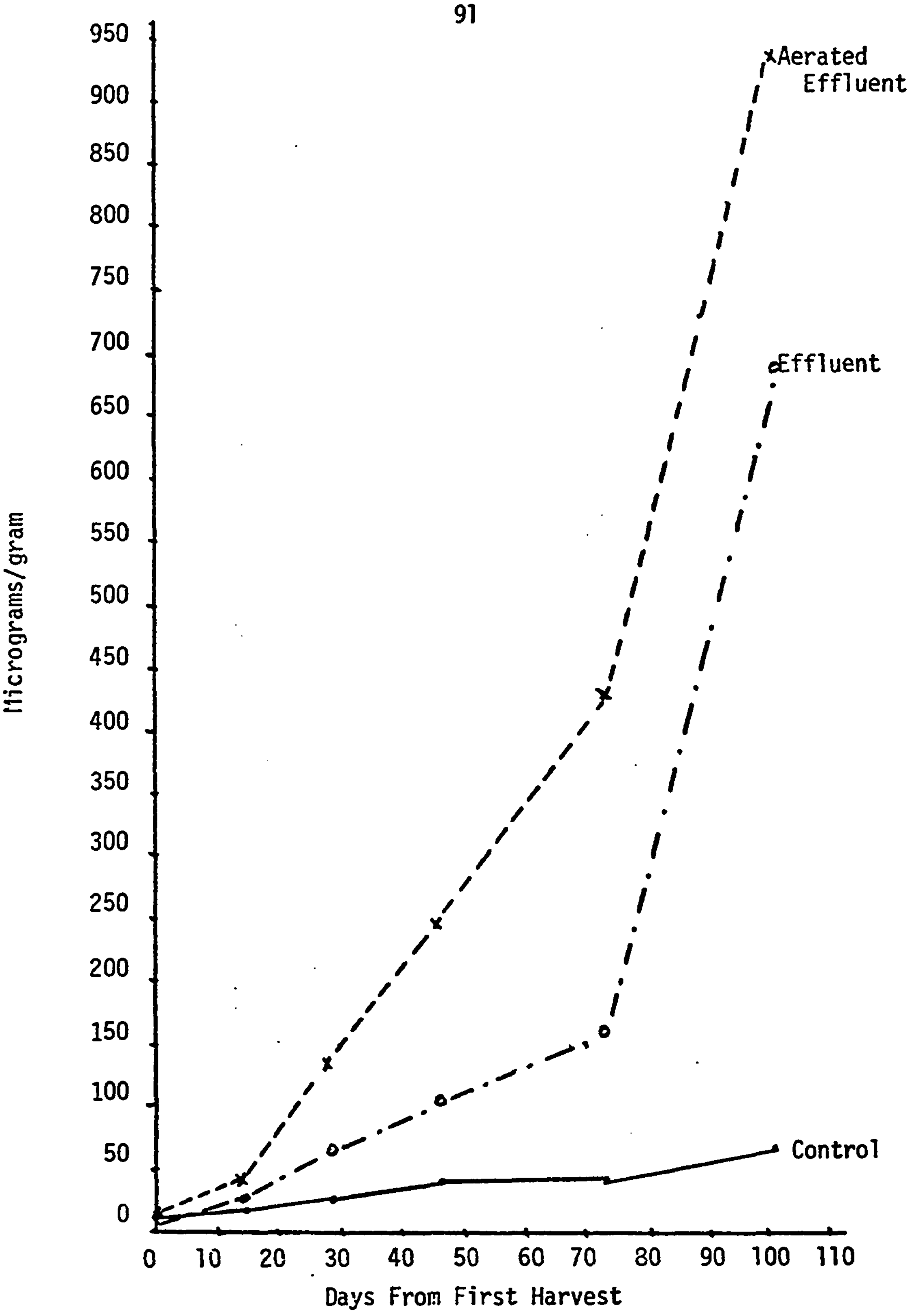

Figure 9. Accumulated Nickel in Wheat

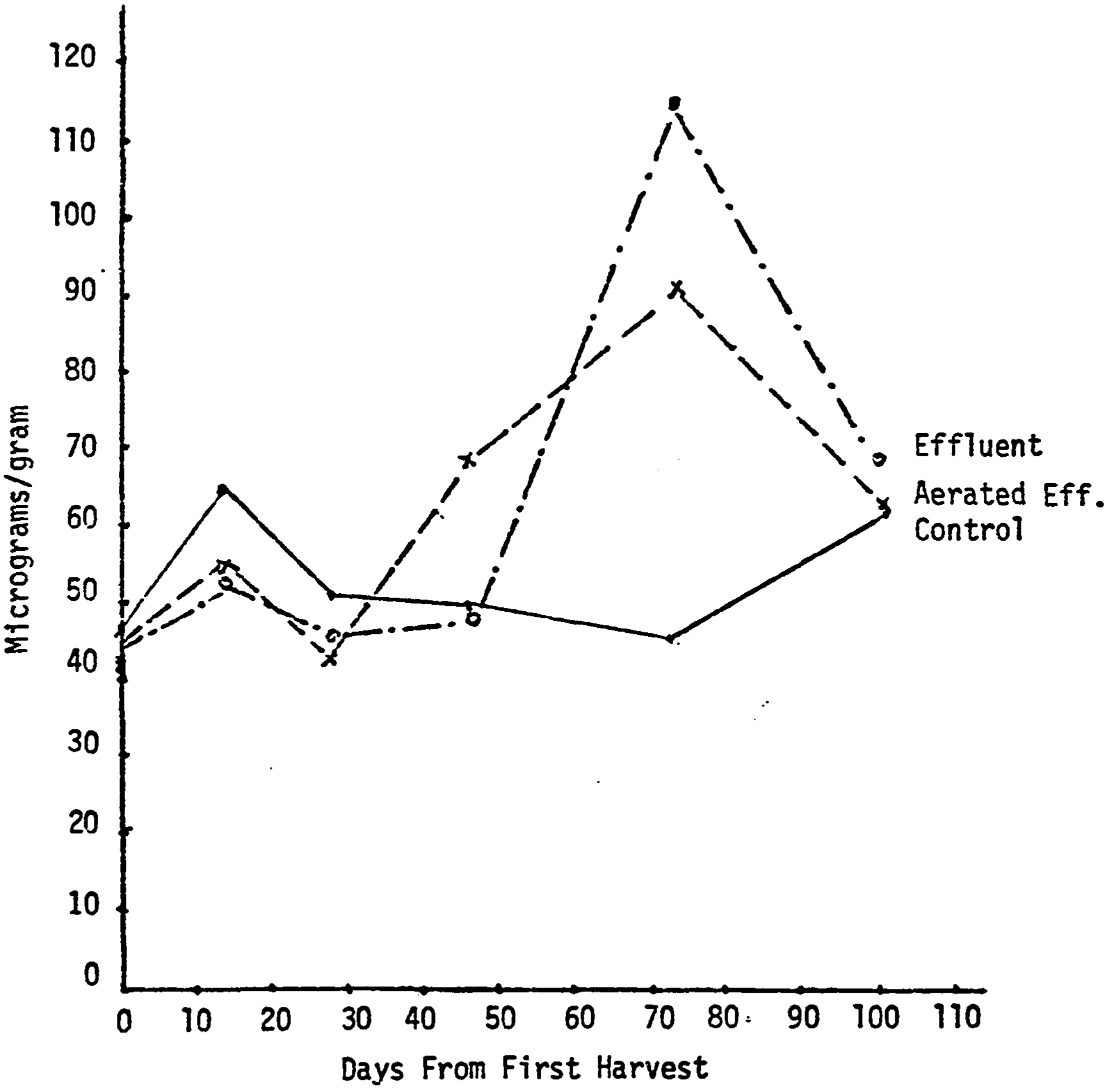

Figure 10. Zinc Concentration in Wheat (Normalized)

The zinc content increased in all three treatments at the second harvest. The increase to 65.87 micrograms/gram in the Control plants was the largest. The Effluent plants increased to 53.34 micrograms/gram and the Aerated Effluent plants increased in zinc content to 55.34 micrograms/gram.

Again at the third harvest the Control plants had the greatest zinc content (51.27 micrograms/gram), although the zinc content in all three treatments decreased somewhat. The Effluent plants (46.28 micrograms/gram) decreased less than did the Aerated Effleutn plants (43.20 micrograms/gram).

There was a further decrease in the zinc content of the Control plants at the fourth harvest. However, both the Effluent plants and the Aerated Effluent plants increased in zinc content. The largest amount of zinc was present in the Aerated Effluent plants. The Control plants and the Effluent plants were quite close in zinc content at this time.

The zinc content of the Control plants continued to decrease at the fifth harvest, while that of the Effluent plants increased sharply and the Aerated Effluent plants increased to a somewhat lesser degree.

The zinc content of all three treatments were very close at the sixth harvest ranging from 62.02 micrograms/gram for the Aerated Effluent plants to 69.12 micrograms/gram for the Effluent plants. The Control plants showed an increase to 62.13 micrograms/gram in contrast to the decreases that occurred in the zinc content of the other two treatments.

The analysis of variance and the new multiple range test (Table 4) indicated no significant differences in zinc content existed in the three treatments at the time of the first harvest. However, significant differences in zinc concentrations existed between the Control plants and the Effluent plants at the second, third, and fifth harvests; between the Control plants and Aerated Effluent plants at the second, third, fourth, and fifth harvests; and between the Effluent and Aerated Effluent plants at the fourth harvest. No significant differences in zinc concentrations existed at the sixth harvest.

The accumulated zinc content of the Control plants remained the highest through the third harvest, with the Aerated Effluent plants and Effluent plants having very similar zinc contents to this point. At the fourth harvest the accumulated zinc content of the Aerated Effluent plants was slightly greater than that of the Control plants. At the fifth harvest the highest accumulated zinc content was found in the Effluent plants with the Aerated Effluent plants having the second highest accumulated zinc content. This trend continued through the sixth harvest. However, the accumulated zinc content was very close in all treatments (Table 5 and Figure 11).

6. Chromium in Bermuda Grass

The bermuda grass was sprigged in 12 containers and three treatments of four pots each were established. All treatments were watered with deionized water for the first 21 days in order to give the plants time to become established. The chromium concentrations for the three treatments for each harvest are presented in Table 6 and Figure 12.

The first harvest was made on the 21st growing day. This is

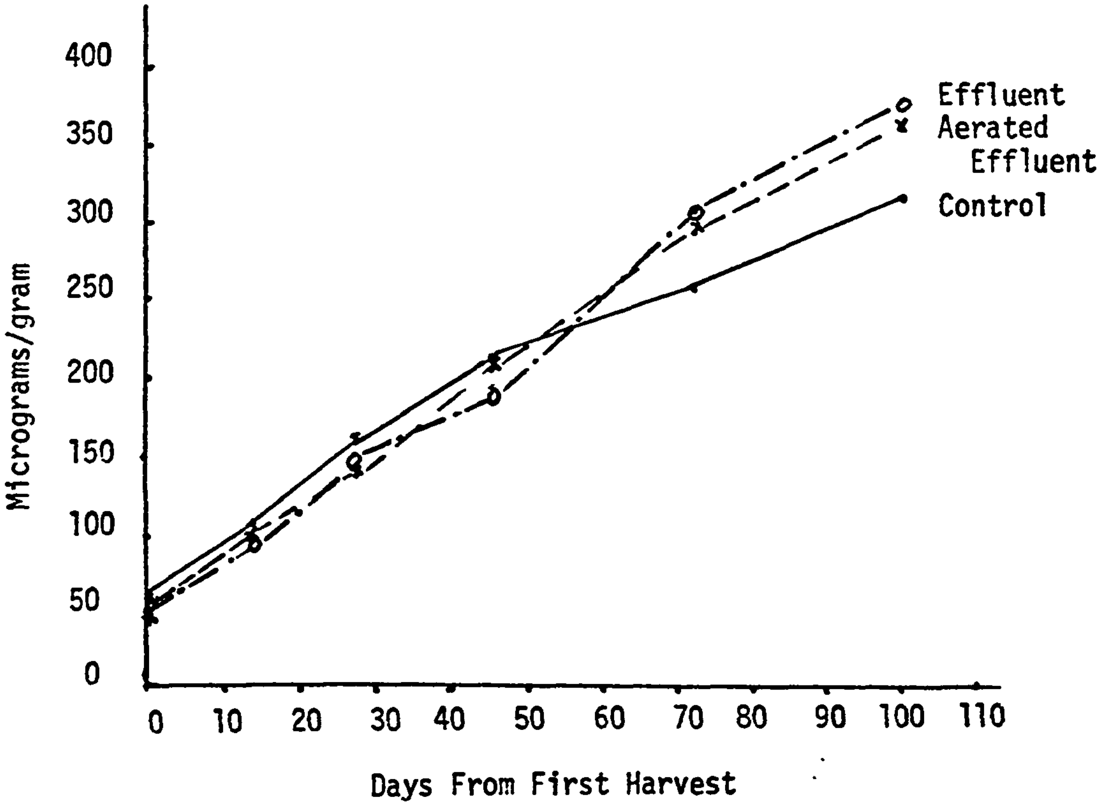

Figure 11. Accumulated Zinc in Wheat

Table 6. Heavy Metals in Bermuda Grass (micrograms/gram)

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Chromium						
Control	9.60±0.40	7.34±2.33	11.58±1.53	15.04±1.26	15.43±0.63	27.14±3.01
Effluent	12.89±2.30	9.85±1.87	43.19±5.03	40.78±4.29	46.71±1.25	53.45±0.00
Aerated Ef.	9.80±5.60	4.42±0.06	19.46±1.74	50.24±0.50	55.02±3.28	83.45±2.94
Copper						
Control	14.57±1.84	14.48±1.09	15.92±0.96	16.42±1.14	19.69±0.56	14.91±2.27
Effluent	12.79±1.77	11.75±0.77	18.77±0.83	13.98±1.08	20.90±2.58	16.12±0.00
Aerated Ef.	13.29±1.43	12.87±0.63	14.44±0.61	11.85±0.44	13.37±1.02	16.65±0.83
Iron						
Control	157.00±19.85	107.35±1.50	84.00±4.57	107.03±1.08	89.46±4.43	386.10±0.00
Effluent	141.64±2.55	114.44±9.31	101.30±12.30	116.35±21.96	146.89±4.55	328.95±0.00
Aerated Ef.	116.34±1.56	123.82±0.82	84.67±5.86	100.37±9.02	139.57±1.44	313.64±0.00
Nickel						
Control	67.34±0.00	19.73±1.17	62.99±2.08	65.86±1.37	59.67±7.85	212.36±0.00
Effluent	58.45±0.00	66.18±8.07	227.19±4.63	140.70±0.70	305.71±0.00	156.25±0.00
Aerated Ef.	44.48±1.58	48.33±2.15	125.14±6.55	122.40±5.90	111.31±0.58	321.37±16.14
Zinc						
Control	60.70±15.80	59.67±5.31	51.57±2.30	66.05±5.34	65.91±0.43	160.86±0.63
Effluent	57.10±8.50	64.46±4.85	83.90±1.56	61.95±4.97	119.54±10.73	183.39±0.00
Aerated Ef.	52.70±4.70	85.44±0.56	97.13±1.41	129.45±4.64	97.90±9.72	123.70±16.15

All values in this table have been normalized

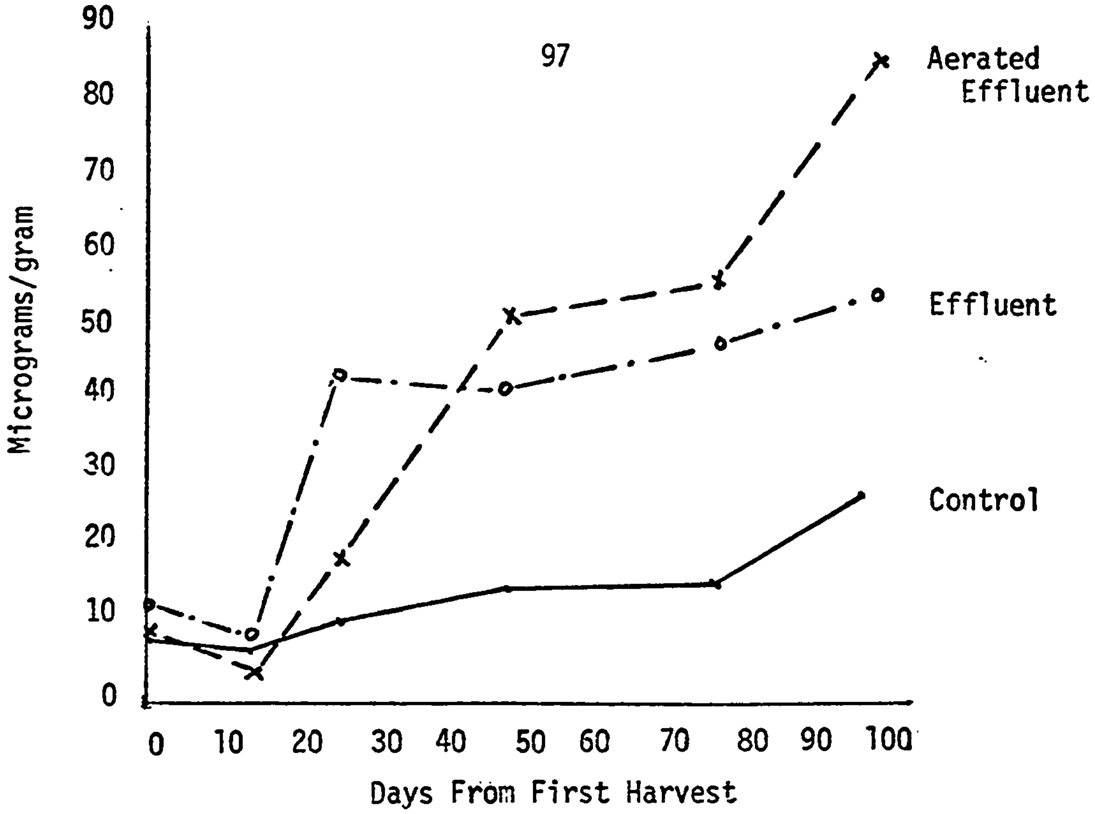

Figure 12. Chromium Concentration in Bermuda Grass (Normalized)

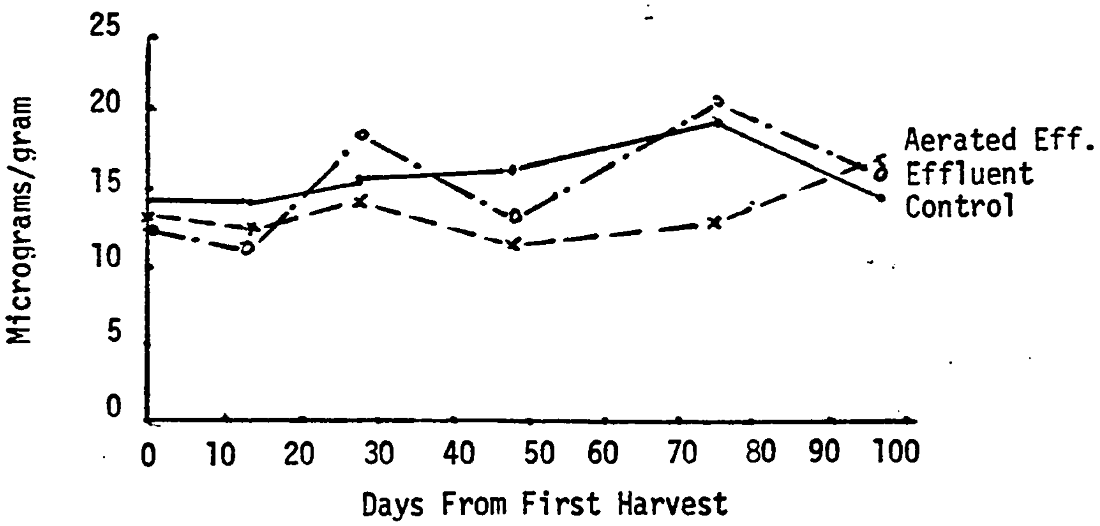

Figure 13. Copper Concentration in Bermuda Grass (Normalized)

shown as day 0 on Figure 12. The chromium content was similar in all three treatments (Control plants - 9.60 micrograms/gram, Effluent plants - 12.89 micrograms/gram, and Aerated Effluent plants - 9.80 micrograms/gram).

The second harvest occurred 14 days later. At this time there was a slight decrease in the chromium content of all three treatments.

The third harvest 14 days later evidenced a sharp rise in the chromium content of the Effluent plants (to 43.19 micrograms/gram) and a lesser increase in the Aerated Effluent plants (19.46 micrograms/gram) and the Control plants (11.58 micrograms/gram).

At the time of the fourth harvest 21 days later the chromium content of the Aerated Effluent plants continued to increase to 50.24 micrograms/gram, while there was a slight drop in the chromium content of the Effluent plants (to 40.78 micrograms/gram. The Control plants continued to increase slowly in chromium content to 15.04 micrograms/gram.

The fifth harvest was taken 27 days later and again there were slight increases in the chromium content of the plants from all three treatments (Control plants - 15.43 micrograms/gram, Effluent plants - 46.71 micrograms/gram, and Aerated Effluent plants - 55.02 micrograms/gram.

Twenty-one days later (118th growing day) the sixth and final harvest was made. There was a fairly large increase in the chromium content of the Aerated Effluent plants (to 83.45 micrograms/gram) and lesser increases in the Effluent plants (to 53.45 micrograms/gram) and Control plants (to 27.14 micrograms/gram).

The analysis of variance and the new multiple range test indicated there were no significant differences in the chromium concentrations of the three treatments at the time of the first harvest (Table 7). This was the expected result since all plants had received the same treatment up to this time. There were significant differences in the chromium content between the Control plants and the Effluent plants at the second, fourth, fifth, and sixth harvests; and between the Control plants and the Aerated Effluent plants at the second, third, fourth, and sixth harvests. No significant differences in chromium concentrations were indicated between the Effluent and Aerated Effluent plants throughout the experiment.

The total accumulated chromium in the Control plants was close to a straight line increase until the sixth harvest when the increase was greater. The Control chromium content was at all times much less than the Effluent plants which at the third harvest began a steep rise and continued this rapid increase through the life of the project. The accumulated chromium concentrations of the Aerated Effluent plants were less than the other two treatments for the first two harvests, but began to increase rapidly after the second harvest and exceeded the Effluent plants by the sixth harvest (Table 8 and Figure 14).

7. Copper in Bermuda Grass

The conditions under which the bermuda grass was grown are discussed in Chapter III, Experimental Design and Procedures. The copper content of the bermuda grass is presented in Table 6 and Figure 13. At the

Table 7. Results From the New Multiple Range Test (Bermuda Grass)

Heavy Metal	Test	First Harvest	Second Harvest	Third Harvest	Fourth Harvest	Fifth Harvest	Sixth Harvest
Chromium	C - E	NS	NS	S	S	S	S
	C - AE	NS	NS	S	NS	S	S
	E - AE	NS	NS	S	S	NS	S
Copper	C - E	NS	S	NS	NS	NS	NS
	C - AE	NS	NS	NS	S	NS	NS
	E - AE	NS	NS	NS	NS	NS	NS
Iron	C - E	NS	NS	NS	NS	S	S
	C - AE	NS	NS	NS	NS	S	S
	E - AE	NS	NS	NS	NS	NS	S
Nickel	C - E	NS	S	S	S	S	NS
	C - AE	NS	S	S	S	NS	NS
	E - AE	NS	NS	S	S	S	NS
Zinc	C - E	NS	NS	S	NS	S	NS
	C - AE	NS	S	S	S	S	NS
	E - AE	NS	S	S	S	NS	NS

C = Control plants
E = Effluent plants
AE = Aerated Effluent plants
S = Significant difference
NS = No significant difference

Table 8. Accumulated Heavy Metals in Bermuda Grass (micrograms/gram)

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Chromium						
Control	9.60	17.54	28.52	43.56	58.99	86.14
Effluent	12.89	22.74	65.93	106.71	153.41	206.87
Aerated Eff.	9.80	14.22	33.68	83.92	138.94	222.39
Copper						
Control	14.57	29.05	44.97	61.39	81.08	96.00
Effluent	12.79	24.53	43.31	57.29	78.19	94.31
Aerated Eff.	13.29	26.16	40.60	52.46	65.83	82.47
Iron						
Control	157.00	264.35	348.35	455.38	544.87	931.79
Effluent	141.64	256.08	357.38	473.74	620.63	949.10
Aerated Eff.	116.34	240.16	324.83	425.20	564.77	878.41
Nickel						
Control	67.34	87.08	150.07	215.94	275.61	487.96
Effluent	58.45	124.64	351.82	492.52	798.23	954.48
Aerated Eff.	44.48	92.81	217.95	340.35	451.66	773.02
Zinc						
Control	60.70	120.37	171.94	237.99	303.90	464.76
Effluent	57.10	121.56	205.46	267.40	386.94	570.33
Aerated Eff.	52.70	138.14	235.27	364.72	462.61	586.31

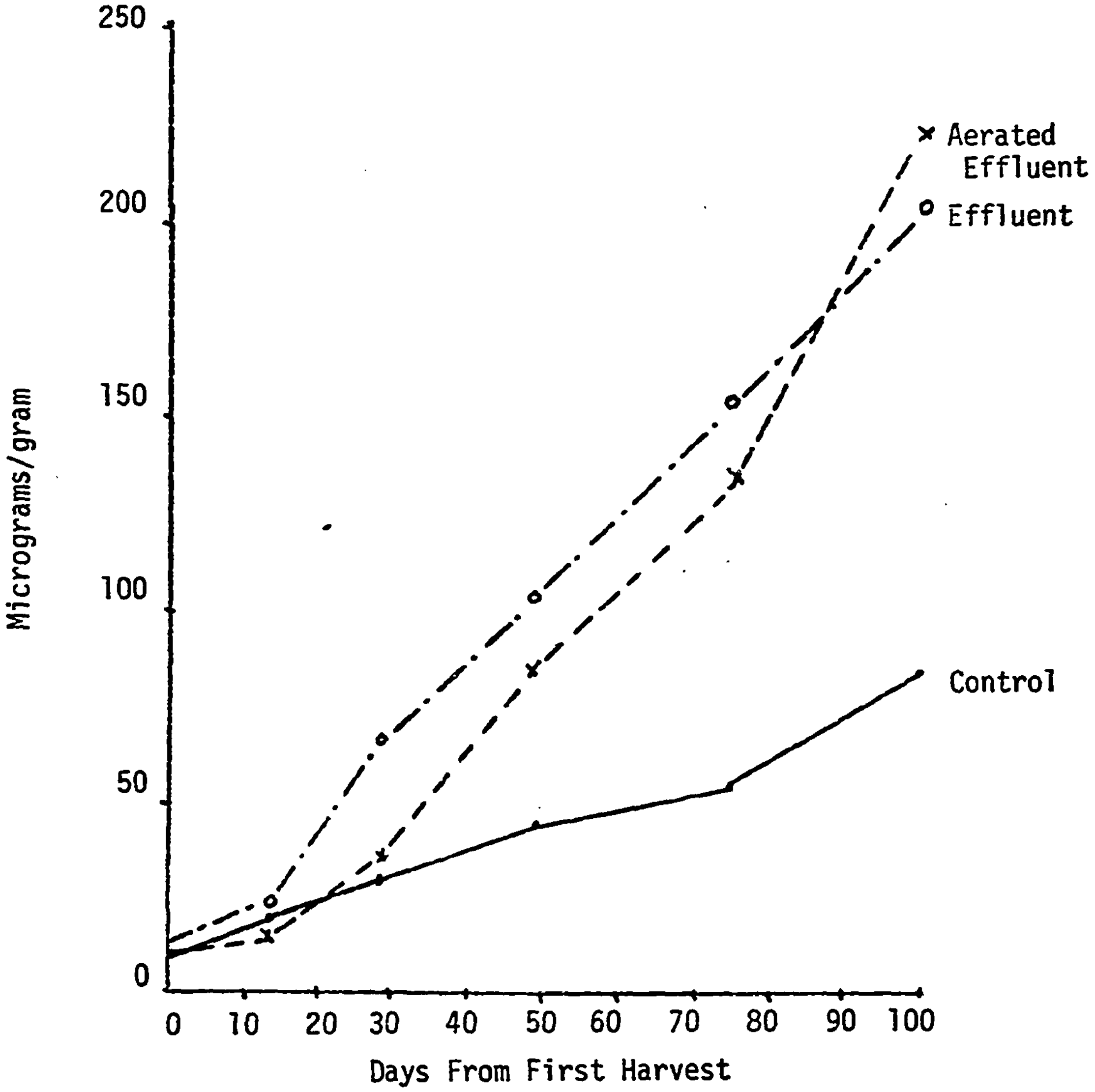

Figure 14. Accumulated Chromium in Bermuda Grass

first harvest the copper content was similar in all three treatments (Control - 14.57 micrograms/gram, Effluent plants - 12.79 micrograms/gram, and Aerated Effluent plants - 13.29 micrograms/gram).

At the second harvest the values were quite similar (Control plants - 14.48 micrograms/gram, Effluent plants - 11.75 micrograms/gram, and Aerated Effluent plants - 12.87 micrograms/gram). While the copper content of the Control plants varied very little from the first harvest, there was a decrease from the first harvest in the other two treatments.

At the third harvest there was an increase in the copper content of all three treatments. However, the increase was most marked in the Effluent plants (18.77 micrograms/gram) compared to the Control plants (15.92 micrograms/gram) and the Aerated Effluent plants (14.44 micrograms/gram).

There was a continued increase in the copper content of the Control plants (16.42 micrograms/gram) at the fourth harvest, while there was a decrease in the copper content of the Effluent plants to 13.86 micrograms/gram.

The copper content of all three treatments continued to increase at the time of the fifth harvest. The largest increase was in the Effluent plants (20.90 micrograms/gram) with a lesser increase in the Aerated Effluent plants (13.37 micrograms/gram) and Control plants (19.69 micrograms/gram).

At the time of the sixth harvest there was a decrease in the copper content of both the Control plants (to 14.91 micrograms/gram) and the Effluent plants (to 16.12 micrograms/gram). There was, however, a continued increase in the copper content of the Aerated Effluent plants (to 16.64 micrograms/gram).

The analysis of variance and the new multiple range test indicated no significant differences existed in the copper content of plants in the three treatments throughout the experiment, except between the Control plants and Effluent plants at the time of the second harvest (Table 7).

The total accumulated copper content of the Control plants evidenced an almost straight line increase on the graph in Figure 15. The other two treatments had slightly flatter slopes on the graph showing a slower rate of increase of copper content. The accumulated copper content of the Effluent and Aerated Effluent plants appeared to be somewhat depressed when compared to the copper content of the Control plants. In general the copper content of the Effluent plants was greater than that of the Aerated Effluent plants (Table 8).

8. Iron in Bermuda Grass

The iron content of bermuda grass is presented in Table 6 and Figure 16. The iron content at the time of the first harvest was very similar in the three treatments (Control plants - 157.00 micrograms/gram, Effluent plants - 141.64 micrograms/gram, and Aerated Effluent plants - 116.34 micrograms/gram).

At the time of the second harvest the iron content had dropped in the Control plants and the Effluent plants (to 107.35 micrograms/gram and 114.44 micrograms/gram respectively). The iron content of the

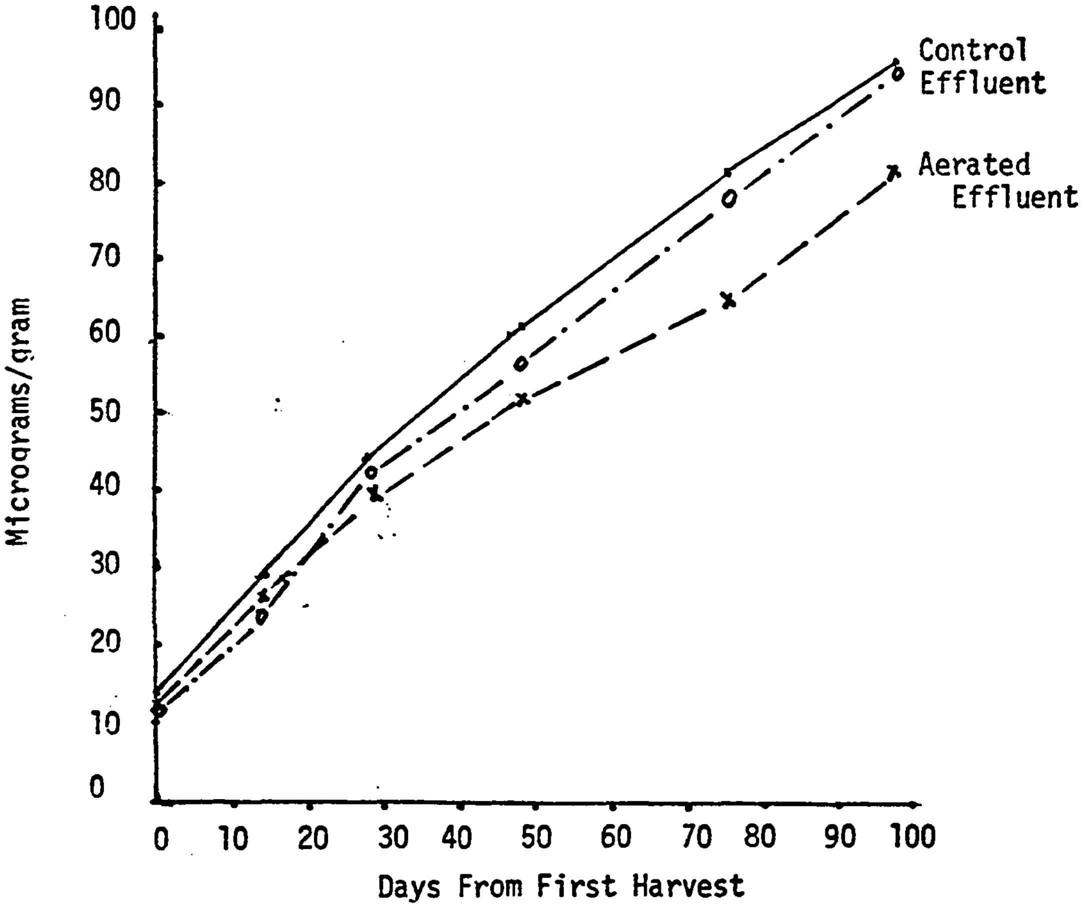

Figure 15. Accumulated Copper in Bermuda Grass

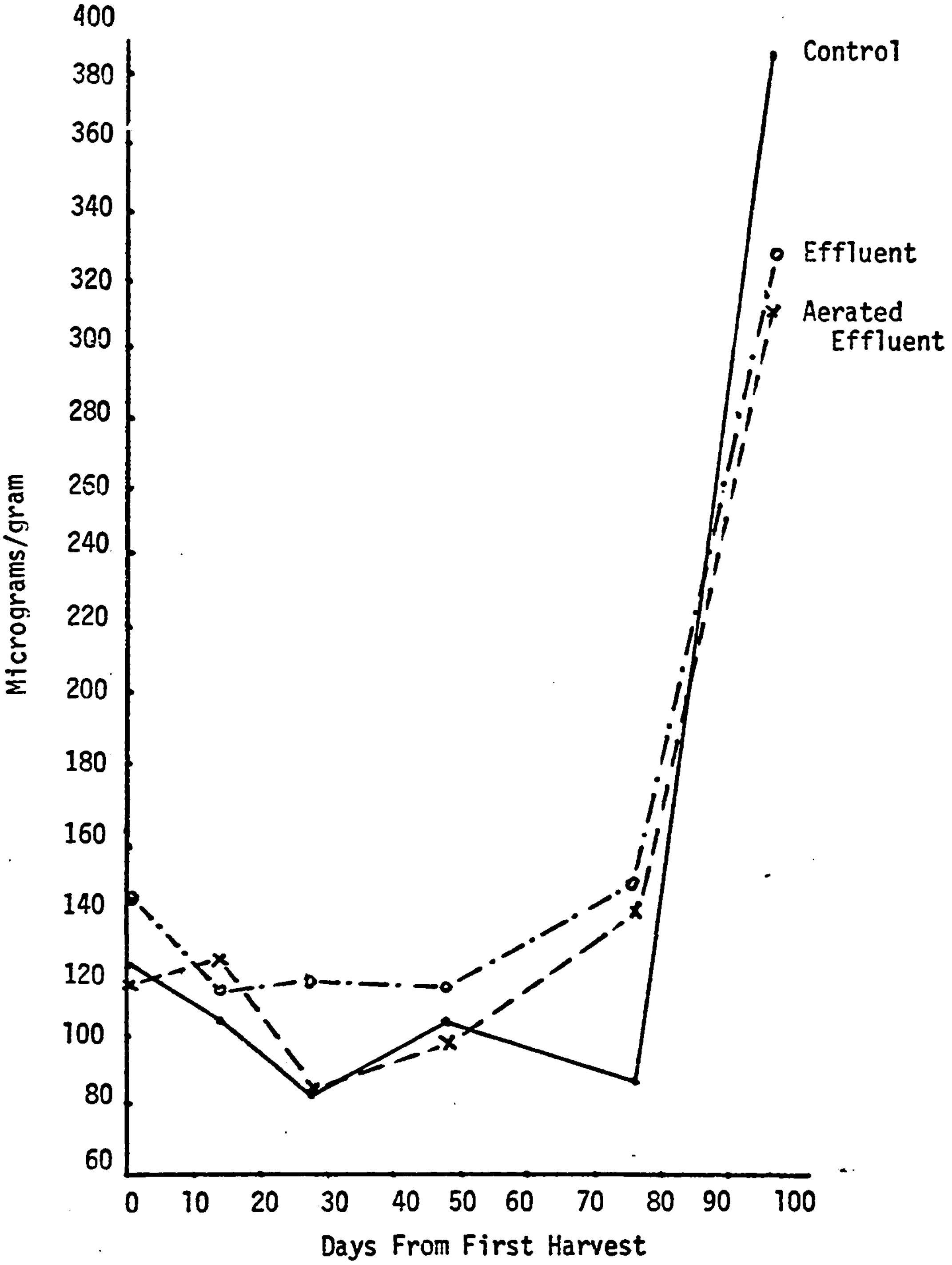

Figure 16. Iron Concentration in Bermuda Grass (Normalized)

Aerated Effluent plants had increased to 123.82 micrograms/gram. Again the values for all three treatments are similar.

At the third harvest there was a decrease in the iron content of the Control plants (84.00 micrograms/gram), the Aerated Effluent plants (84.67 micrograms/gram), and the Effluent plants (101.30 micrograms/gram).

There was an increase in the iron content of the Control plants (to 107.03 micrograms/gram), the Aerated Effluent plants (to 100.37 micrograms/gram), and the Effluent plants (to 116.45 micrograms/gram) at the fourth harvest.

At the fifth harvest there was a decrease in the iron content of the Control plants to 89.46 micrograms/gram. The iron content of the Effluent plants and the Aerated Effluent plants increased to 146.89 micrograms/gram and 139.57 micrograms/gram, respectively.

The sixth harvest showed a large increase in all three treatments (Control plants - 386.10 micrograms/gram, Effluent plants - 328.95 micrograms/gram, and Aerated Effluent plants - 313.64 micrograms/gram). The value of these figures is, however, questionable due to the small sample size.

The analysis of variance and the new multiple range test indicated no significant differences in iron content of the three treatments in harvests one through four (Table 7). At the fifth harvest there were significant differences in the iron concentration between the Control and Effluent plants and between the Control and Aerated Effluent plants. There were significant differences of iron content among all three treatments at the time of the sixth harvest.

The curves on the graph of the accumulated iron content are similar in shape for all three treatments (Table 8 and Figure 17).

9. Nickel in Bermuda Grass

The nickel concentrations of the bermuda grass are presented in Table 6 and Figure 18. The nickel content of the three treatments at the first harvest were very similar (Control plants -67.34 micrograms/gram, Effluent plants 58.45 micrograms/gram, and Aerated Effluent plants - 44.48 micrograms/gram).

At the second harvest there was a decrease in the nickel content of the Control plants (to 19.73 micrograms/gram) and an increase in the Effluent plants (to 66.18 micrograms/gram) and the Aerated Effluent plants (to 48.33 micrograms/gram).

There was an increase in all three treatments at the third harvest (Control plants - 63.00 micrograms/gram, Effluent plants - 227.19 micrograms/gram, and Aerated Effluent plants - 125.14 micrograms/gram).

At the fourth harvest the nickel content remained essentially constant in the Control plants. The nickel content decreased in both the Effluent and the Aerated Effluent plants to 141.27 micrograms/gram and 116.96 micrograms/gram respectively.

There was a decrease in the nickel content of the Aerated Effluent and Control plants at the fifth harvest (Aerated Effluent plants - 111.31 micrograms/gram and Control plants - 59.67 micrograms/gram). The nickel content increased to 305.71 micrograms/gram in the Effluent plants.

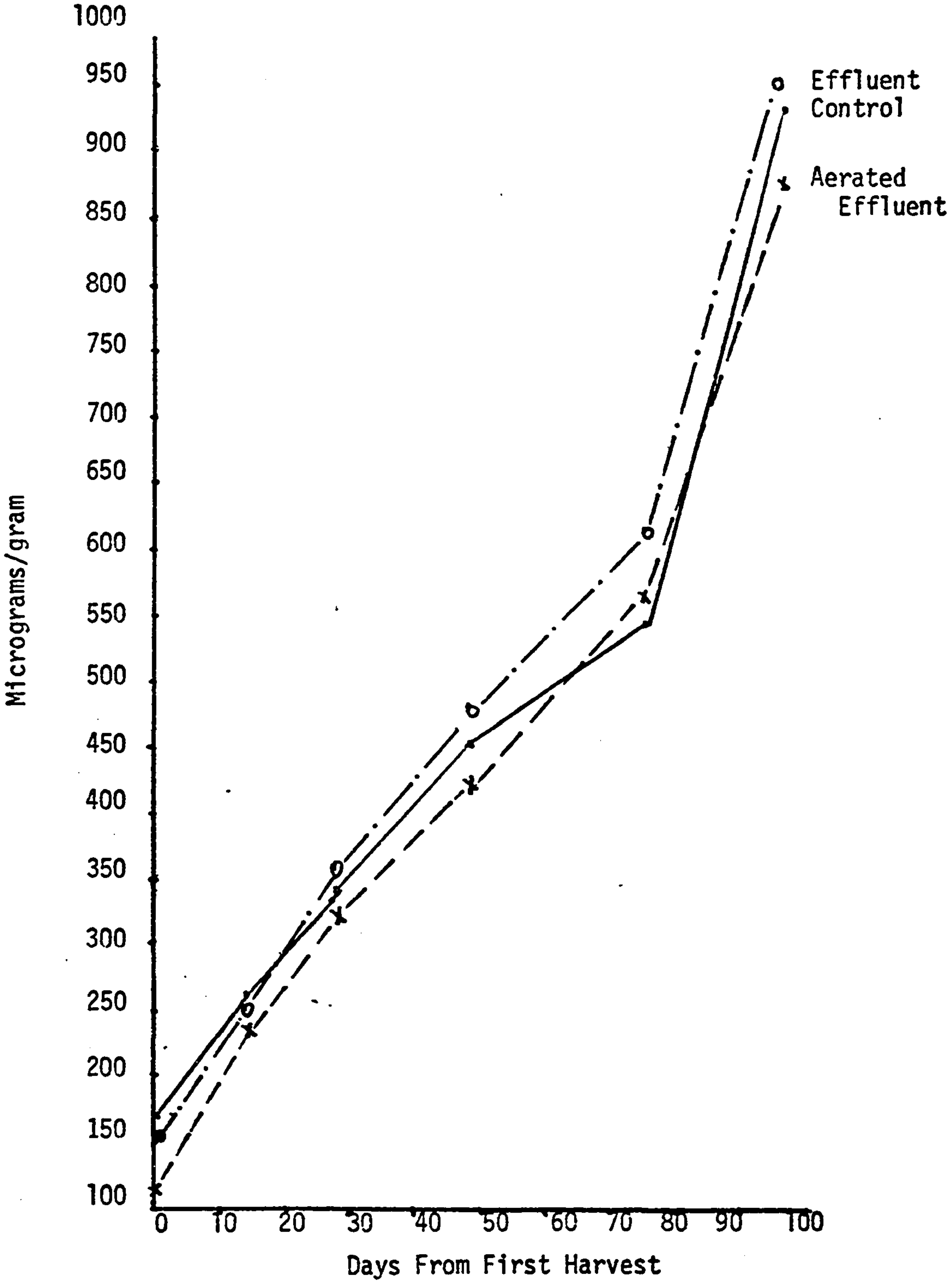

Figure 17. Accumulated Iron in Bermuda Grass

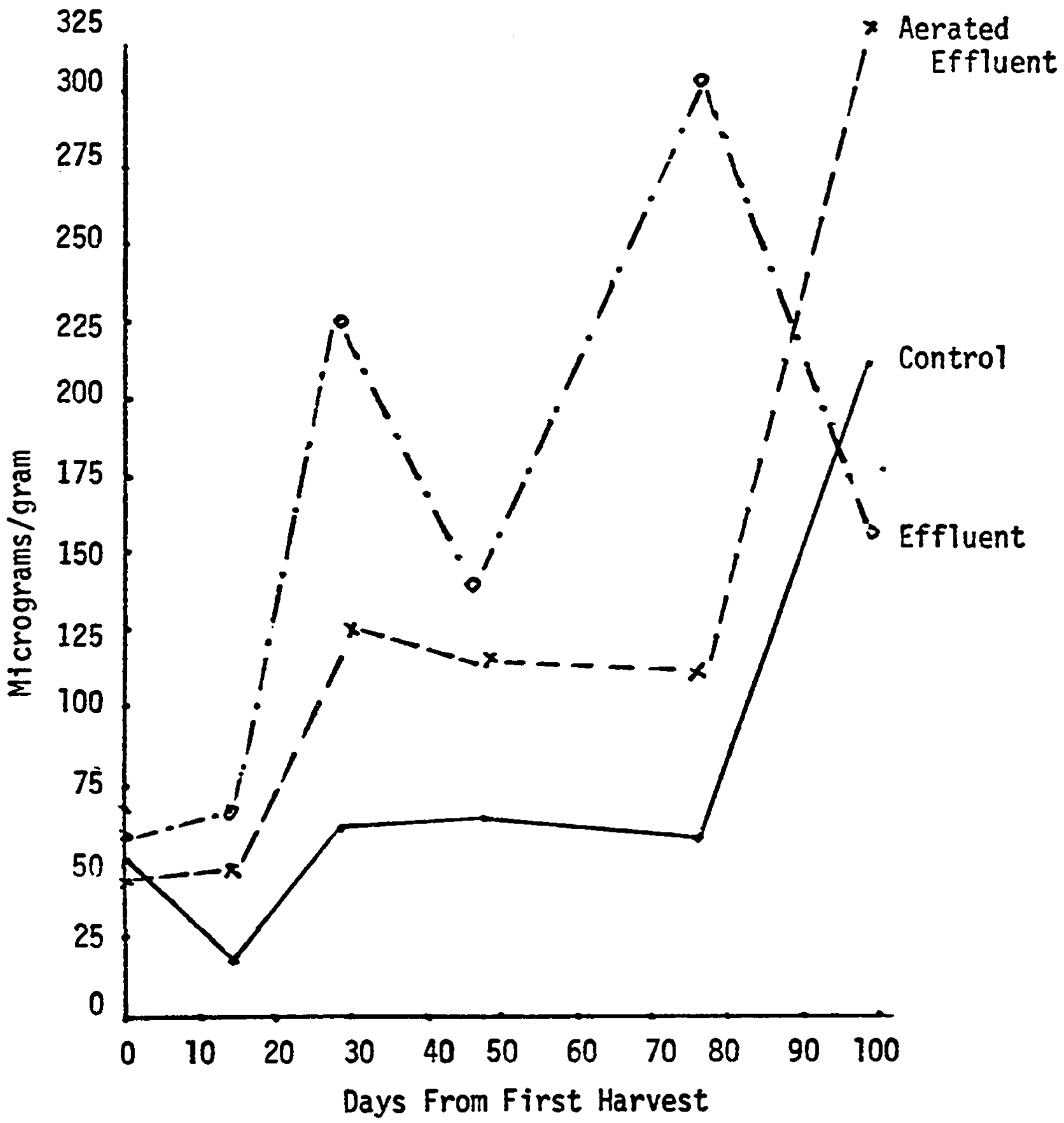

Figure 18. Nickel Concentration in Bermuda Grass (Normalized)

At the final harvest there were large increases in the nickel content of the Control plants and the Aerated Effluent plants to 212.36 micrograms/gram and 321.37 micrograms/gram respectively. There was, however, a decrease in the nickel content of the Effluent plants to 156.35 micrograms/gram. The results of this harvest are suspect due to the small sample size.

The analysis of variance and the new multiple range test indicated no significant differences in nickel concentration existed at the time of the first harvest (Table 7). However, significant differences in nickel concentrations were indicated between the Control plants and the Effluent plants from the second harvest through the fifth harvest; between the Control plants and the Aerated Effluent plants from the seocnd harvest through the fifth; and between the Effluent and Aerated Effluent plants from the third harvest through the fifth harvest.

The shape of the curves on the graph of accumulated nickel content were very similar for all three treatments. However, the Effluent plants accumulated much more nickel than did the plants subjected to the other two treatments (Table 8 and Figure 19).

10. Zinc in Bermuda Grass

At the first harvest the zinc content of the plants of the three treatments was similar (Table 6 and Figure 20). By the second harvest there was a slight increase in the zinc content of the Control plants and the Effluent plants and a much larger increase in the zinc content of the Aerated Effluent plants. THe third harvest showed a

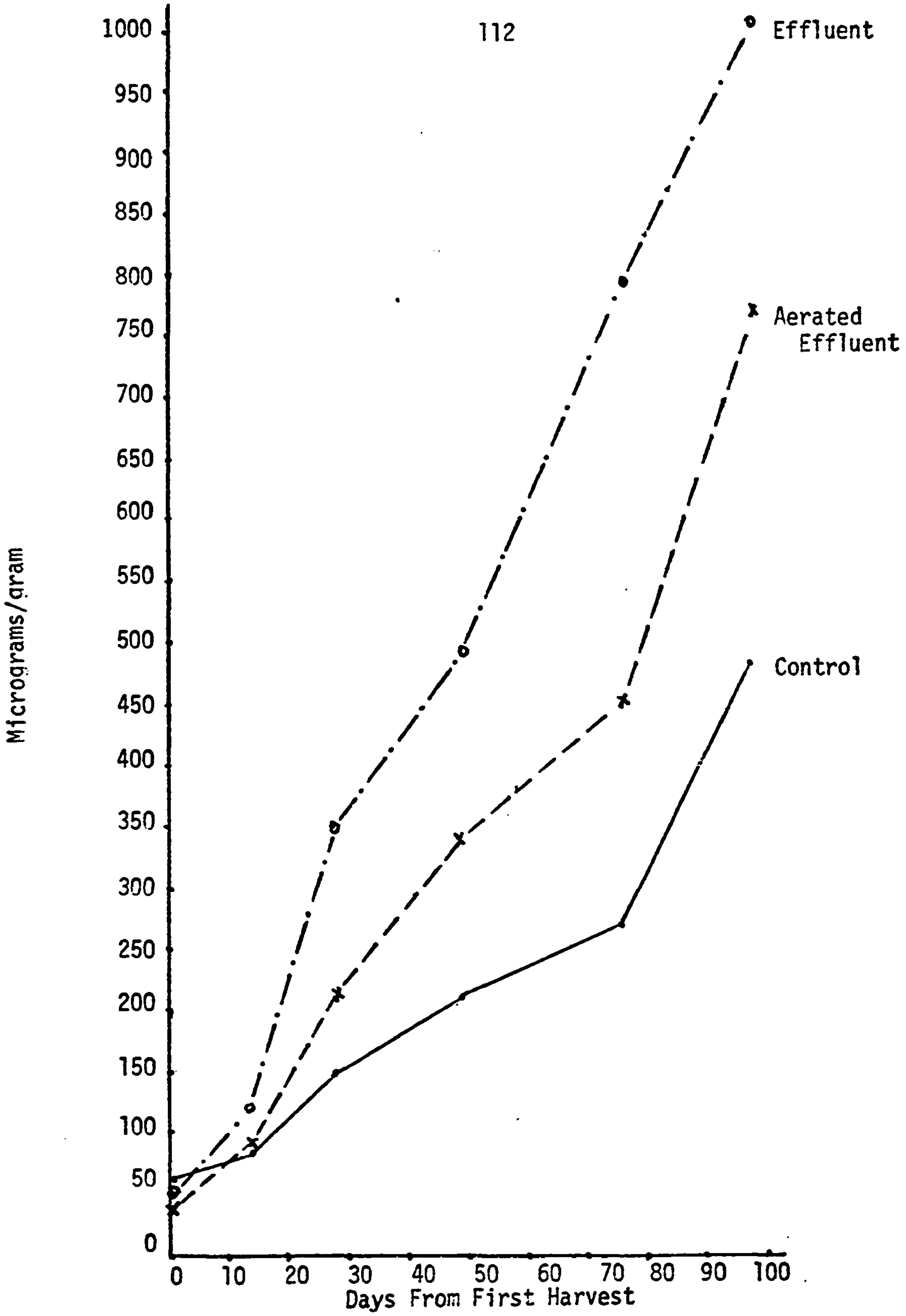

Figure 19. Accumulated Nickel in Bermuda Grass

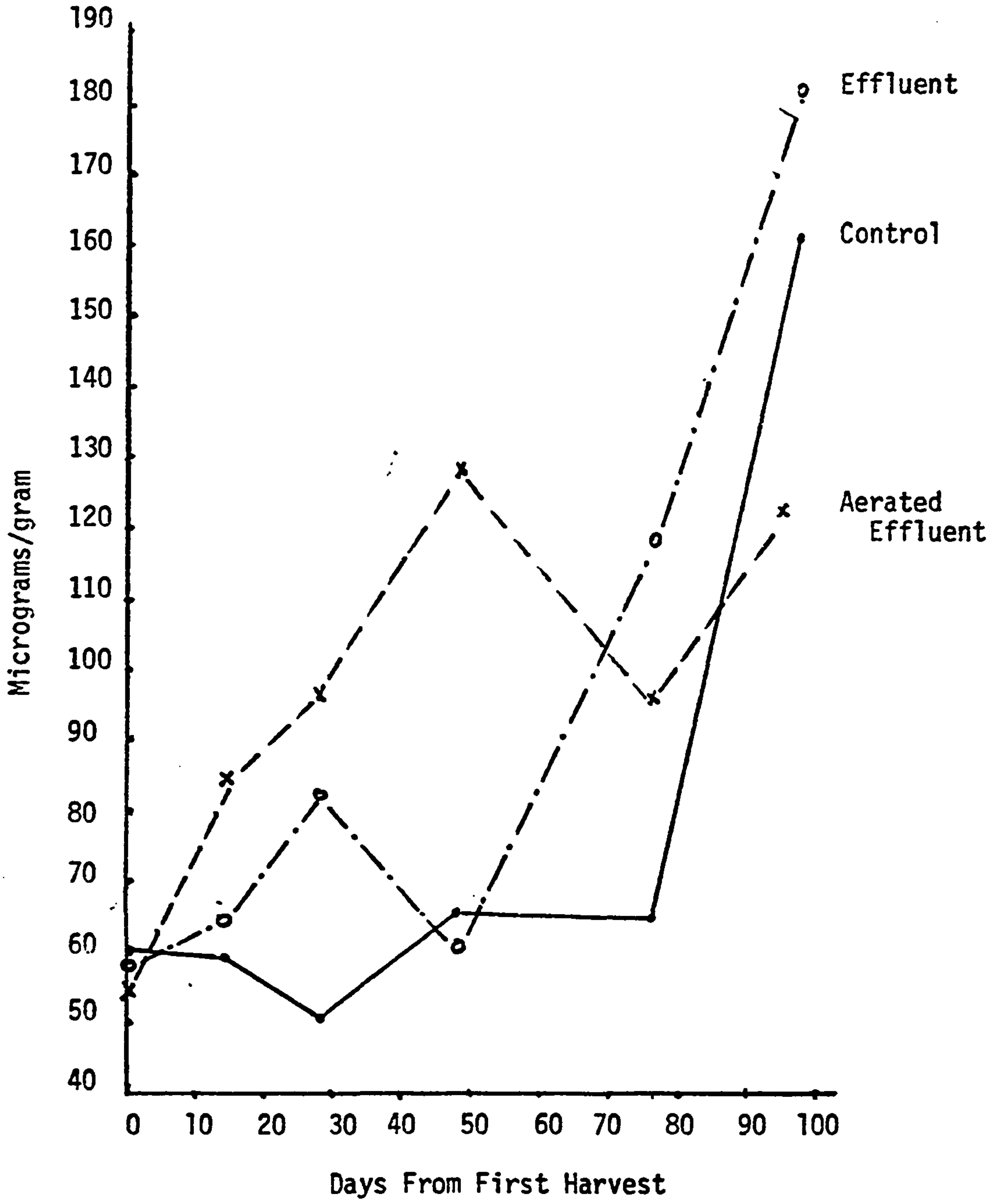

Figure 20. Zinc Concentration in Bermuda Grass (Normalized)

slight decrease in the Control plants, while there were increases in the zinc content of the Effluent and Aerated Effluent plants. There was a decrease in the zinc content of the Effluent plants at the fourth harvest and an increase in the zinc content of the Aerated Effluent and Control plants. A reversal of the trend was noted at the fifth harvest with a decrease in the Control and Aerated Effluent plants and an increase in the zinc contnet of the Effluent plants. At the final harvest there were increases in the zinc content of the plants of all three treatments. The results of the sixth harvest are somewhat unreliable due to the small sample size.

The analysis of variance and the new multiple range test indicated that no significant differences in zinc content existed at the time of the first harvest (Table 7). Significant differences in zinc content were indicated between the Control and Effluent plants for the third and fifth harvests; between the Control and Aerated Effluent plants from the second through the fifth harvests; and between the Effluent and Aerated Effluent plants from the second through the fourth harvests.

The accumulated zinc content was the greatest in the Aerated Effluent plants, followed closely by the Effluent treated plants. The Zinc content was lowest in the Control plants (Table 8 and Figure 21).

11. Chromium-51 in Wheat and Bermuda Grass

Twelve pots each of wheat and bermuda grass were planted and watered with a chromium-51 solution after an initial period of watering with deionized water to allow the plants to establish themselves,

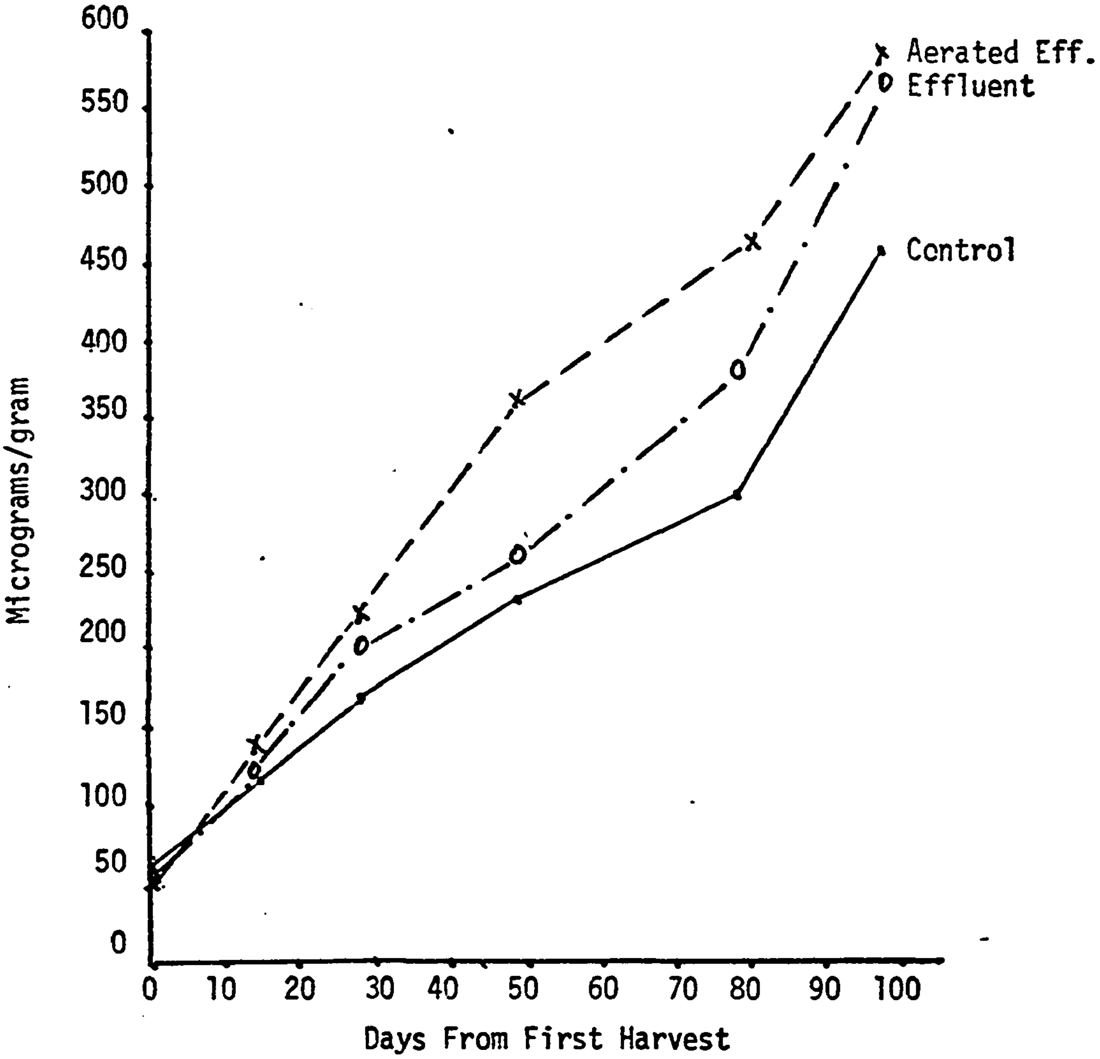

Figure 21. Accumulated Zinc in Bermuda Grass

The data was compiled and a ratio (CPM/100 g plant material)/(CPM/ml Cr-51 solution added) was calculated. The results appear in Table 9 and Figures 22 and 23. The ratio is higher for wheat than bermuda grass except at the first and sixth harvests. The ratio increases and decreases from harvest to harvest in a manner similar to the increase and decrease of chromium concentrations in wheat and bermuda grass previously presented.

The shapes of the curves on the graphs of the chromium-51 ratio compared to that of the chromium concentrations in wheat and bermuda grass appear somewhat different, but most of this difference can be attributed to the fact that eight harvests were made on the chromium-51 wheat compared to six on the non-radioactive wheat. In the main the shape of the curves are essentially the same.

There is less similarity between the graphs of the chromium-51 ratio in bermuda grass and the chromium content in the non-radioactive bermuda grass.

Table 9. Chromium-51 Ratio in Wheat and Bermuda Grass

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest	7th Harvest	8th Harvest
Wheat	0.0	188.62±6.8	352.93±13.8	226.7±13.0	264.35±9.9	201.70±5.2	405.77±11.9	459.11±14.6
Bermuda Grass	0.0	207.12±17.5	110.26±7.4	145.78±10.7	124.04±26.6	354.66±14.8		

Table 10. Accumulated Chromium-51 in Wheat and Bermuda Grass

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest	7th Harvest	8th Harvest
Wheat	0.0	188.62	541.56	768.24	1032.59	1234.29	1640.06	2099.17
Bermuda Grass	0.0	207.12	317.38	463.16	587.20	941.86		

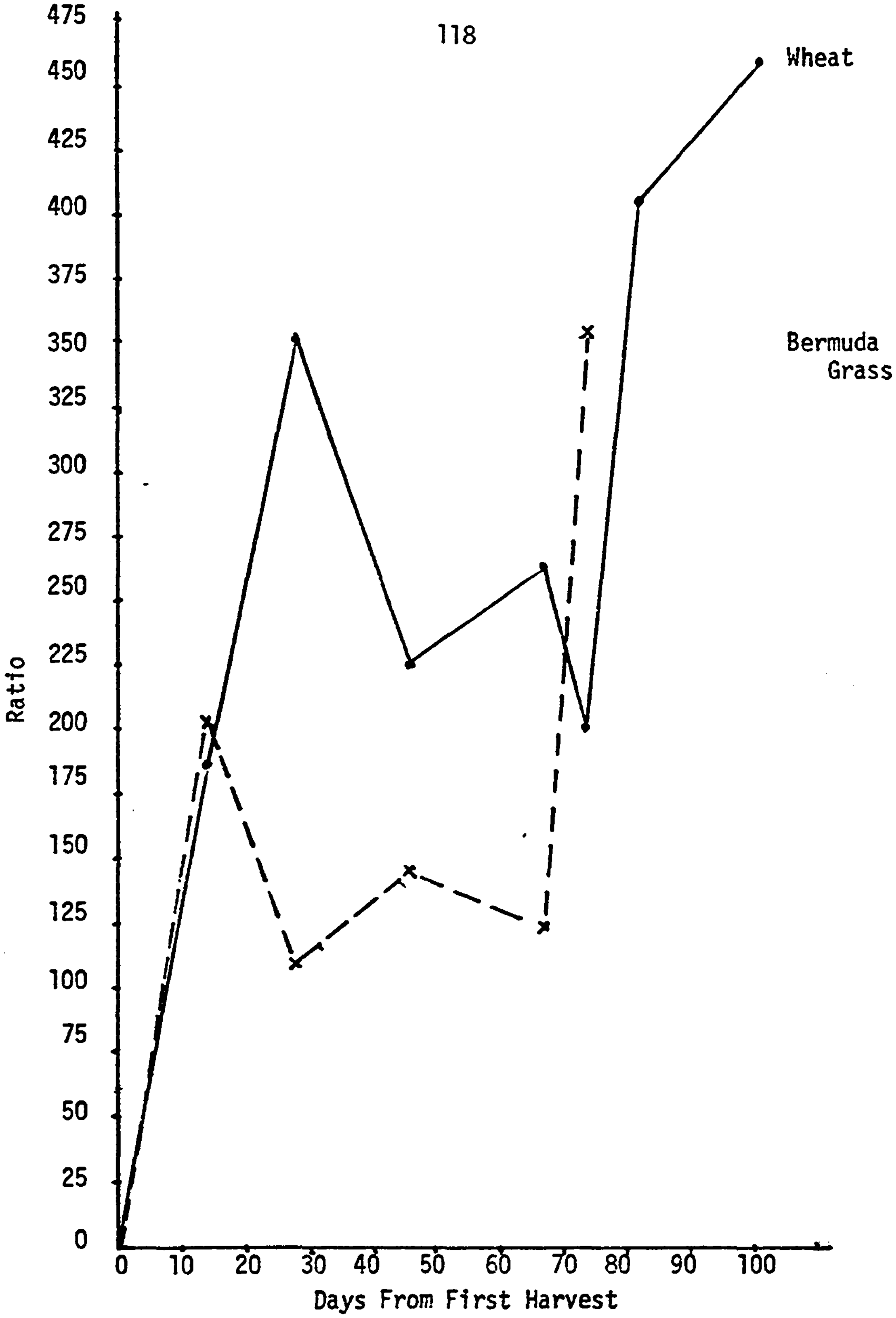

Figure 22. Chromium-51 Ratio in Wheat and Bermuda Grass

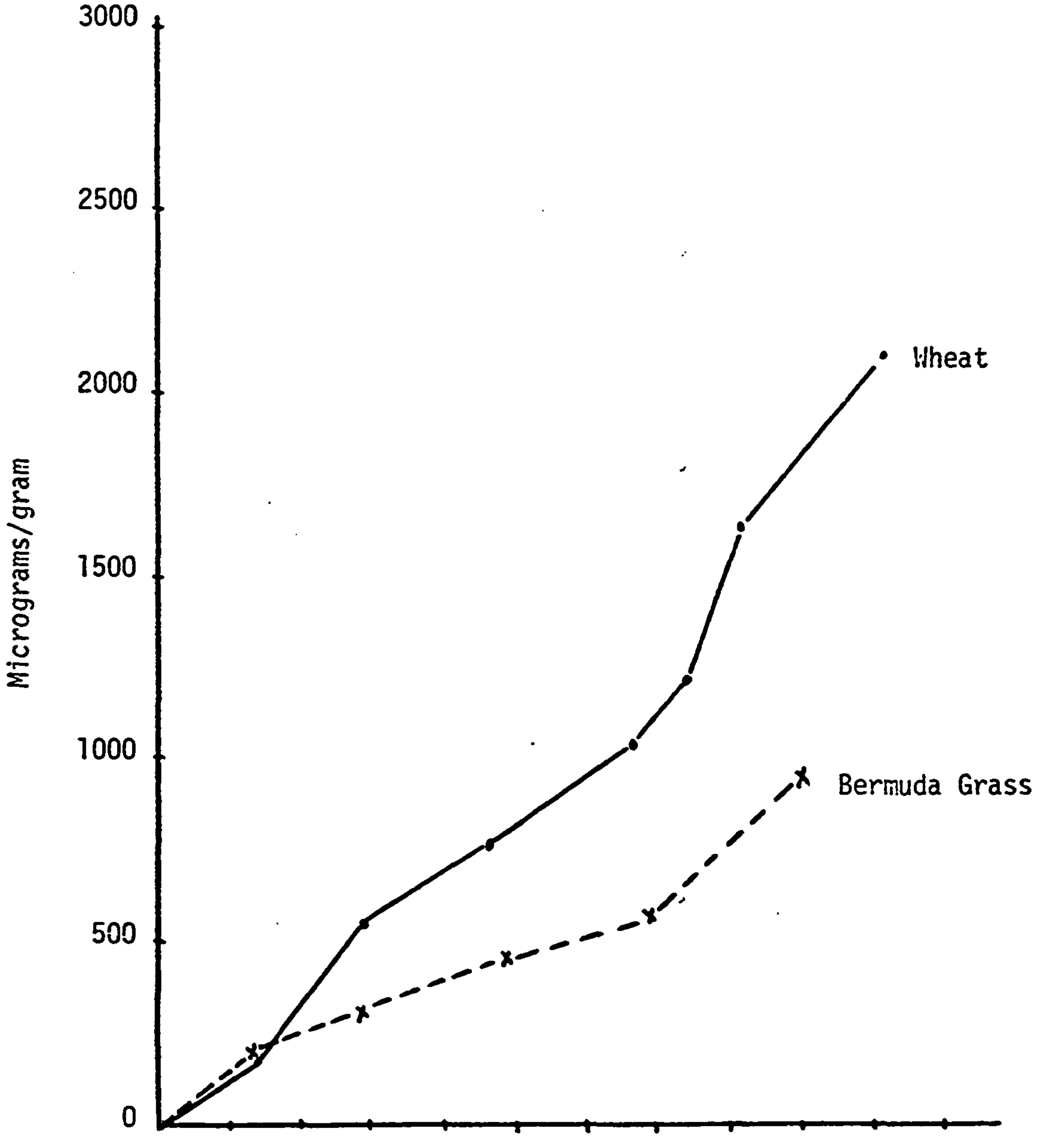

Figure 23. Accumulated Chromium-51 Ratio in Wheat and Bermuda Grass

CHAPTER V

DISCUSSION

A. Introduction

At the beginning of the project the wheat and bermuda grass plants were in good condition. The wheat was thick, green and tall. The bermuda grass was less luxurous, but healthy in appearance. The die off of the wheat plants at the time of the second harvest ranged from 11 to 24 percent. The dead plants were stunted and off-white in color. The living plants were green and showed no visible symptoms of toxicity or deficiency. The mortality rate at this time could have been due partially to natural thinning. The mortality rate at the time of the third harvest ranged from 32 to 37 percent (Table 11), essentially the same for all treatments. However, by the fourth harvest greater differences were detectable. The mortality rates for the Control plants, Effluent plants, and Aerated Effluent plants were 41%, 60% and 80% respectively. At the fifth harvest 61% of the Control plants had died, while 91% of the Effluent plants and 93% of the Aerated Effluent plants were dead. By the sixth harvest 75% of the Control plants were dead, but 99.5% of the Effluent and Aerated Effluent plants had died. As the experiment progressed, the Effluent and Aerated Effluent plants had less growth than the Control

Table 11. Wheat Mortality (%)

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Percent Living						
Control	100	76	68	59	39	25
Effluent	100	85	67	40	9	0.5
Aerated Effluent	100	89	63	20	7	0.5
Percent Dead						
Control	0	24	32	41	61	75
Effluent	0	15	33	60	12	99.5
Aerated Effluent	0	11	37	80	93	99.5

Table 12. Bermuda Grass Yield (% of first harvest)

	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	6th Harvest
Control	100	331	87	108	125	50
Effluent	100	271	63	77	31	13
Aerated Effluent	100	207	56	37	17	11

plants. The living plants, however, remained bright green in all experiments and turned a bleached greyish white upon death. There was no evidence of intervenal chlorosis, necrotic spots, or changes in color of leaf margins. Growth was retarded in the Effluent and Aerated Effluent plants. The high mortality rate in the Control plants could be due to the fact that the pots containing these plants were dropped prior to the fifth harvest.

The bermuda grass was less easy to analyze because the individual plants could not be distinguished. In general the yields decreased more rapidly and to a greater extent in the Effluent and Aerated Effluent plants than in the Control plants (Table 12). As with the wheat the plants appeared healthy until death. At the time of death the leaves were an off-white color. The treated plants had reduced growth.

Throughout the experiment the effluent contained higher concentrations of all five heavy metals than did the aerated effluent. It would appear that aeration caused precipitation of some of these heavy metals and they settled to the bottom of the container (Tables A-1 & A-2).

In spite of the fact that over 10 times as much chromium was added per pot of Effluent wheat as per pot of Aerated Effluent wheat (541.06 micrograms to Effluent Wheat and 45.42 micrograms to Aerated Effluent wheat) the chromium range over the length of the study was remarkably similar (5.40-74.47 micrograms/gram in the Effluent wheat and 4.36-80.12 micrograms/gram in the Aerated Effluent wheat). The same was true for copper, iron, nickel and zinc in both wheat and bermuda grass

(Tables 13 and 14). It would appear that the heavy metals in the aerated effluent were in a more available form to the plants than those in the effluent.

B. Chromium

1. Wheat

The chromium concentration of the leaves and stems of the Control plants ranged from 4.30 ppm to 15.57 ppm. The chromium concentration of the Effluent plants ranged from 4.50 ppm to 74.57 ppm, and that of the Aerated Effluent plants ranged from 4.36 ppm to 80.12 ppm.

The literature is deficient in concentrations that cause toxicity in wheat. Concentrations of chromium in normal wheat plants range from 4.5 ppm to 14.8 ppm. The Effluent plants and Aerated Effluent plants are 6 to 7 times the level reported in the literature. However, from the literature it is not possible to state that the levels of chromium in the treated plants were toxic.

2. Bermuda Grass

The chromium content of the Control plants ranged from 7.34 ppm to 27.14 ppm and the chromium concentration of the Effluent treated plants and the Aerated Effluent plants ranged from 9.85 to 53.45 ppm and 4.42 ppm to 83.45 ppm respectively. As with wheat the literature is incomplete concerning the toxic concentration levels of chromium in bermuda grass. It is not possible to state whether the chromium content of the Effluent and Aerated Effluent plants reached toxic levels prior to the

Table 13. Average Heavy Metals Added to Each Pot (micrograms)

Plant and Treatments	Chromium	Copper	Iron	Nickel	Zinc
Wheat					
Effluent	541.06	358.72	510.39	867.82	209.96
Aerated Effluent	45.42	131.15	315.07	32.22	60.57
Bermuda Grass					
Effluent	683.17	373.12	383.39	1072.85	235.23
Aerated Effluent	23.62	100.47	196.02	17.87	44.00

Table 14. Range of Heavy Metals in Each Treatment (micrograms/gram)

	Chromium	Copper	Iron	Nickel	Zinc
Wheat					
Effluent	5.4-74.5	6.9-21.9	71.7-378.2	13.8-525.2	43.8-115.0
Aerated Eff.	4.4-80.1	11.0-21.9	83.1-301.6	14.2-513.5	42.2-91.4
Bermuda Grass					
Effluent	9.9-52.5	11.8-20.9	101.3-328.9	58.5-305.7	57.1-183.4
Aerated Eff.	4.4-83.5	11.9-16.7	100.4-313.6	44.3-321.4	52.7-219.5

end of the growth period, although it was several times the concentration normally found in many plants.

3. Conclusions

The new multiple range test (Table 4) indicated that the chromium concentrations of the wheat Effluent plants were significantly different from the wheat Control plants at the second, fourth, fifth and sixth harvests and that there were significant differences in the chromium levels between the Aerated Effluent and Control plants at the second through fourth harvests and at the sixth harvest. Although toxic chromium levels for wheat were not listed in the literature, the chromium concentrations in the Effluent and Aerated Effluent plants appear to be toxic when compared to the toxic levels listed for other species. However, it must be noted that the chromium toxicity level is probably species dependent.

The new multiple range test (Table 7) indicated that significant differences in chromium concentrations existed between the bermuda grass Control and Effluent plants from the third harvest through the sixth and that the chromium content was significantly different between the Control and Aerated Effluent plants at the third, fifth and sixth harvests. There were also significant differences in chromium levels between Effluent and Aerated Effluent plants on the third, fourth and sixth harvests. As in the case of wheat, it is possible that the concentrations of chromium in the Effluent and Aerated Effluent plants were toxic. However, this cannot be determined by comparison with the literature. Toxic levels of chromium in bermuda grass have not been reported.

Chromium is one of the few essential elements (for animal life) for which no accumulation against a concentration gradient is evident at any point in the biological cycle from soil to plant to animal (Allaway, 1968). A large increase in available chromium in the soil may result in only a modest increase in the chromium concentration in plants (Allaway, 1968). Chromium appears to be concentrated in the roots of plants (Pratt, 1966 and NSA, 1974).

The irrigation of vegetables with sewage wastewaters containing chromium resulted in increased plant absorption of chromium by a factor of three to ten times over that of controls. The reason for this increase might be the efficient plant absorption of continuously provided chelated chromium (Lisk, 1972). Similar results were obtained in this study.

C. Copper

1. Wheat

The copper concentration of the Control plants ranged from 5.80 ppm to 13.00 ppm. That of the Effluent treated plants ranged from 6.97 ppm to 21.85 ppm and for the Aerated Effluent treated plants the copper concentration range was from 11.00 ppm to 21.89 ppm.

The literature indicates that the copper concentration in wheat straw necessary for normal growth ranges from 9-18 ppm (Reuther and Labanauskas, 1966). Copper deficiency results when copper is less than 8.5 ppm (Reuther and Labanauskas, 1966) and that toxicity symptoms result if the copper content is in excess of 20 ppm (Allaway, 1968).

However, these concentrations may vary from species to species and for varieties within a species.

2. Bermuda Grass

The copper content of the control plants ranged from 14.48 ppm 59.19.69 ppm. The copper concentration of the Effluent plants ranged from 11.75 ppm to 20.90 ppm and that of the Aerated Effluent treated plants ranged from 11.85 ppm to 16.66 ppm.

The literature indicates that the copper concentration necessary for normal plant growth ranges from 5-20 ppm (Allaway, 1968). The copper concentration of the Control plants falls within this range. The literature indicates that copper concentrations in excess of 20 ppm are possibly toxic (Allaway, 1968). The Effluent plants fall very close to this concentration (20.90 ppm).

3. Conclusions

The new multiple range test (Table 4) indicated that no significant differences existed in the three treatments of the wheat plants throughout the project.

The copper content of the wheat plants neared or slightly exceeded the toxic level reported by Allaway (1968) for most plants. It is possible that copper may have played some part in the death of some of the plants. It is impossible to tell from the results of this particular research.

The new multiple range test (Table 7) indicated only one instance of significantly different copper levels throughout the growth period for bermuda grass. This was between the Control and Effluent plants at the time of the second harvest.

The copper concentration in the bermuda grass fell within the range for normal growth of most plants (Allaway, 1968). It is therefore concluded that plant mortality in the bermuda grass was probably not due to copper deficiency or toxicity.

It has been reported by Antonovics (1971) that copper uptake in the above ground parts stayed low and constant at low levels of soil copper, but at certain higher soil copper levels this resistance to uptake appears to break down. Above this level the concentration in the plant tops increased rapidly and at only slightly higher levels of copper in the soil phytotoxicity occurred.

This research bears out the report by Antonovics et al in that the copper content of the leaves varied little throughout the growing period. However, the level of soil copper necessary for a breakdown of the uptake resistance does not appear to have been reached for either wheat or bermuda grass.

The soil copper was relatively low, ranging from 2.0 ppm to 6.8 ppm. The literature indicates that the normal range of copper in soil is from 2 to 200 ppm (Allaway, 1968).

D. Iron

1. Wheat

The iron content of the Control plants ranged from 69.48 to 158.83 ppm. The iron concentration of the Effluent plants ranged from

71.71 ppm to 378.15 ppm and the iron concentration of the Aerated Effluent plants ranged from 83.08 ppm to 301.56 ppm.

The literature indicates that iron concentrations less than 56 ppm cause deficiency symptoms in some plants (Wallihan, 1966). The Control plants fall within the intermediate iron range as shown by Wallihan (1966). Sauchelli (1969) states that the amount of iron in the leaves of a normal plant will average a few hundred parts per million, the amount hardly varying. Melsted (1973) reported the iron content of common agronomic crops as 20-300 ppm with a suggested tolerance level of 750 ppm. Iron toxicity is usually unknown under natural conditions (Wallihan, 1966).

2. Bermuda Grass

The iron concentration in the Control plants varied from 84.00 ppm to 386.10 ppm. The iron concentration in the Effluent treated plants ranged from 114.44 ppm to 328.95 ppm and that in the Aerated Effluent plants ranged from 84.68 ppm to 313.64 ppm.

The literature indicates the average iron concentration of pasture grasses is 100-200 ppm. Melsted (1973) reported concentrations from 20-300 ppm iron in common agronomic crops.

3. Conclusions

The new multiple range test (Table 4) indicated that significant differences existed in the wheat plants between the Control and Effluent plants from the second harvest through the sixth, between the Control and Aerated Effluent plants at all harvests except the first and third, and between the Effluent and Aerated Effluent plants at the second, third and sixth harvests.

It would appear from a comparison of experimental results and literature that the iron content of the plants in all three treatments at all harvest dates was within the range for normal plant growth. However, there was an increase in iron concentrations for the two treated groups as compared to the Control plants.

The same was true for the iron concentration in the bermuda grass. The new multiple range test (Table 7) indicated that significant differences existed in the bermuda grass between the Control and Effluent plants and the Control and Aerated Effluent plants at the fifth and sixth harvests, and between the Effluent and Aerated Effluent plants at the sixth harvest. The iron concentrations of all three treatments fell within the range for normal plant growth.

The iron concentrations in the soil ranged from 4,000 ppm to 7,000 ppm. Most investigators feel that the amount of iron in the leaves cannot be related to the amount of iron present in the soil. There is no acceptable soil extraction procedure for determing iron availability at this time (Sauchelli, 1973 and Wallihan, 1966). The concentration of iron in the dry matter of leaves is usually 10^{-2} to 10^{-4} times that of the soil in which it grows (Wallihan, 1966). The concentration of iron in the dry matter of the leaves in this study was 9.5×10^{-2} to 17.0×10^{-3} times that of the soil on which it grew for the wheat and 9.7×10^{-2} to 21.0×10^{-3} times the iron concentration in the soil for the bermuda grass.

E. Nickel

1. Wheat

The nickel concentration in the leaves and stems of the wheat ranged from 3.45 to 26.17 ppm in the Control plants; 14.43 to 525.20 ppm in the Effluent plants and 14.52 to 513.56 ppm in the Aerated Effluent plants.

It is not possible to fix allowable nickel content of healthy plants because there are too few data from experiments in which nickel excess was the only adverse factor (Vanselow, 1966). The literature indicates that in general nickel concentrations in excess of 50 ppm are toxic to most plants (Allaway, 1968).

2. Bermuda Grass

The nickel concentration of the Control plants ranged from 19.73 ppm to 212.36 ppm. The bermuda grass treated with Effluent had nickel concentrations ranging from 58.45 ppm to 305.71 ppm and the Aerated Effluent treated plants had nickel concentrations ranging from 44.48 ppm to 321.37 ppm. The literature indicates that the nickel concentration in grass ranges from 0.2 to 56.0 ppm (Vanselow, 1966).

3. Conclusions

The new multiple range test (Table 4) indicates that significant differences exist in the wheat plants between the Control and Effluent plants and the Control and Aerated Effluent plants from the second through the sixth harvests and between the Effluent and Aerated Effluent plants from the second through the sixth harvests. The literature indicates that although the nickel concentration in the Control plants was not toxic, the nickel concentrations of the Effluent and Aerated Effluent plants did reach toxic levels.

The new multiple range test (Table 7) indicates that significant differences exist in the bermuda grass between the Control and Effluent plants from the second through the fifth harvests, between the Control and Aerated Effluent plants from the second through the fourth harvests, and between the Effluent and Aerated Effluent plants from the thrid through the fifth harvests. A comparison with the literature indicates that toxic concentrations of nickel were reached in all three treatments. However, the nickel concentrations in the literature were for grass species and not bermuda grass specifically. The toxic concentration varies for different species. With the exception of the nickel concentration at the sixth harvest (which is suspect due to the small sample size), the nickel concentration of the Control plants does not exceed 67.34 ppm, which may be less than toxic level for nickel in bermuda grass. The final concentration of 212.36 ppm nickel in the Control may be in error.

The nickel content of the soil did not vary appreciably among the three treatments for the two species. It ranged from 10.44 ppm to 13.08 ppm in the bermuda grass soil and from 9.64 ppm to 12.74 ppm in the wheat soil. The nickel concentration of the soil prior to planting was 12.57 ppm. The amount of nickel added (average of 867.82 micrograms per container of wheat and 1072.85 micrograms per container of bermuda grass treated with effluent and 32.22 micrograms per container of wheat and 17.87 micrograms per container of bermuda grass treated with aerated effluent) was small compared to the amount of naturally occuring nickel in the soil (37,712.4 micrograms/pot). The reason for the increase in uptake by those plants treated with wastewater may be similar to that for chromium uptake. It may be due to the efficient plant absorption of continuously provided nickel.

F. Zinc

1. Wheat

The zinc content of the leaves and stems of the wheat Control plants ranged from 44.47 ppm to 65.87 ppm during the growing period. These values appear to be normal compared to values found in the literature. The zinc content of the Effluent plants ranged from 43.84 ppm to 115.02 ppm. While these values were somewhat higher than the Control plants, they did not reach what is considered to be toxic concentrations. The same is true for the Aerated Effluent plants, whose zinc concentrations ranged from 43.20 ppm to 91.35 ppm. The literature indicates that zinc concentrations in excess of 150 ppm may be toxic to wheat, while zinc concentrations less than 10 ppm indicate a zinc deficiency in wheat (Sauchelli, 1969). All zinc values in the study appear to be above the deficiency level and below toxicity level.

2. Bermuda Grass

The zinc concentration of the Control plants ranged from 60.70 ppm to 160.85 ppm. The literature shows a variety of concentrations for zinc, due in part to the many species of grass. Zinc concentrations in grasses may be deficient if less than 10 ppm (Sauchelli, 1969) or toxic if greater than 200 ppm (Allaway, 1968). The normal range is between 15 ppm and 80 ppm (Sauchelli. All but one harvest of the Control plants fell within the range of zinc necessary for normal growth (15-30 ppm) reported by Sauchelli (1969). The zinc concentration of the Effluent treated plants ranged from 57.10 to 183.39 ppm. Three of the six harvests fell outside the normal range as reported by Sauchelli (1969), but were less than the 200 ppm concentration that causes toxicity as generalized by Allaway (1968). The same is true of the Aerated Effluent plants whose zinc concentrations ranged from 55.70 ppm to 129.44 ppm.

3. Conclusions

The new multiple range test (Table 4) indicates that the zinc content of the wheat plants was significantly different between the Controls and Effluent plants at the second, third and fifth harvests; between the Control and Aerated Effluent plants from the second through the fifth harvests; and between the Effluent and Aerated Effluent plants at the fourth harvest.

The new multiple range test (Table 7) indicates that significant differences existed in bermuda grass between the Control and Effluent plants at the third and fifth harvests; between the Control and Aerated Effluent plants from the second through fifth harvests; and between the Effluent and Aerated Effluent plants from the second through the fourth harvests.

The zinc concentrations found in the wheat and bermuda grass in all three treatments are within the normal range as reported in the literature (Sauchelli, 1969 and Allaway, 1968). This was to be expected since the soil contained a normal range of zinc (Chapman 1966 and Buckner and Brady, 1969) and the concentrations of zinc added to each container was small (an average of 209.96 micrograms to the wheat and 235.19 micrograms to the bermuda grass treated with effluent and an average of 60.56 micrograms to the wheat and 44.52 micrograms to the bermuda grass treated with aerated effluent) compared to what was already present in the soil (103,800 micrograms zinc per container).

It has been found that the quantity of zinc in plants changes with the growing season and often shows an increase througout the season (Antonovics et al, 1971). The results from this study tend to support this statement. The results of this study do not indicate that the death or reduced yield of the plants was caused by zinc deficiency of toxicity.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

A. Statement of the Problem

The passage of the Federal Water Pollution Control Act Amendments of 1972 presented many municipalities and industries with the problem of upgrading their wastewater treatment methods to meet the 1977 standards and the 1985 no discharge of pollutants to navigable stream standards.

An electric plating plant located in Oklahoma City was faced with the problem of disposal of wastewater containing chromium, cyanide, aluminum, copper, iron, zinc, cadmium, lead, fluorine, nickel and phenols. The company wished to consider the feasibility of land application of this wastewater on land owned by the company.

Land application as an ultimate disposal method with no discharge can be economical compared to tertiary treatment or storage. However, most of the research done to determine the feasibility of irrigation with wastewaters that contain significant levels of heavy metals indicate that such wastewater is unsuitable for land application, primarily due to the accumulation of heavy metals in the soil. The addition of heavy metals could lower crop productivity and lead to phytotoxicity. Another concern is the uptake and accumulation of heavy metals by crops causing higher than normal levels of heavy metals in food chains.

However, the characteristics of industrial wastewaters vary widely. There is no typical industrial wastewater. It has been concluded that in general industrial wastewaters containing heavy metals are not suitable for irrigation on most soils. However, such operations have been successful. Therefore, each case must be considered individually. The response of the soil at a proposed site to the particular wastewater to be applied should be studied carefully prior to full-scale operation.

B. Conclusions and Recommendations

This research project was established to determine the feasibility of using the effluent from the electric plating industry as irrigation waters. The greenhouse experiment was carried out treating the plants with aerated and non-aerated effluent. The concentrations of chromium, copper, iron, nickel, and zinc were determined in plants irrigated with this water and compared to control plants.

The experimental design placed certain restraints on the research. The research was carried out as a pot experiment, therefore, conditions were not the same as for field experiments. The treated plants were watered only with effluent or aerated effluent after the first harvest. In the field plants would receive rainfall as well. A real effluent was used which contained several toxic substances not tested for in this study. The plant mortality and reduction in yield could be due in part to some of these substances. Because of the variety of toxic substances in the effluent, synergestic or antagonistic effects could be occurring, but would be impossible to detect. It is not possible to generalize the uptake of heavy metals and the accumulation by plants from soil type to soil type or from species to species of plant. The data collected are valid only for the particular soil, plants, and waste effluent involved, but may add more information to that available on heavy metal uptake and concentration in plants.

The following conclusions may be drawn from this research:

1. The treated electroplating wastewater effluent should not be used for irrigation purposes. The chemical composition was toxic to the plants, as well as, showing significant increases in heavy metal content in the surviving plants. This supports the general assumption that wastewater effluent with significant concentrations of heavy metals should not be used for irrigation.
2. The mortality rate of plants treated with effluent and aerated effluent was greater than that of the Control plants. The mortality rate in the Control plants could be due in the case of wheat to natural thinning or the fact that the control containers were dropped prior to the fifth harvest. The productivity was less for the Effluent and Aerated Effluent plants.
3. The heavy metal content in both the wheat and bermuda grass reached toxic levels.
    a. It is possible that the chromium concentration in the Effluent wheat plants and Aerated Effluent wheat plants

reached toxic levels. In any event the concentration of chromium increased 6 to 7 times as much in the Effluent and Aerated Effluent wheat as in the Control wheat. The same was true for the bermuda grass.

   b. The copper concentration in the Effluent wheat plants and the Aerated Effluent wheat plants may have reached a toxic level. The copper concentration in the Effluent bermuda grass may have been slightly toxic.
   c. The nickel content of the Effluent wheat, Aerated Effluent wheat, Effluent bermuda grass and Aerated Effluent bermuda grass reached toxic levels.

4. The concentrations of heavy metals added to the soil was in all cases too small for statistical detection as compared to the concentrations already present in the soil. For most of the metals studied the addition by irrigation made them more available than the naturally occurring metals in the soil.
5. Although in all cases the concentration of the heavy metals added to the soil was greater in the Effluent plants than the Aerated Effluent plants, the Effluent plants did not always have higher concentrations of the heavy metals than did the Aerated Effluent plants. This would indicate that much of the heavy metals in the effluent are in an unavailable form.

6. In most instances the addition of the wastewater enhanced the heavy metal content of the plants. However, it would appear that there is little chance of elevated heavy metal concentrations entering the food chain through this route since the effluent proved lethal to both the wheat and the bermuda grass.

C. Recommendations for Further Study

There is a need for knowledge of the chemical forms of the heavy metals in the soil solution and of the forms preferentially absorbed from the soil by plants. More research is needed in the area of tolerance levels and to establish more definite toxicity levels for heavy metals in plants. Research is needed to establish the efficiency with which crops can absorp toxic elements from the soil.

There is almost no data available on the total ecological cycling of most of the heavy metals in the environment. Such research is needed to correlate plant composition with soil analytical values. Permissible upper tolerance levels for heavy metals in plants that represent complete safety with respect to the food chain should be studied.

APPENDIX

Table A-1. Heavy Metal Content of Effluent (micrograms/ml)

Sample Number	pH	Chromium	Copper	Iron	Nickel	Zinc
E 1-1	6.5	0.13	0.18	0.14	0.11	0.10
E 1-2	6.6	0.02	0.08	0.15	0.01	0.03
E 1-3	6.6	0.02	0.08	0.98	0.01	0.05
E 1-4	7.1	0.09	0.17	0.31	0.07	0.08
E 1-5	7.2	0.03	0.08	1.17	0.002	0.03
E 1-6	7.4	0.01	0.07	0.39	0.002	0.02
E 1-7	7.5	0.02	0.09	0.05	0.03	0.03
E 1-8	8.0	0.01	0.06	0.30	0.01	0.02
E 1-9	6.8	0.15	0.27	0.28	0.12	0.13
E 1-10	7.7	0.04	0.13	0.06	0.04	0.08
E 1-11	7.4	0.04	0.10	0.05	0.05	0.06
E 1-12	7.4	0.05	0.12	0.07	0.03	0.06
E 1-13	7.1	0.03	0.08	0.09	0.01	0.03
E 1-14	7.1	0.03	0.09	0.07	0.02	0.03
E 1-15	7.5	0.03	0.09	0.45	0.01	0.04
E 1-16	7.4	0.02	0.07	0.31	0.01	0.05
E 1-17	7.5	0.02	0.10	0.10	0.04	0.02
E 1-18	7.4	0.03	0.12	0.17	0.03	0.07
E 2-1	7.9	1.49	0.55	0.15	1.80	0.41
E 2-2	7.9	0.91	0.38	0.12	1.50	0.29
E 2-3	7.8	1.34	0.54	0.26	1.77	0.46
E 2-4	7.9	0.54	0.22	0.06	1.20	0.06
E 2-5	7.9	0.86	0.39	0.06	1.40	0.08
E 2-6	7.9	0.41	0.17	0.08	1.09	0.15
E 2-7	7.9	0.36	0.13	0.07	0.78	0.13
E 2-8	7.9	0.97	0.30	0.12	1.38	0.25
E 2-9	7.9	0.48	0.10	0.05	0.98	0.21

Table A-2. Heavy Metal Content of Aerated Effluent (micrograms/ml)

Sample Number	pH	Chromium	Copper	Iron	Nickel	Zinc
A 1-0	6.5	0.02	0.08	0.62	0.004	0.03
A 1-1	6.6	0.01	0.05	0.10	0.003	0.06
A 1-2	6.6	0.06	0.08	0.45	0.01	0.03
A 1-3	6.7	0.01	0.09	0.64	0.01	0.03
A 1-4	6.7	0.01	0.08	0.09	0.01	0.03
A 1-5	6.8	0.01	0.07	0.03	0.01	0.03
A 1-6	6.9	0.08	0.09	0.05	0.10	0.03
A 1-7	7.0	0.09	0.11	0.03	0.04	0.03
A 1-8	8.0	0.01	0.07	0.31	0.01	0.03
A 1-9	7.1	0.02	0.07	0.38	0.01	0.03
A 1-10	7.4	0.02	0.08	0.01	0.01	0.03
A 1-11	7.8	0.01	0.06	0.08	0.01	0.03
A 1-12	7.7	0.02	0.07	0.01	0.01	0.02
A 1-13	7.4	0.03	0.12	0.12	0.01	0.05
A 1-14	7.7	0.03	0.06	0.01	0.01	0.03
A 1-15	8.0	0.01	0.06	0.18	0.01	0.03
A 1-16	7.7	0.01	0.06	0.16	0.01	0.04
A 2-0	9.0	0.02	0.04	0.13	0.01	0.02
A 2-1	8.2	0.01	0.04	0.15	0.001	0.02
A 2-2	7.7	0.01	0.04	0.07	0.001	0.01
A 2-3	7.7	0.01	0.04	0.12	0.001	0.03
A 2-4	7.6	0.01	0.02	0.04	0.03	0.01
A 2-5	7.8	0.01	0.04	0.10	0.01	0.03
A 2-6	7.8	0.01	0.05	0.03	0.002	0.04
A 2-7	7.9	0.01	0.04	0.06	0.01	0.01
A 2-8	7.9	0.01	0.02	0.09	0.01	0.01
A 2-9	7.9	0.005	0.03	0.02	0.01	0.02
A 2-10	7.9	0.02	0.04	0.11	0.01	0.02
A 2-11	7.9	0.02	0.02	0.01	0.03	0.01

Table A-3. Heavy Metals in the Soil (micrograms/gram)

	Chromium	Copper	Iron	Nickel	Zinc
Wheat (After final harvest)					
Control	8.79±0.3	6.83±0.5	7687.50±20.4	12.74±0.0	37.22±0.6
Effluent	3.06±0.2	4.50±0.5	7999.43±19.2	11.60±1.5	40.40±0.5
A. Eff.	2.53±2.2	2.04±2.0	2053.69±240.9	10.50±0.7	30.00±1.6
Bermuda Grass (After final harvest)					
Control	4.44±0.3	3.12±0.6	2882.88±28.6	11.39±2.1	33.46±2.1
Effluent	5.46±2.3	4.14±0.4	3652.86±30.0	13.08±2.1	34.31±1.3
A. Eff.	4.27±0.6	3.67±0.3	2294.79±25.5	11.50±0.8	34.36±1.2
Untreated Soil (Sampled prior to treatment)					
Soil	5.13±0.6	4.94±0.3	4203.33±22.8	12.57±2.8	34.58±1.4

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