The uptake in inorganic arsenic by wheat and alfalfa
by Gwen Dee Gray

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University
© Copyright by Gwen Dee Gray (1994)

Abstract:
The Madison and the Upper Missouri Rivers typically have 50 - 200 ppb arsenic concentration which exceeds the Federal EPA drinking standard of 50 micrograms per liter for arsenic. Water from these sources has been used for the irrigation of crops in Montana since the late 1800's. There is great concern that crops grown with this water may deliver arsenic to the human population. The goal of the project is to determine arsenic uptake in wheat and alfalfa and the soil in which they were grown as a function of arsenic concentration in irrigation water. In a greenhouse experiment, irrigation water of 4 different arsenic trioxide concentrations of 0,10,50, and 100 ppb, were applied to wheat and alfalfa plants for 11 weeks. The plants were then harvested and analyzed for arsenic concentration levels in roots, stems, leaves, and for wheat, seed heads. Soil samples at 0.5 and 10 cm depths were also analyzed for arsenic.

Wheat roots accumulated more arsenic than other plant sections for all application concentrations. No alfalfa plant part consistently accumulated more arsenic than other plant parts, however at the 1 ppm treatment, leaves had significantly higher concentrations. Wheat as a whole stored more arsenic than alfalfa. Regardless of plant species, more arsenic accumulated near the surface than at the 10 cm depth. When alfalfa was grown, soil arsenic concentrations increased more readily at the surface, for all treatments greater than zero, than when wheat was grown. No statistically significant differences were observed in measured plant heights for either species as a function of applied arsenic concentration. No conclusive cellular structure differences could be found between the treated and control, alfalfa and wheat groups except for a possible growth reduction in the wheat leaves. The wheat and alfalfa both appear to be accumulating arsenic but at very low concentrations. Approximately 90% of the arsenic in this experiment is flowing out with the drainage. Therefore at risk populations should be more concerned about the arsenic in drinking water than in food supplies.

THE UPTAKE OF INORGANIC ARSENIC BY WHEAT AND ALFALFA

by

Gwen Dee Gray

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Environmental Engineering

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 1994

T378
G7925

APPROVAL

of a thesis submitted by

Gwen Dee Gray

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

April 20, 1994
Date

Chairperson, Graduate Committee

Approved for the Major Department

20 April 1994
Date

Head, Major Department

Approved for the College of Graduate Studies

4/21/94
Date

Graduate Dean

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under the rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Signature Gwen D. Gray

Date 4/21/94

ACKNOWLEDGEMENTS

To Dr. Otto Stein for his trust in my abilities; to Dr. Sharon Eversman for her tolerance of basic questions; to Gil Alexander for his love and personal interest; to Marilyn Alexander for keeping me in the lab; to Ray Hamilton for reteaching me everything I forgot; to my parents for instilling the drive in my soul that has gotten me everything I've strived for in life and bribing me to attend science camp as a high school freshman.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF TABLES vii

LIST OF FIGURES ix

ABSTRACT xi

INTRODUCTION 1

- Inorganic Arsenic General Chemistry 2
- Inorganic Arsenic in Water 5
- Inorganic Arsenic in Soils 10
- Inorganic Arsenic in Plants 11
- Sources of Arsenic in the Montana Area 13
- Human Response to Inorganic Arsenic 16
  - Exposure 16
  - Uptake 17
  - Distribution 18
  - Metabolism 18
  - Reactive Products 19
  - Mutation 20
  - Human Cancer Data concerning Arsenic 21

MATERIALS AND METHODS 23

- Planters 23
- Growth Period 23
- Digestion and Filtration 24
- Analysis 25
- Stained Slides 27

RESULTS AND DISCUSSION 28

- Plant Heights 28
- Soil Results 28
- Plant Arsenic Results 34
- Microscopic Analysis 37

CONCLUSIONS 47

TABLE OF CONTENTS-Continued

Page

BIBLIOGRAPHY.......... 49

APPENDIX.......... 54

Appendix A- Statistical Analysis.......... 55

LIST OF TABLES

Table Page

1. Common arsenic species found in environmental samples.................... 4
2. Free energies of formation for arsenic species at 25° C and 1 atmosphere are listed.. 6
3. Stability constants for the arsenic acid system and some soluble arsenates (25° C).. 8
4. Arsenic concentrations in the water of the upper Madison and Missouri mainstreams.. 16
5. Arsenic concentrations measured in soil in which wheat was grown as a function of applied arsenic concentration......................... 30
6. Significant differences of arsenic concentration means of the different treatment groups in the soil in which wheat was grown.. 31
7. Arsenic concentrations measured in soil in which alfalfa was grown as a function of applied arsenic concentration......................... 31
8. Significant differences of arsenic concentration means of the different treatment groups in the soil in which alfalfa was grown.. 31
9. Arsenic concentrations measured in different wheat plant organs as a function of applied arsenic concentration......................... 35

LIST OF TABLES-Continued

Table Page

10. Significant differences of arsenic concentration means of the different treatment groups in the different wheat plant organs........ 36

11. Arsenic concentrations measured in different alfalfa plant organs as a function of applied arsenic concentration........ 36

12. Significant differences of arsenic concentration means of the different treatment groups in the different alfalfa plant organs........ 37

LIST OF FIGURES

Figure | Page

1. The abbreviated Periodic Table displaying arsenic.............................. 3
2. The Eh-pH diagram for As at 25° C and 1 atm with total arsenic 10^{-5} M and total sulfur 10^{-3} M. Solid species are enclosed in parentheses in cross-hatched area, which indicates solubility less than $10^{-5.3}$ M.. 7
3. The local cycle of arsenic in a stratified lake.................................... 9
4. Sketch map showing rivers and major hydrothermal areas sampled in Yellowstone National Park, Wyoming............................. 15
5. Breakdown of methylated arsenic.. 19
6. Schematic of the effect on DNA of oxygen radicals............................. 20
7. Height of alfalfa and wheat plants in different arsenic treatment groups... 29
8. Arsenic concentrations measured in soil in which wheat was grown as a function of applied arsenic concentration........................... 32
9. Arsenic concentrations measured in soil in which alfalfa was grown as a function of applied arsenic concentration........................... 33
10. Arsenic concentrations measured in different wheat plant organs as a function of applied arsenic concentration........................... 38
11. Arsenic concentrations measured in different alfalfa plant organs as a function of applied arsenic concentration........................... 39

LIST OF FIGURES-Continued

Figure Page

12. Photographs of wheat control roots under the microscope.................... 41
13. Photographs of wheat treatment roots under the microscope................. 41
14. Photographs of wheat control stems under the microscope................... 42
15. Photographs of wheat treatment stems under the microscope................ 42
16. Photographs of wheat control leaves under the microscope.................. 43
17. Photographs of wheat treatment leaves under the microscope............... 43
18. Photographs of alfalfa control roots under the microscope................... 44
19. Photographs of alfalfa treatment roots under the microscope................ 44
20. Photographs of alfalfa control stems under the microscope.................. 45
21. Photographs of alfalfa treatment stems under the microscope............... 45
22. Photographs of alfalfa control leaves under the microscope.................. 46
23. Photographs of alfalfa treatment leaves under the microscope............... 46

ABSTRACT

The Madison and the Upper Missouri Rivers typically have 50 - 200 ppb arsenic concentration which exceeds the Federal EPA drinking standard of 50 micrograms per liter for arsenic. Water from these sources has been used for the irrigation of crops in Montana since the late 1800's. There is great concern that crops grown with this water may deliver arsenic to the human population. The goal of the project is to determine arsenic uptake in wheat and alfalfa and the soil in which they were grown as a function of arsenic concentration in irrigation water. In a greenhouse experiment, irrigation water of 4 different arsenic trioxide concentrations of 0, 10, 50, and 100 ppb, were applied to wheat and alfalfa plants for 11 weeks. The plants were then harvested and analyzed for arsenic concentration levels in roots, stems, leaves, and for wheat, seed heads. Soil samples at 0.5 and 10 cm depths were also analyzed for arsenic.

Wheat roots accumulated more arsenic than other plant sections for all application concentrations. No alfalfa plant part consistently accumulated more arsenic than other plant parts, however at the 1 ppm treatment, leaves had significantly higher concentrations. Wheat as a whole stored more arsenic than alfalfa. Regardless of plant species, more arsenic accumulated near the surface than at the 10 cm depth. When alfalfa was grown, soil arsenic concentrations increased more readily at the surface, for all treatments greater than zero, than when wheat was grown. No statistically significant differences were observed in measured plant heights for either species as a function of applied arsenic concentration. No conclusive cellular structure differences could be found between the treated and control, alfalfa and wheat groups except for a possible growth reduction in the wheat leaves. The wheat and alfalfa both appear to be accumulating arsenic but at very low concentrations. Approximately 90% of the arsenic in this experiment is flowing out with the drainage. Therefore at risk populations should be more concerned about the arsenic in drinking water than in food supplies.

INTRODUCTION

The Madison and the Upper Missouri Rivers frequently exceed the federal Environmental Protection Agency (EPA) drinking water standard for total arsenic of 50 micrograms per liter. Natural geothermal springs are the major source of arsenic in the Madison River. Water from the Madison River has been used for irrigation since the late 1800's and this arsenic laden water is believed to be the source of extensive ground water contamination and chronic (long term) arsenic poisoning of resident animal and human populations. The Madison and Missouri Rivers are drinking water sources for many Montana cities including Helena and Great Falls. Chronic health problems in humans have been documented when arsenic levels in drinking water exceed 80 micrograms per liter (Water Management Bureau, 1992). EPA may soon lower the acceptable arsenic limit for drinking water to 15 micrograms per liter. In a personal communication, Dr. Abraham Horpestad of the Montana Water Quality Bureau estimated the increased risk of cancer due to arsenic for water consumers near the upper Missouri is 1 per 2000 people. Gallatin County health officers attribute chronic arsenic poisoning in the Gallatin valley to combined effects of arsenic in drinking water, garden produce, locally grown chicken and beef, and dermal contact.

The experiment described herein was designed to answer four questions:

1. In what plant tissues does arsenic accumulate when introduced through water in a typical Montana monocot, e.g. wheat, and dicot, e.g. alfalfa?
2. Where in the soil profile does arsenic accumulate?
3. Does arsenic accumulation affect plant growth characteristics?
4. Is there discernible damage in the plant cell tissues due to accumulation?

Inorganic Arsenic General Chemistry

Arsenic is a toxic metalloid found in almost all natural waters and rocks. Determining the pathways of arsenic mobility through the environment is essential if we are to understand arsenic toxicity effects. The abbreviated periodic chart (Figure 1) shows that arsenic is the thirty-third element and is located in the same group column as phosphorus. Elements in the same group have the same number of electrons in their valence shell. The Group V elements, arsenic and phosphorus, therefore behave similarly in chemical reactions in biochemical pathways. Arsenic (As) is a conservative member, an element that cannot be removed by nuclear decay, of Group V, the metalloid group. Metalloids are elements that are technically non-metals but often behave like metals (Schulman, 1992). Inorganic arsenic is stable in four oxidation states: -3, 0, +3, and +5. Arsenic (0) is very rare and As (-3) only occurs at low redox values, which do not commonly occur in Montana river waters (Baudo,1990). Arsenic (+3) is volatile, but oxidizing conditions can prevent loss. Organoarsenic compounds differ in their stability to oxidation. It should be recognized that arsenic exists in several oxidation states of limited interconvertability (Penrose, 1974). A list of arsenic species commonly found in environmental samples are listed in Table 1.

Figure 1. Abbreviated Periodic table displaying arsenic (Schulman, 1992).

IV	V	VI
7 2, 4, 0, −4 C Carbon 12.011	8 1, 2, 3, 4, 5, 0, −1, −2, −3 N Nitrogen 14.007	9 −2 O Oxygen 15.999
14 2, 4, 0, −4 Si Silicon 28.086	15 3, 5, 0, −3 P Phosphorus 30.974	16 4, 6, 0, −2 S Sulfur 32.064
32 2, 4, 0 Ge Germanium 72.59	33 3, 5, 0, −3 As Arsenic 74.922	34 4, 6, 0, −2 Se Selenium 78.96
50 2, 4, 0 Sn Tin 118.69	51 3, 5, 0, −3 Sb Antimony 121.75	52 4, 6, 0, −2 Te Tellurium 127.60

Table 1. Common arsenic species found in environmental samples (Holm et. al., 1979).

Species	Name(s)	Oxidation State
AsO_4^{3-}	Arsenate	+5
AsO_3^{3-}	Arsenite	+3
$CH_3AsO(OH)_2$	Methanearsonic Acid	+3
	Monomethyl Arsonic Acid	
$(CH_3)_2AsOOH$	Hydroxydimethyl Arsine Oxide	+1
	Dimethyl Arsinic Acid	
	Cacodylic Acid	
AsH_3	Arsine	-3
$(CH_3)_2AsH$	Dimethyl Arsine	-3
$(CH_3)_3As$	Trimethyl Arsine	-3

Arsenic is difficult to categorize because its chemistry is so complex. It can exist in either a pentavalent (+5) or trivalent (+3) state and can combine with other elements to form many different compounds and ions, the most common being arsenic trioxide (+3). Conversion from the trivalent state (arsenite) to the pentavalent state (arsenate) is reversible in solution without the use of catalysts, and both species can occur simultaneously. Free energies of formation for arsenic species at 25° C and 1 atmosphere are listed in Table 2. An Eh-pH diagram for arsenic in a system including oxygen, water, and sulfur is shown in Figure 2, the Eh is a measure of the amount of electrons measure in voltage units. At the high Eh values encountered in oxygenated waters, arsenic acid species (H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-}) are stable. At Eh values characteristic of mildly

reducing conditions, arsenous acid species (H_3AsO_3, $H_2AsO_3^-$, and $HAsO_3^{2-}$) become stable. Except at extremely low Eh values, organic arsenics are unstable with respect to oxidation of the organic part of the molecule. Very little information about the rates of arsenic reactions in solution exists, and specific rate constants are unknown (Ferguson and Gavis, 1972). Arsenite is approximately ten times more toxic than arsenate and about thirty-five times more toxic than organic arsenic forms (Schulman, 1992). Table 3 gives the solubility product for some soluble arsenates that may limit the arsenic concentration in the aqueous phase. Due to arsenic's complexity, the inorganic form is assumed in this paper unless noted otherwise.

Inorganic Arsenic in Water

In well oxygenated water at a pH of 7-9, $HAsO_4^{2-}$ is the dominant form of dissolved arsenic (Schulman, 1992). The solubility of arsenic in freshwater depends on oxygen concentration, ferric and sulfur hydroxides, pH, and suspended particle content. Increasing concentrations of these substances cause arsenic to precipitate into the sediment of natural water bodies (Baudo et al., 1990; Brannon et al., 1987). In seawater, under normal pH and dissolved oxygen conditions, arsenic should exist at equilibrium as arsenate, but marine bacteria can reduce added arsenate to arsenite, therefore creating a dynamic equilibrium between chemical oxidation and biological reduction. In aerated streams, arsenate is the dominant species; in anaerobic reservoirs, it is arsenite (Penrose, 1974).

Table 2. Free energies of formation for arsenic species at 25° C and 1 atmosphere (Ferguson and Gavis, 1972).

Species	State	dG_t
H_3AsO_4	aq.	-184.0
$H_2AsO_4^-$	aq.	-181.0
$HAsO_4^{2-}$	aq.	-171.5
AsO_4^{3-}	aq.	-155.8
H_3AsO_3	aq.	-154.4
$H_2AsO_3^-$	aq.	-141.8
$HAsO_3^{2-}$	aq.	-125.3
$HAsS_2$	aq.	-11.61
AsS_2^-	aq.	-6.56
AsS	s.	-16.81
As_2S_3	s.	-40.25
As	s.	0.0
AsH_3	aq.	23.8
AsH_3	g.	16.5
As_2O_3	s.	-140.8
As_2O_5	s.	-186.9

Legend

aq = aqueous
g = gas
s = solid

Figure 2. The Eh-pH diagram for As at 25 ^{0} C and 1 atm with total arsenic 10^{-5} M and total sulfur 10^{-3} M. Solid species are enclosed in parentheses in cross-hatched area, which indicates solubility less than $10^{-5.3}$ M (Ferguson and Gavis, 1972).

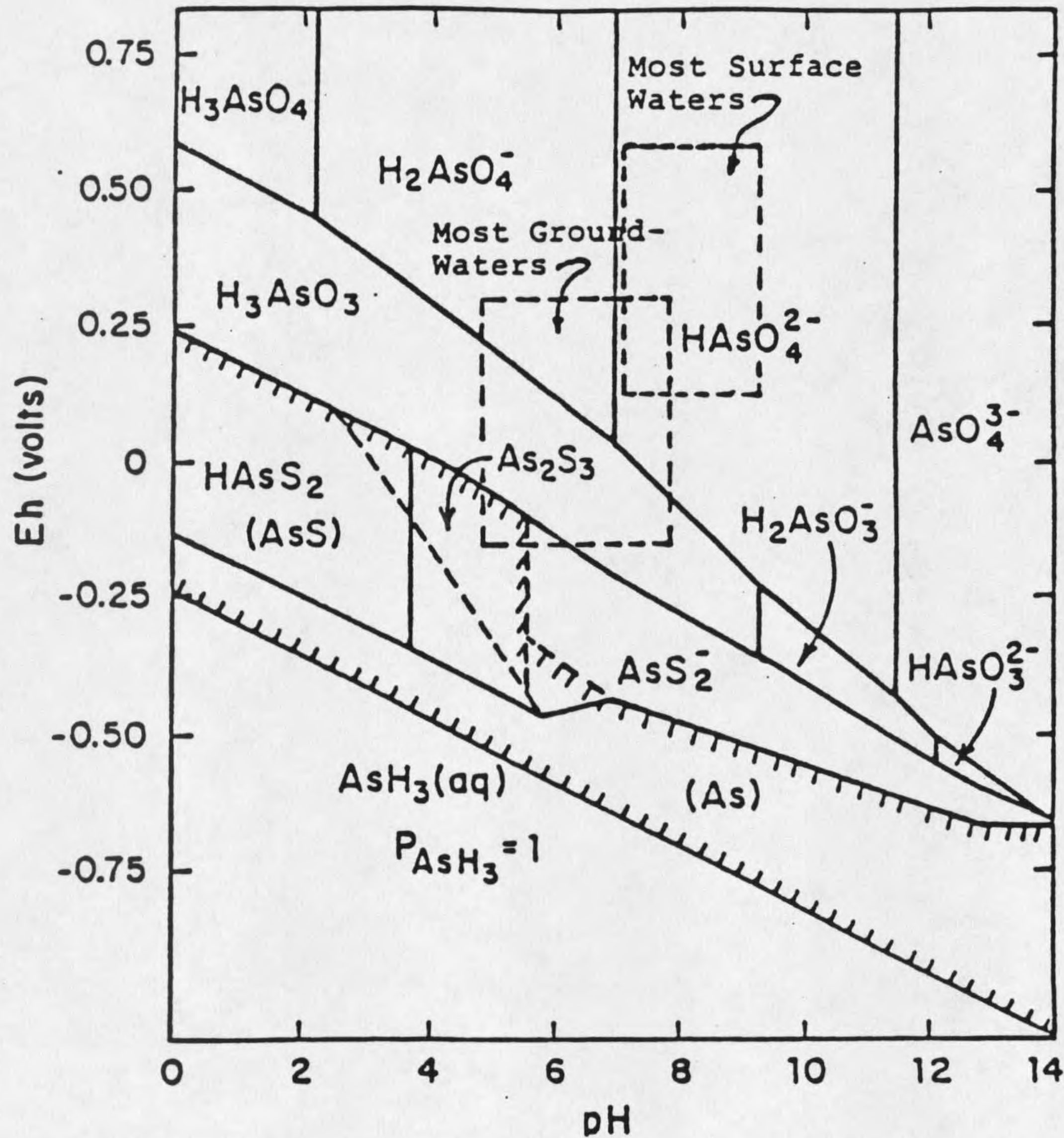
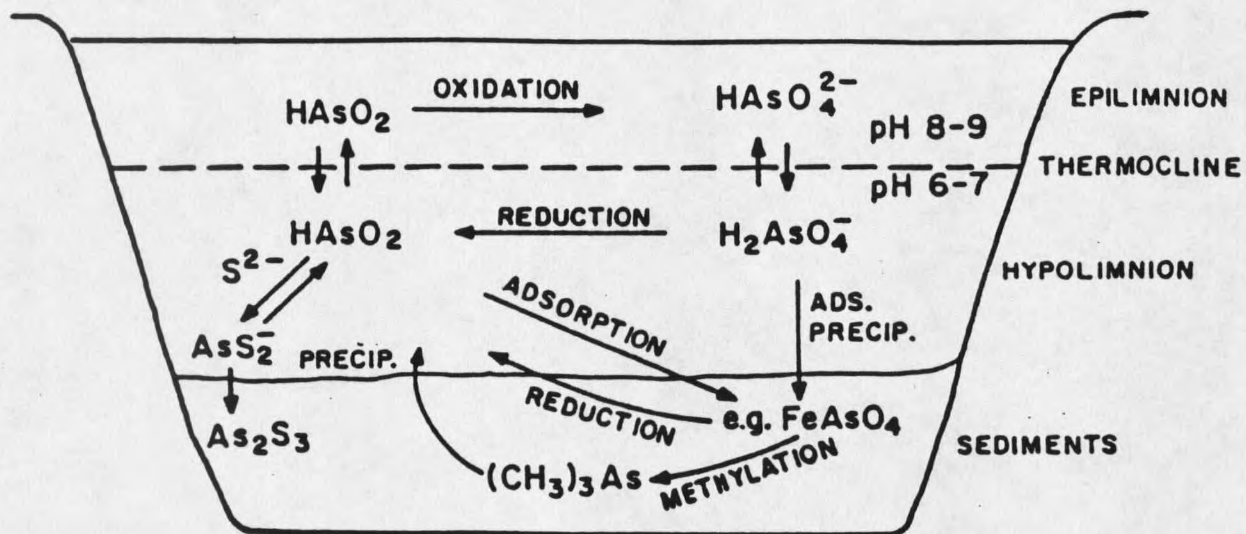


Table 3. Stability constants for the arsenic acid system and some soluble arsenates at 25° C (Allard and Grimvall, 1988).

Reaction		log K
$H^+ + H_{n-1}AsO_4^{n-4} = H_nAsO_4^{n-3}$	n=1	11.5
	n=2	6.9
	n=3	2.2
$AlAsO_4$ (s) = $Al^{3+} + AsO_4^{3-}$		-15.8
$FeAsO_4$ (s) = $Fe^{3+} + AsO_4^{3-}$		-20.2
$Ca_3(AsO_4)_2$ (s) = $3Ca^{2+} + 2AsO_4^{3-}$		-18.2

The local cycle of arsenic in a stratified lake is shown in Figure 3. In natural waters, arsenic can be absorbed by microorganisms, which in some cases can detoxify arsenic by methylating it (the compound becomes lipid soluble). In this form, arsenic is 35 times less toxic than in the inorganic form. The methylated compounds produced in these phytoplankton are readily ingested by fish or shellfish and accumulated in their tissues. Concentrations of 2 milligrams per kilogram (mg/kg) to 50 mg/kg of arsenic have been measured in shellfish (Baudo et al., 1990). Stonefly and caddisfly larvae also accumulate arsenic and are currently being used as arsenic indicators in freshwater streams (Bedwell, 1992). Even though arsenic is rapidly excreted by the ingesting organism, the rate of uptake can be greater than the rate of elimination. Biomagnification through other parts of the food web has yet to be scientifically proven (Ferguson and Gavis, 1972).

Figure 3. The local cycle of arsenic in a stratified lake (Ferguson and Gavis, 1972).



Inorganic Arsenic in Soils

Arsenic forms stable, covalent bonds with most nonmetals and with some metals (Penrose 1974). The affinity to bond with other compounds causes arsenic to coprecipitate with ferric hydroxide and form complexes with sulfur compounds. Therefore, when clay containing ferrous and sulfur compounds is present in soils, inorganic arsenic is often bound to these sites. This binding inhibits the mobility of arsenic through the soil column (Pierce and Carleton, 1982).

The behavior of arsenic in soil is controlled by the redox conditions in the soil environment. An oxidized zone is usually created when irrigation water flows down through the soil profile. In this zone, iron, sulfur, and manganese oxides, hydroxides, and organics bind to the arsenic. As(+5) is the dominant species in this zone.

If a reduction zone exists, adsorbed and coprecipitated metals are released into the pore water and the hydroxides dissolve. The predominant species of As(+5) is reduced to As(+3). There is a dynamic interchange of ions between pore water and sediments controlled by redox conditions, sulfide concentration, and organic matter concentration (Moore et. al., 1990).

Arsenic adsorption is also dependent on pH. At pH less than point of zero charge (PZC), the surface of the solid is positive and adsorption of As(+5) anions is facilitated by coulombic attraction. Therefore at low pH's, there is a dissolution of metal hydroxides, immobilizers of arsenic by coprecipitation. At pH greater than PZC, the surface of the solids is negative and anion adsorption must then compete with coulombic repulsion. Therefore at high pH's, adsorption of arsenic to hydroxides decreases. The PZC values ranging from pH of 6 to 7 are found in literature and are compound specific, e.g. Al_2O_3 and Fe_2O_3 (Allard et. al., 1988). At extremely high or low pH values, arsenic mobility is increased (Allard and Grimvall, 1988).

Arsenic also competes with phosphorus for adsorption sites on solids such as sediments and roots. Therefore, increasing phosphorous concentration decreases the adsorption of arsenic which increases arsenic's mobility in the same soils (Roy et. al., 1986). Either arsenic or phosphorus is mobilized as the other removed from the soil by plant uptake (depending on the plant's uptake efficiency of phosphorus) and the arsenic volatility (Otte et.al., 1990).

Small soil particles, primarily clay, which have large surface areas per volume are the main reaction sites for arsenic. The major mechanism for collection is adsorption (the condensation of atoms, ions, or molecules on the surface of particles) without a chemical reaction. Soil grains near the surface often contain iron and sulfur oxides which become significant collectors of arsenic (Horowitz, 1991; Livesly and Huang, 1981). Numerous studies have shown that the inorganic form of arsenic accumulates in the soil when applied repeatedly to crops. Most of this accumulation occurred in the top 10 centimeters of soil (Anastasia and Kender, 1973). The range of total arsenic content in soils of the United States is <0.1 to 69 ppm with a mean of 6.7 ppm (Kabata-Pendias and Pendias, 1984).

Inorganic Arsenic in Plants

Anastasia (1973) found a correlation between the concentration of arsenic in the lowbush blueberry plant tissue and the soil in which the plant was grown. Arsenic accumulation was greatest in the roots, with lesser amounts in the stems, and even less in the leaves. Additionally, a reduction in plant growth occurred when the arsenic concentration was greater than 6.7 milligrams per liter. Barley seedlings, apple trees, and garden vegetables have also been shown to accumulate arsenic (Chisholm, 1972; Asher and Reay, 1979).

Arsenic uptake in plants occurs in four stages: 1) arsenic adsorption on the plant surfaces, 2) movement of arsenic from the exterior to the interior of the roots or tops, 3)

arsenic translocation to the site of action, and 4) a toxic biochemical reaction. Arsenate is more readily adsorbed and translocated than arsenite because it is less toxic to the roots. Adsorption is limited only by arsenic availability. The second stage is a metabolically driven, selective transfer to symplasm. The translocation pathway is from roots to xylem to leaves to phloem or from leaves to phloem to roots or xylem depending on the source of arsenic introduction (Lederer and Fensterheim, 1983).

Little is known of the biochemical role of arsenic in plants. Evidence of translocation of arsenic from roots to leaves and seed grains has been observed (Kabata-Pendias and Pendias, 1984). The arsenic concentration found in edible plant parts grown in uncontaminated soil ranges from 0.009-1.5 ppm dry weight with fruits in the lower range and leafy vegetables in the upper. Symptoms of arsenic toxicity in plants are wilted leaves, violet coloration, root discoloration, cell plasmolysis, and, most commonly, growth reduction.

Different plants have different tolerances to arsenic (Otte et al., 1990). Some plants in the nettle family are so tolerant of arsenic that they have been used to locate arsenic laden ore deposits (Siegel, 1987). These tolerant plants may have developed specific mechanisms which enable the plant to detoxify the metal. One possible mechanism is the production of compounds which safely bind the metal in the compounds contained within the plant's cytoplasm.

Arsenic can chemically substitute for other elements such as phosphorous and be preferentially absorbed through plant roots. Most plants will suffer from stunted growth if phosphorus deficient. Wheat and alfalfa, two of the most important agronomic plants in Montana, are among these types of plants.

Spring wheat of the genus *Triticum*, family Poaceae, is an annual crop widely used for human and livestock consumption. Wheat produces a grain that is ground for flour and

used for cereals, breads, and other baking. One half of an average (age 30-40) adult's caloric intake is supplied by wheat and it supplies vital nutrients.

Triticum resembles many other monocots in basic anatomy. It has a fibrous root system, with a primary exarch stele. The roots have a thin epidermis and cortex that are joined by one pericycle. The stem provides structure for the plant and transports minerals, hormones, and water through widely spaced vascular bundles. Transpiration is carried out through the stomata which are primarily on the lower surface of the leaves. The upper surface is also the location of most photosynthesis. Wheat has spikelets at the tip of the stem which contain the pistillate flowers that form the seed grains. These grains, including the endosperm, are the parts of the wheat used in food production. Wheat is a self-pollinating plant and adaptable to varying amounts of agronomic care (Metcalf, 1960; Peterson, 1965).

Alfalfa of the genus *Medicago* , family Fabaceae, is a perennial forage crop cultivated since before recorded history. This plant is used to produce hay, processed feeds for livestock, and drugs. Alfalfa, a dicot with a legume fruit, has a taproot system that penetrates deeply into the soil in search of water. The *Medicago* stem is woody at the base and transports water to the leaves through the xylem and minerals to the roots through the phloem. Alfalfa leaves have the same function as do wheat leaves. *Medicago* cross-pollinates primarily through insect transport (Bolton, 1962).

Sources of Arsenic in the Montana Area

Naturally generated arsenic usually comes from geothermal sources. Geothermal waters are a mixture of waters from a high enthalpy magma dome, 2-3 kilometers below the surface and meteoric water from the surface (Stauffer et al., 1980). As the hot water comes to the surface, it dissolves minerals in the rock layers (Stauffer et al., 1980). Since most minerals including arsenic are more soluble in warm water than they are in cool water,

this warm geothermal water contains a higher concentration of minerals than cold springs or surface waters. When this mineral laden water reaches the surface it is cooled rapidly reducing the solubility of many minerals and becomes supersaturated, causing minerals to be deposited (Schulman, 1992).

In Yellowstone Park, a dome of molten rock a mile below the surface heats water to a temperature of 240 degrees Celsius under a high pressure (Schulman, 1992). This water travels to the surface through rock laden with arsenic. This water enters the Firehole River with an arsenic concentration of 252 micrograms per liter (parts per billion-ppb). The Firehole River then joins the Gibbon River to form the Madison River (Figure 4). The Madison enters Montana and joins with the Jefferson and Gallatin Rivers to form the Missouri River.

Soils in the Madison River drainage typically are highly aerobic and have high clay concentrations, making them ideal for arsenic uptake and accumulation. Of sixty-five wells drilled in the Madison Valley, more than forty exceed the EPA drinking water limit of 50 micrograms per liter for arsenic, some by as much as six times.

Arsenic attached to sediment is removed from rivers and reservoirs, such as Canyon Ferry reservoir, when sedimentation occurs. When the river enters the reservoir its velocity is greatly reduced decreasing the available kinetic energy. Thereby gravity forces on the suspended, arsenic-rich sediment exceed their buoyancy forces, causing the particles to settle to the bottom. Therefore a reservoir has sediments with higher concentrations of arsenic than riverbed sediments (Schulman, 1992). Table 4 provides arsenic concentration values in the water along the upper Madison and Missouri mainstreams.

Figure 4. Sketch map showing rivers and major hydrothermal areas sampled in Yellowstone National Park, Wyoming (Stauffer et. al., 1980).

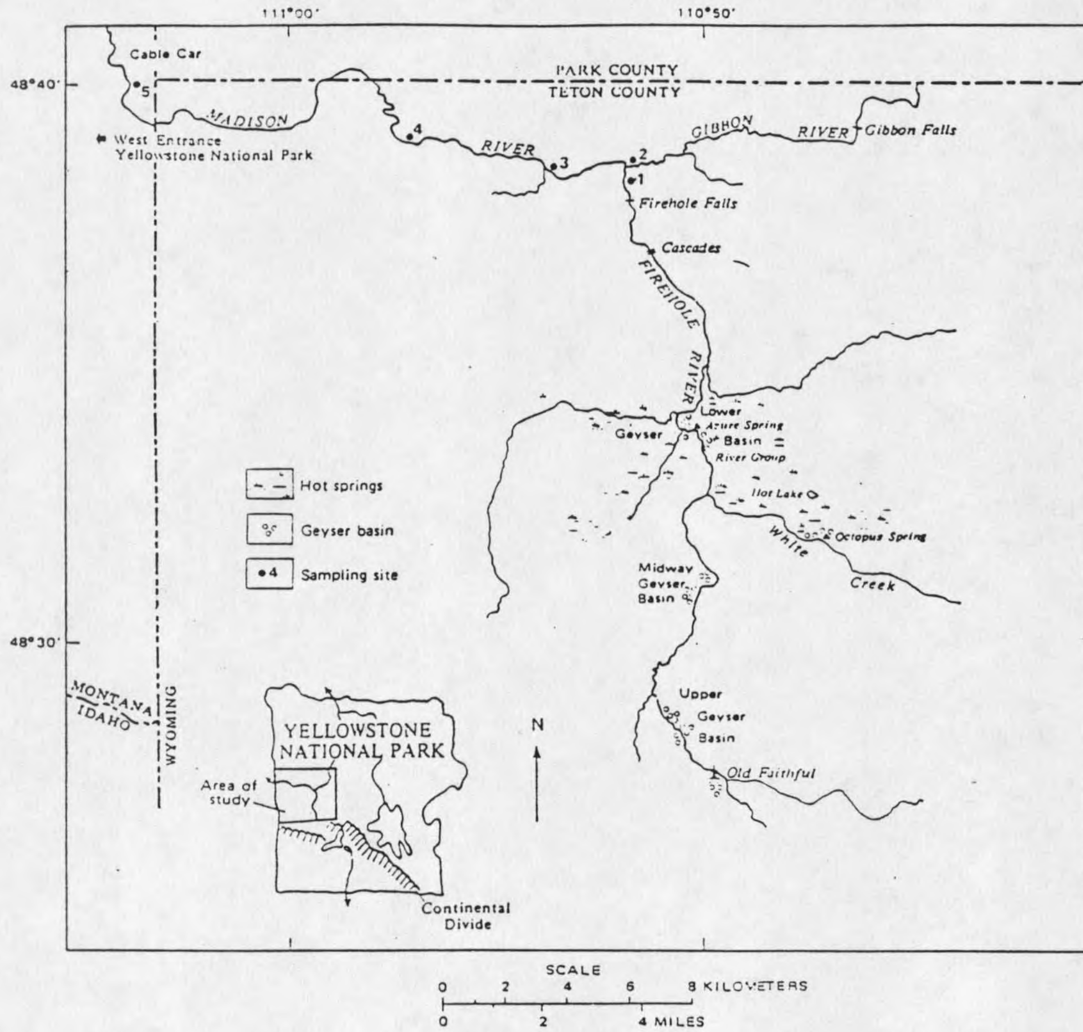


Table 4. Arsenic concentrations in ppb in the water of the upper Madison and Missouri mainstreams (Water Management Bureau, 1992).

Location	minimum	median	maximum
Madison River:			
near west Yellowstone	137	260	370
below Hebgen Reservoir	78	120	240
below Ennis Reservoir	48	73	100
at Three Forks	42	68	88
Missouri River:			
near Toston	10	30	100
below Canyon Ferry Reservoir	23	28	34
near Virgelle	9	15	20
at Landusky	7	14	28
below Fort Peck Reservoir	2	4	8

Human Response to Inorganic Arsenic

The most common type of arsenic poisoning is through acute intoxication. Acute (an immediate threat) arsenic toxicity occurs when high concentrations of arsenic are ingested and is characterized by immediate damage to the central nervous system. This poisoning is rare in naturally contaminated water because arsenic concentrations are too low. Chronic (long duration threat) toxic effects of arsenic generally result in carcinogenic growths due to long term exposure to relatively low concentrations.

Exposure

Exposure to arsenic can be from air, food, and water sources. Copper, lead, silver, and zinc smelters are the primary source of air-borne arsenic (Council on Scientific Affairs, 1985); arsenic trioxide is the primary arsenic species in this method of dispersal. A secondary source for arsenic trioxide is coal burning power plants (Leonard et al., 1980); approximately 0.01 to 0.75 micrograms per cubic meter is released from these sources.

Food is contaminated with arsenic in one of two ways, by pesticides or water. Arsenic is a common constituent in agriculturally useful herbicides and insecticides (Leonard et al., 1980). Plants sprayed with pesticides and harvested for human consumption are a direct source for human contamination. Plants can also introduce arsenic indirectly into the human food chain through herbivore grazing. With the exception of seafood, the average arsenic level in food is usually 1 mg/kg. Arsenic trioxide insecticide used by the tobacco industry has increased the level of arsenic to 40 mg/kg (Council on Scientific Affairs, 1985).

Food is also contaminated by arsenic in sea water. Sea water contains approximately 2-5 micrograms per liter of arsenic. Phytoplankton readily ingest inorganic arsenic, introducing it into the aquatic food web. The arsenic-contaminated phytoplankton are eaten by zooplankton which are then consumed by fish and crustaceans. Fish usually contain between 1 and 10 mg/kg of arsenic and shellfish can contain concentrations up to 100 mg/kg.

Groundwater can be contaminated with arsenic leached from soils and rocks (Leonard et al., 1980). Water is the main transporter of this metalloid in the environment (Weir, 1988). In an extreme case, high-arsenic artesian well water has been related to skin cancer and to the blackfoot disease, a "unique peripheral vascular disorder confined to the southwest coast of Taiwan Island" (Weir, 1988).

Uptake

The average intake by humans of arsenic through food is 0.5 to 4.2 mg of arsenic/day (Leonard et al., 1980) with the regulation level of arsenic at 2.6 mg/day. Inhalation can also be a significant source if the human is a smoker or works or resides near a smelter releasing arsenic trioxide as a byproduct (Leonard et al., 1980). Inhaled arsenic enters the respiration system and is coughed up and transferred to the digestive system.

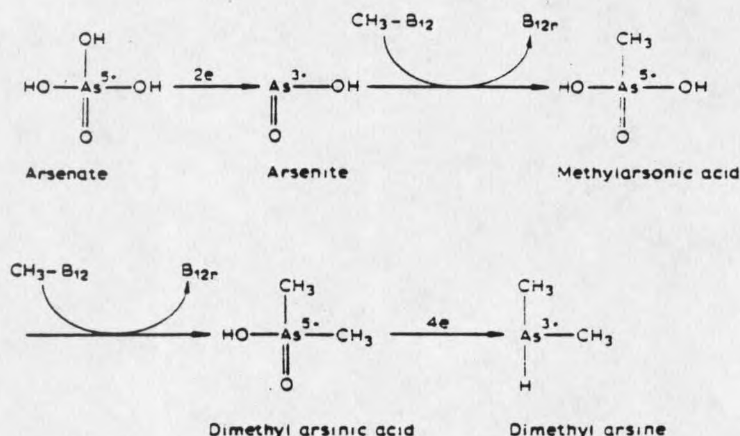
Distribution

After arsenic is taken in by the digestive system, it is absorbed from the small intestine by the blood. In blood, pentavalent arsenate As(+5), a water soluble compound, is reduced to arsenite As(+3), a lipid soluble compound. It is then transported by the blood to the liver where inorganic arsenic is partly metabolized. Metabolized arsenic is lipid soluble and is distributed to other tissues of the body. The unmetabolized arsenic is water soluble and readily excreted. Over 50 percent of the inorganic arsenic taken in by the body is excreted through the kidneys within two days (Leonard et al., 1980).

Metabolism

Metabolism takes place primarily in the liver. Arsenite is methylated first to methanearsonic acid which is then further methylated to dimethylarsinic acid, DMAA (Yamanaka et al., 1991). DMAA is then metabolized to dimethylarsine and trimethylarsine (Figure 5). This metabolic reduction is caused by the donation of a proton by NADPH (Yamanaka et al., 1991). After extensive studies, trimethylarsine was not found to be mutagenic while dimethylarsine was found to be mutagenic. Dimethylarsine also has a high affinity for sulfhydrols, a characteristic argued by some to be important for the carcinogenicity of arsenic (Yamanaka et al., 1989). Dimethylarsine is believed to be a metabolic derivative of arsenite because dimethylarsine is volatile and has a garlic odor. Arsenic intoxicated humans have been reported to have breath resembling garlic in odor (Yamanaka et al., 1989).

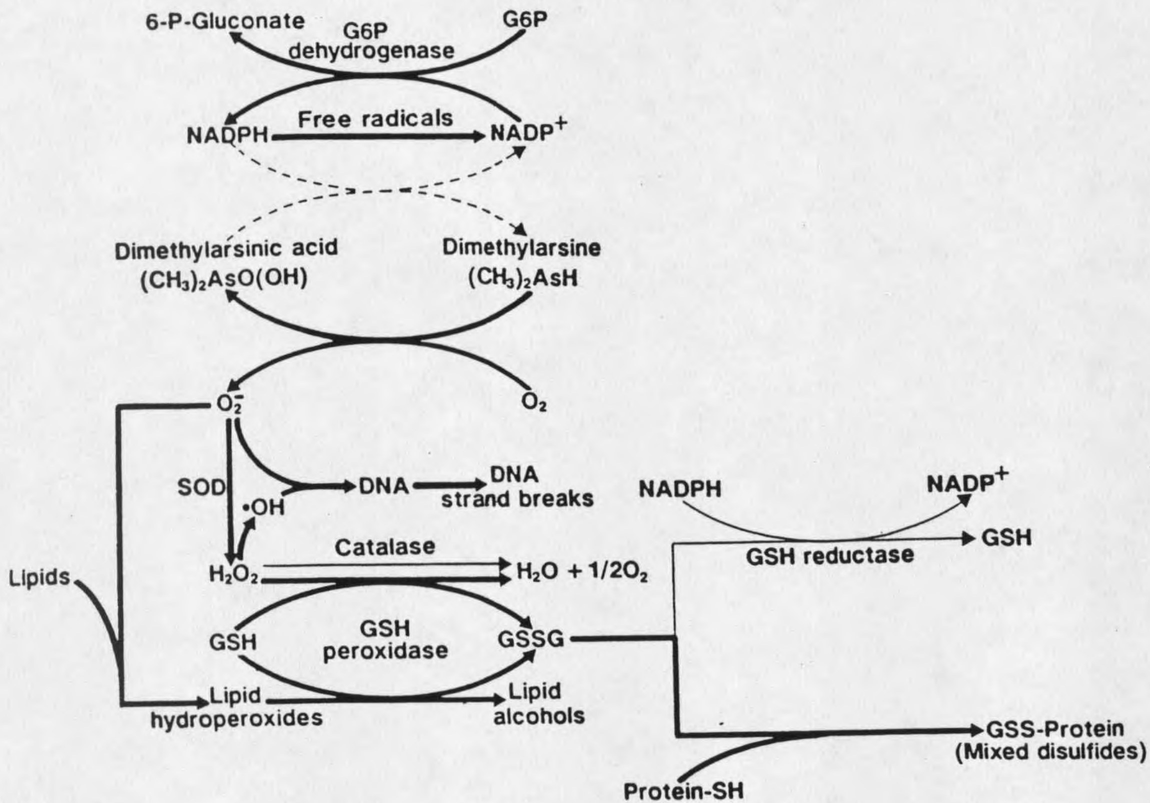
Figure 5. Breakdown of methylated arsenic (Fishbein, 1972)



Reactive Products

In the mitochondria of the cell, dimethylarsine reacts readily with molecular oxygen to form active oxygen species and the dimethylarsenic peroxyl radical. Mutation is induced by these radicals (Yamanaka et al., 1989). One of the active oxygen radicals is believed to be molecular oxygen minus one electron (Yamanaka et al., 1991). Another free oxygen radical produced is the bond of an oxygen and hydrogen with a negative charge. The reaction product of these two active oxygen species causes a DNA-damaging reaction (Figure 6).

Figure 6. The schematic of the effect on DNA of oxygen radicals (Yamanaka et al., 1991).



Mutation

Inorganic arsenic has been found to be an inhibitor of DNA synthesis and repair (Enterline and Marsh, 1982) and a clastogen causing chromosomal aberrations (Fong, 1989). In particular, the main pathway of DNA repair, known as "cut and patch" has been shown to be inhibited by dimethylarsine. Through this pathway, DNA has the ability to cut and remove sections, such as lesions and mutations, and synthesize a new strand to replace the one removed. The dimethylarsine binds to a dithiol group in a lipoic acid of gamma-keto glutacate dehydrogenase complex which inhibits enzymic reactions. This results in a decrease in respiration which decreases the production of ATP, essential for the incision process in the cut and patch repair of DNA (Yamanaka et al., 1989).

Arsenite also inhibits the synthesis of DNA by replacing the phosphorus in the phosphate group of DNA, forming a weak bond in the DNA chain which increases the probability of DNA strand breaks (Nakamuro and Sayato, 1981). The chromosomal aberrations of gaps and breaks induced by arsenite are in the chromatids (Nakamuro and Sayato, 1981). These aberrations are caused by the byproduct of active oxygen radicals or by the dimethylarsinic peroxyl radical (Yamanaka et al., 1991). The exact mechanism of how these radicals cause mutation is still unknown.

Human Cancer Data Concerning Arsenic

Many studies have been conducted to determine arsenic's role in cancer development. The data relating arsenic to cancer in humans are taken from case studies on a population of people exposed to an abnormally high amount of arsenic, near smelters producing base metals or water with high arsenic levels. One smelter study was done on workers in a gold mine in Rhodesia (Yamanaka et al., 1991). A six fold increase was observed in the incidence of lung cancer from these workers and researchers attributed it to arsenic exposure. In Taiwan, skin cancer was prevalent in the natives from the southwest coast. This incidence has been related to the high amount of arsenic in local artesian well water supplies (Yamanaka et al., 1991).

Approximately 50% of arsenic consumed by humans is absorbed and about 50% of the absorbed arsenic is methylated. Assuming that arsenic is carcinogenic, the dose of arsenic taken in by humans is compared to the number of chromosomal breaks incurred by each dose amount. This relationship shows how many breaks an average person could incur with a known dose of arsenic. The breaks caused by the arsenic make people less able to repair mutations caused by other sources, such as ultraviolet rays.

In cases where arsenic is the main cause of cancer, the cancer located in the lungs, liver, and epidermal area. These organs are all rich in molecular oxygen which promote the

oxygen and dimethylarsine combination to produce DNA-damaging radicals. In other tissues of the body, arsenic is a co-carcinogen which inhibits the DNA repair and synthesis essential for fighting other cancers (Yamanaka et al., 1991).

MATERIALS AND METHODS

Planters

Twenty-four planters,18 inches long, were constructed using sections of 8 inch diameter PVC piping. Three layers of mosquito netting were connected on one end with oversized hoseclamps. These planters were then filled with Bozeman silt loam soil collected from the Gallatin Gateway area, provided by the Plant and Growth Center (PGR) at Montana State University. This soil was analyzed by Dr. Jon Wraith of Plant and Soil Sciences Department at MSU and found to contain 2.8% by volume of organic matter. Each of the planters was placed, mosquito netting side down, in a bucket full of water to saturate the soil column and minimize the air pockets. After saturation, they were placed on racks made of 2x4 inch dimension lumber in a PGR greenhouse lit naturally from 9 am to 5 pm and artificially three hours before and three hours after the period of natural light. Total photoperiod was 14 hours. For the wheat experiment, three to four seeds were planted in each of 12 planters and for the alfalfa, 15 seeds were planted in each of the remaining 12 planters. Dr. Howard Bowman of the Plant and Soil Sciences Department provided the spring wheat strain (1992 Certified Dalen) and Dr. Raymond Ditterline of the same department provided the alfalfa strain (1984 IAYT Wrangler).

Growth Period

Each group of twelve planters was then divided into four groups of three replications of different arsenic concentrations. Group A (the control group)was watered with untreated Bozeman tap water provided by the PGR. Groups B, C, and D were all watered with specific concentrations of arsenic spiked water. This water was spiked by

adding specific amounts of arsenic trioxide to the control water (Anastasia, 1973; Arnot, 1967) Treatment Group B was watered with a 10 ppb (microgram per liter) concentration of arsenic, Group C was watered with a 50 ppb concentration, and Group D was watered with a 1.0 ppm (milligrams per liter) or 1000 ppb concentration. These arsenic concentrations were selected because they bracket the range of arsenic concentrations in the Madison River. Plants were fertilized with a common houseplant fertilizer every four weeks.

These four groups were watered with the same volume of 200 milliliters every 1 to 2 days for a total of 7.4 liters per planter. During this growing phase the plants were observed and the heights of their stems plus roots measured with a metric ruler. The heights of each plant were measured from the top of the stem or leaf to the bottom root tip after harvesting. The wheat was harvested when it turned gold, to simulate when farmers would harvest it in the fields. The alfalfa was harvested at the same time as the wheat. When harvested, each plant was dug up and air dried. The plants were harvested exactly 11 weeks after their seeds were planted. Each plant was then separated into roots, stems, leaves, and seed heads (in wheat) to be analyzed later. At the harvest time, two soil samples were also taken from each planter. One sample was taken at the level of the roots, approximately 0.5 cm in depth, and one more was taken at the 10 centimeter depth (Otte, 1990). These samples were also allowed to dry before analysis. After each soil and plant sample was dried, it was placed in an individual teflon container for digestion.

Digestion and Filtration

Soil and plant samples were digested with nitric acid to break down the organic matter into a liquid form for the analysis of arsenic. Five milliliters of trace metal grade nitric acid were pipetted into the teflon containers holding the dried soil and plant samples (Hewitt and Reynolds, 1990). A torque wrench was used to secure the lids of these

containers to a torque of seventeen pounds/ square inch. The containers were then microwaved for thirty seconds on high power. After heating, the containers were placed in a refrigerator for fifteen minutes to cool. The caps were then loosened to release pressure, retorqued, and placed back into the microwave for ninety seconds at high power. This process was repeated three times. After the last cooling, the supernatent and nitric acid were poured into 50 ml centrifuge tubes.

For filtering, each sample was then diluted with distilled water to the 50 ml line and capped tightly. The samples were then filtered using a 40 micron vacuum filter followed by a 25 micron syringe filter. The supernatent was then analyzed for arsenic content. This technique, developed by Shaun Hurley, Canyon Ferry Limnological Institute, was found to have a 99.9% recovery rate for arsenic within a 95% confidence interval (Hewitt and Reynolds, 1990).

Analysis

The digested samples were then analyzed for arsenic using a Varian AA-5 Atomic Absorption Spectrophotometer with a hydride generating system and a standard hollow cathode AA lamp. This instrument was located at the Canyon Ferry Limnological Institute and has an accuracy of one microgram per liter as measured by Dennis Braun of the Montana Environmental Health Department Chemistry Lab. Standards, spiked samples, and blanks were prepared with each set of samples.

The standards were 0.001 ppm, 0.005 ppm, 0.01 ppm, 0.100 ppm, and 1.0 ppm of arsenic (+3). These standard solutions were prepared from a 1000 ppm standard solution by adding arsenic trioxide to distilled deionized water in a 1000 ml volumetric flask using the equation (Volume A)*(Concentration A)=(Volume B)*(Concentration B). These standards were prepared daily for accurate analysis. The standards were analyzed and their peak heights graphed for use in the sample analysis. The blanks of digested nitric

acid were analyzed to determine interference and background noise in the analysis. Spiked samples were prepared by adding one ml of an arsenic standard to 19 ml of a sample. The arsenic (+3) concentration of the resulting solution was calculated and was measured to determine the accuracy and reliability of the AA-hydride instrument. The desirable percentage recovery of each spiked sample was 90 -110%. One spiked sample was analyzed for every ten samples.

Fifteen mls of each sample, standard, blank, spike, and distilled water were placed into a 50 ml centrifuge tube using a 15 ml class A pipette. Five ml of hydrochloric acid, 1.0 ml of 25% potassium iodide, and one ml of 25% urea solutions were added to each tube. These mixtures were allowed to react for 50 minutes. The HCl and KI were added to insure that all arsenic (+5) was reduced to arsenic (+3), since arsenic (+5) is not detected by the hydride method. Urea was added to reduce interference by NO_2 gas. Three milliliters of each mixture were placed into individual sample cups and placed in the autosampler. Distilled water washes followed the standards and each set of samples.

The hydride method was developed by the staff of the Chemistry Laboratory Bureau of the Montana Department of Health and Environmental Sciences. This method uses a 1.0 % sodium borohydride solution reacting with a 0.5% nitric acid solution to generate hydrogen gas necessary to produce arsine, AsH_3. The arsenic (+3) in the mixtures was converted to arsine and the resultant mixture flowed into a quartz tube wrapped with nichrome wire and heated to 900° C. The peak heights of the standards were measured at least twice, entered into a spreadsheet, and graphed. The calibration curve and linear regression equation were used to calculate the concentration of arsenic in each sample (Hatfield, 1987). The MSU Stat program was used for statistical analysis.

Stained Slides

Slides for light microscopy were made for the control group and the 1.0 ppm arsenic (the highest concentration treatment group) treated replications. For each group a separate slide was prepared for the cross section of the roots, stems, and leaves. Each slide was prepared using standard methods developed by Mischke and Berlin in 1976. Plants were separated into roots, stems, and leaves, cut into 0.5 cm lengths, and fixed with formalin-acetic acid-alcohol (FAA). After 24 hours the FAA was removed and a dehydration process was begun, using increasing concentrations of ethyl alcohol. After all water was removed from the tissues, paraffin was infiltrated and embedded into the plant parts using ethyl alcohol and xylene. These tissues were then thin sectioned with a microtome and the sections mounted on new slides and dried for 48 hours. To stain the slides, the samples were hydrated with water, stained with Safranin-fast green, and then dehydrated. These tissues were analyzed for anomalies in cell structure in the treated group as compared to the control group.

RESULTS AND DISCUSSION

The results of the atomic adsorption spectrophotometer data are recorded in Appendix A. These include the total arsenic concentration in each sample calculated from the line equations determined from the standards groups and their concentration averages. All treatment groups, plant sections, and soil depths were analyzed statistically using an analysis of variance test and t-test (Appendix A).

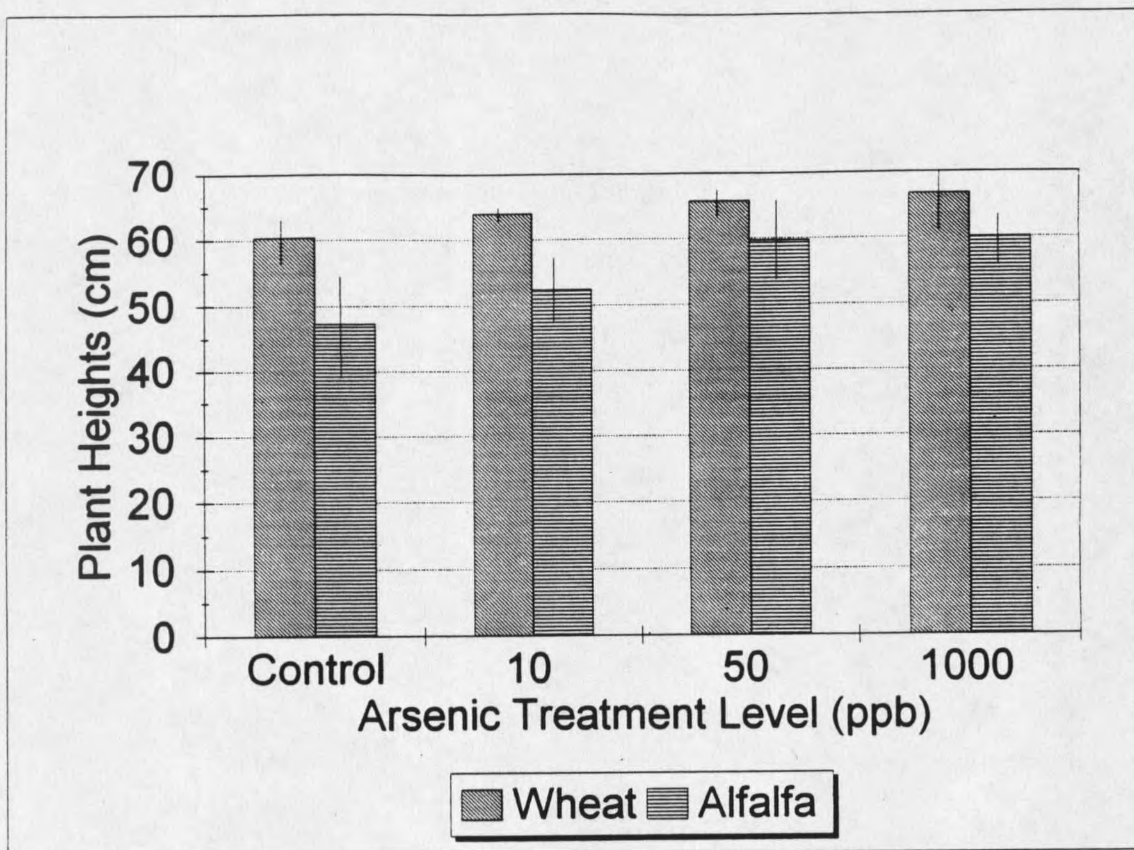
Plant Heights Results

The plant heights of the different treatment groups were compared and no significant differences were observed. In both the wheat and alfalfa the heights of the different treatment groups were not significantly different from each other (Figure 7). Previous studies show growth reduction in crops due to arsenic at levels of 6 ppm or greater. Therefore this study reconfirms that growth reduction does not occur at arsenic levels of 1 ppm or less. This study does not include the effects of annual arsenic accumulation.

Soil Arsenic Results

Soil samples were taken from the surface and from a depth of 10 cm at the time of harvest. The soil had an average original arsenic value of 19 ppb. After 11 weeks of treatment with arsenic laced water, the surface soil samples were found to have retained a greater amount of arsenic than the soil at a 10 cm depth (Tables 5-8). There was no significant difference in arsenic concentration at the soil surface when wheat was grown among the treatments. At the 10 cm depth in the wheat growing samples, the control was

Figure 7. Measured alfalfa and wheat plant heights as a function of applied arsenic concentration. The vertical line indicates the 95 % confidence interval of each sample series.



significantly lower and the 1000 ppb treatment group was significantly higher than the other treatments. There was a significantly greater arsenic concentration at the soil surface in the A and B treatment groups than the 10 cm depth where wheat was grown (Figure 8). In the alfalfa soils, all four treatment groups had a higher arsenic accumulation at the surface than at 10 cm except in the control group which showed no difference between the arsenic values at the surface and 10 cm depth (Figure 9).

Significantly more arsenic accumulated at the soil surface when alfalfa was grown but less accumulated at 10 cm depth as compared to samples when wheat was grown (Tables 5-8). This is possibly due to differing root systems of each plant species. Alfalfa has a taproot which consists of one root 2 to 3 times longer than the height of the plant. This root has tiny root hairs (the youngest ones) that aid in absorption in soil. Wheat has a fibrous root system that is a matrix of many small roots which extend horizontally and generally less than 10 cm vertically. Therefore, wheat would presumably take up more arsenic near the surface, reducing arsenic concentrations in soil near the surface. Conversely, alfalfa would absorb more arsenic from lower soil depths, reducing the arsenic concentrations in soils at lower depths.

Table 5. Arsenic concentrations measured in soil in which wheat was grown as a function of applied arsenic concentration.

	Original Soil	A	B	C	D
Surface (ppb)	19.0	23.93	26.27	28.83	39.82
10 cm (ppb)	19.0	20:15	22.30	21.78	29.07

Legend
Treatment Groups: A : Control Group; B : 10 ppb; C : 50 ppb; D : 1000 ppb

Table 6. Significant differences of arsenic concentration means of the different treatment groups in the soil in which wheat was grown.

Samples	Arsenic Concentrations (ppb)
Wheat Surface Soil	no differences
Wheat 10 cm Soil	A < (B, C) < D
Wheat Soil A	10 cm < Surface
Wheat Soil B	10 cm < Surface
Wheat Soil C	no differences
Wheat Soil D	no differences

**no differences => p-value > 0.05

Legend
Treatment Groups: A : Control Group C : 50 ppb
B : 10 ppb D : 1000 ppb

Table 7. Arsenic concentrations measured in soil in which alfalfa was grown as a function of applied arsenic concentration.

	Original Soil	A	B	C	D
Surface (ppb)	19.0	21.28	23.18	37.53	63.46
10 cm (ppb)	19.0	21.45	22.12	23.00	24.49

Legend
Treatment Groups: A : Control Group C : 50 ppb
B : 10 ppb D : 1000 ppb

Table 8. Significant differences of arsenic concentration means of the different treatment groups in the soil in which alfalfa was grown.

Samples	Arsenic Concentrations (ppb)
Alfalfa Surface Soil	(A, B) < C < D
Alfalfa 10 cm Soil	(A, B) < C < D
Alfalfa Soil A	no differences
Alfalfa Soil B	10 cm < Surface
Alfalfa Soil C	10 cm < Surface
Alfalfa Soil D	10 cm < Surface

**no differences => p-value > 0.05

Legend
Treatment Groups: A : Control Group C : 50 ppb
B : 10 ppb D : 1000 ppb

Figure 8. Arsenic concentrations measured in soil in which wheat was grown as a function of applied arsenic concentration. The arrow represents the original arsenic soil concentration. The vertical line indicates the 95 % confidence interval of each sample series.

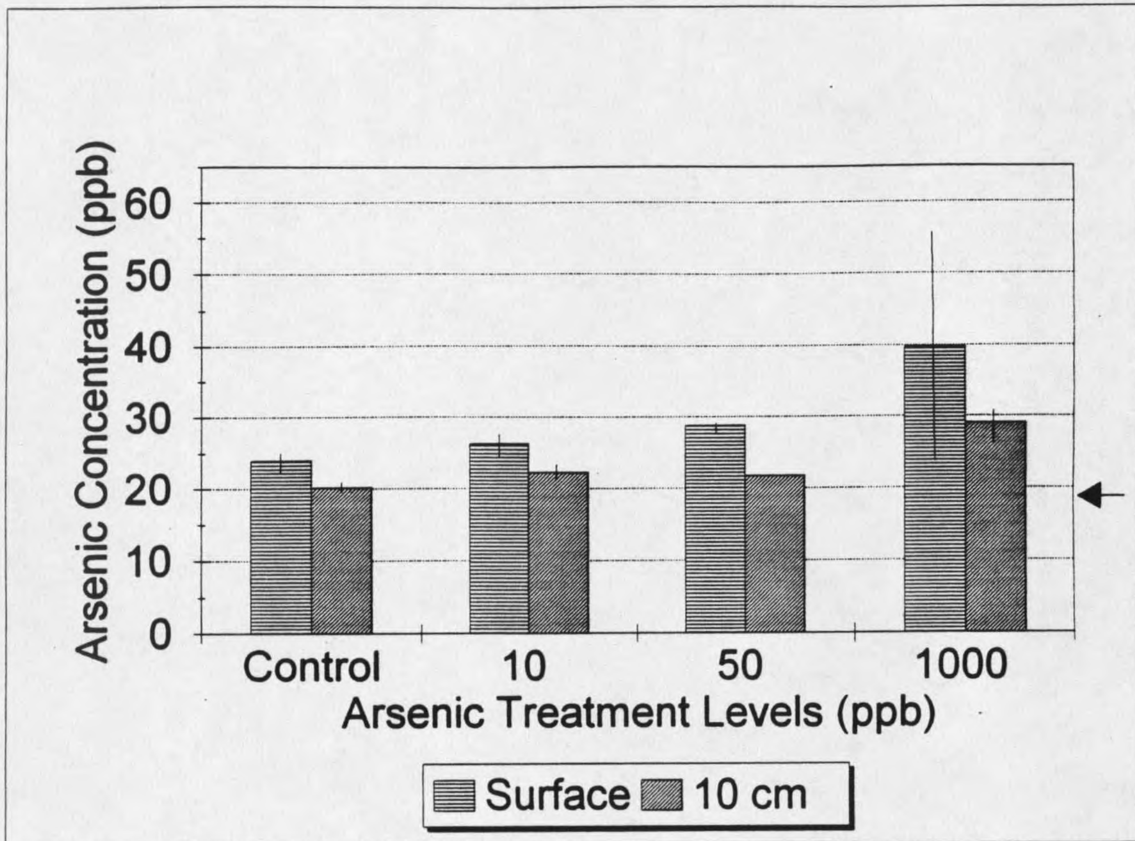
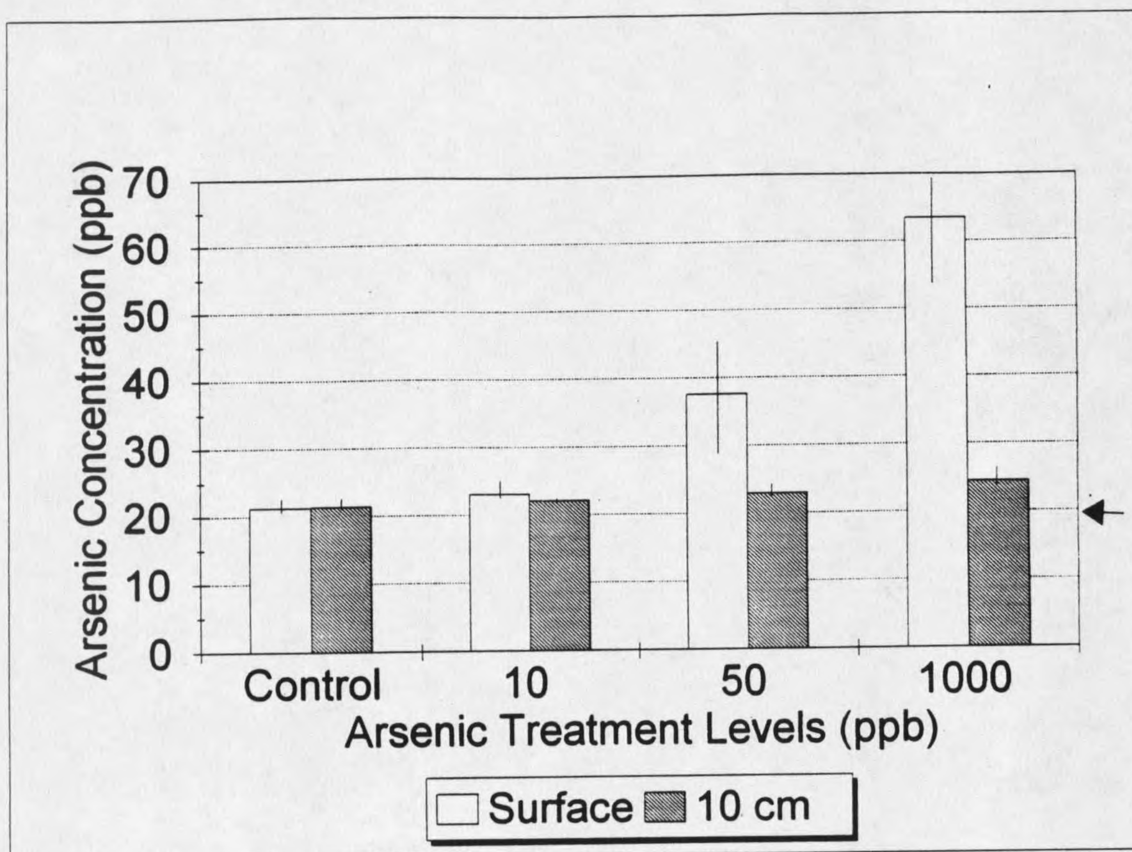


Figure 9. Arsenic concentrations measured in soil in which alfalfa was grown as a function of applied arsenic concentration. The arrow represents the original arsenic soil concentration. The vertical line indicates the 95 % confidence interval of each sample series.



Plant Arsenic Results

The arsenic concentration measurements of digested nitric acid averaged 17.22 ppb of arsenic concentration. The control group averaged 16 ppb of arsenic concentration.

Comparison in arsenic concentration between different plant organs (roots, stems, leaves, and seed heads,for wheat) for a given applied arsenic concentration and between different treatments within a given plant structure are shown in Tables 9-12. Arsenic concentrations in wheat roots were not significantly different in the 95 % confidence range ($p < 0.05$) but were all significantly different in the 90 % confidence range among the four treatment groups. The only significant difference in the concentrations of the wheat stems was between Groups A and D. The wheat leaves and seed heads showed a significant difference in arsenic accumulation among all of the treatment groups. In the control group (A), the root arsenic concentration was significantly higher than the leaves, seed heads, and stems (Tables 9,10, Figure 10). In Group B, the roots had a higher arsenic level than the leaves, seed heads, and stems and the leaves had higher levels than the stems (Tables 9,10, Figure 10). The roots, leaves, and seed heads had a higher concentration than the stems in Group C (Tables 9,10, Figure 10). In the wheat plants, Group D was found to have no significant differences between the arsenic concentration means (Tables 9,10, Figure 10).

In the alfalfa roots, Groups A and B had a significantly lower arsenic concentration than Group D. In the alfalfa stems, none of the treatment group arsenic accumulations were significantly different. In the alfalfa leaves, Groups B, C, and D were higher than the treatment control Group A, and Group D was higher than B and C (Tables 11,12, Figure 11). The alfalfa roots had a higher arsenic concentration than the stems in treatment Groups A, B, and C (Tables 11,12, Figure 11). The arsenic concentrations in alfalfa roots were higher than in the leaves in Group A (Tables 11,12, Figure 11). The leaves had higher arsenic levels than roots and leaves in Group D. The leaves also collected the most

arsenic having a concentration of 23.20 ppb in Group D. This value was 1. 2 times the value in stems which has a concentration of 18.79 ppb which was 1.3 times the value for roots of 18.22 ppb (Tables 11,12, Figure 11).

The wheat roots consistently appeared to absorb more arsenic than the rest of the plant. This may be because the roots are a fibrous system designed for maximum absorption. Alfalfa plants do not have a part that consistently absorbs more arsenic than the rest at different arsenic treatment levels. The leaves make the most dramatic change from almost undetectable levels in Group A to the highest level in Group D. Wheat as a whole stores more arsenic than the alfalfa plant.

Table 9. Arsenic concentrations measured in different wheat plant organs as a function of applied arsenic concentration.

	A	B	C	D
Roots (ppb)	18.20	18.08	18.51	34.17
Stems (ppb)	16.08	16.08	16.46	16.98
Leaves (ppb)	16.20	16.98	17.53	23.02
Seed Heads (ppb)	15.89	16.72	17.79	20.07

Legend
Treatment Groups: A : Control Group; B : 10 ppb; C : 50 ppb; D : 1000 ppb

Table 10. Significant differences of arsenic concentration means of the different treatment groups in the different wheat plant organs.

Samples	Arsenic Concentrations (ppb)
Wheat Roots	no differences (difference if p-value>0.1)
Wheat Stems	A < D
Wheat Leaves	A < B < C < D
Wheat Seed Heads	A < B < C < D
Wheat Plants A	Stems, Leaves, Seed Heads < Roots
Wheat Plants B	Stems , Seed Heads, Leaves < Roots Stems < Leaves
Wheat Plants C	Stems < Roots, Leaves, Seed Heads
Wheat Plants D	no differences

**no differences => p-value > 0.05

Legend
Treatment Groups: A : Control Group, B : 10 ppb, C : 50 ppb, D : 1000 ppb

Table 11. Arsenic concentrations measured in different alfalfa plant organs as a function of applied arsenic concentration.

	A	B	C	D
Roots (ppb)	16.848	16.715	17.515	18.217
Stems (ppb)	15.829	16.203	16.456	18.787
Leaves (ppb)	15.890	16.715	17.177	23.200

Legend
Treatment Groups: A : Control Group, B : 10 ppb, C : 50 ppb, D : 1000 ppb

Table 12. Significant differences of arsenic concentration means of the different treatment groups in the different alfalfa plant organs.

Samples	Arsenic Concentrations (ppb)
Alfalfa Roots	A, B < D
Alfalfa Stems	no differences
Alfalfa Leaves	A < (B, C) < D
Alfalfa Plant A	Leaves, Stems < Roots
Alfalfa Plant B	Stems < Leaves, Roots
Alfalfa Plant C	Stems < Roots
Alfalfa Plant D	Roots, Stems < Leaves

**no differences => p-value > 0.05

Legend
Treatment Groups: A : Control Group; B : 10 ppb; C : 50 ppb; D : 1000 ppb

Plant Microscopic Analysis

The wheat grown for this study exhibited typical monocot features. The roots have vascular bundles arranged in a ring with a pith in the middle and a cortex and epidermis on the outside. The bundles contain the xylem and phloem (Figures 12, 13). The stems have vascular bundles containing xylem and phloem sporadically dispersed throughout the tissue (Figures 14, 15). The leaves have C3 photosynthesis; stomata are on the upper surface. A midrib and parallel vascular bundles with a double bundle sheath are located along the leaf crossection (Figures 16, 17). No visible difference was found between the control and treated roots and stems. The width of the leaves of the control and treated wheat leaves were measured using an objective micrometer under the microscope; the control was found to be approximately 25 percent larger than the treated leaf. This difference could have been caused by a lack of phosphate uptake by the plant. No cell structural difference was found.

Figure 10. Arsenic concentrations measured in different wheat plant organs as a function of applied arsenic concentration. The arrow represents the tap water arsenic concentration. The vertical line indicates the 95 % confidence interval of each sample series.

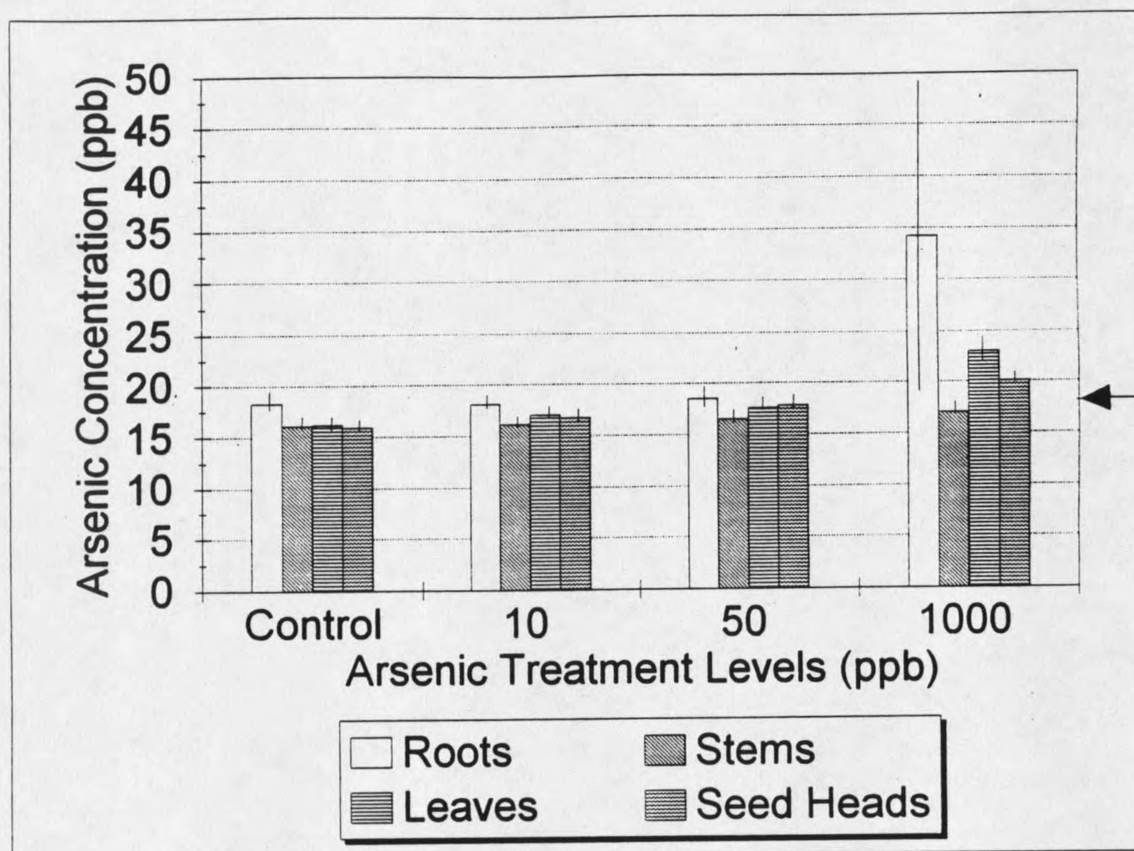
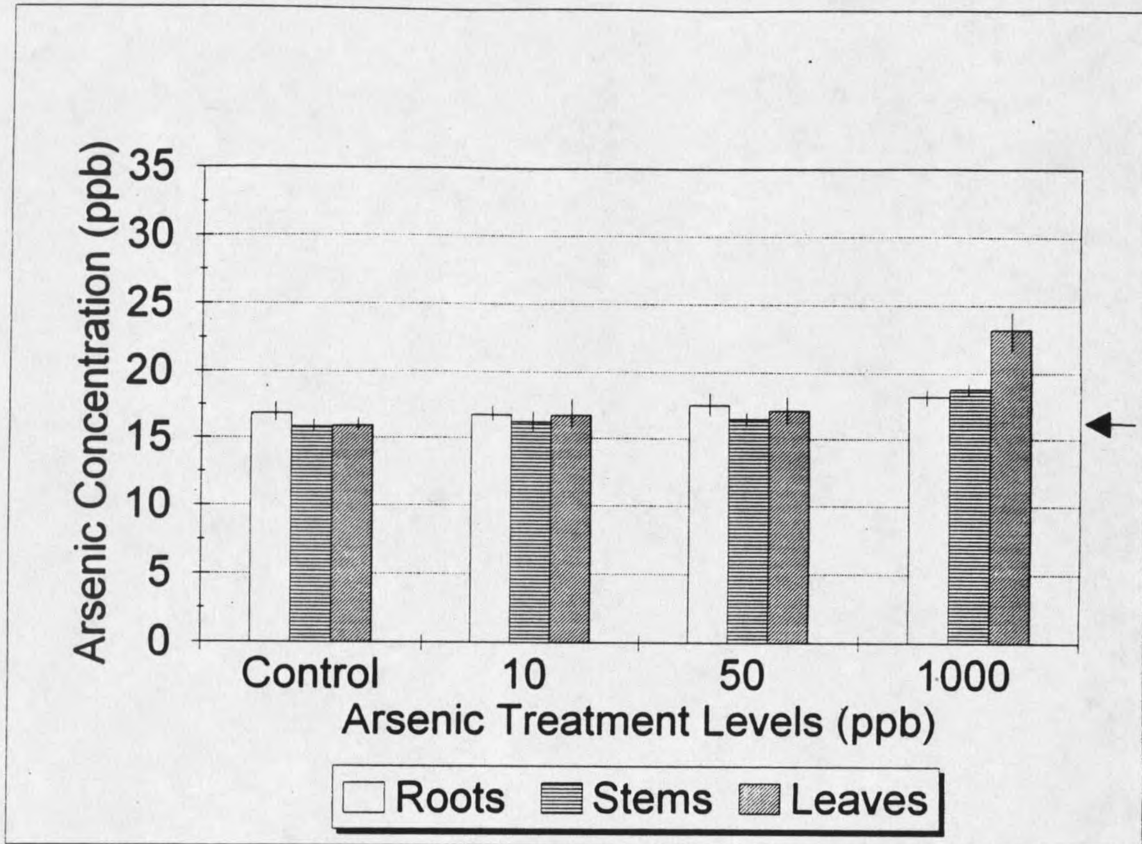


Figure 11. Arsenic concentrations measured in different alfalfa plant organs as a function of applied arsenic concentration. The arrow represents the tap water arsenic concentration. The vertical line indicates the 95 % confidence interval of each sample series.



The alfalfa grown for this study exhibited typical features of a dicot. The roots have large xylem and parenchyma cells in a starlike structure in the center of the root with phloem between the arms. The starlike structure is surrounded by a pericycle, a ring of endodermis, a cortex, an epidermis, and some secondary growth (Figures 18, 19). The stems have a ring of vascular bundles with the xylem on the inside and the phloem on the outside separated by a vascular cambium. The alfalfa stems also have a large pith of parenchyma cells and a small section of cortex and epidermis on the outside (Figures 20, 21). The leaves have a palisade and spongy layer of parenchyma cells with vascular bundles and stomata (Figures 22, 23).

No conclusive differences could be found between the treated and control, alfalfa and wheat groups except for a possible growth reduction in the wheat leaves. These results could be because only a light microscope was used to study the cross-sections. From the studies conducted, the only conclusion would be that the cellular structures of arsenic polluted wheat and alfalfa plants are not visibly affected by the introduction of arsenic into their irrigation water.

Figure 12. Photographs of wheat control roots under the light microscope

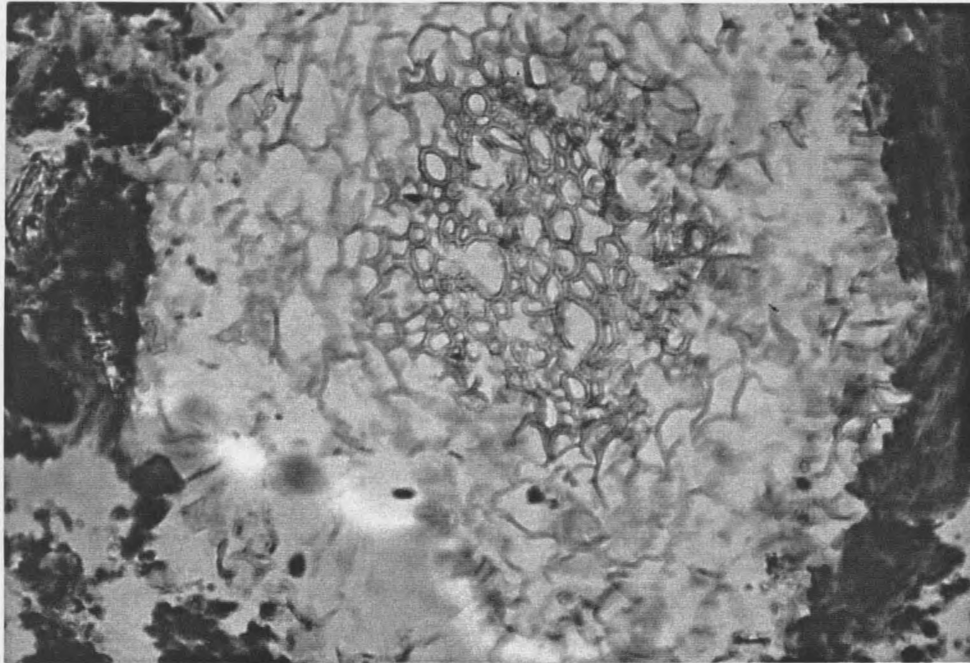


Figure 13. Photographs of wheat treatment roots under the light microscope

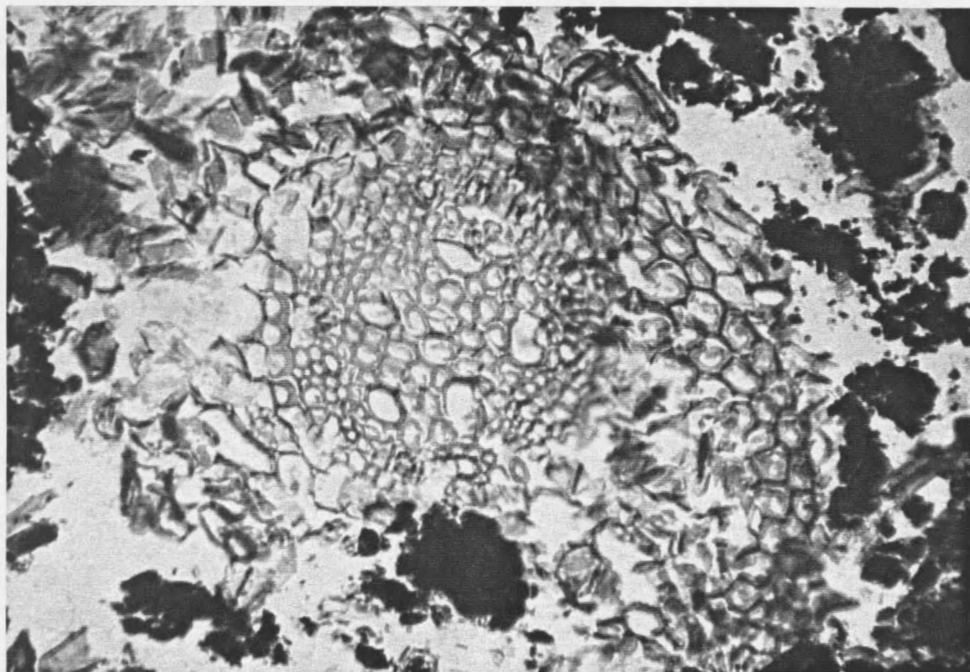


Figure 14. Photographs of wheat control stems under the light microscope

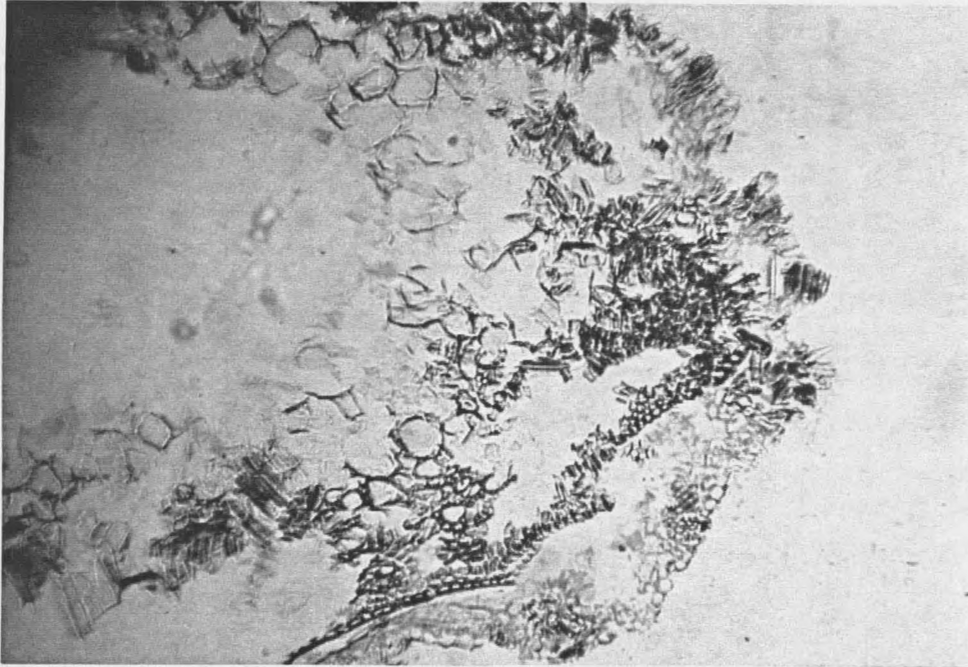


Figure 15. Photographs of wheat treatment stems under the light microscope

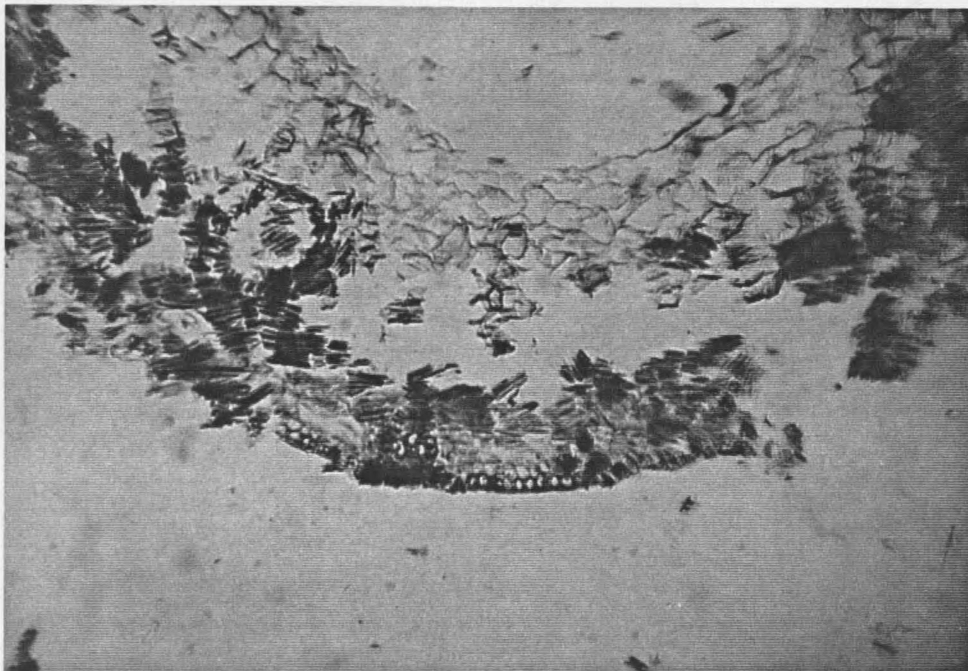


Figure 16. Photographs of wheat control leaves under the light microscope

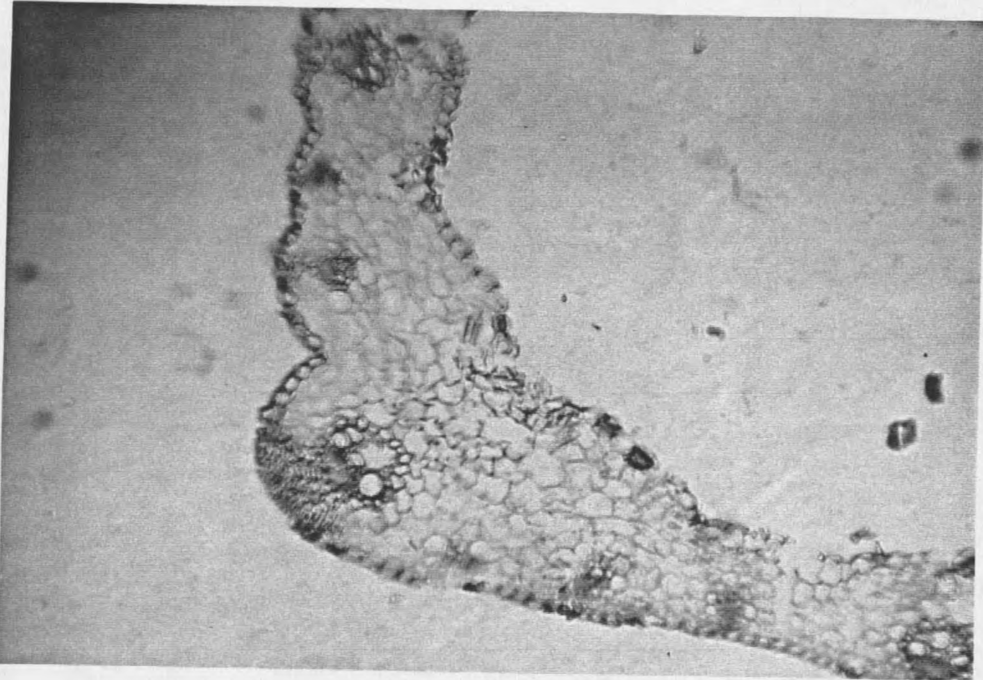


Figure 17. Photographs of wheat treatment leaves under the light microscope

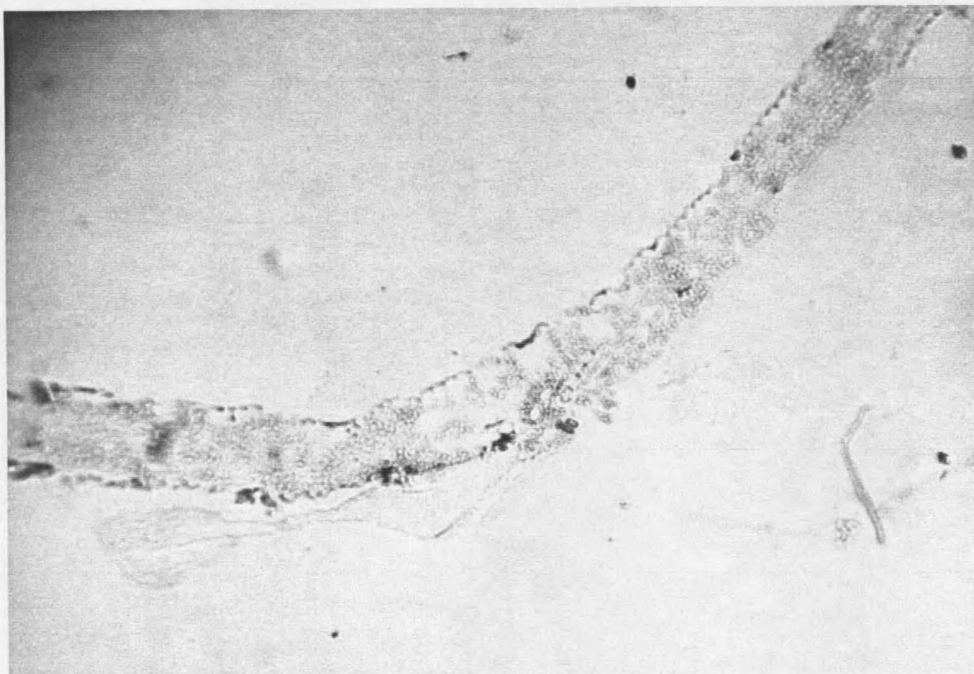


Figure 18. Photographs of alfalfa control roots under the light microscope

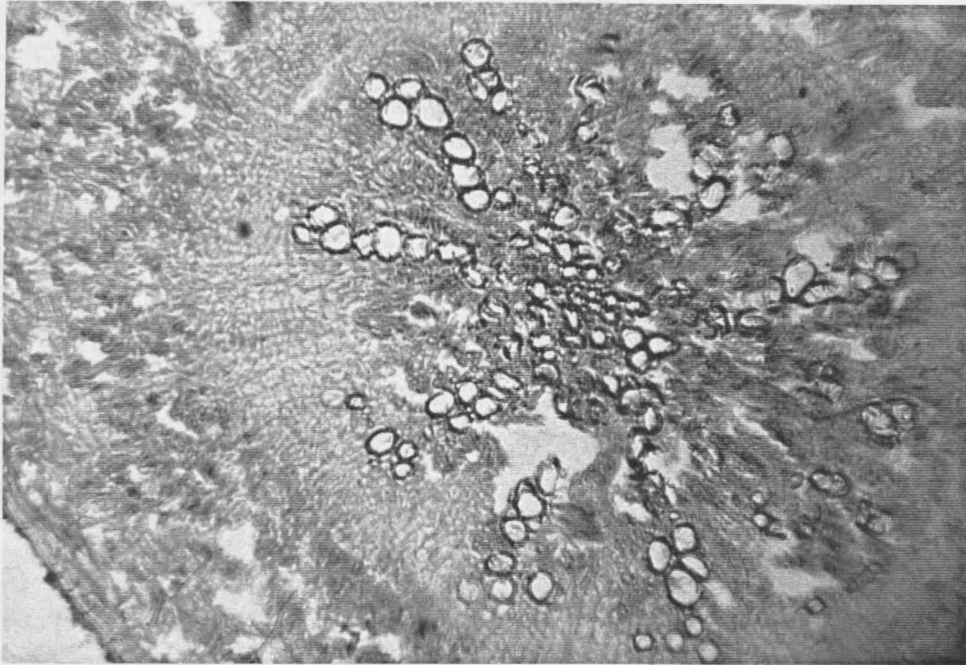


Figure 19. Photographs of alfalfa treatment roots under the light microscope

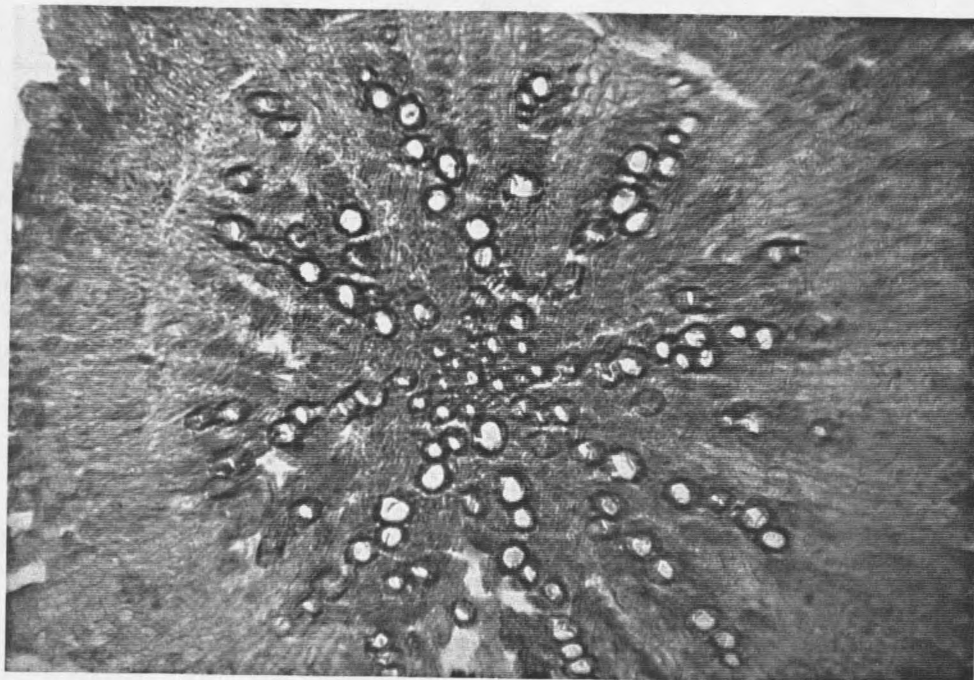


Figure 20. Photographs of alfalfa control stems under the light microscope

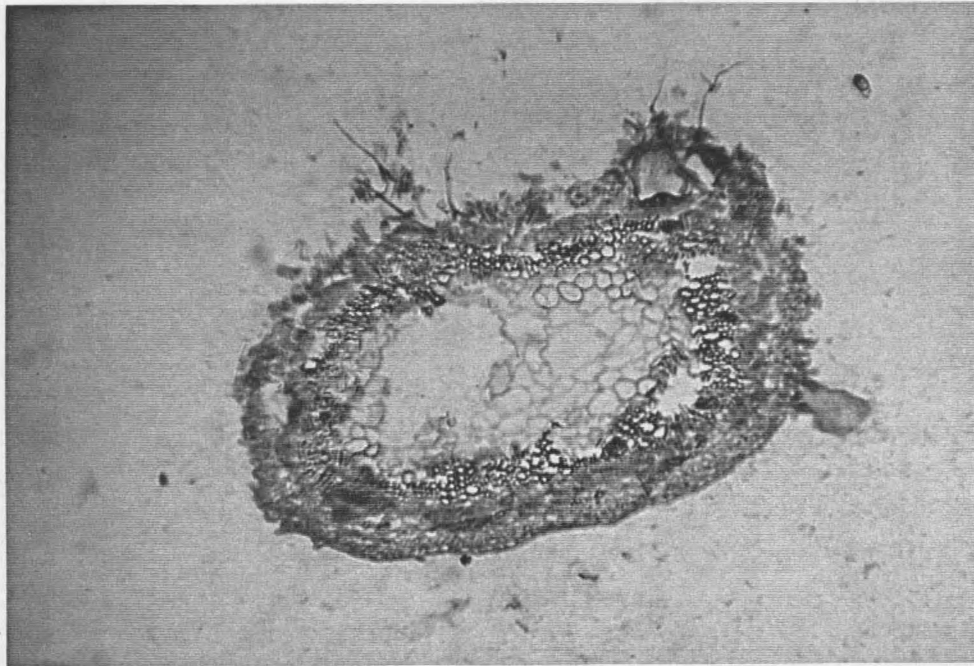


Figure 21. Photographs of alfalfa treatment stems under the light microscope

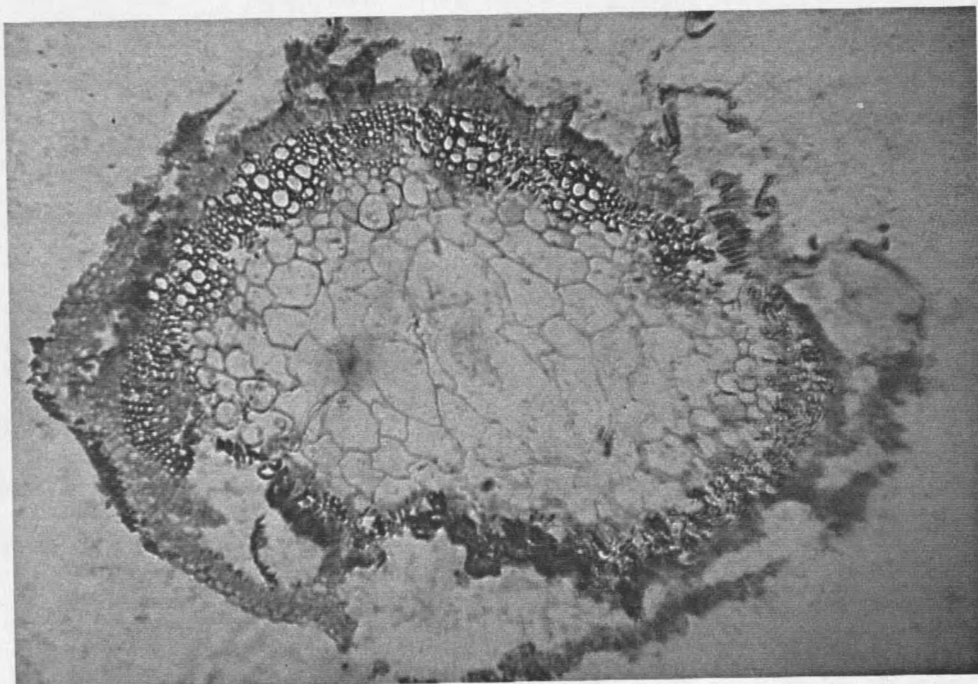


Figure 22. Photographs of alfalfa control leaves under the light microscope

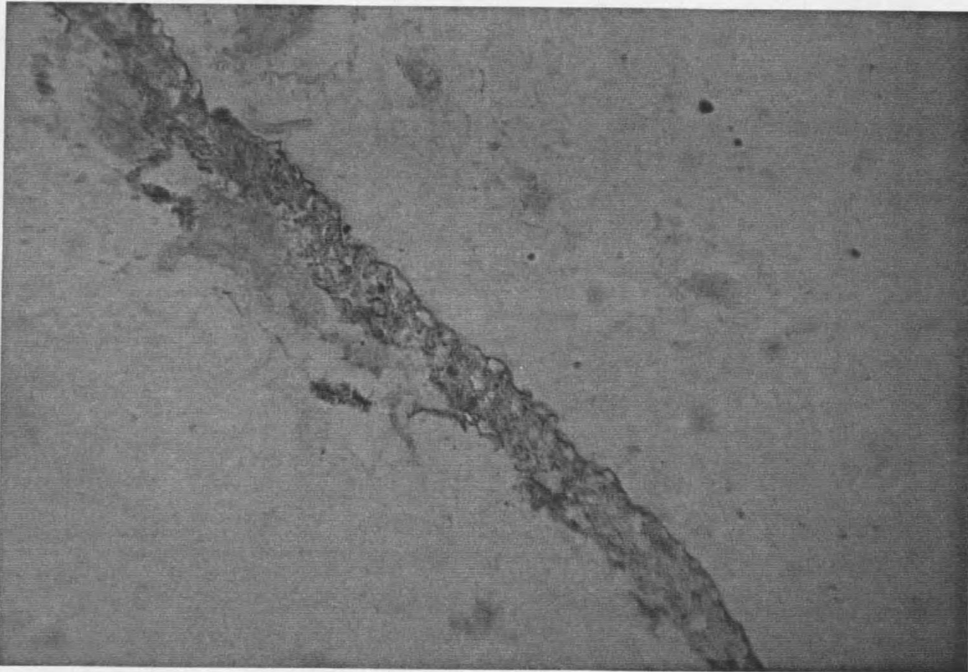
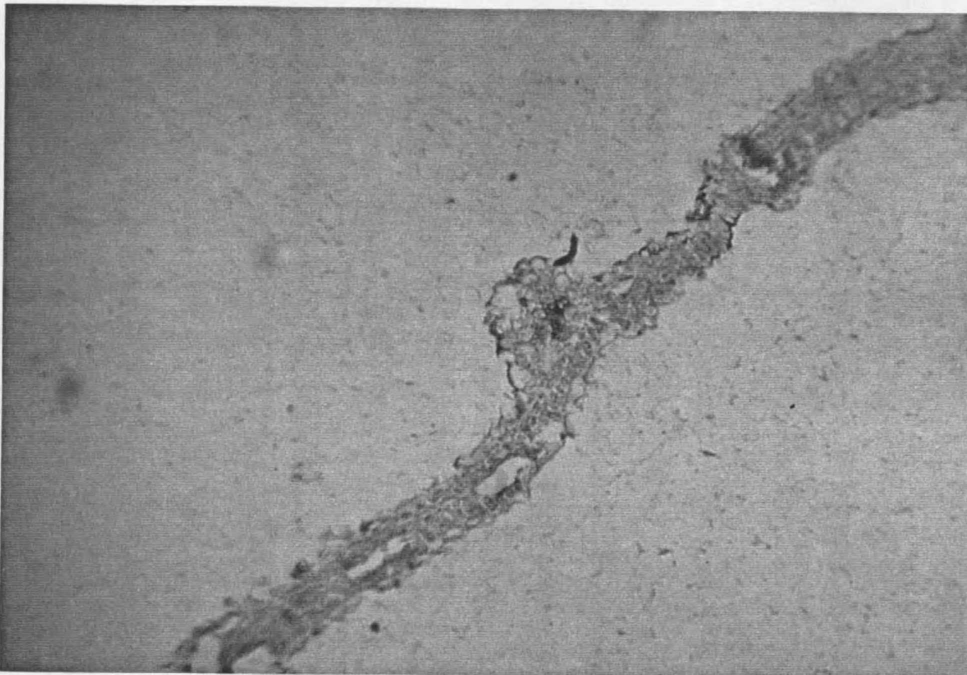


Figure 23. Photographs of alfalfa treatment leaves under the light microscope



CONCLUSIONS

The initial questions were answered about the effects of arsenic on wheat and alfalfa plants.

1. In what plant tissues does arsenic accumulate when introduced through water in a typical Montana monocot, e.g. wheat, and dicot, e.g. alfalfa?

Wheat roots consistently appear to accumulate more arsenic than the rest of the plant. Alfalfa plants do not have a plant organ that consistently accumulates more arsenic than the rest at different arsenic levels. Alfälfa leaves exhibit the most dramatic change from almost undetectable levels of arsenic in Group A to the highest level in Group D (Tables 9-12, Figures 10, 11). Wheat as a whole stores more arsenic than alfalfa.

2. Where in the soil profile does arsenic accumulate?

Surface soil in which alfalfa was grown had more arsenic than the soil in which wheat was grown but had less arsenic at 10 cm depth than the soil in which wheat was grown. The arsenic concentrations in surface soil were significantly higher than the concentrations in 10 cm depth soils for both wheat and alfalfa (Tables 5-8, Figures 8,9).

3. Does arsenic accumulation affect plant growth characteristics?

The arsenic accumulation of both the alfalfa and wheat did not appear to be affected by the concentration of the arsenic added through the irrigation water containing concentrations between 0 and 1.0 mg/l of arsenic (Figure 7).

4. Is there discernible damage in the plant cell tissues due to accumulation?

No conclusive differences could be found between the treated and control alfalfa and wheat groups except for a possible growth reduction in the wheat leaves. These results

could be because only a light microscope was used to study the cross-sections. Further study could include examining the plant cells with an electron microscope or measuring the photosynthetic rates as the different groups grew.

From these results, the wheat and alfalfa both appear to be accumulating arsenic but at very low concentrations. This implies that approximately 90% of the arsenic in this experiment is flowing out with the drainage. Therefore at risk populations should be more concerned about the arsenic in drinking water than in food supplies.

This study brings up many questions,

1. To what concentrations does arsenic accumulate in the soils and perennials over several years?

2. How much arsenic is eliminated as water percolates out of the soil containers?

3. Does arsenic affect any of the plant's physical and chemical processes?

4. Does arsenic accumulate in the same plant sections in other similar monocots and dicots?

Results from these questions could be used to conduct further studies.

1. Conduct a field study of arsenic concentration in 11 week old alfalfa and wheat from fields near the Madison River.

2. Conduct a study to measure the photosynthetic and respiration rates of the plants as they take up arsenic from irrigation water. Determine if there is any correlation between different metabolic rates and arsenic added to the system.

3. Measure the arsenic content in the groundwater while conducting experiment #1 and determine the rate of transport of arsenic through those soils.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allard, H. Xu B. and Grimvall, A., "Influence of pH and Organic Substance on the Adsorption of As (V) on Geologic Materials," *Water, Air, and Soil Pollution* 1988, Volume 40, p 293-305.

Anastasia, Frank B. and Kender, Walter J., "The Influence of Soil Arsenic on the Growth of Lowbush Blueberry," *Journal of Environmental Quality* 1973, Vol. 2, no. 3, p. 335-337.

Arnot, James T. and Leaf, Albert L., "The Determination and Distribution of Toxic Levels of Arsenic in a Silt Loam Soil," *WEEDS* 1967, Vol. 15, p. 121-124.

Asher, C.J. and Reay, P.F., "Arsenic Uptake by Barley Seedlings," *Australia Journal of Plant Physiology* 1979, Vol. 6, p. 459-466.

Baudo, Renato, Giesy, John P., and Muntau, Herbert, Sediments: Chemistry and Toxicology of In-Place Pollutants, Ann Arbor, MI: Lewis Publishers, INC., 1990, p. 169-171, 185-187, 194-199.

Bausch and Lomb, "Basic Spectrophotometry," Analytical Systems Division.

Bedwell, Gayleen, "Arsenic Accumulation in Macroinvertebrates of the Missouri River Drainage System," Canyon Ferry Limnological Institute November 1992.

Bolton, J.L., Alfalfa; Botany, Cultivation, and Utilization, Leonard Hill Books Limited, London 1962.

Brannon, James M. and Patrick, William H., "Fixation, Transformation, and Mobilization of Arsenic in Sediments," *Environmental Science Technology* 1987, Vol. 21, p. 450-459.

Chisholm, D., "Lead, Arsenic, and Copper Content of Crops Grown on Lead Arsenate-treated and Untreated Soils," *Canadian Journal of Plant Science* 1972, p. 583-587.

Collier, Graham F., "Spectrophotometric Estimation of Arsenic in Nitric Acid Extracts of Soil and Soil Additives," *Journal of Science Food Agriculture* 1973, Vol. 24, p. 1115-1117.

Council on Scientific Affairs, "Effects of Toxic Chemicals on the Reproductive System," *JAMA*, 253:23, June 21, 1985, p. 3435-3436.

Enterline and Marsh, "Cancer Among Arsenic-Exposed Workers," *American Journal of Epidemiology*, 116 1982, p. 905-910.

Ferguson, John F. and Gavis, Jerome, "A Review of the Arsenic Cycle in Natural Waters," *Water Research* 1972, Volume 6, p. 1259-1274.

Fishbein, L., "Natural Non-nutrient Substances in the Food Chain," *The Science of the Total Environment* 1972, p. 211-244.

Hatfield, D. Brooke, "Electrically Heated Quartz Atomization Cell for Hydride Generation-Atomic Absorption Spectrophotometry," *Analytical Chemistry* 1987, Vol. 59, p. 1887-1888.

Hewitt, Alan D. and Reynolds, C.M., "Dissolution of Metals from Soils and Sediments with a Microwave-Nitric Acid Digestion Technique," *Atomic Spectroscopy* September/October 1990, Vol. 11, No. 5, p. 187-192.

Hoaglin, David C., Mosteller, Frederick, and Tukey, John W., Fundamentals of Exploratory Analysis of Variance, New York, NY: John Wiley & Sons, Inc. 1991.

Holm, Thomas R., Anderson, Marc A., Iverson, Dennis G., and Stanforth, Robert S., "Heterogeneous Interactions of Arsenic in Aquatic Systems," *American Chemical Society* 1979, p. 711-736.

Horowitz, Arthur J., A Primer on Sediment-Trace Element Chemistry, 2nd ed., Ann Arbor, MI: Lewis Publishers 1991, p. 32-42, 71-79.

Horpestad, Abe, "Arsenic Briefing Paper," electronic mail, Montana State Water Quality Bureau, 1992, p. 1-4.

Johns, Carolyn and Moore, Johnnie, "Trace Metals in Reservoir Sediments of the Lower Clark Fork River, Montana," Montana Water Resources Center, Montana State University, Bozeman, MT, April 1987, p. 1-52.

Kabata-Pendias, Alina and Pendias, Henryk, Trace Elements in Soils and Plants, Boca Raton, FL.: CRC Press, Inc. 1984, p. 171-177.

Knapton, J.R. and Brosten, T.M., Arsenic and Chloride Data for Five Stream Sites in the Madison River Drainage, Montana, 1988, U.S. Geological Survey Open-File Report 88-722, January 1989.

Knapton, J.R. and Horpestad, A.A., Arsenic Data for Streams in the Upper Missouri River Basin, Montana and Wyoming, U.S. Geological Survey Open-File Report 87-124, March 1987.

Knapton, J.R. and Brosten, T.M., Supplemental Arsenic Data for Selected Streams in the Missouri River Basin, Montana, 1987, U.S. Geological Survey Open-File Report 87-697, December 1987.

Lederer, William and Fensterheim, Robert, Arsenic, Van Nostrand Reinhold Company 1983.

Leonard, A. and Lauwerys, RR., "Carcinogenicity, Tetratogenicity, and Mutagenicity of Arsenic," *Mutation Research*, Vol 75, 1980, p. 45-50.

Levit, S.M. and Munshower, F.F., "A Comparison of the Infectivity of Vesiculararbuscular Mycorrhiza from Three Grass Species," Reclamation Research Unit Publ. N. 8902 1989, p.1-12.

Lichte, F.E. and Skogerboe, R.K., "Emission Spectrometric Determination of Arsenic," *Analytical Chemistry*, July 1972, Vol. 44, No. 8, p.1480.

Livesey, N.T. and Huang, P.M., "Adsorption of Arsenate by Soils and its Relation to Selected Chemical Properties and Anions," *Soil Science*, Feb. 1981, Vol.131, No. 2, p. 88-94.

Metcalfe, C.R., Anatomy of the Monocotyledons, Clarendon Press, Oxford 1960, p. 519-524.

Moore, Johnnie, Ficklin, Walter H., and Johns, Carolyn, "Partitioning of Arsenic and Metals in Reducing Sulfic Sediments," *Environmental Science Technology* 1990, Vol. 22, p. 432-437.

Nakamuro, K. and Sayato, Y., "Comparative Studies of Chromosomal Abberation Induced by Trivalent and Pentavalent Arsenic," *Mutation Research,* Vol. 88, 1981, p.74-81.

Otte, M.L., Rozema, J., Beek, M.A., Kater, B.J., and Broekman, R.A., "Uptake of Arsenic by Estuarine Plants and Interactions with Phosphate, in the Field (Rhine Estuary) and Under Outdoor Experimental Conditions," *The Science of the Total Environment* 1990, Vol. 97/98, p. 839-854.

Penrose, W.R., "Arsenic in the Marine and Aquatic Environments: Analysis, Occurrence, and Significance," *CRC Critical Reviews in Environmental Control* October 1974, p. 465-482.

Peterson, R.F., Wheat: Botany, Cultivation, and Utilization, London: Leonard Hill Books 1965.

Pierce, Matthew L. and Moore, Carleton B., "Adsorption of Arsenite and Arsenate on Amorphous Iron Hydroxide," *Water Research* 1982, Vol. 16, p. 1247-1253.

Roy, W.R., Hassett, J.J., and Griffin, R.A., "Competitive Interactions of Phosphate and Molybdate on Arsenate Adsorption," *Soil Science* October 1986, Vol. 142, No. 4, p. 203-210.

Sakata, Masahiro, "Relationship between Adsorption of Arsenic (III) and Boron by Soil and Soil Properties," *Environmental Science Technology* 1987, Vol. 21, p. 1126-1130.

Schulman, Jon, "Mobility and Transport of Arsenic in the Madison and Missouri River Basins of Montana," Montana College of Mineral Science and Technology Thesis, December 1992.

Siegel, Frederic R., "Prospecting with Plants," *Earth Science* Fall 1987, p.18-19.

Stauffer, R.E., Jenne, E.A., and Ball, J.W., "Chemical Studies of Selected Trace Elements in Hot-Spring Drainages of Yellowstone National Park," USGS Geologic Survey Professional Paper 1044-F, 1980.

Tammes, P.M. and de Lint, M.M., "Leaching of Arsenic from Soil," *Netherlands Journal of Agriculture Science* 1969, Vol. 17, p. 128-132.

Thurman, David and Hardwick, Keith, "How Plants Survive and Overdose of Metal," *New Scientist* March 10, 1988, p. 44-45.

Water Management Bureau, "Arsenic Transport and Mobility in Surface Water, Irrigated Soils and Shallow Groundwater of the Upper Missouri River Basin," May 15, 1992.

Weir, Shelagh and Thuriaux, M.C., "Arsenic and Cancers," **The Lancet**, February 20, 1988.

Yamanaka, Kenzo, Hasegawa, A., Sawamura, R., and Okada, S., "Cellular Response to Oxidative Damage in Lung Induced by the Administration of Dimethylarsinic Acid, a Major Metabolite of Inorganic Arsenics, in Mice," *Toxicology and Pharmacology*, 108 1991, p.205-220.

Yamanaka, Kenzo, Ohba, H., Hasegawa, A., Sawamura, R., and Okada, S., "Mutagenicity of Dimethylated Metabolites of Inorganic Arsenics," *Chemical Pharmaceutical Bulletin*, 37:10 1989, p.2755-2760.

APPENDIX

APPENDIX A. Statistical Analysis

F and p values and confidence intervals for arsenic concentrations of wheat and alfalfa and the soils in which they were grown. All units are in ppb (micrograms per liter) in the confidence interval table. The F and p values are dimensionless.

	T	x	Avg x	x^2	sum(x)	sum(x^2)	sum(x)^2/	Sum d^	Sigma	K*Sigma	Upper Li	Low Lim
Roots	0	18.933	18.288	358.4585	54.865	1004.21	1003.389	0.8218	0.641	0.725375	19.01371	17.56296
		17.651		311.5578								
		18.281		334.195								
	1	18.933	18.078	358.4396	54.235	981.555	980.4603	1.0948	0.7399	0.837223	18.91539	17.24094
		17.651		311.5578								
		17.651		311.5578								
	2	18.933	18.506	358.4585	55.517	1028.47	1027.379	1.0957	0.7402	0.83755	19.34322	17.66812
		17.651		311.5578								
		18.933		358.4585								
	3	24.48	34.166	599.2704	102.5	3906.89	3501.878	405.01	14.23	16.10281	50.26848	18.06286
		27.513		756.9652								
		50.504		2550.654								
Stems	0	16.076	16.076	258.4378	48.228	775.313	775.3133	1.1E-13	2E-07	2.7E-07	16.076	16.076
		16.076		258.4378								
		16.076		258.4378								
	1	15.705	16.079	246.647	48.237	775.885	775.6027	0.282	0.3755	0.424916	16.50392	15.65408
		16.456		270.7999								
		16.076		258.4378								
	2	16.845	16.459	283.7641	49.377	813.002	812.7059	0.2959	0.3847	0.435271	16.89437	16.02383
		16.076		258.4378								
		16.456		270.7999								
	3	17.244	16.981	297.3555	50.944	865.511	865.097	0.414	0.455	0.514812	17.49615	16.46652
		16.456		270.7999								
		17.244		297.3555								
Leave	0	16.076	16.203	258.4378	48.608	787.675	787.5792	0.0963	0.2194	0.24826	16.45093	15.95441
		16.456		270.7999								
		16.076		258.4378								
	1	16.845	16.978	283.7641	50.934	864.874	864.7676	0.1061	0.2303	0.260575	17.23867	16.71753
		16.845		283.754								
		17.244		297.3555								
	2	17.286	17.529	298.7989	52.588	921.914	921.8256	0.0889	0.2108	0.238591	17.76786	17.29068
		17.651		311.5578								
		17.651		311.5578								
	3	21.781	23.019	474.412	69.058	1592.1	1589.651	2.4524	1.1073	1.253035	24.27223	21.76617
		23.362		545.783								
		23.915		571.9081								
SH	0	16.076	15.89	258.4378	47.671	757.577	757.5081	0.0688	0.1855	0.209907	16.10024	15.68043
		15.705		246.647								
		15.89		252.4921								
	1	16.456	16.715	270.7999	50.146	838.308	838.2071	0.1009	0.2246	0.254139	16.96947	16.46119
		16.845		283.754								
		16.845		283.754								
	2	18.068	17.79	326.4526	53.37	949.568	949.4523	0.1159	0.2408	0.272432	18.06243	17.51757
		17.651		311.5578								
		17.651		311.5578								
	3	19.838	20.072	393.5462	60.216	1208.77	1208.656	0.11	0.2345	0.265356	20.33736	19.80664
		20.071		402.845								
		20.307		412.3742								
Blank	0	15.89	15.89	252.4826	47.671	757.587	757.5176	0.0689	0.1856	0.210021	16.10045	15.68041
		15.705		246.6533								
		16.076		258.4506								
Root	0	17.244	16.848	297.3555	50.545	851.909	851.599	0.3105	0.394	0.445852	17.29419	16.40248
		16.845		283.754								
		16.456		270.7999								
	1	16.845	16.715	283.754	50.146	838.308	838.2071	0.1009	0.2246	0.254139	16.96947	16.46119

		16.845		283.754								
		16.456		270.7999								
	2	17.244	17.515	297.3555	52.546	920.471	920.3607	0.1104	0.235	0.265899	17.78123	17.24943
		17.651		311.5578								
		17.651		311.5578								
	3	18.933	18.217	358.4585	54.652	996.469	995.6137	0.8552	0.6539	0.739955	18.95729	17.47738
		17.651		311.5578								
		18.068		326.4526								
Stems	0	15.705	15.829	246.647	47.486	751.732	751.6401	0.0918	0.2142	0.24238	16.07105	15.58629
		15.705		246.647								
		16.076		258.4378								
	1	16.456	16.203	270.7999	48.608	787.675	787.5792	0.0963	0.2194	0.24826	16.45093	15.95441
		16.076		258.4378								
		16.076		258.4378								
	2	16.456	16.456	270.7999	49.368	812.406	812.4064	2.7E-08	0.0001	0.000131	16.4562	16.45594
		16.456		270.7999								
		16.456		270.8065								
	3	18.933	18.787	358.4585	56.361	1058.98	1058.854	0.1279	0.2529	0.286152	19.07315	18.50085
		18.495		342.065								
		18.933		358.4585								
Leave	0	16.076	15.89	258.4378	47.671	757.567	757.4985	0.0688	0.1855	0.209908	16.10014	15.68033
		15.705		246.647								
		15.89		252.4826								
	1	16.845	16.715	283.754	50.146	838.308	838.2071	0.1009	0.2246	0.254139	16.96947	16.46119
		16.845		283.754								
		16.456		270.7999								
	2	17.244	17.117	297.3555	51.351	879.713	878.9751	0.7382	0.6075	0.687475	17.80447	16.42963
		16.456		270.7999								
		17.651		311.5578								
	3	22.823	23.2	520.8893	69.599	1617.27	1614.669	2.5975	1.1396	1.28957	24.4892	21.91006
		22.296		497.1116								
		24.48		599.2655								
W Sur	0	25.059	23.928	627.9284	71.783	1719.49	1717.576	1.9187	0.9795	1.108349	25.03585	22.81915
		23.362		545.783								
		23.362		545.783								
	1	25.059	26.272	627.9535	78.815	2072.81	2070.601	2.2058	1.0502	1.18838	27.46005	25.08329
		26.878		722.4269								
		26.878		722.4269								
	2	28.829	28.829	831.1112	86.487	2493.33	2493.334	4.5E-13	5E-07	5.4E-07	28.829	28.829
		28.829		831.1112								
		28.829		831.1112								
	3	22.823	39.819	520.8893	119.46	5212.74	4756.603	456.14	15.102	17.08905	56.90782	22.72971
		51.697		2672.611								
		44.936		2019.244								
W10S	0	20.307	20.151	412.3742	60.452	1218.29	1218.148	0.1466	0.2708	0.306405	20.45707	19.84426
		19.838		393.5462								
		20.307		412.3742								
	1	21.781	22.3	474.412	66.9	1492.41	1491.87	0.5429	0.521	0.589563	22.88956	21.71044
		22.296		497.1116								
		22.823		520.8893								
	2	21.781	21.781	474.412	65.343	1423.24	1423.236	2.3E-13	3E-07	3.8E-07	21.781	21.781
		21.781		474.412								
		21.781		474.412								
	3	28.829	29.067	831.1112	87.2	2536.79	2534.613	2.1757	1.043	1.180244	30.24691	27.88642
		30.208		912.5233								
		28.163		793.1546								

OrigS	0	18.933	18.929	358.4585	56.788	1074.96	1074.959	8.1E-05	0.0064	0.007186	18.93652	18.92215
		18.922		358.0421								
		18.933		358.4585								
A Surf	0	21.278	21.282	452.7533	63.846	1359.26	1358.771	0.494	0.497	0.562406	21.84441	20.71959
		21.781		474.412								
		20.787		432.0994								
	1	22.813	23.179	520.433	69.537	1612	1611.798	0.2009	0.317	0.35867	23.53767	22.82033
		23.362		545.783								
		23.362		545.783								
	2	29.51	37.534	870.8401	112.6	4346.04	4226.479	119.56	7.7318	8.749138	46.28347	28.7852
		38.157		1455.957								
		44.936		2019.244								
	3	51.697	63.464	2672.58	190.39	12323.5	12083.04	240.43	10.964	12.40676	75.87076	51.05724
		65.302		4264.351								
		73.393		5386.532								
A10So	0	21.278	21.446	452.7533	64.337	1379.92	1379.75	0.1687	0.2904	0.328617	21.77428	21.11705
		21.781		474.412								
		21.278		452.7533								
	1	22.296	22.124	497.1116	66.373	1468.64	1468.458	0.1768	0.2973	0.336457	22.46079	21.78788
		21.781		474.412								
		22.296		497.1116								
	2	22.823	23.003	520.8893	69.008	1587.56	1587.368	0.1937	0.3112	0.352137	23.3548	22.65053
		22.823		520.8893								
		23.362		545.783								
	3	23.915	24.485	571.9061	73.454	1799.13	1798.472	0.6549	0.5722	0.647503	25.132	23.837
		25.059		627.9535								
		24.48		599.2655								
Blank	0	15.89	15.89	252.4826	47.671	757.587	757.5176	0.0689	0.1856	0.210021	16.10045	15.68041
		15.705		246.6533								
		16.076		258.4506								
Blank	1	15.89	15.89	252.4826	47.671	757.587	757.5176	0.0689	0.1856	0.210021	16.10045	15.68041
		15.705		246.6533								
		16.076		258.4506								
Blank	2	15.89	15.89	252.4826	47.671	757.587	757.5176	0.0689	0.1856	0.210021	16.10045	15.68041
		15.705		246.6533								
		16.076		258.4506								
Blank	3	15.89	15.89	252.4826	47.671	757.587	757.5176	0.0689	0.1856	0.210021	16.10045	15.68041
		15.705		246.6533								
		16.076		258.4506								

Wht H	0	57	60.333	3249	181	10945	10920.33	24.667	3.5119	3.973959	64.30729	56.35937
		64		4096								
		60		3600								
	1	64	64	4096	192	12288	12288	0	0	0	64	64
		64		4096								
		64		4096								
	2	67	65.667	4489	197	12941	12936.33	4.6667	1.5275	1.728508	67.39518	63.93816
		64		4096								
		66		4356								
	3	72	66.667	5184	200	13378	13333.33	44.667	4.7258	5.347612	72.01428	61.31905
		65		4225								
		63		3969								
Alf Ht	0	47	47.333	2209	142	6834	6721.333	112.67	7.5056	8.493092	55.82643	38.84024
		55		3025								
		40		1600								
	1	48	52.333	2304	157	8257	8216.333	40.667	4.5092	5.102552	57.43588	47.23078
		57		3249								
		52		2704								
	2	65	59.667	4225	179	10725	10680.33	44.667	4.7258	5.347612	65.01428	54.31906
		56		3136								
		58		3364								
	3	58	60	3364	180	10824	10800	24	3.4641	3.919689	63.91969	56.08011
		64		4096								
		58		3364								

	A	B	C	D	E	F
1	Group	DF	DF	F-value	Expected F	p-value
2	Wheat Ht	3	8	2.51	4.07	0.1325
3	Alf Ht	3	8	3.99	4.07	0.0521
4	Wheat Rts	3	8	3.71	4.07	0.0614
5	Wheat Stm	3	8	4.43	4.07	0.0409
6	Wheat Lves	3	8	84.37	4.07	0
7	Wheat SH	3	8	199.21	4.07	0
8	Alf Rts	3	8	8.32	4.07	0.0077
9	Alf Stm	3	8	0.97	4.07	0.4513
10	Alf Lves	3	8	76.91	4.07	0
11	Wht Surf Soil	3	8	2.58	4.07	0.1265
12	Wht 10 Soil	3	8	129.77	4.07	0
13	Alf Surf Soil	3	8	25.22	4.07	0.0002
14	Alf 10 Soil	3	8	34.62	4.07	0.0001
15	Wht Plnt A	3	8	30.69	4.07	0.0001
16	Wht Plnt B	3	8	10.54	4.07	0.0037
17	Wht Plnt C	3	8	10.83	4.07	0.0034
18	Wht Plnt D	3	8	3.3	4.07	0.0787
19	Alf Plnt A	2	6	12.49	5.14	0.0073
20	Alf Plnt B	2	6	5.29	5.14	0.0474
21	Alf Plnt C	2	6	6.07	5.14	0.0362
22	Alf Plnt D	2	6	37.38	5.14	0.0004
23	Wht Soil A	1	4	41.42	7.71	0.003
24	Wht Soil B	1	4	34.43	7.71	0.0042
25	Wht Soil C	1	4	*	7.71	*
26	Wht Soil D	1	4	1.51	7.71	0.286
27	Alf Soil A	1	4	0.24	7.71	0.6482
28	Alf Soil B	1	4	17.67	7.71	0.0137
29	Alf Soil C	1	4	10.58	7.71	0.0313
30	Alf Soil D	1	4	37.81	7.71	0.0035
31						
32	* error					

HOUCHEN
BINDERY LTD
UTICA/OMAHA