



Uptake and phytotoxicity of arsenic III and V in four grass species
by Stephanie Wagner Tice

A thesis submitted in partial fulfillment of the requirements for the degree of Masters of Science in
Land Rehabilitation
Montana State University
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Abstract:

Inorganic arsenicals including arsenic trioxide and arsenic pentaoxide have been used or liberated for many years due to one or more of the following: smelting, coal burning, arsenical pesticide use, and even glass manufacturing. After years of application or exposure, this arsenic accumulates in soils to levels which are often toxic to plants.

Western wheatgrass, basin wildrye, hard sheep fescue, and Canada bluegrass were grown in sand and irrigated with four concentrations of arsenic III (0, 1, 2, and 5 mg/L) and four concentrations of arsenic V (0, 7, 14, and 28 mg/L) in diluted nutrient solution for 75 days in a greenhouse. Plant height, shoot weight, root weight, and arsenic concentration in the above ground material were measured for each grass species. Plants were observed for changes in color, vigor, burn, percent red, and survival four times during the growing period.

For all grass species treated with either oxidation state of arsenic, symptoms of phytotoxicity usually appeared in plants that were treated with the highest arsenic concentrations. Plants irrigated with solutions containing lower arsenic III and V levels developed symptoms of arsenic toxicity later during the study period. Higher levels of arsenic V were added to the irrigation solution than were used for arsenic III. Plant responses to these higher concentrations of arsenic V were more pronounced than the responses to arsenic III.

Shoot weight of basin wildrye was stimulated by the addition of 7 mg As V/L, but overall the shoot weight, root weight, and plant height of the other grass species decreased as arsenic concentration in the nutrient solution increased. Height of Canada bluegrass irrigated with 5 mg As III/L was reduced at 75 days. This was the only measured response produced by any of the arsenic III treatments in any of the grass species. Basin wildrye has been observed growing on sites containing elevated arsenic concentrations, and the evidence provided by this investigation supports the use of basin wildrye for rehabilitation of lands contaminated with arsenic.

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A thesis submitted in partial fulfillment
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in
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APPROVAL

of a thesis submitted by

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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.

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Date September 7, 1995

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ABSTRACT

Inorganic arsenicals including arsenic trioxide and arsenic pentaoxide have been used or liberated for many years due to one or more of the following: smelting, coal burning, arsenical pesticide use, and even glass manufacturing. After years of application or exposure, this arsenic accumulates in soils to levels which are often toxic to plants.

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For all grass species treated with either oxidation state of arsenic, symptoms of phytotoxicity usually appeared in plants that were treated with the highest arsenic concentrations. Plants irrigated with solutions containing lower arsenic III and V levels developed symptoms of arsenic toxicity later during the study period. Higher levels of arsenic V were added to the irrigation solution than were used for arsenic III. Plant responses to these higher concentrations of arsenic V were more pronounced than the responses to arsenic III.

Shoot weight of basin wildrye was stimulated by the addition of 7 mg As V/L, but overall the shoot weight, root weight, and plant height of the other grass species decreased as arsenic concentration in the nutrient solution increased. Height of Canada bluegrass irrigated with 5 mg As III/L was reduced at 75 days. This was the only measured response produced by any of the arsenic III treatments in any of the grass species. Basin wildrye has been observed growing on sites containing elevated arsenic concentrations, and the evidence provided by this investigation supports the use of basin wildrye for rehabilitation of lands contaminated with arsenic.

INTRODUCTION

Up until the early part of this century, fumes were liberated into the air from smelters. This activity frequently destroyed or damaged vegetation as much as 40 miles downwind and deposited large quantities of smoke-borne metals including arsenic on surfaces of leaves (Goodman et al. 1973). The installation of smoke-cleaning devices during this century reduced these effects, but dumps of arsenic containing mine and smelter wastes still remain. The composition and quantity of these waste materials varies considerably between dumps. It is necessary to separately study each site and identify the adverse characteristics of the material present (ie. acidity, nutrient deficiencies, toxic substances, etc.) to determine the treatments required to enhance plant growth (Peterson and Nielson 1978).

Vegetation in the vicinity of the copper smelter near Anaconda, Montana, has been severely altered by several factors, including water and airborne wastes from the local copper smelter (Taskey et al. 1972). Taskey's study dealt with the ecological problems that are created when soils are contaminated with high concentrations of smelter-emitted heavy metals including arsenic, copper, lead, and zinc. Although appreciable quantities of these elements are remnants of earlier emissions from this smelter, they have remained concentrated near the soil surface and are a limiting factor to plant growth. Although the most serious damage to vegetation occurred in the late 1800's and early 1900's, Taskey notes that the region's plant cover and diversity are still low, and revegetation attempts have met with limited success. Since the closure of the smelter many sites are

recovering naturally, but the process is extremely slow, and weed species are often dominant.

An economic and permanent method of stabilizing mine wastes and soils containing toxic levels of metals is needed, both to prevent pollution and to improve the appearance of mine workings (Smith and Bradshaw 1972). A possible reclamation alternative is the use of naturally occurring metal-tolerant plant populations. The metal tolerance of these species permits them to grow in environments that are otherwise metal toxic. Knowledge of arsenic III and V toxicity to grasses is needed for successful revegetation. The objective of this study was to determine the effects of arsenic III and V on the growth, productivity, and arsenic loading of four grass species often used to revegetate disturbed lands.

REVIEW OF LITERATURE

Sources of Arsenic

Arsenic is a relatively mobile element, and is transported in gaseous, dissolved, and solid states within the environment (Woolson 1983). A tentative biogeochemical cycle for arsenic was estimated by Mackenzie et al. (1979). They considered movement to and from land, oceans, air, sediments, volcanos, and rivers. Arsenic is lost from sediments ($1,129 \times 10^8$ g/yr) while there are gains on land (660×10^8 g/yr) and in the oceans (566×10^8 g/yr). Land gains are mainly influenced by emission from coal and oil burning, cement manufacturing, and roasting of sulfide ores (779.3×10^8 g/yr). The loss of arsenic from sediments is a result of mining, burning fossil fuels, roasting of sulfide ores for metals, and the roasting of shale and limestone in cement manufacturing. Current anthropogenic practices have modified the global arsenic cycle. The burning of fossil fuel and industrial activities of society appear to have caused a departure from the presumed preindustrial steady-state cycle of arsenic, but on a global scale the element is not being accumulated to a significant extent by living biota.

After many years of using inorganic insecticides there was a shift to inorganic and organic herbicides which were applied at lower rates therefore reducing the amount of arsenic applied to the soil (Woolson 1983). Arsenical pesticides were once one of the largest classes of biocontrol agents, but these have been replaced with organic phosphates. Growth regulator arsenicals continue to be important with

arsanilic acid, 3-nitro-4-hydroxy-phenylarsonic acid, 4-nitro-phenylarsonic acid and carbarsone the most important in animal production (Woolson 1983).

Natural Occurrences of Arsenic

Arsenic, (As), a relatively scarce element in the earth's crust, ranks twentieth in elemental abundance and has concentrations in the continental crust of 1.5 to 2 mg As/kg (Woolson 1983). It is a major constituent of at least 245 different minerals. Arsenic is frequently found in association with sulfur, and arsenopyrite (FeAsS) is one of the most common minerals. Arsenic occurs naturally in all soils as a result of the composition of the parent rock material from which the soil was formed. It is normally present in low concentrations in the soil, less than 2 mg As/kg (Woolson et al. 1971). Plants growing in these soils also contain concentrations of less than 2 mg As/kg in dry plant tissue.

Williams and Whetstone (1940) analyzed a variety of soils and found naturally occurring arsenic to range between 0.3 and 40 mg As/kg. Vegetation growing on these soils ranged from less than 0.1 to 10 mg As/kg.

Arsenic, widely diffused in many types of mineral deposits, is highly concentrated in those deposits containing sulfide and sulfosalt deposits (Boyle and Jonasson 1973). Arsenic-bearing sulfides and sulfosalts oxidize readily when exposed to air to yield arsenic trioxide and finally arsenate (Woolson 1983). The most common elemental associates of arsenic are copper, gold, silver, zinc, cadmium, mercury, uranium, tin, lead, phosphorus, antimony, bismuth, molybdenum, iron, cobalt, nickel, platinum metals, selenium, and sulfur. Arsenic is, therefore, a good indicator in

geochemical prospecting surveys for some twenty elements of commercial importance (Boyle and Jonasson 1973). For example, soils overlying sulfide ore deposits usually contain arsenic at several hundred mg As/kg of soil, with a reported average of 126 mg As/kg of soil and the range extending from 2 to 8,000 mg As/kg (NRCC 1978). Naturally occurring arsenic anomalies occur in Rhodesia often in association with gold deposits and often accompanied by antimony (Wild 1974a). The arsenic values in these soils usually vary between 300 and 5,000 mg As/kg of soil, rarely reaching 20,000 mg As/kg. The analysis of plant species growing on these soils revealed values up to 242 mg As/kg of dry plant tissue with high levels in roots and shoots, depending on the plant species. Since the soil arsenic content can be a reflection of the soil's parent material composition, areas near arsenic mineral deposits may reveal total soil levels of 400 to 900 mg As/kg (NRCC 1978).

Arsenic in Soils

Arsenic levels in uncontaminated, nontreated soils seldom exceed 10 mg total As/kg. Selby et al. (1974) demonstrated that 1,140 soil samples collected from 114 counties in Missouri had a mean concentration of 8.7 mg total As/kg. The arsenic concentrations of soils of the Russian Plain were estimated to be 1 to 10 mg As/kg, with an average of 3.6 mg As/kg (Vinogradov 1959). Based on analysis of soils from various parts of the world, Berrow and Reaves (1984) reported a mean arsenic content of 10 mg total As/kg of soil.

Total arsenic is not a valid indicator of possible arsenic toxicity in the soil environment because other soil constituents may bind the arsenic and limit its uptake by plants (NRCC 1978). When it is necessary to determine the amount of arsenic that is likely to be taken up by plants from contaminated soil, Berrow and Reeves (1984) recognize that the soil extractable content is a better guide. Mitchell (1964) noted that the effective or available-to-plant form of arsenic (water-soluble), is not related to total arsenic and may be very low in soils with relatively high amounts of total arsenic. Arsenic retention or fixation in soils varies with the adsorptive capacity of the soil colloidal system (Jacobs et al. 1970a). An increase in arsenic sorption by soil colloids decreases the amount of water-soluble arsenic available to vegetation and reduces the phytotoxic effects. Johnson and Hiltbold (1969) determined that 85% of the arsenic content in a sandy loam surface soil was adsorbed on clay micelles. In subsurface soil (15-30 cm below the surface), they found 94% of the arsenic associated with the clay fraction. The arsenic retention capacity of sandy soils and soils with a high silica:sesquioxide ratio was relatively low, while fine textured soils such as clays tended to retain greater amounts of arsenic (Akins and Lewis 1976). Regardless of the soil type, arsenic toxicity becomes pronounced when the ion-exchange capacity of the soil is exceeded.

The lowest arsenic levels are found in sandy soils and, in particular, in those derived from granites. Higher arsenic concentrations are related most often to alluvial soils and soils rich in organic matter (Shacklette et al. 1974, Woolson 1983, Jacobs et al. 1970a, and Reed and Sturgis 1936). Because arsenicals tend to accumulate in the upper layer of the soil, shallow-rooted plants are most likely to accumulate and be injured by it

(Machlis 1941). Thus, a given total soil concentration of arsenic is: i) most toxic in coarse (sandy), gritty soils containing little colloidal material; ii) moderately toxic on loams, silt loam, and clay loams; and iii) least toxic on fine-textured soils of high clay, high organic matter and high available iron, aluminum, calcium, and phosphorus contents (Jacobs et al. 1970a).

Arsenic toxicity is partly determined by its solubility, which is dependent upon the chemical and biological properties of the soil (Greaves 1934, Walsh and Keeney 1975, and Hiltbold 1975). Since phytotoxicity of arsenic is highly dependent on soil properties, in high clay content soils about 90% growth reduction occurs at 1000 mg total (H_2SO_4 and HClO_4 extracted) As/kg soil. In sandy soils 100 mg As/kg is equally toxic (Woolson et al. 1973). Woolson et al. (1971) reported that correlation was better between extractable arsenic and plant growth than between total arsenic and plant growth. Several reports on the linear relationship between arsenic content of vegetation and concentrations in soil of both total and soluble arsenic suggest that plants take up arsenic passively with the water flow (Porter and Peterson 1975). Since arsenic toxicity to plants is directly related to the soluble level in the soil, a measure of the soluble arsenic content should be more informative than the total arsenic content (Deuel and Swoboda 1972).

Arsenic in Plants

The phytotoxicity of arsenic varies with: i) the plant species; ii) the soil arsenic levels and other soil characteristics; iii) the rate and nature of arsenical application; iv) the

arsenic levels in plants; and v) the temperature and humidity (NRCC 1978). Both organic and inorganic forms of arsenic, at some level, are phytotoxic to plants whether the arsenic is incorporated in the soil or sprayed on the plants (Woolson 1983). In general, much higher applications or soil levels are required in order to affect the plant through root uptake rather than through leaf uptake.

There is no evidence that arsenic is essential for plant growth, although stimulation of root growth with small amounts of arsenic in solution culture was reported by Albert and Arndt (1931), Liebig et al. (1959), and Stewart and Smith (1922). Liebig (1966) noted that stimulation does not always occur, is sometimes only temporary, and may result in the reduction of top growth. Two possibilities exist for growth stimulation by arsenic: first, stimulation of plant systems by small amounts of arsenic, since other pesticides, like 2,4-D, stimulate plant growth at sublethal dose levels (Woolson et al. 1971); second, displacement of phosphate ions from the soil by arsenate ions, with a resultant increase in phosphate solubility (Jacobs et al. 1970b).

The highest arsenic levels occur in plant roots, followed by those in plant tops, and lastly by those in edible seeds and fruits. Root tissue is often a useful indicator of high levels of available arsenic in the soil. Walsh and Keeney (1975) pointed out that the exposed portion of a plant may be contaminated by adherence of the arsenic-containing dust particles to its surface. In general, though, the edible seeds and fruit of the plant seldom accumulate highly elevated levels of arsenic as most plant growth is severely retarded prior to this occurrence.

Weaver et al. (1984) grew bermudagrass on three soils amended with arsenic to determine uptake and concentrations of arsenic in the leaves, stems, and roots. Plant growth was reduced on all soils containing 90 mg soluble As/kg. Concentrations of arsenic in leaves, stems, and roots of plants grown on soil containing 45 mg soluble As/kg, often exceeded 15, 25, and 200 mg As/kg, respectively, on a dry weight basis. The maximum concentration of arsenic in plant tops was 45 mg As/kg. Marcus-Wyner and Rains (1982) found that the uptake and translocation of arsenic by cotton grown in solution culture were influenced by the source of arsenic. Arsenic trioxide was readily taken up by the roots, but was not translocated to the shoots.

Many studies have shown differences in the sensitivity of plants to arsenic. For instance, Cooper et al. (1931) observed growth reduction responses of 11.3, 18.0, 34.0, 72.1, and 105.8% of control for vetch, oats, barley, wheat, and rye, respectively, grown on a sandy clay loam containing 1,131 mg total As/kg. Similar differences in plant responses were seen in studies by Clements and Munson (1947), Jacobs et al. (1970b), and Deuel and Swobada (1972). Woolson (1973) measured the response of a variety of crops to different arsenic levels and different soils. Plants have different sensitivities since it took 6.2, 10.9, 10.6, 48.3, 25.4, and 19.0 mg soluble As/kg of soil to reduce growth 50% in green beans, lima beans, spinach, cabbage, tomatoes and radishes, respectively. Green beans are most sensitive and cabbage the least sensitive of the crops tested. If one assumes that one-tenth of the total arsenic present is available (Woolson et al. 1971), levels needed to reduce growth 50% would range from 62 to 483 mg total As/kg soil for these crops on this soil.

Deuel and Swoboda (1972) found that the yield-limiting arsenic concentration in plant tissues was 4.4 mg (H_2SO_4 , HNO_3 , and $HClO_4$ digested) As/kg dry weight in cotton and 1 mg As/kg in soybeans. The critical level for barley was 20 mg As/kg in the leaves and shoots (11-26 mg As/kg range) as determined by sand culture studies (Davis et al. 1978). In rice, the critical level in tops ranged from 20 to 100 mg As/kg, and in roots 1,000 mg As/kg (Chino 1981). Normal leaves from fruit trees contained 0.9 to 1.7 mg As/kg, but leaves from trees suffering from arsenic excess contained 2.1 to 8.2 mg As/kg (NAS 1977). Woolson (1973) found that the soil for the GR_{50} level (50% reduction in growth) for an unpeeled radish contained about 19 mg As/kg. In applying the GR_{50} technique he found that limiting concentrations were 76 mg As/kg dry weight (8 mg As/kg fresh weight) with unpeeled, washed radish and 10 mg As/kg dry weight (1 mg As/kg fresh weight) with spinach. These values exceeded the tolerance limit of 2.6 mg As/kg dry weight for vegetables treated with calcium arsenate.

When Machlis (1941) grew Sudan grass and bush bean for varying lengths of time in nutrient solutions containing several concentrations of sodium arsenate, it was found that concentrations of up to 0.5 or 0.6 mg As/kg dry weight had no effect on the growth of either plant as measured by increases or decreases in dry weight. Growth was effectively reduced by concentrations of 1.2 and 12.0 mg As/kg for bean and Sudan grass, respectively. The concentrations of arsenic in all parts of the plant except the reproductive structures were directly proportional to the concentrations in the nutrient solutions.

Overley (1950) seeded crops in arsenic toxic soil, 10 to 42 mg water soluble As/kg in a mature apple orchard, and found a wide range of stand and growth quality among the

species planted. The common grasses in general proved to be more tolerant of arsenic toxicity in the soil than legumes or cereals, except possibly rye. On the basis of tolerance Italian rye grass, Kentucky bluegrass, fescues, redtop, orchardgrass, and quackgrass showed the greatest promise for permanent grass cover crops. Benson and Reisenauer (1951) and Liebig (1966) compared the sensitivity of various forage crops to arsenic and also found that: i) alfalfa, bromegrass, clover, vetch, and other legumes have a low arsenic tolerance; ii) crested wheatgrass and timothy are moderately arsenic tolerant; and 3) sudangrass, Italian ryegrass, Kentucky bluegrass, meadow fescue, and redtop are very arsenic tolerant.

With increasing soil arsenic, the highest arsenic concentrations were always recorded in the old leaves and roots of barley (Thoresby and Thornton 1979). The degree and kinetics of arsenic translocation and absorption in vegetation are influenced by: i) the plant species; ii) the temperature; iii) the arsenical compound; iv) the level of available arsenic in soil; and v) the point and rate of arsenical application (NRCC 1978). Several plant species are known to tolerate a high level of arsenic in tissues. Arsenic tolerance has been commonly noted in colonial bentgrass and little bluestem growing on mine waste, on soils treated with arsenical pesticides, and on soils with arsenic added by sewage sludge treatment (Rocovich and West 1975, and Vincent 1944).

Wild (1974b) recorded seventy-two species from 15 Rhodesian arsenic containing dumps. Of these plants five were introduced, 13 were African weed species, and 50 were indigenous non woody species. Of the indigenous species 28 had been recorded on natural arsenic anomalies (Wild 1974a) and 22 had not been previously recorded.

Bermudagrass was the most important grass species on these dumps. The arsenic concentrations of some of the dumps (e.g. Banshee dump with 30,000 mg total As/kg) were once assumed to be incapable of supporting vegetation. A few of these naturally bare dumps were planted successfully with tolerant species, such as bermudagrass (selected races), although at the time of publication it could not be determined if they would survive indefinitely (Wild 1974b). However, some mine dumps (e.g. Reliance, 5,000 mg total As/kg) do carry a moderately well established vegetative cover.

Arsenic phytotoxicity symptoms include wilting of new growth. This is often followed by inhibited root and top growth (Liebig 1966). This inhibited root and top growth is often accompanied by shoot and root discoloration and necrosis of leaf tips and margins. Arsenic often causes injury to roots, resulting in interference with the plant water uptake (Clements et al. 1939). Growth is reduced progressively as the arsenic concentrations increase, and arsenic is found in the plants in concentrations roughly proportional to the concentrations of arsenic in the soil solutions.

Deep plowing to dilute the arsenic concentration of the surface soil and expose arsenic to more fixation sites appears to be one of the most economical methods of alleviating soil arsenic toxicity (Walsh and Keeney 1975). The addition of iron, aluminum, or zinc compounds, lime, manure, or organic matter to the soil minimizes soluble (available) arsenic content and hence reduces arsenic toxicity (Liebig 1966). Vincent (1944) suggested growing tolerant cover crops which if removed or plowed under, could reduce arsenic phytotoxicity.

Arsenic III and V-General

Arsenic, a crystalline metalloid belonging to group V-A, has an atomic weight of 74.922. The more common oxidation states available to it are -3, 0, 3, and 5. Arsenic bonds covalently with most nonmetals and metals, and forms stable organic compounds in both its trivalent (III) and pentavalent (V) states. "Speciation is important in the study of the environmental behavior of arsenic since the major features affecting movement and toxicity of arsenic are associated with changes in oxidation states and the resulting differences in chemical properties of the various chemical forms" (Masscheleyn et al. 1991).

Arsenic is a labile element present in practically all environmental matrices and can exist in several forms and oxidation states (Adriano 1986). In strongly reducing environments, elemental arsenic and arsine can exist, but arsenate (arsenic V) is the stable oxidation state in aerobic environments. Under moderately reducing conditions, such as flooded soils, arsenite (arsenic III) may be the dominant form (Deuel and Swoboda 1972). The reduction of arsenate to arsenite, a more toxic form, is carried out under aerobic conditions by *Pseudomonas fluorescens*, a common aquatic bacterium (Myers et al. 1973). Activated sewage sludge reduces arsenate to arsenite under anaerobic conditions.

Arsenite is a common commercial form of arsenic and one of the most toxic arsenic compounds. This reduced state of arsenic has been reported to be 4 to 10 times more soluble in soils than the oxidized state (arsenate) (Brenchley 1914, Morris and Swingle 1927, and Woolson 1983). Keaton and Kardos (1940) demonstrated that the

oxidized form of arsenic was fixed to a much greater extent than the reduced form, thereby proposing that the oxidation state of arsenic influences its sorption capacity by soils.

Oxidation/reduction reactions can be a combination of chemical and microbiological processes. Chemical redox reactions are governed by iron and pH levels (Keaton and Kardos 1940). High iron levels favor the oxidation of arsenite to arsenate, while aluminum does not affect the redox reactions. However, the redox potential in soils is independent of any individual oxidant or reductant.

Arsenic III and V in Soils

Analysis of the forms of arsenic in water soluble extracts of six mine waste samples from southwest England showed that the major form of this mineral was arsenate (Porter and Peterson 1977). Arsenate comprised 60 to 94% of the arsenic, while arsenite comprised less than 10%. The form of the remainder of the water soluble arsenic was not investigated but was likely organic. The arsenic in the mine and smelter waste studied by these investigators was originally inorganic and mainly trivalent due to input from either ore materials or smelting and roasting which produces arsenic trioxide. Much of this arsenic had been oxidized to arsenate under the aerobic conditions and the wastes have gradually been colonized by a limited flora and presumably an associated microfauna.

Keaton and Kardos (1940) were able to show that the potential of an arsenate-arsenite system in a 0.05 N H_2SO_4 -quartz medium conformed with the theoretical formula for the potential of an oxidation-reduction system. When another component was added,

such as iron oxide, the trend of the potential may be obtained from theoretical considerations of the components. With the substitution of a 0.05 N H_2SO_4 -soil medium, there was an interference due to the complexity of the medium, and consequently the potential was no longer a direct function of the arsenate-arsenite system alone. The results of the study of oxidation-reduction potentials of arsenate-arsenite systems in soil mediums suggest that the treatment of arsenic-contaminated soils with some agent, preferably one mildly oxidizing and at the same time capable of arsenic fixation, such as iron, should produce beneficial effects on plant growth.

Misra and Tiwari (1963) were able to show that at least three factors affected the adsorption of arsenate: i) the amount of iron oxide (Fe_2O_3) or sesquioxides in the soils; ii) the amount of calcium or some other ion; and iii) the pH of the soil. The adsorption of arsenite, however, is adversely affected by these same three factors. The conversion of arsenite to arsenate is enhanced by the addition of Fe_2O_3 or by increasing the alkalinity of the system. This conversion of arsenite to arsenate is an oxidation process but the reduction of arsenate to arsenite is also a possibility at low pH values. A large percentage of adsorbed arsenate remains in a fixed form and persists in soils. The higher the pH of the soils, the less the adsorption of arsenate ions by the soil, which may be due to the formation of soluble arsenate such as sodium arsenate, especially in alkali soils (Keaton and Kardos 1940).

Arsenic III and V in Plants

Marcus-Wyner and Rains (1982) treated cotton plants with arsenic III supplied as arsenic trioxide (As_2O_3). In the 8 mg As III/L arsenic treatment, 352 mg As/kg was observed in the roots and only 81 mg As/kg in the shoots. These plants displayed wilted leaves, curled leaf margins, and had stubby roots that were brown on the tips. These effects increased with arsenic concentration.

Sachs and Michael (1971) examined root adsorption of four arsenical herbicides: monosodium methanearsonate (MSMA) cacodylic acid (hydroxydimethylarsine oxide) (CA), sodium arsenate, and sodium arsenite from nutrient solution. The order of concentration in the roots was arsenate > arsenite > MSMA > CA, while concentrations in the tops was arsenite > arsenate > MSMA > CA. However, according to these authors, if the ratio of arsenical concentration in the tops to that in the roots was a measure of transport, CA was transported to the tops 5 to 10 times more rapidly than MSMA, arsenite, or arsenate.

Porter and Peterson (1977) studied *Agrostis tenuis* plants growing on mine and smelter waste containing high concentrations of arsenic (60 to 94% arsenite). The rooting tests and growth experiments used clonal material and showed that plants from the high arsenic site exhibited tolerance to arsenate but not to arsenite in solution. Clonal material from the low arsenic site showed no tolerance to either form of arsenic. At low levels of arsenate, tillers of both clones were able to root although those of the low arsenic tolerance clone showed a lower index of tolerance. The difference in the mean indices of

tolerance of the two clones was significant at treatment levels of 5 mg water soluble As V/kg and higher. At 20 mg soluble As V/kg of soil only tillers from the high arsenic clone could root and tillers from the low arsenic clone died during the course of the experiment. Thus tillers of the high arsenic clone were tolerant of arsenic at this extreme level while those of the low arsenic tolerance clone were not. When Porter and Peterson supplied arsenic as the arsenite ion, neither clone rooted in solutions of 1 mg soluble As III/kg or higher. A significant decrease in the growth of non-tolerant plants occurred at treatments of 10 mg soluble As V/kg or higher. This was associated with a shoot concentration of 140 mg As/kg dry weight and a root concentration above 1000 mg As/kg. The tolerant plants accumulated generally less arsenic than the non-tolerant plants for a specific treatment. Significant growth effects for tolerant plants occurred only at soil levels of 50 mg soluble As V/kg and above, when plant accumulation resulted in 565 mg As/kg in the shoots and 2,880 mg As/kg in the roots. Even at very low levels, arsenite depressed plant growth. Significant effects occurred at the 0.05 mg As III/kg treatment level. Associated plant concentrations were 4 mg As III/kg in the shoots and 60 mg As III/kg in the roots. The grasses which had originated from sites with elevated arsenite concentrations had exhibited tolerance specifically to the arsenate ion rather than to arsenite.

Wallace et al. (1980) grew bush bean plants in solution culture with varied levels of arsenate. The level of 7.5 mg As V/L in solution resulted in considerable plant damage. Plant concentrations of arsenate at this application rate were 3.6, 18.8, and 41.7 mg/kg respectively for leaves, stems, and roots.

The results of transpiration studies conducted by Morris and Swingle (1927) with oats in water cultures showed that arsenic added as As_2O_3 decreased transpiration even when added at the rate of 1 mg/L, and resulted in narrower leaf blades and a lighter color. In the same study, when sand was used instead of soil, the other environmental conditions remaining the same, the injury was apparent in a shorter time. The rate of arsenate uptake by non-tolerant plant roots was much greater than the uptake rate in the roots of tolerant plants. It was postulated that the difference in the rate of uptake between tolerant and non-tolerant plants was due to an altered phosphate and arsenate uptake system (Meharg and Macnair 1991). The rate of arsenate uptake in the non-tolerant genotype decreased more rapidly with increasing pH (4 to 8) than did uptake in the tolerant plants. In the tolerant plants about 75% of assimilated arsenate was transported to the shoots, with this figure being about 50% in non-tolerant plants.

The toxicity of arsenic compounds to citrus plants grown in solution cultures began to be apparent at concentrations of about 5 mg As V/L in solution and at about 2 mg As III/L (Liebig et al. 1959). Concentrations of either 10 mg As V/L or 5 mg As III/L, though not always lethal, caused very marked depression of top and root growth. In general, the amounts of arsenic found in or on the roots increased with increasing concentrations in the solution. The arsenic contents of roots treated with 2 mg/L as As III or V were not different, however the roots treated with 5 mg As III/L contained more than twice as much arsenic as roots treated with 5 mg As V/L. Small amounts of this mineral, 1 mg/L as As III or V, had a stimulating effect on the root growth of citrus plants in solution culture. The arsenic content of burned-spot leaves was 3.25 mg/kg in leaves

from the 5 mg As V/L treatment, and 3.30 mg/kg in those from the 2 mg As III/L treatment.

Approximately 10 times as much pentavalent arsenic is required in culture solution and in plant tissue as trivalent arsenic to produce equivalent injuries to tomato plants (Clements and Munson 1947). The two forms of arsenic differ not only in lethal concentrations, but also in their immediate action on plant tissue. Trivalent arsenic has a violent action, causing complete disintegration of the roots and burning the tops in one or two days in lethal concentrations. Pentavalent arsenic, on the other hand, often takes several days to produce any response other than wilting, even in concentrations that eventually prove lethal. When arsenite is applied to a leaf, it causes wilting due to loss of turgor (suggesting an alteration of membrane integrity), and in contrast arsenates cause chlorosis but not rapid turgor loss (Woolson 1983).

Arsenate has the necessary structure and chemical properties to take part in biochemical mechanisms, often by acting as a phosphate substitute (Huang and Mitchell 1972). Arsenate has no affinity to thiols and thus, unlike arsenite does not affect many enzyme systems (Johnstone 1963). Therefore, any tolerance mechanism for arsenite would require either exclusion of the ion or rapid adsorption once inside the plant to avoid enzyme inactivation, and no plant has yet been shown to be tolerant to arsenite.

Arsenic and Phosphorus

Arsenic and phosphorus are in the same periodic family and have similar chemical and physical properties. Phosphorus competes for arsenic fixation sites in soil and may affect arsenic availability. Therefore high levels of phosphorus may overcome arsenic toxicity by an antagonistic action (Woolson et al. 1973). At low levels of arsenical application, small increases in crop yields may occur. This may be due to the displacement of phosphate by arsenate, increasing phosphate availability to vegetation (NRCC 1978). Tsutsumi (1983) found that the toxicity of arsenite to rice plants was almost independent of added phosphate, where arsenate could be antagonistically affected by phosphate. In a nutrient solution, Hurd-Karrer (1939) found that the phytotoxicity of arsenic was a function of the phosphorus concentrations. At phosphorus/arsenic ratios of 4:1 or greater, arsenic phytotoxicity on wheat was reduced. However, at a ratio of 1:1, growth reductions occurred at concentrations of 10 mg As/L and higher.

Carrow et al. (1975) studied the influence of soil phosphorus level on the growth of several cool season turfgrasses and on the Bray P_1 extractable arsenic. In the first study five rates of arsenic (0, 0.44, 0.88, 1.76, and 3.53 kg/100m²) were supplied as 0, 2.45, 4.9, 9.7, and 19.4 kg powdered 48% tricalcium arsenate/100m², and four rates of phosphorus (0, 1, 2, and 4 kg monocalcium phosphate/100m²) were applied to soil low in natural phosphorus. In the second study four rates of arsenic (0, 0.88, 1.76, and 3.53 kg/100m²) were applied to soil collected from a long term phosphorus study. In the first experiment,

a significant reduction in arsenic toxicity with increasing phosphorus concentration was observed in annual bluegrass and creeping bentgrass between the 0 and 1 kg/100m² treatments. However, among the three highest phosphorus treatments no significant reduction in arsenic toxicity occurred even though soil phosphorus levels increased 4.3-fold up to an extremely high level of 278 mg phosphorus. No significant effect of phosphorus on arsenic toxicity to annual bluegrass or creeping bentgrass was evident at any soil phosphorus level. In the second experiment the three grasses grown on this soil exhibited no effect of phosphorus on arsenic toxicity. On soils which are very low in phosphorus, a slight reduction in arsenic toxicity may occur after phosphorus fertilization. Also, Bray P₁ extractable arsenic was not affected by applied phosphorus at the rates used in these investigations.

Because the concentrations of phosphorus have an important influence on arsenic toxicity, comparisons between arsenate and arsenite must be made within series of similar phosphorus concentration (Clements and Munson 1947). Comparisons of the arsenate and arsenite experiments at the medium phosphorus level, 60 mg/kg, indicated marked differences between the action of pentavalent and of trivalent forms. The authors noted that an increase in phosphorus level significantly reduced the amount of pentavalent arsenic adsorbed, and resulted in better growth, while the action of phosphorus on the adsorption of trivalent arsenic was quite different from its action on pentavalent arsenic. The investigators showed that for a given concentration of trivalent arsenic, plants adsorb approximately the same amount of the toxic element, irrespective of the phosphorus level, and showed equal degrees of injury. These studies confirm the supposition that

phosphates would be expected to have less effect on the toxicity of arsenite than on that of arsenate.

Studies carried on elsewhere have yielded some treatments which may be useful in correcting arsenic toxicity: the use of heavy phosphate applications (Hurd-Karrer 1939), lime (Paden and Albert 1930), iron oxide (Keaton and Kardos 1940), and organic matter (Keaton 1938) have shown promise of reducing the toxicity of arsenic excess, although none of these very costly treatments reduces the arsenic content of the soil (Clements and Munson 1947). Adsorbed arsenate can easily be replaced by citrate and phosphate ions because a repression in arsenate adsorption is observed in the presence of these ions (Misra and Tiwari 1963). The release of soil arsenate by phosphate and citrate ions showed that at least one third of the adsorbed arsenate is in the exchangeable or replaceable form, while the rest of the arsenate was locked up in the form of calcium compounds or sesquioxide complexes.

In a study involving rice seedlings in solution culture, the toxicity of arsenite was almost independent of added phosphate and the lethal concentration was found to be 7.5 mg As III/L (Tsutsumi 1983). Arsenate toxicity was antagonistically reduced by phosphate when applied in the range of 1 to 100 mg/L without the addition of other nutrients and to 310 mg/L with an increase in the supply of other nutrients. At 1 mg of phosphate/L of solution the growth response to arsenate was similar to that without phosphate and the antagonism seemed to stop. The lethal concentration of arsenite (7.5 mg As III/L) was found to be equal to that of arsenate in the middle range of 1 to 10 mg of phosphate/L, irrespective of the existence of other nutrients. Below this phosphate

level, therefore, arsenate exceeded arsenite in toxicity to rice seedlings. These observations lead to the tentative conclusion that the common view that arsenite is more toxic than arsenate without regard to the status of co-existing phosphate was questionable.

Nutrient Solution Culture

All plants should be grown with a balanced nutrient solution providing specified concentrations of all the elements known at present to be essential (Hewitt and Smith 1974). Water is, of course, always the main component of growing plants, but the major portion, usually about 90%, of the dry matter of most plants is made up of three chemical elements: carbon, oxygen, and hydrogen. Carbon comes from the air, oxygen from the air and water, and hydrogen from water, while other elements such as nitrogen, phosphorous, potassium, calcium, magnesium, sulfur, chlorine, iron, boron, manganese, zinc, copper, and molybdenum are obtained from the soil (Hoagland and Arnon 1950). These authors developed a formula for a nutrient solution which contains all of these minerals. Elemental proportions were based on the approximate proportions of the elements as they were found to be adsorbed by the tomato plant.

The nutrient solution or water culture method is a technique in which plants are grown with their roots submersed in a solution containing the mineral nutrients essential for plant growth. The roots must be aerated to provide for root respiration, the pH of the nutrient solution must be monitored and adjusted accordingly, and periodically the solution must be replaced (Hoagland and Arnon 1950). In the sand culture method plants

are grown in sand that is irrigated with nutrient solution. The sand culture method provides for more efficient natural aeration of roots than the water culture method (Hewitt 1966). The sand must be cleaned or minute quantities of elements in the sand may alter elemental concentrations in the nutrient solution and thus pose a problem to quantitative studies of micronutrients.

MATERIALS AND METHODS

Western wheatgrass, basin wildrye, hard sheep fescue, and Canada bluegrass were grown from seed, placed in a sand culture, and then treated with four concentrations of arsenic III (0, 1, 2, and 5 mg/L) and four concentrations of arsenic V (0, 7, 14, 28 mg/L) for 75 days. Plant height, above ground biomass, below ground biomass, and shoot arsenic concentration were measured for each species. Plant color, vigor, leaf burn, amount of red discoloration, and survival were also observed and recorded.

Sand Culture

Four sand filled flats were planted each with a different grass species (Table 1). Prior to planting, however, the greenhouse sand had been washed to remove any fine material.

Table 1. Species used in the greenhouse study*.

Scientific name	Common name
<i>Pascopyrum smithii</i>	western wheatgrass
<i>Leymus cinereus</i>	basin wildrye
<i>Festuca ovina duriuscula</i>	hard sheep fescue
<i>Poa compressa</i>	Canada bluegrass

*Nomenclature from Rumley and Lavin 1991.

Table 2. Hoagland's nutrient solution*.

Compound	g/L	mg/L
KNO ₃	0.656	
Ca(NO ₃) ₂ · 4H ₂ O	0.994	
NH ₄ HPO ₄	0.115	
MgSO ₄ · 7H ₂ O	1.004	
H ₃ BO ₃		2.68
MnCl ₂ · 4H ₂ O		1.81
CuSO ₄ · 5H ₂ O		0.08
ZnSO ₄ · 7H ₂ O		0.22
H ₂ MoO ₄ · H ₂ O		0.09
FeEDTA		3.98

*Hoagland and Arnon (1950).

The germinating seeds were watered with Hoagland's nutrient solution (Table 2) diluted 1:4 with tap water. Once the slowest growing species had reached a height of 5 cm, five plants of a single species were transplanted into 10 cm wide and 12 cm deep sand filled plastic pots (1,000g of sand). These pots were watered with the 1:4 diluted Hoagland's nutrient solution. Because the age of the plant might affect its ability to withstand the elevated arsenic levels, the plants were allowed to reach substantial growth before the arsenic treatment was started (Stewart and Smith 1922). The amount of time between the emergence of the plants above the soil and the start of arsenic treatment varied from three to seven weeks. Arsenic was added to the nutrient solution as As₂O₃ (arsenic III) and As₂O₅ (arsenic V). Four concentrations of arsenic III (0, 1, 2, and 5 mg/L) and four concentrations of arsenic V (0, 7, 14, 28 mg/L) were prepared. Each treatment was replicated eight times for each grass species.

The plants were grown and watered in a greenhouse within the Plant Growth Center at Montana State University. Full spectrum lamps were used to extend the daylight period to 14 hours. The average daytime temperature during the growing period was 22°C, ranging from 14°C to 30°C. The average night temperature for the same period was 18°C, ranging from 12°C to 24°C. For the first 40 days pots were watered every other day, and for the remainder of the study the pots were watered daily. The pots were irrigated with 85 ml of solution, an amount calculated and observed to saturate the sand. The irrigation solution was applied directly to the sand to minimize solution contact with the plant. As the experiment progressed and the plants wilted, it became impossible to completely prevent solution contact with the shoot and leaf material.

Measurements

Height of all five plants in each pot was measured 0, 25, 50, and 75 days after arsenic treatments began. In addition, symptoms assumed to be influenced by arsenic including plant color, vigor, leaf burn, amount of red discoloration, and survival were observed at these times and a numeric value was assigned to each pot (Table 3).

For each pot of each species above ground plant material was observed and a pot average for color was determined on a scale of dark green to yellow. Plants were studied for thickness of stems and leaves, number of stems, amount of new growth, height of plants, and posture of the plants. Together these several attributes were combined to produce

Table 3. Explanation of the five observation indices.

Assigned value	Color	Vigor	Burn	Red (%)	Survival (plant/pot)
0	---	---	none	0%	0
1	dark green	excellent	tip	<5%	1
2	green	good	marginal	5-25%	2
3	pale green	acceptable	dead areas	25-50%	3
4	yellow	poor	---	50-75%	4
5	---	dead	---	75-95%	5
6	---	---	---	>95%	---

a vigor rating on a scale of excellent to dead. As with the color of the plants a numeric value was assigned to each pot. Leaves were studied for tip and marginal burn as well as dead areas. Tip burn was found on the end of leaf blades, marginal burn was found along the perimeter of the leaf blades, and dead areas were actual dead spots on the surface areas of the leaves. As with the previous plant parameters a numeric value was assigned to each category of this evaluation. The percent of above ground material in each pot that had turned red was divided into six categories. The classes were ranges of values which made it much easier to place a label on the amount of red above ground material in a pot. Plant survival was determined by the number of plants that were alive in each pot at every observation period.

At the end of the 75 day study, above and below ground plant tissues were harvested. Below ground biomass was determined after the roots were washed to remove sand particles. Tissues were dried for 72 hours at 47°C, and weighed. The eight replicates

of dried above ground tissues for each treatment were composited into one sample and ground in a Wiley mill to pass a 20 mesh screen. Two grams of each sample were digested with concentrated nitric acid (HNO_3) followed by 30% hydrogen peroxide (H_2O_2) (Neuman 1994). This acid-peroxide digestate solution was analyzed for arsenic content using Graphite Furnace Atomic Absorption Spectrometry.

To monitor the precision of analyses, laboratory duplicates and blind field replicates were entered into the sample set at approximately a five percent (1 in 20) rate. Laboratory duplicate relative percent difference (RPD) averaged 23.5% total arsenic. Blind field replicate RPD averaged 28% total arsenic. Laboratory matrix spikes and laboratory control samples were utilized at a five percent rate to monitor the accuracy of arsenic analysis. Laboratory matrix spike recoveries for arsenic averaged 75%. Laboratory control samples of National Bureau of Standards citrus leaves standard reference material (#1572) averaged 14.5% RPD. The certified arsenic concentration of these leaves was 3.1 mg/kg. Arsenic concentration in the citrus leaves as determined in this study was 3.5 mg/kg.

Statistical Design

Replicated pots ($n=8$) each containing five plants of a single species were planted for each treatment and arranged in a randomized complete block design. The eight blocks were rotated every two weeks to minimize variation across the blocks. Plant height on days 0, 25, 50, and 75 were measured. In addition, mean shoot weight, and mean root weight were determined for each grass species in each arsenic treatment. Mean dry

weights were calculated by dividing the total shoot or root weights per pot by the number of plants in that pot. For each grass species, oxidation state of arsenic, and arsenic concentration a relative mean percent plant height, shoot weight, and root weight was calculated. This was accomplished by dividing the average value per pot (across the eight blocks) by the maximum mean value per pot (of all the treatments) for that species and multiplying the quotient by 100.

Skewness and kurtosis data are presented in Table 13 (Appendix B), and show that all the data for all the variables fit a normal distribution. Analysis of variance (ANOVA) was used to detect the effects of arsenic treatment on plant height on all four days, and mean shoot and root dry weight per plant. Arsenic III and V treatments were analyzed as two separate experiments. The least significant differences (LSD) test with $p < 0.05$ was used to determine differences between treatment means of those variables which had a significant treatment effect as determined by ANOVA. Data were analyzed with Montana State University Statistical Computer program (MSUSTAT) (Lund 1993).

RESULTS

Arsenic III treatments*Pascopyrum smithii*

Western wheatgrass survival was not affected by arsenic III concentrations in the nutrient solution (Figure 1a). With very few exceptions, all five plants within a treatment survived the 75 day trial period. There was no significant effect of arsenic III treatment on western wheatgrass height nor was there any significant arsenic III effect on above ground or below ground biomass (Table 4). Results of the ANOVA are presented in Tables 14-16 (Appendix C).

Plants irrigated with the highest arsenic III level displayed a reduction in vigor from excellent to a good/acceptable range (Figure 1b). The reduction in vigor of plants treated with the higher levels of arsenic III occurred faster and was more severe than the vigor change in plants treated with lower arsenic III levels. The vigor of plants growing in the 1, 2, and 5 mg As III/L treatments had decreased at the 25 day measurement period. The control began to decline in vigor at 50 days. Because the control did decrease in vigor during the 75 day growth period, not all of the change in vigor of the treatments can be accounted for by arsenic III concentration alone. Some other environmental factors must have had an influence on plant vigor (e.g. water stress).

Plant color changed from dark green to either green or pale green over time depending on the arsenic III concentration (Figure 1c). The color change of plants

