EXTRACTABILITY AND BIOAVAILABILITY OF ARSENIC IN SOILS AND THE EFFECT OF IRON REMEDIATION EFFORTS

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FORWARD

Arsenic (As) is a ubiquitous element found in the earth's crust all over the world. It has been used in medicines, cosmetics, bronzing, as wood preservative, herbicides, desiccants, and in the production of solid-state devices. Concern over arsenic in the soil has stemmed from the reduction of the maximum contaminant level for As in drinking water. The threat of As leaching in to groundwater or surface waters has caused great concern. Furthermore there are concerns over ecological effect of As in soils. Soil properties have the greatest influence on the extractability and bioavailability of As from the soil. Soil screening levels based on soil properties can be developed to determine the threat of leaching and bioavailability to plants and soil organisms. Amending soil that have a high potential of As leaching and bioavailability with chemical immobilzation treatments may help to reduce clean-up activities while reducing the ecological and human health risk. The first part of this manuscript investigates soil properties that influence leaching and bioavailability to plants. Extraction methods are evaluated as indirect measurements of plant and earthworm bioavailability. Chapter 2 explores several iron immobilization treatments to reduce As extractability and toxicity with emphasis on the affect to multiple pathways. The leaching potential (extractability), plant bioavailability and earthworm bioavailability are addressed. In Chapter 3 human availability pathways are examined in the iron remeditated arsenic soils including potential reductions in risk for cancerous and non-cancerous effects. The manuscript is arranged as defined by CBE Style Manual (Council of Biological Editors).

CHAPTER I

ARSENIC EXTRACTABILITY AND BIOAVAILABILITY FROM SOILS WITH VARIOUS PROPERTIES

ABSTRACT

There is growing concern over arsenic contaminated soil affect on ecological health. Currently there are no set ecological soil screening levels for arsenic in soils. Total arsenic content is not an accurate indicator of the extractability or bioavailability to soil organisms. Application of toxicity test and soil modifying factors can be used to established arsenic levels that adequately protect the soil ecosystem. Soil properties that have been identified as modifying factors are pH, clay content, organic carbon and Fe-oxides. Identifying which properties have the greatest influence on arsenic extractability and bioavailability can prove essential for ecological health. Therefore the objective of this study was to examine the soil properties and their relationship to arsenic extractability and plant and earthworms bioavailability. The reduction of plant yield is most related to the amount of Fe-oxides in the soil (r^2 = 0.71). Uptake of arsenic by plants is more complex with influences from Fe-oxides, amount of clay and organic carbon. These relationships were found in all statistical methods; linear regression, backwards multiple regression and path analysis for measured plant endpoints. In general, arsenic extractability was most reduced by the presence of Fe-oxides. Simple regression and backwards multiple regression indicate that Fe-oxides,

amount of clay and pH can all influence how much arsenic can be extracted from the soil. Path analysis results indicate that amount of clay is the most significant soil property followed by Fe-oxides and pH. Relating extractability with plant and earthworm endpoints revealed several extractions that have the potential to be used to predict toxicity. Pore water arsenic and Bray-1 extractable arsenic had strong relationship to plant yield and accumulation and earthworm mortality. Modifying factors for arsenic in soils could be applied for amount of Fe-oxides found in a soil followed by amount of clay and to a less extent amount of organic carbon and pH. Furthermore, due to the strong relationship between Bray-1 extractable arsenic and plant yield, this would be a good predictor of soil arsenic phytotoxicity. These results can aid in development of ecological soil screening levels.

Keywords— Arsenic Arsenic extractability Plant bioavailable arsenic Earthworm toxicity Soil properties Phytotoxicity

INTRODUCTION

Arsenic (As) is a ubiquitous element found in the earth's crust all over the world. It has been used in medicines, cosmetics, bronzing, as wood preservative, herbicides, desiccants, and in the production of solid-state devices. All these industries contribute to the increase of arsenic containing compounds in the environment. Natural uncontaminated soils in the U.S. can contain from 1 to 40 mg/kg arsenic depending on the parent material from which the soil was formed (O'Neill, 1995, Adrianno, 1986). Igneous rocks contain As concentrations from 1-15 mg/kg. Sedimentary rocks such as

shales, mudstones and slates, can contain significantly more As, up to 900 mg/kg. Sandstones and limestones range from 1-20 mg/kg As (O'Neill, 1995, Adrianno, 1986). Arsenic is found as a constituent in many minerals and is a frequent component of sulfide ores in the form of arsenides of nickel, cobalt, copper, and iron. Arsenicpyrite (FeAsS) is the most abundant and common As-containing mineral. Other common As minerals are engargite ($CuAsS_4$), orpiment (As_2S_3) and realgar (As_4S_4) (Tamaki and Frankenberger 1992, Thorton and Farago 1997, Francesconi and Kuehnelt 2002).

Arsenic is a known human carcinogen causing skin, liver, bladder, kidney and lung cancers. Low level As exposure can cause stomach and intestinal irritation, fatigue, abnormal heart rhythm, darkening of the skin as well as impaired nerve function (US ATSDR 1999). Exposure to As can be through inhalation or ingestion of soil, intake of contaminated groundwater, dermal exposure through soil or water and indirectly through consumption of plants grown on contaminated soil. There are concerns not only for human health but there are great concerns in soil ecosystems where organisms are in direct contact with the soil. Arsenic has detrimental effects on soil organisms and plants causing stunted growth, lack of reproduction and even death.

Arsenic is a metalloid of Group Va of the periodic table with properties that allow it to form alloys with various metals and covalent bond with carbon, hydrogen, oxygen and sulfur. The oxidation states of As are –3, 0, +3, and +5. The complex ions AsO_2^-, AsO_4^{3-}, $HAsO_4^{2-}$, and $H_2AsO_3^-$ are the most common mobile forms of As in soil (Kabata-Pendias and Pendias 1992, O'Neill 1995.). There is a similarity between As and phosphorus chemistry. The oxidation states and electron orbitals ($4s^24p^3$) are similar between As and phosphorus, as well as their dissociation constants and solubility products from their salts (Adriano 1986, Kabata-Pendias and Pendias 1992). Both

arsenate and phosphate form oxyanions in the +5 oxidation state in soils. However phosphate is stable over a wider range of Eh and pH conditions than arsenate. Because of their chemical similarity, arsenates and phosphates in soil are assumed to react similarly forming insoluble compounds with Fe, Al and Ca. Arsenic (V) can become immobilized by coprecipitation with hydrous iron oxides (Adriano 2002).

In soils under oxidizing conditions, arsenate (V) is the most stable species (Thornton and Farago, 1997). The reaction for As acid (As V) in aqueous solutions are:

(i) $H_3AsO_4 + H_2O \rightarrow H_2AsO_4^- + H_3O^+$ pKa 2.20

(ii) $H_2AsO_4^- + H_2O \rightarrow HAsO_4^{2-} + H_3O^+$ pKa 6.97

(iii) $HAsO_4^{2-} + H_2O \rightarrow AsO_3^{3-} + H_3O^+$ pKa 11.53

The pKa values indicate that As(V) would be thermodynamically stable over normal soil pH ranges; from pH range 2-7 as (ii) H_2AsO_4- form and at pH 7.0 as (iii) $HAsO_4^{2-}$ form. Arsenic has been found to readily leach and be highly bioavailable in soils with low amounts of clay and oxides (O'Neill 1995, Woolson et al. 1988, Jacobs et al. 1970, Manning and Goldberg 1997). The mechanisms in reduction of arsenic solubility by soil components may be due in part to adsorption reactions. Adsorption reactions are complex and mainly occur on the surfaces of clay, oxides or hydroxides of Fe, Al and Mn, calcium carbonates and/or organic matter (Sadiq 1997). In general there is a decrease in As(V) adsorption with increasing pH (Smith 1998, Lumsdon et al. 2001, Pierce and Moore 1980, Xu et al. 1988). This can be attributed to two factors 1) the increasing pH causes the negative surface potential to increase and 2) the increasing amount of negatively charged As(V) species present in the soil solution (Adriano 2002, Xu 1988, Hingston et al 1968).

Smith et al. (1999) found that Australian soils high in oxides adsorbed three-times as much As(V) than soils low in oxides. Like phosphate, As is strongly adsorbed by amorphous Fe-oxide and to a lesser extent to Al-oxides. The adsorption of As in soils has been positively correlated to ammonium oxalate extracted Fe (amorphous Fe-oxides) (Livesey and Huang 1981, Jones et al. 1999 Manning and Goldberg 1997). Generally clay particles are negatively charge silicate minerals that preferentially adsorb positively charged species, not As oxyanions. However As mobility and bioavailability are greater in sandy soils than clayey soils and significant correlations have been found with As(v) adsorption and clay content (Woolson 1973, Livesey and Huang 1981). The positive charges originating from the crystal edges of the silicate clay minerals may contribute to the adsorption of As and its reduced bioavailability (Livesey and Huang 1981). It has also been reported that sorption of As occurs by chemisorption or ligand exchange on clay surfaces, mainly by replacing or competing with phosphate (Sadiq 1997, Piece and Moore 1980). Clays are often coated with Fe and Al oxides as well, thereby varying directly with the clay content of a soil (Adriano 2002, Smith et al. 1998, Schutless and Huang 1990)

Arsenates behave much like phosphate in the plant-soil system, but As is phytotoxic. Arsenate (AsO_4^{3-}) can be taken up via the phosphate transport system by most organisms. Arsenate is thought to replace phosphate in energy transfer phosphorylation reactions (Tamaki and Frankenberger 1992, Bhumbla and Keefer 1994). In general the transfer of As from soil to plant is usually low. Arsenic induces phytotoxicity resulting in restricted plant growth, and in essence protects humans and animals from plants with high As content (Smith et al. 1998). The effect of phytotoxicity

is influenced by As source, As species, and the soil type, with sandy soils being more toxic than clayey soils (Sheppard 1992).

Earthworms have a particularly intimate contact with the soil, ingesting large amount of soil and having a limited barrier between soil solution and organisms. For this reason, and with their importance in terrestrial food webs, earthworms are ideal test organisms for toxicity test in contaminated soils (Langdon et al. 1999). Sodium arsenate has been shown to be highly toxic to earthworms (Langdon et al., 1999, Fisher and Koszorus 1992, Meharg et al. 1998) causing yellow discoloration, lesions and swelling along the body and death.

To adequately protect soil ecosystems and restore them when necessary, characterization of soil in As contaminated areas need to be identified and define what levels of As in these soils constitute a hazard to soil organisms. Currently there is no set ecological soil screening levels for soils in the United States. Ecological soil screening levels are amounts of chemicals that have been identified to pose no or little risk to soil organisms (USEPA 2000). If site measurements of As are found to be lower than the ecological soil screening level, then the site can be removed from further evaluation from the ecological risk assessment process. Soil screening levels are based on toxicity test performed on ecological indicators such as lettuce or earthworms. Current guidelines are based on total As in the soil, which is not a good indicator of toxicity. Soil modifying factors affect the toxicity of As to soil organisms. Arsenic can be divided up in to different fractions or pools based on availability or association with solid phases. The different fraction of As can be identified using different extractions. Because of the chemical similarity between As and phosphorus, the same type of extraction procedures have been utilized to describe As in soils.

Several properties have been identified that affect As extractability and bioavailability. These properties include pH, clay content, organic matter and Fe-oxides. Identifying which properties have the greatest influence on As extractability and bioavailability can prove essential for soil screening levels and clean-up activities. Therefore the objective of this study was to examine the soil properties pH, clay, organic carbon and Fe-oxide and determine their relationship to As extractability and bioavailability. The second objective is to determine which extraction method most readily predicts or models the toxicity of As to plants and soil organisms (earthworms).

MATERIALS AND METHODS

Twenty-two soils from Oklahoma and Iowa were selected based on their soil properties. Soil properties used for the selection process were pH, %organic carbon, % clay, cation exchange capacity, and amount of iron and aluminum oxides (Table 1.0). Soil pH was determined using 1:1 soil:water ratio (Thomas SSSA, 1996). The hydrometer method (Gee and Bauder 1986) was used to determine texture (% clay). All soils were pretreated with H_2O_2 to remove organic matter. The amount of Fe and Al-oxides were determined using a modified Tamm's reagent (Loeppert and Inskeep 1996, McKeague and Day, Schwertmann SSSA 1996). Percent organic carbon was determined using the acid dichromate digestion method (Heanes, 1984, Nelson and Sommers, SSSA 1996). And cation exchange capacities of non-calcarious soils were determined using $BaCl_2$ replacement method (Hendershot and Duquette 1986). Cation exchange capacity of calcareous soils was determined according to the method of Polemio and Rhoades (1977).

The soils were spiked with 250 mg/kg As using $H_2NaAsO_4 \bullet 7H_2O$. The soils were saturated with the As solution, mixed thoroughly, and then dried at 105°C for 24 hours. The wetting/drying process was repeated two more times, for a total of three wet/dry cycles to promote reaction between the soil and As. Complex, slow solution and precipitation reactions are affected by soil wetting/drying cycles (Wauuchope 1983). The electrical conductivity (EC) was measured (1:1, soil:water ratio) at the conclusion of the wet/dry cycles followed by leaching with de-ionized water when EC exceeded 1.5 dS/m. Leaching was done by transferring the soil into a 5 gallon bucket, flooded with de-ionized water, allowing the mixture to settle for 30 h, then removing the excess water. This was repeated until the EC was reduced to below 1.5 dS/m. All soils were then dried at 105°C, ground using a ball grinder, sieved to pass 2mm sieve and stored in plastic containers. Texture was checked on random samples to insure there were no texture changes due to grinding. Total soil As content was determined by EPA method 3051 (USEPA, 1994).

Plant Growth and Arsenic Uptake with Soil Properties

Several plant methods were used for evaluation. All test were done with lettuce, *Lactuca sativa var. Paris Island Cos.*

Germination Test

Fifty grams of soil was placed into a deep petri dish and temperature adjusted to 24±2°C (EPA 600/3-88/029, ASTM E 1598). One day prior to planting, water was added to 120% of water-holding capacity (field capacity at $-^1/_3$ bar). Twenty lettuce seeds (*Latuca sativa var. Paris Is.*) were placed into the petri dish and covered with 25g artificial soil (water added to 25% or 6.2 mL/25g). Artificial soil was composed of

69.5% silica sand, 20% kaolin clay 10% 2-mm sieved *Sphagnum* peat moss and approximately 0.5% $CaCO_3$ added to adjust the pH to 7.0. The petri dishes were covered and incubated at 24± 2°C in the dark for 48 hours followed by sequencing 16 h of light and 8 h of dark until termination of the test (Baud-Grasset 1993). Germination was first determined after 120 hours (5 days) by counting the number of seedlings (leaves or stems) that protruded above the soil surface. After the germination count, soils were watered to 120% of field capacity. Petri dishes were placed on a tray and put into large clear plastic bags to retain moisture and allow the seedlings to grow for another 120 hours (5 days) without the hindrance of a lid. After 10 days, a second seed germination count was taken. (EPA 600/3-88/029, ASTM E 1598).

Shoot Elongation Test/Early seedling growth

The germination study was continued in the growth chamber for a total of 17 days of growth (12 days after more than 50% relative seed germinated) (EPA 600/3-88/029, ASTM E 1598). After counting the total number of plants, each plant was removed and measured from the hypocotyl to the leaf tip.

Phytotoxicity/Bioaccumulation in Lettuce – 8 Week Bioassay

The first plant bioassay was an eight-week growth test utilizing lettuce plants (*Lactuca sativa var. Paris Island Cos).* The plants were grown in triplicate with 750 g of dried soil/pot and a volume of 100 mL of vermiculite. The vermiculite was mixed with the soils to improve with water infiltration and drainage. Plastic pots (15 cm diameter) without drain holes were used to grow the lettuce. Each soil was tested for available N-P-K prior to planting and fertilized with the needed nutrients of an equivalent 120 lbs/acre N (60 mg/kg), 60 lbs/acre P (30 mg/kg), and 60 lbs/acre K (30 mg/kg) (Lorenz

and Maynard, 1988). Ten seeds were placed into each pot and slightly covered with soil. The pots were watered to 120% of field capacity ($-^1/_3$ bar) and placed in a constant temperature room modified with a mix of grow lights, incandescence and soft white florescent bulbs to achieve the light spectrum needed for lettuce growth. The plants were grown for 8 weeks with watering every 2-3 days to 120% of field capacity and fertilized every two weeks with 45 lbs/acre N-P-K to ensure adequate plant nutrients. Lighting was cycled with 16 hours light and 8 hours dark and temperature maintained at 20 ± 4° C. At the conclusion of the test the plants were harvested washed 3 times with de-ionized water, dried at 60°C for 48 hours and weighted. Plants were mechanically ground using a Wiley mill (Jones and Case 1990) stainless steel grinder, or hand ground when plant matter was small. The ground plant material was placed in plastic sample bags and stored at room temperature.

To determine As accumulation, a sample of dried plant material (<0.2 g) was mixed with 5mL of 20% $Mg(NO_3)_2$ and 5mL of conc. trace metal grade nitric acid. The samples were covered with watch glasses and digested at 80°C overnight (16-20 hours). The watch glasses were removed the next day and temperature was gradually increased to 200°C to dry the samples. Drying time took 12-14 hours and was spread over two days. Preliminary analysis indicated that samples must be totally dry to achieve As recovery from the plant material. The samples were placed in a muffle furnace and heated to 450°C at a rate of 0.8°/min then held at 450°C for 6 hours (Ybanez et al. 1992). The ashed samples were mixed with 4mL of 50% trace metal HCl and heated until resolubilized (~80°C for 1 hour). The samples were cooled, then 0.25mL of 40% Potassium iodide - 4% ascorbic acid solution was added 24 hours prior to As analysis by

inductively coupled plasma atomic emission spectroscopy with hydride generation (ICP-HG).

Modeling & Statistics

All statistics were done using SAS for Windows V8 (SAS Institute 2002) and GraphPad Prism 3.0 (1999). Three different statistical models were used to compared and determine the soil properties that most influence As bioavailability. Simple (e.g. linear, exponential) regression models were run separately for each soil property and each lettuce endpoint. Lettuce endpoints used included % seed germination, % relative seed germination, shoot length, % relative shoot length, yield, and % relative yield. Where '% relative' is a ratio of the lettuce endpoint in arsenic spiked soil to control soils expressed as a percent. A backwards step-wise multiple regression technique was used to derive empirical model that capable of preciting effects of bioavailabile As to soil properties. The backwards stepwise multiple regression is also known as step down multiple regression. This modeling procedure starts with a full model and eliminates variable that do not significantly ($P<0.05$) enter the regression equation. The initial model composed of single variable (e.g. pH), squared variable term (e.g. pH^2) and inter-correlated variables (e.g. pH x OC, pH x clay and pH x Fe) for the soil properties pH, OC, Fe-oxide and clay.

Lastly, path analysis was done for each lettuce endpoint to determine indirect and direct effects. Path analysis is a statistical method that partitions simple correlation coefficients between dependent variables (lettuce endpoints) and independent variables (soil properties) into direct and indirect effects. Soil properties and graphical representation of path analysis are shown in Fig. 1. Path analysis direct effects (P_{ij}) are derived from multiple linear regression of soil properties on lettuce endpoints and

indirect effects (r_{ij}) from simple correlation values (Pearson correlation) between soil properties (Basta et al. 1993). The multiple regression format includes Y = a(A) + b(B) + c(C) + d(D). Where Y = lettuce endpoint as previously defined, A, B, C, and D are soil properties pH, % organic carbon, % clay and Fe-oxide content and a, b, c, and d are coefficents

Mathematically path analysis is determined by the equations:

$$r_{15} = P_{15} + r_{12}P_{25} + r_{13}P_{35} + r_{14}P_{45} \quad [1]$$

$$r_{25} = r_{12}P_{15} + P_{25} + r_{23}P_{35} + r_{24}P_{45} \quad [2]$$

$$r_{35} = r_{13}P_{15} + r_{23}P2_5 + P_{35} + r_{34}P_{45} \quad [3]$$

$$r_{45} = r_{14}P_{15} + r_{24}P_{25} + r_{34}P_{35} + P_{45} \quad [4]$$

Where r_{ij} corresponds to the simple correlation coefficient between soil property and lettuce endpoint. P_{ij} are path coefficients or direct effects of soil properties on endpoints (Fig. 1) derived from multiple regression. Subscript designation (1) corresponds to pH, (2) to % organic carbon, (3) to % clay and (4) to amount of Fe-oxide as determined by modified ammonium oxalate and (5) corresponds to the lettuce endpoints. Therefore $r_{ij}P_{ij}$ are the indirect effects of soil properties on lettuce endpoints. For example, P_{15} = direct effect of pH on lettuce endpoint, $r_{12}P_{25}$ = indirect effect of OC on pH effecting lettuce endpoint, $r_{13}P_{35}$ = indirect effect of clay on pH effecting lettuce endpoint, and $r_{14}P_{45}$ = indirect effect of Fe-oxide on pH effecting lettuce endpoint. In addition an uncorrelated residual (U) was determined from this model using the equation:

$$U = \sqrt{1 - R^2}$$

Low uncorrelated residual (U) and high R^2 values will indicate that the path analysis model explains most of the variation.

The results are presented in a matrix format with the main diagonal indicating direct effects and off-diagonal elements designating indirect effects. The position of each element in the matrix corresponds to the respective normal equations above (Eq. (1), (2), (3), and (4)).

Soil Arsenic Extractability

Soils were extracted with five different soil extractions and digestion for total metal content (EPA 3051). All samples were extracted in duplicate and included reagent blanks and spikes (Appendix A). Modeling and statistics were done using SAS for Windows V8 (SAS Institute 2002) and GraphPad Prism 3.0. Three different statistical approaches were used to determine the soil properties that most influence As extractability. Simple regression models were run for each soil property and each extractant. A backwards step-wise multiple regression technique was used to derive a model that related As extractability to multiple soil properties. And path analysis, as described previously, was done for each extraction method to determine indirect and direct effects of soil properties on As extractability.

Pore Water

To measure the amount of As in the soil pore water, 40 g of soil was saturated with DDW until a slurry or paste was formed as described by J. Rhoades (1996) when measuring electrical conductivity. The soil slurry was allowed to sit for 36-48 hours to equilibrate, mixing intermittedly. The soil solution removed by centrifugation at 12,500 RPM for 10 minutes. The supernant was decanted and filtered through 0.45 micron syringe filter, acidified and analyzed for As (mg/L) using an inductively coupled plasma atomic emission spectroscopy (ICP).

Bray-1 Extraction

An extracting solution of 0.03M NH_4F and 0.025M HCl was used to determine the plant available As. One gram of soil was mixed with 10 mL of Bray-1 solution and shaken on a reciprocating shaker for 5 minutes. The solution was centrifuged at 7500 RPM for 5 minutes then syringed filtered through 0.45 micron filters and analyzed on ICP for plant available As and phosphorus (Kuo 1996).

Sodium Phosphate Extraction

The amount of As associated with water soluble, weakly adsorbed and strongly adsorbed can be measured using this method (Yamamoto 1975). A volume of 600 mL of 0.1M Na_2HPO_4 was added to 400 mL of 0.1M NaH_2PO_4. A soil:solution ratio of 1:10 (2g:20mL) was mixed and shaken for 1 hour on a reciprocating tabletop shaker. The solution was filtered through 0.45 micron syringe filter and analyzed on inductively coupled plasma atomic emission spectroscopy with hydride generation (ICP-HG) for As (Yamamoto, 1975).

Modified Hydroxylamine HCl Extraction

Hydroxylamine Hydrochloride solution is used to extract the water soluble, weakly and strongly adsorbed As, Mn-oxide and some amorphous Fe-oxide As (Ross and Wang, 1993). The extracting solution, 0.25M $NH_2OH{\bullet}H_2O$ and 0.25M HCl is modified by adding 0.025M H_3PO_4 (Amacher and Kotuby-Amacher 1994) to prevent re-adsorption of the As. Soil (4 g, <250 micron fraction) is mixed with 100 mL of solution and shaken for 18 hours on a tabletop recipricating shaker. Samples were suction filtered through 0.45 micron filters and analyzed using ICP-HG. (Amacher and Kotuby-Amacher 1994, Ross and Wang 1993)

Modified Acid Ammonium Oxalate Extraction

To measure the amount of As associated with water soluble, weakly adsorbed, strongly adsorbed and amorphous Fe-oxide. An acid ammonium oxalate method (0.2M ammonium oxalate + 0.2M oxalic acid and 0.1M ascorbic acid) was modified with added 0.025M H_3PO_4 to prevent the re-adsorption of As. The final pH of the solution was 2.7. Soil (0.5 g, <250 micron fraction) was mixed with 20 mL of the modified acid ammonium oxalate solution and shaken on a reciprocating tabletop shaker for 4 hours (Loeppert and Inskeep 1996, McKeague and Day, Schwertmann, SSSA 1996). The soil-solution mixture was centrifuged and filtered through 0.45 micron syringe filter. Analysis for As, iron, and other elements of interest was performed on ICP.

Total Content of Arsenic

Total As was done by nitric acid microwave digestion, EPA Method 3051 (USEPA 1994). This confirms the total amount of As added to each soil. Certified reference material (CRM020-050, RTC Corporation, Laramie, WY, USA) as well as blanks and spikes were digested and analyzed on ICP (Appendix A).

Extractability of Arsenic with Biological Endpoints

Several biological endpoints were used to evaluate the ability of soil extractions to predict As toxicity and accumulation. Biological endpoints that were used includes lettuce seed germination, lettuce shoot elongation, 8-week lettuce bioassay, 5-week lettuce bioassay and earthworm toxicity test. Seed germination, shoot elongation and the 8-week lettuce bioassay are described in the previous section.

Phytotoxicity/Bioaccumulation in Lettuce – 5 Week Bioassay

The 5-week lettuce bioassay was performed by Department of Plant and Soil Sciences at Oklahoma State University with use of the Center of Environmental Research Lab (CERL) constant temperature chamber. The 8-week lettuce bioassay had loss of several plants in the test soils due to arsenic content. To further determine the ability of phosphate to reduce arsenic toxicity and increase yield, this 5-week test was performed. Lettuce plants (*Lactuca sativa var. Paris Island Cos)* were grown in triplicate using 700 g dried soil/pot with added vermiculite to help with water infiltration and drainage. A 15 cm plastic pot without drain holes were used to grow the lettuce. Prior to planting, soil in each pot was fertilized with a (15-30-15, N-P-K) commercial fertilizer to supply 400 mg P/kg (800 lbs/acre), 200 mg N/kg (400 lbs/acre) and 200 mg K/kg soil (400 lbs/acre). Ten seeds were placed into each pot and slightly covered with soil. The pots were watered and placed in a growth chamber. The plants were grown for 40 days (referred to as 5 week bioassay) with a light cycle of 16 hours light and 8 hours dark and temperature maintained at 20°C ± 4° C. At the conclusion of the test the plants were harvested washed and dried at 60°C for 48 hours then weighted. Plants were hand ground or crushed and placed in plastic sample bags and stored at room temperature. Nitric acid digestions were done of all plant material, and analyzed for As on ICP.

Earthworm Toxicity Test

The earthworms were cultivated and tests were run by the Department of Zoology at Oklahoma State University. The earthworm, *Eisenia andrei,* was used in a 28-day toxicity test to determine the ability of extractions to predict toxicity and uptake by earthworms. Each test was done in triplicate for each soil. Canning jars (473 mL)

were used as the environmental chambers to house the worms. Two hundred grams of soil was placed into each jar and wet to just over field capacity ($-^1/_3$ bar). The soils were mixed and water added as needed. Earthworms were removed from growth chambers and allowed to depurate for 24 h by placing in a clean environment (no soil) and allowing the worms to eliminate soil from the gut. Ten worms were weighted out for each jar and placed into the container. At least one hole was poked into the tops of the lids to allow air exchange. The jars were placed on trays and put into a constant temperature chamber with 16 hours light, 8 hours dark and 20 ± 4° C. The worms were check daily for mortality for the first 5 days, then about every 3 days after that. Mortality was determined if the worms failed to respond when gently poked or prodded. Dead worms were removed, rinsed in de-ionized water and frozen at 4°C. Every seven days the worms were fed 1 tsp of manure. At the end of the toxicity test (28 days), all worms were removed from the jars and rinsed in de-ionized water. Worms were depurated for 24 hours then weighted, and frozen at 4°C.

To determine the amount of bioaccumulated As one worm from each jar (total of 3 worms per soil) was digested. The worms were placed into pre-weight crucibles and oven dried at 80°C overnight. The dried worms were weighted and digested as describe by Ybanez et al. (1992) with noted modifications as described for 8-week plant digest.

ICP-Hydride Generation

To determine low level As concentration and to remove potential interferences found on direct analysis of sample digest, ICP-hydride generation was used. The hydride generator is a batch type produced by Thermo Jarrell Ash (TJA) for the IRIS TJA ICP. Reaction rates for hydride generation are controlled by several variables: 1) chemical forms of the As, 2) oxidation state of the hydride-forming element, 3) acid

concentration, and 4) concentration of the $NaBH_4$ reducing agent. The least error occurs when these condition are set so that the reaction goes to completion instantaneously. Inorganic As, arsenate (V) and arsenite (III), can be reduced to arsine gas through the hydride process, although reduction of arsenate is more time consuming. Therefore all samples (10-50 mL) are pre-treated with 40% potassium iodine + 4% ascorbic acid solution (0.5 mL) to reduce all As to arsenite (III), the faster reacting arsenic form (TJA, Dedina and Tsalev, 1995). Hydrochloric acid is used as the sample medium to form hydrides. Samples were mixed with concentrated HCl to a concentration of 3 molar. The efficiency of hydride formation is constant at HCl concentrations above one molar (Dedina and Tsalev 1995). A 0.5% $NaBH_4$ solution with 0.42% NaOH for stability was used as the reducing agent. Acidified sample and base were pumped to the reactor chamber at a rate of 3.25 mL /minute with a 2 minute rinse between samples to eliminate carry over. Arsine gas was separated in a gas-liquid separator via nebulizer directly into the ICP argon plasma.

RESULTS AND DISCUSSIONS

Plant Growth and Arsenic Uptake with Soil Properties

Germination Test and Shoot Elongation

A count of the number of seeds germinated was taken at two points: 5-days after planting and 10-days after planting. The 5-day count tended to be inconsistent among repetitions. At 10-days, repetitions were much more consistent with no loss of seedlings. Therefore a 10-day germination test was used for all analysis. Extremely

high levels of arsenate are known to inhibit seed germination (Wauchoe 1983). Although in this test, mean seed germination was 60% with a range from 0 % to 96.7% (Table 3) indicating that less than half the soils had toxic levels of available As to inhibit seed germination. Simple regressions with soil properties pH, % organic matter, % clay and Fe-oxide content indicate that the amount of clay was the only measured soil property to be significantly (P=0.001) correlated with germination (Fig. 2). The mean % relative germination (% germination in As spiked soil/ % germination from control soils *100) was 64.5% and also correlated with % clay (P<0.0001, Fig. 3). Shoot lengths ranged from 0 to 2.96 cm with an overall mean length of 1.69 cm (Table 3). In comparison, the shoot length from the control soil was much higher with an overall mean of 3.2 cm (1.91-4.33 cm). The mean % relative shoot length (shoot length from As spiked soil/shoot length from control soils *100) was 50% with a range of 0 to 99.8% relative shoot length (Table 3). Both shoot length from As spiked soil and % relative shoot length, results in the same relationship with soil properties. The amount of clay showed a strong correlation (P<0.01) to this test without regard to the way the data is expressed (Fig. 4 and 5). Shoot length seems to be more sensitive in detecting variation is As toxicity in the early stages of lettuce development with more variability in response from the soils.

Multi-regression and Path Analysis for Germination and Shoot Elongation Tests

The results of the backwards stepwise multiple regressions (Table 4) indicate that Fe, soil pH × Fe interaction and pH × clay interaction, were the soil properties that were important for influencing the effect of added As on seed germination and shoot elongation (R=0.84, P<0.0001 and R=0.76, P=0.0012 respectively). Linear regressions did not show any relationship with pH and germination or shoot elongation. The

influence of pH on these results may be due to indirect effects of pH on the clay and Fe-oxide adsorption sites. The multiple regressions for the % relative germination and % relative shoot length were somewhat different. The results (Table 4) indicate that clay along with a pH × Fe interaction explains most of the variability in % relative germination (R=0.89, $P<0.0001$). Whereas clay, pH and interactions between pH × clay and Fe × clay explained most of the variability in the empirical model for % relative shoot length (R=0.84, $P=0.006$, Table 4).

Path analysis provides a somewhat different explanation than correlation analysis for seed germination (Table 5). Both path analysis and simple regressions indicate that clay strongly affects seed germination this is also seen in the empirical model for % relative germination (Table 4). However, path analysis indicates an additional relationship between Fe-oxides and seed germination ($P<0.05$) that due to indirect effects is not apparent with Simple regressions. The indirect effect of clay on Fe-oxide is very large signifying a close relationship between these two soil properties. The % relative germination path analysis results were nearly identical to germination (Table 5).

The results for path analysis and simple correlation analysis for shoot elongation indicate that the amount of clay is the soil property with the most influence (Fig. 4, Table 5). Soil pH, found in multiple regression equations, shows a small direct and no indirect effect suggesting that this soil property is not important for germination or shoot elongation. The path analysis direct-effects indicate that clay and Fe-oxide affect % relative shoot length ($P<0.05$ and $P<0.01$ respectively). The % relative shoot length may be a better representation of the potential effects of soils properties on early lettuce growth. Overall, the property with the most significant effect on germination

and early seedling growth in As contaminated soils is the amount of clay followed by Fe-oxide content in a soil.

8-Week Bioassay: Lettuce Yield and Arsenic Accumulation

The yield of the 8-week lettuce bioassay was lost in about ¼ of the test soils possibly due to a combination of inherit fertility deficiencies and As content. It has been shown that in As contaminated soils plants live longer and have higher yields at high soil P concentrations (Meharg and Macnair 1991, Carbonell-Barrachina 1998). Heeraman et al. (2001) found that increase of added phosphorus on mine soil increased the yield of fescue grass significantly. If the plants are P-sufficient, phosphate is a very effective competitive inhibitor of arsenate by suppressing the root uptake system (Meharg and Macnair 1991, Carbonell-Barrachina et al. 1999). The presence of phosphate inhibits the uptake of arsenate whereas the presence of arsenate only mildly inhibits the uptake of phosphate (Tamaki and Frankenberger 1992, Burlo et al. 1999). A P:As ratio of 4:1 or less causes phytotoxicity on wheat with reduced yield (Adriano 1986). At a P:As ratio of 1:1 and available As concentrations above 10 mg/kg, stunting occurred in the wheat. Woolsen et al. (1973) found reduced phytotoxicity at available P:As ratio of 0.7:1 to 42.5:1. In this study the P:As ratio for lettuce 8-week bioassay ranged from 3.34:1 to 0.02:1 as determined by Bray-1 soil extraction. Fig. 6 shows the linear relationship ($P<0.001$) between yield from 8-week lettuce bioassay and P:As ratio as determined by Bray-1 extraction. There is strong evidence ($r^2= 0.70$) that with added P-fertilizer additional yield would have ensued.

The uptake of As reduces plant growth and at higher availability causes various detrimental effects (Onken and Hossner 1995). Abnormalities with regard to growth, chlorosis and necrotic spots and leaf tips were seen for about half of the soils tested. In

several soils (Mansic A, Mansic B, Hanlon, Perkins, Pratt B) the seeds would germinate, grow for 2-3 weeks then die. This suggests that the level of As is not immediately toxic to inhibit seed germination in most soils. This is supported by germination test results with high germination rates. High As availability has been shown to result in poor shoot survival of lima beans (Woolsen 1973). As arsenic accumulates in the plant tissue and phosphate uptake is inhibited, the result is detrimental effects and reduced growth as compared to the controls (Appendix A).

Lettuce yield from 8-week lettuce bioassay ranged from 0 to 5g dry weight with a overall mean of 0.84g. The linear regressions indicate that Fe-oxide ($P<0.0001$), amount of clay ($P=0.005$) and amount of organic carbon ($P=0.008$, Fig. 7) have a significant relationship to total yield. The % relative yield ranged from 0 to 75.6 % relative. This is the ratio of the yield from the As spiked soils to the yield from the controls expressed as a percent (Appendix A). There is a reduction of yield from the As spiked soils compared to the control soil due to the increase of As in the soil. The soil properties that have the strongest correlation to the % relative yield are the amount of Fe-oxides ($r^2=0.76$, $P<0.0001$), followed by clay ($r^2=0.28$, $P=0.01$) and organic carbon ($r^2=0.21$, $P=0.03$, Fig. 8).

The amounts of As accumulated in the leaves after 8-weeks ranged from 2 to 30 mg/kg dry weight with a mean of 11 mg/kg As (Appendix A). Control lettuce plants had As levels below detection limits. Wauchope (1983) reported that As concentrations in plant tissue above 3.4 to 10 mg/kg are toxic to lettuce and other leafy vegetables. In general, lettuce with higher As content had lower yields as compared to the controls. The main difference in how much As is accumulated by plants is controlled by the availability of As in soils (Wauchope 1983). The exponential regression explain the

relationship best between soil Fe-oxide (R^2=0.65) and clay (R^2=0.37) and the amount of As accumulated in lettuce (Fig. 9). Both clay and Fe-oxides are sinks for As providing adsorption sites and possible complexation with As that would reduce As availability and toxicity of added As (Adriano 1986, Woolson 1973).

Multi Regression and Path Analysis for Lettuce Yield and Accumulation

Backwards multi-regression for (8-week bioassay) lettuce yield, % relative yield and As accumulation is shown in Table 4. The regression for yield indicates that Fe-oxide and amount of clay are the main soil properties affecting yield (R = 0.95, P<0.0001). There are also interactions between pH × Fe-oxide and organic carbon × Fe-oxide. The multiple regression for % relative yield specifies that Fe-oxide and amount of clay are the main soil properties that explain most the variability (R = 0.95, P<0.0001). There are also significant interactions between pH × Fe-oxide, pH × clay and Fe-oxide × clay. The multi-regression equation for As accumulation (R= 0.91, P=0.0008) includes organic carbon, Fe-oxide and amount of clay with and interaction between pH × clay (Table 4).

Unlike either simple regression or the multiple regressions, path analysis indicates that only Fe-oxide (P<0.01) is important for lettuce yield all the other soil properties were found to be a combination of indirect effects (Table 5). The same conclusions are found with % relative yield. Path analysis for As accumulation in lettuce indicates that several properties have an affect on lettuce uptake of As. Soil pH (P<0.05), organic carbon (P<0.01) and Fe-oxide (P<0.01) all have an affect on the amount of As taken up by the plant. Correlation analysis does not show the relationship between organic carbon and As accumulation due to the indirect effect of Fe-oxides on this parameter. Low uncorrelated residual (U) values and significant R^2 values in path

analysis suggest that the model explains most of the variation in lettuce yield and As accumulation.

Soil properties are modifying factors that help define the way a biological organism reacts to and accumulates nutrients and contaminants. A summary (Table 6) of soil properties found to explain variability in each of the lettuce endpoints indicates that a variety of results can be acquired depending on the type of statistical analysis performed, although certain trends in the results can be established. The amount of clay and Fe-oxide in the soil have a dominant effect on the ability of a plant to survive, grow and accumulate As.

Arsenic Extractability and Soil Properties

The average amount of As extracted by the various methods followed the order: pore water (20.6 mg/L, 7.52 mg/kg) < Bray-1 extraction (45.5 mg/kg) < Na-phosphate extraction (65.3 mg/kg) < hydroxylamine HCl extraction (102.5 mg/kg) < ammonium oxalate extraction (217.5 mg/kg). The amount of As extracted by each method and the percent of total As extracted are shown in Table 7. The % of total for pore water As was determined from 40 g of spiked soil and the measured weight of water added to each soil to make a slurry (Appendix). Spiked recoveries ranged between 94 to 102% recovery with a mean of 98.7% As recovered. Result of each extraction method including repetitions, spike recoveries and detections limits are reported in Appendix A. The pore water extraction only measured the most readily available/water soluble As in the soil. This fraction is important since it is considered to be the most readily available to plants and soil organisms and the most easily leached to groundwater. Pore water As ranged from < 0.2 to 162.6 mg/L, with a mean of 20.6 mg/L As in solution or 3.8% of total As, normally less than 5% of total As is water-soluble (Adrianno 2001). The

dissimilarity in pore water As indicates that soil properties modify the As levels. Only three soils had > than 5% pore water As, Dougherty, Pratt A and Pratt B. All three soils were both low in % clay and Fe-oxide content.

The Bray-1 extractant is commonly used to determine plant available phosphorus. Arsenate, since is chemically similar, can be extracted by Bray-1 to determine the amount of plant available As (Wauchope, 1983, Huang and Fuji, 1996). The Bray-1 extraction measures the water soluble, weakly adsorbed (non-specific) which is the plant available fraction of As in the soil. This extraction causes the slight dissolution of Fe and Mn oxides and extracts As associated with poorly ordered alumino silicate gels and allophane (Lombi et al. 2000). Bray-1 extraction ranged from 2.9 to 142 mg As/kg soil with a mean of 45.5 mg/kg. This is 21.4% of the total As extracted (1.3 to 65% extractable). The Na-phosphate extraction measures, in addition to the previous fractions, the strongly (specifically) adsorbed As. Phosphate competes with arsenate for adsorption sites and is more effective than other anions (e.g. nitrate and sulfate) to extract arsenate from soils (Lombi et al. 2000). The range of extractable As was 11.9 to 201.6 mg As/kg, with an average of 30% of the total As extracted (5.1 to 95.8%). The hydroxylamine extraction is a more aggressive extraction resulting in 80.7 to 156 mg As/kg, 45.9% of the total As extracted (37-75.4%). This extraction is able to release the surface bonded and some of the occluded As from Mn, Al and Fe-oxide As. The ammonium oxalate extraction is able to dissolve the amorphous and crystalline Fe and Al–oxides releasing the associated As. An average of 96.7% of the total As extracted (78.2 to 115%), with a range of 173.9 to 277 mg As/kg was extracted. This indicates that the majority of the As in these soils resided in the amorphous Al and Fe-

oxide fraction (~40 to 60%). The variability in extractability is due to the range of soil properties.

In general, As mobility is greater in sandy soil than clayey soils (Adriano 2002). Pore water and Bray-1 extractions measure the most loosely bound As and that with the greatest potential to leach from soil. Exponential regressions describe the relationship between pore water As and clay (R^2=0.67) and Fe-oxide (R^2=0.79, Fig 10). Similarly, exponential regression also describe the relationship between Bray-1 As and clay (R^2=0.48) and Fe-oxide (R^2=0.43, Fig. 11). The soil pH (R^2=0.49) and Fe-oxide (R^2=0.43) had the strongest expoenential regression with Na-phosphate extractable As (Fig. 12). In general, as the amount of Fe-oxides in soil increased, the amount of extractable As decreased. Fe-oxides are attributed to being the major adsorption/complextion site for As in most soils (Chen et al., 2002, Smith et al. 1998, Lombi et al. 2000).

The linear regressions for ammonium oxalate suggest a more complex relationship (Fig. 13). The amount of clay (r^2=0.41, P=0.002), Fe-oxide (r^2=0.41, P=0.001) and organic carbon (r^2=0.49, P=0.0004) all have significant linear relationships to the amount of As extracted. In contrast Hydroxylamine HCl extracted As does not relate to any of the measured soil properties (Fig. 14).

Multiple Regression

A backwards, stepwise multi-regression was done to further investigate the relationship with multiple soil properties and As extractability and to develop a model to describe this relationship (Table 8). The soil properties pH, Fe-oxide and amount of clay were all found to be significant with pore water extractable As (R=0.88, P=0.0004). Bray-1 extraction produced a bit more complex relationship with significant interactions

between organic carbon × clay and organic carbon × Fe-oxide and significant properties of clay and organic carbon (R=0.81, P=0.0008). Organic carbon is not thought to be a significant sink for As in soils. The significance of organic carbon maybe due to the indirect effect it can have on clay and Fe-oxides by the introduction of strong reducing agents which influence the processes that control mobilization and extractability of As (Chen et al. 2002).

The multi-regression for Na-phosphate extractable As also has a more complex equation with significant soil properties clay, organic carbon and pH and interaction existing between pH × organic carbon, organic carbon × clay and iron × organic carbon (R=0.94, P<0.0001, Table 8). The final equation, for ammonium oxalate extractable As, is only significant for Fe-oxide, with an interaction between organic carbon × Fe-oxide (R=0.78, P=0.0001). The multi-regression technique eliminated all soil properties (P<0.05) for hydroxylamine HCl extractable As. This extraction appears to extract about 50% of the As in soil not relating to any measured soil properties.

Path Analysis

Path Analysis pulls apart the influence of each soil property and identifies the direct and indirect affect of each soil property on the extractability of As. Table 9 shows the direct and indirect effects for the soil properties pH, organic carbon, Fe-oxide and clay content and the regression significance (r) for each extractant. To determine the weight of indirect effects, they can be compared to an r-table where n=22, this will indicate the strength of the indirect effects and are noted on Table 9. Simple regressions for pore water indicated that Fe-oxide and clay content had significant relationship to the amount of As in this fraction. In path analysis, (Table 9) only clay content is found to be significant to the amount of pore water As. Fe-oxide, which was

also found in multiple regression, can be attributed to the indirect affect of clay. Therefore the soil property with the greatest influence on pore water As is clay. This similar result was also found with Bray-1 extractable As. The amount of Fe-oxide was significant using simple regression but in path analysis it is found to be due to the indirect affect of clay (Table 9). Na-phosphate is able to remove arsenate from strong adsorption sites. Both pH and Fe-oxide had significant linear relationships with Na-phosphate extractable As and the same conclusions are found with path analysis. Arsenate adsorption has been found to be pH dependent with the amount of arsenate adsorbed decreasing with increasing pH (Liu et al. 2001, Darland and Inskeep 1997).

The path analysis for ammonium oxalate As indicates that the amount of extractable As is related to the soil properties organic carbon and clay content. Iron, found to be significant with simple regression, is attributed to the indirect affect of organic carbon and clay content on Fe-oxide. Organic matter generally has a low affinity for As, however, humic substances in the soil can serve as strong reducing agents and can influence the processes that control mobilization and extractability of As (Chen et al. 2002).

Table 10 shows a summary of simple simple regression, backwards multi-regression and path analysis for the select soil properties and soil extraction methods.

Extractability of Arsenic with Biological Endpoints

Total As in soils is not an accurate representation of the fraction of As that is bioavailable to plants and soil organisms. Fig. 15 shows the plant and earthworm response to total As concentration in soil. There are no significant relationships between total As and lettuce bioassay and earthworm toxicity test. This is consistent with the finding of Bech et al. (1997) that found there were no relationships between total As in

soil and available As to plants. An extracted fraction of As can gives an indirect measurement of As bioavailability to earthworms and plants. To determine which extraction best correlated with bioavailable As, simple regressions were completed between multiple endpoints and each extractant.

5-Week Bioassay

Bray-1 extracted P:As ratio (Fig. 6) indicated that with additional P fertilizer more yield would have ensued. To test the effect of added P on lettuce yield in As contaminated soil, a 5-week bioassay was conducted with excess P fertilizer added. The resulting yield was increased and toxicity reduced in nearly all soils as a result of the added fertilizer (Table 11). The 5-week lettuce bioassay had an average yield of 2.4g dry weight with a range of <0.01g to 7.2g dry weight. This is almost three times the average yield from the previous study (0.84g). The mean % relative yield also increased from 22.8% to 30.8% with a range of 0.15% to 99.2%. The As accumulation in lettuce tissue ranged from <1 mg/kg to 40.6 mg/kg. This range is high as compared to leafy and other vegetable. Woolsen (1973) tested green beans, lima beans, radish, tomato, cabbage and spinach and found As concentrations <10 mg/kg in leaf tissue. Wauchope (1983) reported <0.4 mg/kg in leafy vegetables, tobacco 1-78 mg/kg, and legumes from 1-14 mg/kg. The reason for the higher arsenic levels may be due to increase in soluble As (Table 11). Figure 21 shows the strong relationship between plant available (Bray-1) As from the fertilized soils and arsenic concentration in lettuce. Arsenate can be taken up via the phosphate transport system and is thought to replace phosphate in energy transfer phosphorylation reactions (Tamaki and Frankenberger 1992, Bhumbla and Keefer 1994). The addition of phosphate fertilizers to As contaminated soils promotes the release of soluble As a result of competitive adsorption between phosphate and

arsenate (Darland and Inskeep 1997, Peryea and Kammereck 1997). The results of the addition of phosphate fertilizers may increase plant yield but also has the potential to increase soluble As for plant uptake and increased potential As leaching to groundwater.

Arsenic Extractability and Lettuce Endpoints

Pore water As had a significant correlation to early stages of plant growth including germination and shoot length (Fig. 16). This fraction of As is the most readily available and it has the most effect on early plant growth. An exponential regression of pore water and lettuce yield and % relative yield from the 8-week lettuce bioassay (R^2=0.30 and R^2=0.59 respectively) and 5-week lettuce bioassay (R^2=0.84 and R^2=0.90 respectively, Fig. 17) best explains this relationship. A linear relationship between As content in lettuce and soil pore water are significant (r^2=0.42, P = 0.01 and r^2=0.67, P = 0.0004) for both lettuce bioassays (Fig. 18). Arsenic content in corn plants grown in As contaminated soil has been shown to have a strong linear correlation to water-extracted As with total As soil levels < 25mg/kg (Sadiq 1986).

Bray-1 has previously shown a relationship between plant yield and As added to soil (Wauchope 1983, Jacobs et al. 1970). Jacobs et al. (1970) applied 0 - 710 kg/ha sodium arsenate to a field then planted potatoes, peas, snap beans and sweet corn. Every crop showed a significant correlation between Bray-1 As and yield. Similarly, lettuce yield, arsenic accumulation and to a lesser extent germination and shoot length had a significant correlation to Bray-1 extractable As (Fig. 19, 20 and 21).

Na-phosphate had the best relationship with the lettuce yield (R^2=0.60) from 5-week bioassay and % relative yield from both 8-week and 5-week bioassays (R^2=0.53 and R^2=0.61 respectively, Fig. 23). The other lettuce endpoints (Fig. 22 and 24) had no significant relationship to this fraction of As. Na-phosphate is able to release the As

bound to sorption sites, similar to the way a phosphate fertilizer would release As bound to sorption sites. For maximum plant growth, major nutrients must be in ample supply. The 5-week bioassay received additional phosphate fertilizer where as the other tests received none or little fertilizer. Arsenate and phosphate compete for adsorption sites. With added phosphate some of the adsorption sites held by arsenate will be replaced by phosphate. Once the sites are filled the remaining arsenate and phosphate will be in solution and available for plant uptake. With the limited number of adsorption sites and twice as many ions competing for them, this allows for more phosphate in solution. At low available As concentration (e.g. arsenic bound to Fe-oxides) this can stimulate plant growth by the increase in soluble phosphate for plant uptake (Carbonell-Barrachina 1995, Carbonell-Barrachina 1998, Marin et al. 1992, Jacobs et al. 1970.). When phosphate concentrations are low, the plant is unable to take-up the needed phosphates and arsenate is accumulated by the plant. This creates a situation in which the plant reacts as if there is a P-deficiency and in turn takes up more arsenate. Arsenic can substitute for P in the plant, but is unable to carry out the role of P in energy transfer; therefore the plant reacts as if there is a P deficiency (Burlo et al. 1999, Heeraman et al. 2001).

Ammonium oxalate extractable As had a significant correlation to shoot elongation (r^2=0.20, P=0.04, Fig. 25) and lettuce yield in the 8-week lettuce bioassay (r^2=0.47, P=0.0005, Fig. 26). The As accumulation in lettuce had no relationship to ammonium oxalate As (Fig. 27). Hydroxylamine Hydrochloride extracted As has no significant relationships to any of the measured lettuce endpoints (Appendix A).

An important step in determining toxicity of a chemical is generating a dose-response curve. A dose-response or concentration-response curve establishes the

relationship between exposure to a substance and the incidence and severity of an effect. Concentration-response curves are used to plot the results of this experiment. The x-axis plots the log concentration of As extracted by Bray-1 and the y-axis plots the response from each test. Bray-1 extracted As was selected because it had the strongest relationship with the most lettuce endpoints or responses. The equation used to produce these curves (GraphPad 3.0):

$$Y = \frac{100}{1+10^{(\log IC50 - X)*HillSlope}} \quad (1)$$

Where Y = Lettuce endpoint expressed as the % relative yield or germination

X = log Bray-1 extracted As mg/kg

Hill Slope = the steepness of the curve

Concentration-response curves were produced to find an IC_{50} (inhibitory concentration which induced a response in 50% of the samples) or and IC_{20} (inhibitory concentration that induced a response in 20%) (GraphPad 3.0). For % relative yield the resulting IC_{50} was 7.98 and 72.4 mg/kg Bray-1 As for the 8-week and 5-week lettuce bioassays respectively (Fig. 28). The IC_{20} can be calculated using the equation (Motulsky 1999):

$$IC_{20} = \left(\frac{80}{100-80}\right)^{\frac{1}{H}} * IC_{50}$$

Where H = Hill slope

The IC_{20} for % relative yield were 1.92 and 42.0 mg/kg Bray-1 As for the 8-week and 5-week lettuce bioassays respectively (Fig. 28). The R^2 was 0.44 and 0.70 for the 8-week and 5-week bioassays respectively. The large difference in the IC_{20} from these bioassay is most probably a result of the lowered As toxicity in the 5-week bioassay due to the addition of phosphate fertilizer. The IC_{50} and IC_{20} for % relative germination are

69.2 mg/kg and 17.2 mg/kg Bray-1 As respectively. This curve has a very shallow slope indicating that germination requires a large change in Bray-1 As concentration before a resulting lower germination rate.

Earthworm Toxicity Test and Extractable Arsenic

Toxicity test are effective way of determining the level of toxicity of a particular soil to the test organism. It also gives an indication of the amount of contaminant that might be transferred up the food chain. The ability to characterize a soil and its contaminant as hazardous can be difficult. Currently total content of As in soil is used to determine clean-up levels. This does not always prove to be the best choice for the soil ecosystem. Toxicity test help to determine whether the soil is hazardous to the local fauna. But these test are time consuming and require support personnel. Finding a quick extraction method that can determine if a soil needs to be further assessed can aid in the clean-up process. The five extractions described previously, are used in simple regression models from a 28-day earthworm toxicity test. Earthworm mortality and As accumulation are correlated to each extraction and examined. Earthworm mortality ranged from no mortality to 100% mortality with a mean of 21.2 % mortality (Appendix A). Arsenic body burdens ranged from 32.3 to 628.5 mg/kg dry weight, with a mean of 300.3 mg/kg. The test soils were all spiked to obtain a target level of 250 mg/kg total As with a mean result of 226 mg/kg As. Therefore the variability in response and As body burden indicates that there is a difference in toxicity dependent on the soil and not the total As in the soil. A fraction, or extractable portion of As, may describe the toxicity of As to earthworm. Earthworm mortality had the best relationship to pore water As (R^2=0.41, Fig. 29) and Bray-1 As (r^2=0.35, P=0.004, Fig. 30). Earthworm exposure to soil As is through soil contact with the epidermis and through

adsorption across the gut (Meharg et al. 1998, Langdon et al. 2001). With greater soluble As in the soil, there is greater As exposure to the earthworm. The earthworms are much more affected by the soluble forms of As than the strongly adsorbed or complexed As. There was also a weak relationship (P=0.09) with ammonium oxalate extracted As and earthworm mortality (Fig. 33). This may be associated with the relationship between amount of Fe-oxide and earthworm mortality found in correlation analysis (Table 12). The remaining extractions are much more aggressive thereby removing a greater fraction of As from the soil consequently not a good representation of the fraction of As affecting earthworm viability (Fig. 31 and 32).

Concentration-response curves were produced for earthworm mortality and pore water and Bray-1 As (Fig. 34). The equation used for this curve (uphill curve) was (GraphPad 3.0):

$$Y = \frac{100}{1+10^{(\log LC50 - X)*HillSlope}}$$

The lethal concentration at which 50% (LC_{50}) of the worms died was 120.2 mg/kg Bray-1 As and 79.4 mg/L pore water As. The LC20, the concentration at which 20% of the worms died, was 37.1 mg/kg Bray-1 As and 4.05 mg/lL Pore water As Where:

$$LC_{20} = \left(\frac{20}{100-20}\right)^{\frac{1}{H}} * LC_{50}$$

The Hill slope for Bray-1 (1.44) is much steeper than pore water (0.47). This indicates that smaller changes in Bray-1 As concentration can cause greater earthworm mortality. A steep slope or higher slope factor reflects the magnitude of the range of "doses" between non-affected concentration and a lethal or effective concentration. The steeper, the curve the slighter the margin of safety or the more toxic the substance.

Earthworm As accumulation did not correlate to any extraction method at the $P<0.05$ level. Bray-1 As (Fig. 30) and Na-phosphate As (Fig. 31) had a weak correlation ($r^2=0.17$, $P=0.07$ and $r^2=0.18$, $P=0.06$ respectively) to As accumulation in the earthworm. More data would be needed before using these methods for toxicity assessment. Living earthworms are able to regulate their burdens by homeostatic controls over As accumulation and through detoxification processes (Meharg et al. 1998.) In addition edaphic conditions affect arsenate bioavailability (Meharg, et al. 1998). Table 12 shows correlation analysis for the soil properties pH, % organic carbon, Fe-oxide and % clay. These results indicate the potential of pH ($P=0.09$) to influence As accumulation by earthworms. As pH increases the amount of As that becomes available increases due to reduction of sorption sites and increase of negatively charged As species thereby increasing the amount of labile As (Manning and Goldberg, 1996). Peijnenburg et al. (1999) found that pH had a direct influence on the elimination rate of As from the earthworm. At soil pH ($CaCl_2$) greater than 5, As is elimination from the earthworm. But at soil pH ($CaCl_2$) above 6.75 there is no further elimination of As from the earthworm and body concentration increase linearly. Evidence suggests that As is taken up primarily from the solid soil matrix or via an exposure route related to the soil matrix e.g. uptake from the labile or extractable portion (Peijnenburg et al. 1999). It has been shown that soil properties can reduce the labile or extractable portion of As from the soil matrix. Properties that have the greatest influence of reducing the labile portion of As would be clay and Fe-oxides.

CONCLUSIONS

Plant bioavailable As varied depending on soil properties. Results indicate that Fe-oxide and amount of clay have the most influence on lettuce yield in As contaminated soils. Arsenic accumulation by lettuce was much more complex relationship indicating several soils properties; Fe-oxide, amount of clay and pH, all have an influence on As uptake. The results illustrate that at different stages of lettuce growth and development, a range of soil properties can affect shoot growth, yield and accumulation in As contaminated soils. Models may be useful for predicting potential risk of As in soil. Path analysis models provided quantitative causative influence of As bioavailability for lettuce yield and accumulation of As.

The majority of As in spiked soils was found in association with Fe-oxides. The soil properties that influence extractability of As differed depending on the regression model and extraction method. But overall the extractability of As is dependent on Fe-oxides and clay.

There are several extractants that have a high correlation to plant bioavailable As. Extractions have more value than soil properties alone when determining the amount of As that is posing a risk to the environment. Soil properties act as modifying factors and influence the amount of As that is extracted. Pore water and Bray-1 have strong relationship to seed germination, early shoot growth, and lettuce yield. In addition Bray-1 extraction estimates As accumulation in lettuce. Earthworm mortality is strongly correlated to pore water and Bray-1 As. Na-phosphate extraction had the best relationship ($P<0.08$) with As accumulation in earthworms. More investigations are needed to confirm use of this extraction to predict As uptake. Basing all bioavailability on one or more soil properties is not adequate in determining the risk associated with As

content in soil. Just as soil fertility is based on the amount of a nutrient that is extracted, so should bioavailability and risk due to As in soils.

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Table 1. Soil properties pH, %Organic carbon, % Clay, Cation exchange capacity (CEC), and Fe and Al – oxide as determined by ammonium oxalate extractant from selected soils (Controls).

Soil Name	pH	$pH^{\mp}_{sp}$	%OC	%Clay	% Silt	CEC cmol/kg	Fe-Oxide mg/kg	Al-Oxide mg/kg
Bernow B	5.6	4.45	0.30	26.3	19.4	6.70	873	2270
Cannisteo	7.8	7.55	3.00	38.8	51.3	30.5	1680	1510
Dennis A	6.2	4.90	1.90	23.8	40.0	9.80	1680	8070
Dennis B	6.5	5.60	0.80	45.0	41.9	14.6	2080	8510
Dougherty	5.7	5.00	1.20	11.3	21.3	3.30	291	594
Efaw	4.1	4.00	1.20	17.5	54.4	4.60	1210	4100
Hanlon	7.7	7.00	1.60	17.5	23.8	16.3	832	2850
Haskell	4.9	4.65	1.20	11.3	70.6	4.80	863	3570
Kirkland	5.5	5.10	1.45	31.3	57.5	14.0	1420	2980
Luton	7.4	7.15	2.00	71.3	38.8	32.4	3110	9730
Mansic A	8.0	7.95	1.50	30.0	43.8	16.5	839	752
Mansic B	8.4	8.00	0.53	35.0	42.5	11.7	873	2210
Osage A	7.2	6.00	2.60	55.7	53.8	28.3	2710	14200
Osage B	6.6	6.15	2.00	61.3	47.5	27.5	2740	14800
Perkins	4.4	4.30	0.85	10.0	30.0	3.00	759	2180
Pond Creek A	5.2	4.65	1.90	28.8	62.5	10.7	1330	2890
Pond Creek B	6.0	5.95	0.80	32.5	48.8	12.5	1390	3270
Pratt A	6.7	6.30	0.90	5.00	3.75	4.40	250	382
Pratt B	6.1	6.00	0.50	6.25	1.25	3.40	240	349
Richfield	7.2	7.55	1.10	41.3	51.3	22.3	1420	1870
Summit A	7.6	7.25	2.40	45.7	53.8	29.4	4210	11400
Summit B	7.0	6.65	1.25	56.8	48.8	27.6	2690	5670

$\mp$ – pH of soil after spiking with 250 mg/kg arsenic and leaching of the soil

Table 2. Arsenic chemical extractants and their respective literature references.

Chemical Extractant	Arsenic Species Extracted	References
Bray-1 0.03 *M* NH_4F + 0.025 *M* HCl	Water Soluble Weakly Adsorbed (Plant Available)	Bray and Kurtz, 1945 as described by Kuo, 1996
Sodium Phosphate 0.06 *M* Na_2HPO_4 + 0.04 *M* NaH2PO4	Water Soluble Weakly Adsorbed Strongly Adsorbed	Yamamoto, 1975
Hydroxylamine HCl 0.25 *M* $NH_2OH•H_2O$ and 0.25 *M* HCl and 0.25 *M* H_3PO_4	Water Soluble Weakly Adsorbed Strongly Adsorbed Mn-oxide and Some Amorphous Fe-oxides	McKeague and Day, 1966 as descrribed by Ross and Wang, 1996, modified by Amacher and Kotuby-Amacher, 1994
Acid Ammonium Oxalate Tamm's Reagent 0.175*M* ammonium oxalate $(NH_4)2C_2O_4$, 0.1 *M* oxalic acid $(H_2C_2O_4)$ + 0.025 *M* H_3PO_4	Water Soluble Weakly Adsorbed Strongly Adsorbed Amorphous Fe-oxides	McKeague and Day, 1966, as describe by Loeppert and Inskeep 1996: Schwertmann, SSSA, 1996,

Table 3. Ten day % seed germination, %relative seed germination, shoot length, and % relative shoot length results.

Soil	10-day % Germ	% Relative Germ	Shoot Length, cm	% Relative Shoot Length
Bernow B	80.0	85.3	1.95	56.8
Canisteo A	86.7	98.1	2.12	54.5
Dennis A	30.0	31.6	0.63	26.5
Dennis B	90.0	96.4	2.48	63.4
Dougherty A	0	0	0	0
Efaw A	83.3	NA	1.88	NA
Hanlon A	38.3	44.2	1.75	40.5
Haskell	73.3	NA	2.27	NA
Kirkland A	62.5	47.2	1.37	57.3
Luton A	95.0	121.3	2.63	64.6
Mansic A	58.3	62.5	1.58	71.2
Mansic B	96.7	112.1	2.05	61.5
Osage A	61.7	67.3	2.36	58.7
Osage B	93.3	102.6	2.96	91.6
Perkins A	6.7	7.4	0.36	9.18
Pond Creek A	48.3	56.9	2.20	83.1
Pond Creek B	90.0	105.9	2.65	63.6
Pratt A	13.3	17.8	0.53	16.1
Pratt B	1.7	2.1	0.17	9.43
Richfield B	96.7	105.5	2.35	99.8
Summit A	26.7	29.6	0.58	14.4
Summit B	88.3	96.4	2.18	58.4
Overall Mean	60.0	64.5	1.7	50.0

Table 4. Backwards stepwise multiple regression using the soil properties pH, % organic carbon, Fe-oxide (ammonium oxalate extracted), and % clay for lettuce bioassay endpoints: % germination, %relative germination, shoot length, % relative shoot length, 8-week lettuce yield, 8-week % relative yield, and 8-week arsenic accumulation (n=22, $P < 0.05$).

Method	Regression Equation for soil properties pH, %OC, Fe-oxide, and %Clay	R	*P*
% Germ	[%Germ] = 4.73 + (0.025*Fe) – (0.005* pH*Fe) + (0.33* pH*Clay)	**0.84**	**<0.0001**
% Relative Germination[2]	[%Relative Germ] = -1.69 + (2.80*Clay) - (9.52E-4*pH*Fe)	**0.89**	**<0.0001**
Shoot Length	[Shoot] = 0.305 + (0.00062*Fe) – (0.0001* pH*Fe) + (0.007* pH*Clay)	**0.76**	**0.0012**
% Relative Shoot Length[2]	[%Relative Shoot] = -77.0 + (7.97*Clay) + (2.06*pH^2) - (0.96*pH*Clay) - (7.15E-5*Fe*Clay)	**0.84**	**0.0006**
Total Yield (8-week)	[Yield] = 406.29 + (0.41*Fe) – (45.96*Clay) + (0.96*$Clay^2$) – (0.097* pH*Fe) + (0.17* OC*Fe)	**0.95**	**<0.0001**
% Relative Yield (8-week)	[%Rel. Yield] = 13.5 + (1.86E-6*Fe^2) + (0.104*$Clay^2$) + (0.0025*pH*Fe) - (0.49*pH*Clay) - (8.6E-4*Fe*Clay)	**0.95**	**<0.0001**
As Accumulation.[1]	[As] = 21.22 + (8.71*OC) – (0.002*Fe) + (0.01*Clay2) – (0.11* pH*Clay)	**0.91**	**0.0008**

1 For this regression n=15, P=0.01
2 For this regression n=20

Table 5. Path analysis indicating direct effects (underlined) and indirect effects of the soil pH, %organic carbon, %clay and Fe-oxide on lettuce endpoints: % germination, % relative germination, shoot length, % relative shoot length, 8-week lettuce yield, 8-week % relative yield, and 8-week arsenic accumulation. (*designates a r-table significance at $P<0.05$, ** designates a r-table significance at $P<0.01$).

Endpoint		pH	OC	Fe	Clay	r	R^2	U
	pH	**-0.21**	-0.05	0.01	0.53*	0.27	0.59**	0.64
%Germination	**OC**	-0.06	**-0.20**	-0.25	0.58**	0.06		
n=22	**Fe**	0.00	-0.11	**-0.49***	0.85**	0.26		
	Clay	-0.10	-0.10	-0.35	**1.20****	0.66**		
% Relative	**pH**	**0.02**	-0.06	0.02	0.46	0.44	0.79**	0.46
Germination	**OC**	0.01	**-0.25**	-0.25	0.59	0.09		
n=20	**Fe**	0.00	-0.13	**-0.49***	0.89	0.28		
	Clay	0.01	-0.12	-0.35	**1.23****	0.76**		
	pH	**-0.23**	-0.03	0.00	0.46*	0.21	0.50*	0.71
Shoot	**OC**	-0.06	**-0.10**	-0.17	0.50*	0.18		
Elongation	**Fe**	0.00	-0.05	**-0.32**	0.74**	0.37		
n=22	**Clay**	-0.10	-0.05	-0.23	**1.04****	0.66**		
% Relative	**pH**	**-0.12**	-0.02	0.02	0.42	0.29	0.53**	0.68
Shoot	**OC**	-0.03	**-0.08**	-0.28	0.53	0.14		
Elongation	**Fe**	0.01	-0.04	**-0.55***	0.81	0.23		
n=20	**Clay**	-0.05	-0.04	-0.40	**1.12****	0.63**		
Lettuce Yield	**pH**	**-0.01**	0.05	-0.01	-0.04	-0.01	0.74**	0.51
8-week	**OC**	-0.00	**0.17**	0.43	-0.05	0.55**		
Bioassay	**Fe**	0.00	0.09	**0.83****	-0.07	0.85**		
n=22	**Clay**	-0.00	0.08	0.59**	**-0.09**	0.57**		
% Relative	**pH**	**-0.05**	0.01	-0.01	-0.07	-0.12	0.78**	0.46

Endpoint		pH	OC	Fe	Clay	r	R^2	U
Yield	**OC**	-0.01	**0.05**	0.50*	-0.08	0.46*		
8-week	**Fe**	0.00	0.03	**0.96****	-0.11	0.87**		
n=22	**Clay**	-0.02	0.02	0.68**	**-0.16**	0.53**		
Lettuce	**pH**	**-0.53***	0.13	-0.20	0.11	-0.49	0.76**	0.49
Arsenic	**OC**	-0.09	**0.78****	-0.78**	0.08	-0.01		
Accumulation	**Fe**	-0.09	0.54*	**-1.14****	0.14	-0.55*		
n=15	**Clay**	-0.29	0.32	-0.79**	**0.20**	-0.56*		

Table 6. Summary of Linear Regression, Backwards multiple regression and Path Analysis illustrating the relationship between soil properties: pH, % organic carbon, iron oxide, % clay and lettuce endpoints: % germination, % relative germination, shoot length, % relative shoot length, 8-week lettuce yield, 8-week % relative yield, and 8-week arsenic accumulation (*designates and r-table significance at $P<0.05$, ** designates and r-table significance at $P<0.01$).

	Simple Regression (* = $P<0.05$, ** = $P<0.01$)				**Backwards Multiple Regression (* = $P< 0.05$)**					**Path Analysis**			
	pH	**OC**	**Fe**	**Clay**	**pH**	**OC**	**Fe**	**Clay**	**Interaction**	**pH**	**OC**	**Fe**	**Clay**
Germination				**			*		pH × Fe pH × clay			*	**
%Rel. Germ	*			**				*	pH × Fe			*	**
Shoot Length				**			*		pH × Fe pH × clay				**
% Rel. Shoot Length				**	*			*	pH × clay Fe × clay			*	**
Lettuce yield (8-week bioassay)		*	**	**			*	*	pH × Fe OC × Fe			**	
%Rel. Yield		*	**	*			*	*	pH × Fe pH × clay Fe × clay			**	
As Accumulation (8-week bioassay)			*	*		**	**	**	pH × Clay	*	**	**	

Table 7. Arsenic concentrations and amount and percent of total As (italics) extracted by pore water, Bray-1, Na-phosphate, hydroxylamine HCl and ammonium oxalate procedures from 22 soils spiked with arsenic.

Soil	Pore water As mg/L	*% Pore water Extracted As*	Bray - 1 As mg/kg	*% Bray-1 Extracted As*	Phosphate Extract, mg/L	*% Phosphate Extracted As*	Hydroxyl-amine HCl mg/L	*% Hydroxy HCl Extracted As*	Ammonium Ox. As, mg/L	*% Ammon Ox Extracted As*	Total As, mg/kg
Bernow B	0.19	*0.03*	26.3	*10.3*	37.3	*14.6*	81.4	*31.9*	223	*87.4*	255
Canisteo A	8.44	*2.62*	37.8	*16.8*	102	*45.3*	111	*49.3*	239	*106*	225
Dennis A	0.30	*0.07*	10.6	*4.5*	11.9	*5.1*	94.0	*40.2*	232	*99.4*	234
Dennis B	0.003	*0.001*	2.91	*1.3*	15.6	*7.0*	79.4	*35.7*	174	*78.2*	222
Dougherty A	98.8	*13.8*	90.5	*44.3*	64.3	*31.4*	103	*50.3*	194	*95.1*	205
Efaw A	4.06	*0.80*	51.2	*22.9*	36.0	*16.1*	102	*45.5*	215	*96.3*	223
Hanlon A	13.9	*2.95*	77.0	*33.8*	61	*26.9*	105	*46.2*	227	*99.5*	228
Haskell	3.81	*0.94*	35.7	*17.2*	25.8	*12.4*	157	*75.4*	215	*103*	208
Kirkland A	2.01	*0.47*	37.3	*16.5*	40.5	*17.9*	103	*45.5*	223	*98.7*	226
Luton A	1.49	*0.51*	40.6	*16.8*	98.7	*40.9*	112	*46.3*	278	*115*	242
Mansic A	16.9	*4.87*	29.3	*14.5*	95.2	*47.2*	110	*54.8*	209	*104*	201
Mansic B	20.3	*3.71*	8.6	*4.1*	202	*95.8*	113	*53.6*	193	*91.5*	210

Soil	Pore water As mg/L	% Pore water Extracted As	Bray - 1 As mg/kg	% Bray-1 Extracted As	Phosphate Extract, mg/L	% Phosphate Extracted As	Hydroxyl-amine HCl mg/L	% Hydroxy HCl Extracted As	Ammonium Ox. As, mg/L	% Ammon Ox Extracted As	Total As, mg/kg
Osage A	0.23	*0.05*	12.2	*4.6*	25.3	*9.6*	109	*41.3*	275	*104*	265
Osage B	0.12	*0.03*	10.1	*4.3*	27.3	*11.7*	108	*46.1*	250	*107*	234
Perkins A	10.1	*1.26*	62.7	*27.4*	57.9	*25.3*	101	*44.2*	210	*91.8*	229
Pond Creek A	7.49	*1.86*	43.0	*18.9*	39.9	*17.6*	93.6	*41.2*	222	*97.8*	227
Pond Creek B	0.49	*0.10*	40.3	*17.7*	45.4	*19.9*	103	*45.3*	218	*95.3*	228
Pratt A	94.4	*24.1*	113	*75.7*	93.3	*62.7*	81.8	*55.0*	151	*101*	149
Pratt B	163	*23.7*	142	*65.0*	130	*59.4*	80.7	*36.9*	186	*85.2*	218
Richfield B	8.61	*2.27*	110	*47.1*	154	*65.9*	105	*44.9*	219	*93.4*	234
Summit A	0.31	*0.08*	7.0	*2.7*	45.4	*17.6*	107	*41.6*	233	*90.5*	258
Summit B	0.02	*0.005*	12.3	*5.1*	27.7	*11.4*	93.9	*38.6*	210	*86.2*	243
Overall Mean	20.6	*3.8*	45.5	*21.4*	65.3	*30.1*	102	*45.9*	218	*96.7*	226

Table 8. Backwards stepwise multiple regression for various arsenic extraction methods using soil properties pH, % organic carbon, Fe-oxides (mg/kg, ammonium oxalate), and % clay (n=22, $P < 0.05$).

Method	Regression Equation for soil properties pH, %OC, Fe-oxide, and %Clay	R	*P*
Pore water	[As] =– 281.0 + (134.6*pH) – (0.014*Fe) - (3.54*Clay) – (10.9*pH^2) + (7.6x10-7 * Fe^2) + (0.04*$Clay^2$)	**0.88**	**0.0004**
Bray-1	[As] = -132.3 - (4.3*Clay) – (26.4*OC^2) + (2.77*OC*Clay) – (0.003*OC*Fe)	**0.81**	**0.0008**
Na-Phosphate	[As] =49.68–(3.2*Clay)+(4.7*pH^2)+(20.82*OC^2)–(23.8*pH*OC)+(2.4*OC*Clay)-(0.003*Fe*OC)	**0.94**	**<0.0001**
Hydroxylamine HCl	*NA*	**0.0**	
Ammon Ox	[As] = 197.6 - (5.12x10-7*Fe^2) – (0.005*OC*Fe)	**0.78**	**0.0001**

Table 9. Path analysis indicating direct effects (underlined) and indirect effects of the soil pH , % organic carbon, % clay and Fe-oxide (ammonium oxalate extracted) on extractability of arsenic using five different extractions (n=22, * designates r-table significance at $P<0.05$, ** designates r-table significance at $P<0.01$).

Extraction		pH	OC	Fe	Clay	r	R^2	U
Pore Water	pH	**0.40**	-0.05	-0.00	-0.35	-0.001	0.41	0.77
	OC	0.11	**-0.19**	0.10	-0.37	-0.35		
	Fe	-0.00	-0.10	**0.19**	-0.55**	-0.46*		
	Clay	0.18	-0.09	0.14	**-0.78****	-0.55**		
Bray-1	pH	**0.23**	-0.01	0.00	-0.24	-0.02	0.44*	0.75
	OC	0.06	**-0.05**	-0.10	-0.26	-0.35		
	Fe	-0.003	-0.03	**-0.18**	-0.38	-0.60**		
	Clay	0.10	-0.03	-0.13	**-0.54***	-0.59**		
Na-Phosphate	pH	**0.68****	-0.06	0.00	0.01	0.64**	0.70**	0.55
	OC	0.18	**-0.21**	-0.22	0.01	-0.24		
	Fe	-0.01	-0.11	**-0.42***	0.02	-0.52*		
	Clay	0.30	-0.10	-0.30	**0.02**	-0.07		
Hydroxylamine HCl	pH	**0.07**	0.10	0.00	-0.04	0.13	0.11	0.94
	OC	0.03	**0.36**	-0.03	-0.04	0.31		
	Fe	-0.001	0.19	**-0.05**	-0.06	0.08		
	Clay	0.03	0.18	-0.04	**-0.08**	0.09		
Ammon Oxalate	pH	**-0.24**	0.14	0.00	0.21	0.11	0.68**	0.57
	OC	-0.06	**0.54****	0.01	0.23	0.72**		
	Fe	0.00	0.28	**0.02**	0.34	0.64**		
	Clay	-0.11	0.26	0.01	**0.47***	0.64**		

Table 10. Summary of Linear Regression, Backwards multiple regression and Path Analysis illustrating the relationship between the extractability of arsenic and soil properties: pH, % organic carbon, iron oxide and % clay (*designates and r-table significance at $P<0.05$, ** designates and r-table significance at $P<0.01$).

	Simple Regression (*=$P<0.05$, **=$P<0.01$, ^{E}=Exponential, ^{L}=Linear)				**Backwards Multiple Regression (* = $P< 0.05$)**					**Path Analysis**			
Extraction	**pH**	**OC**	**Fe**	**Clay**	**pH**	**OC**	**Fe**	**Clay**	**Interaction**	**pH**	**OC**	**Fe**	**Clay**
Pore Water			$**^{E}$	$**^{E}$	*		*	*					**
Bray-1			$**^{E}$	$**^{E}$		*		*	OC × Clay OC × Fe				*
Na-Phosphate	$**^{LE}$		$*^{E}$		*	*		*	pH × OC Fe × OC OC × Clay	**		*	
Hydroxyl-amine HCl													
Ammonium Ox.			$**^{L}$	$**^{L}$	$**^{L}$			*		OC × Fe		**		*

Table 11. Lettuce yield and Bray-1 extracted arsenic from 8-week and 5-week bioassays.

Soil	8-Week Bioassay				5-Week Bioassay			
	Yield, mg	Lettuce As Conc. mg/kg	Bray-1 As mg/kg	Bray-1 P mg/kg	Yield, mg	Lettuce As Conc. mg/kg	Bray-1 As mg/kg	Bray-1 P mg/kg
Canisteo A	0		37.8	9.69	129		167	570
Dennis A	1396	5.14	10.6	36.20	6837	39.3	41.8	120
Dennis B	49.3	2.14	2.91	2.50	5725	2.42	19.3	36.5
Dougherty A	0		90.5	26.80	38.0	0.76	192	489
Efaw A	280	16.6	51.2	85.72	1771		91.4	347
Hanlon A	43.0	16.5	77.0	94.36	224	26.5	169	534
Haskell	2042	22.72	35.7	88.15	1381	28.5	112	437
Kirkland A	429	16.48	37.3	54.85	2178	29.6	104	326
Luton A	2196	9.30	40.6	69.88	2901	14.8	139	573
Mansic A	0		29.3	1.10	41.3	4.58	207	269
Mansic B	1.33		8.60	0.19	17.7		53.2	2.5
Osage A	5037	6.27	12.2	58.73	4503		51.4	265
Osage B	3783	3.80	10.1	34.43	4924	5.16	45.9	285
Perkins A	17.0		62.7	52.47	268	5.60	120	304
Pond Creek A	505	29.8	43.0	123.65	1827		138	635
Pond Creek B	47.3	15.1	40.3	36.22	2200	40.6	99.6	301
Pratt A	0		113	22.64	14.7	9.42	201	317
Pratt B	0		142	31.71	8.00		216	327
Richfield B	33.3		110	28.25	53.7		217	378
Summit A	1464	3.24	7.02	10.06	7179	1.6	51.6	170
Summit B	1135	5.87	12.3	4.96	4310	1.12	43.8	87.6
Overall Mean	842	11.5	45.5	52.3	2154	15.0	118	323

Table 12. Linear regression coefficients for earthworm mortality and accumulation and the soil properties pH , % organic carbon, iron (ammonium oxalate, mg/kg) and % clay.

Soil Properties		Earthworm Mortality	Earthworm As Accumulation
pH (1:1 water)	r^2	0.10	0.15
	P	*0.15*	*0.09*
		y = -7.09x + 63.8	y = 56.3x – 43.52
% Organic Carbon	r^2	0.07	0.03
	P	*0.23*	*0.50*
		y = -10.7x + 36.3	y = 41.2x + 240
Fe-oxide	r^2	**0.24**	0.14
	P	***0.03***	*0.12*
		y = -0.003x + 36.8	y = -0.015x + 383
% Clay	r^2	**0.34**	0.001
	P	***0.005***	*0.89*
		y = -0.88x + 49.2	y = -0.32x + 311

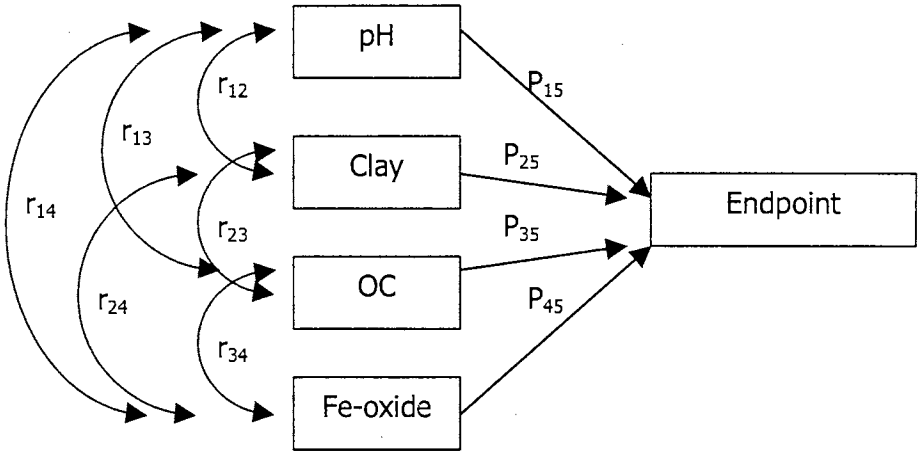

Fig. 1. Graphical representation of path analysis for the relationship between soil properties pH, clay content, organic carbon, Fe-oxide and endpoint.

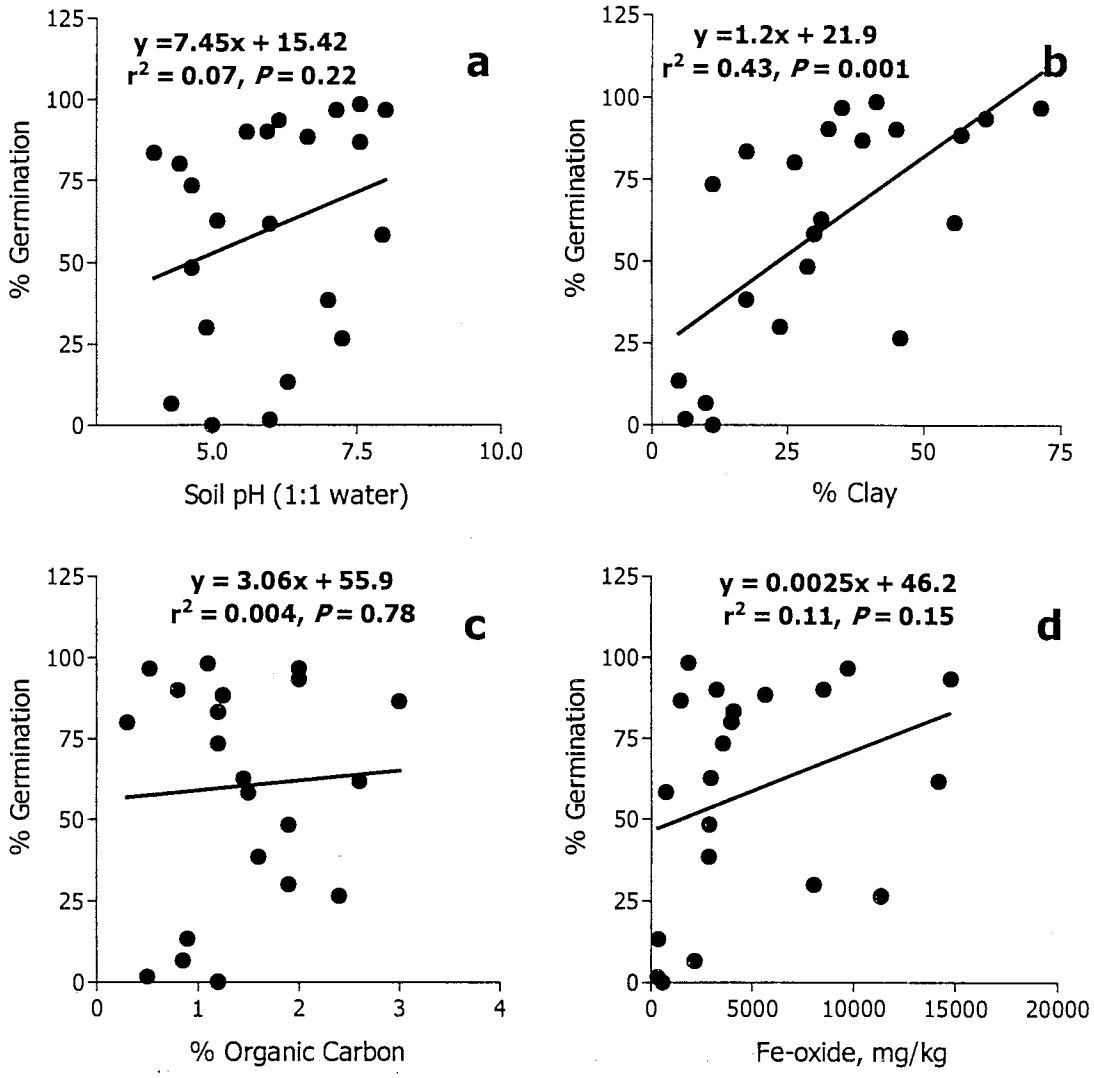

Fig. 2. Ten-day Germination rates on 22 arsenic spiked soils with soil properties soil a) soil pH, b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

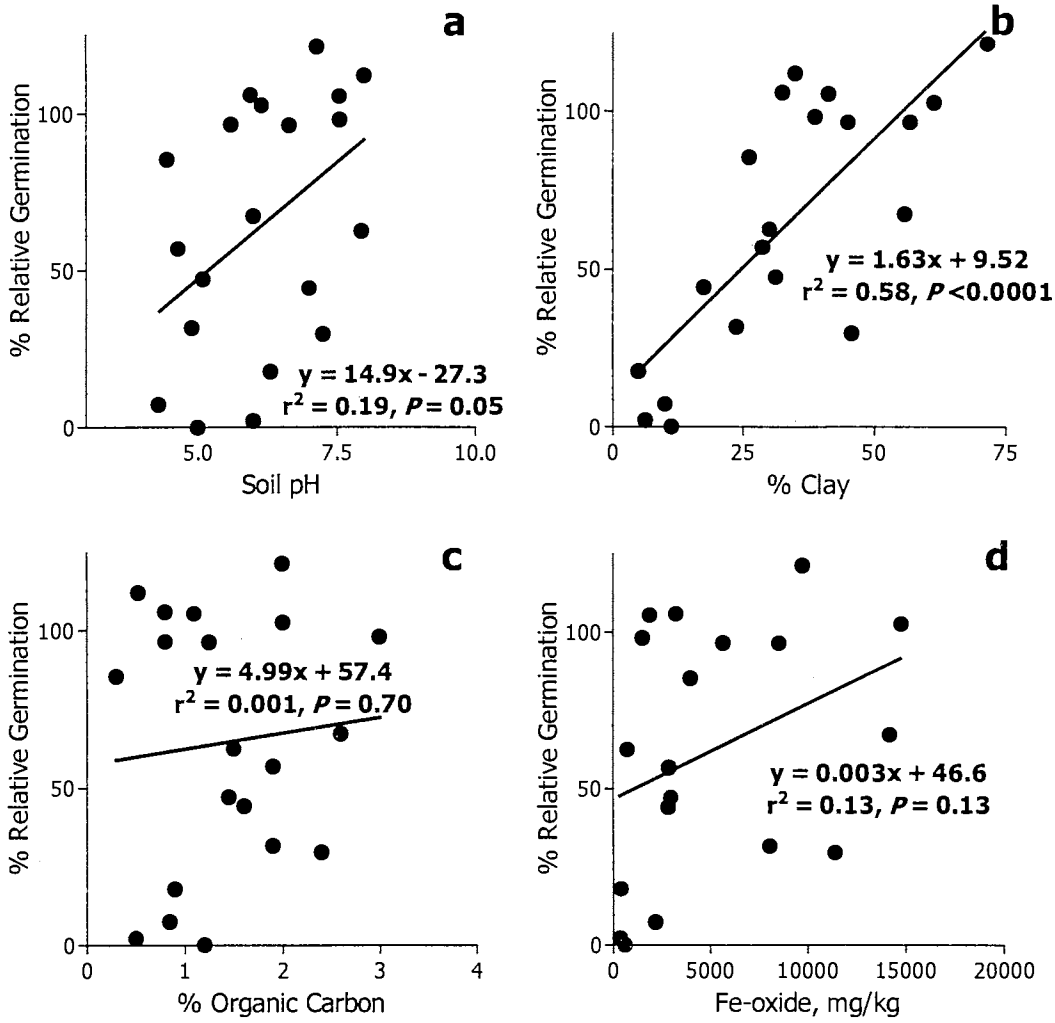

Fig. 3. % Relative Germination from ten-day germination tests on 22 arsenic spiked soils with soil properties a) soil pH, b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

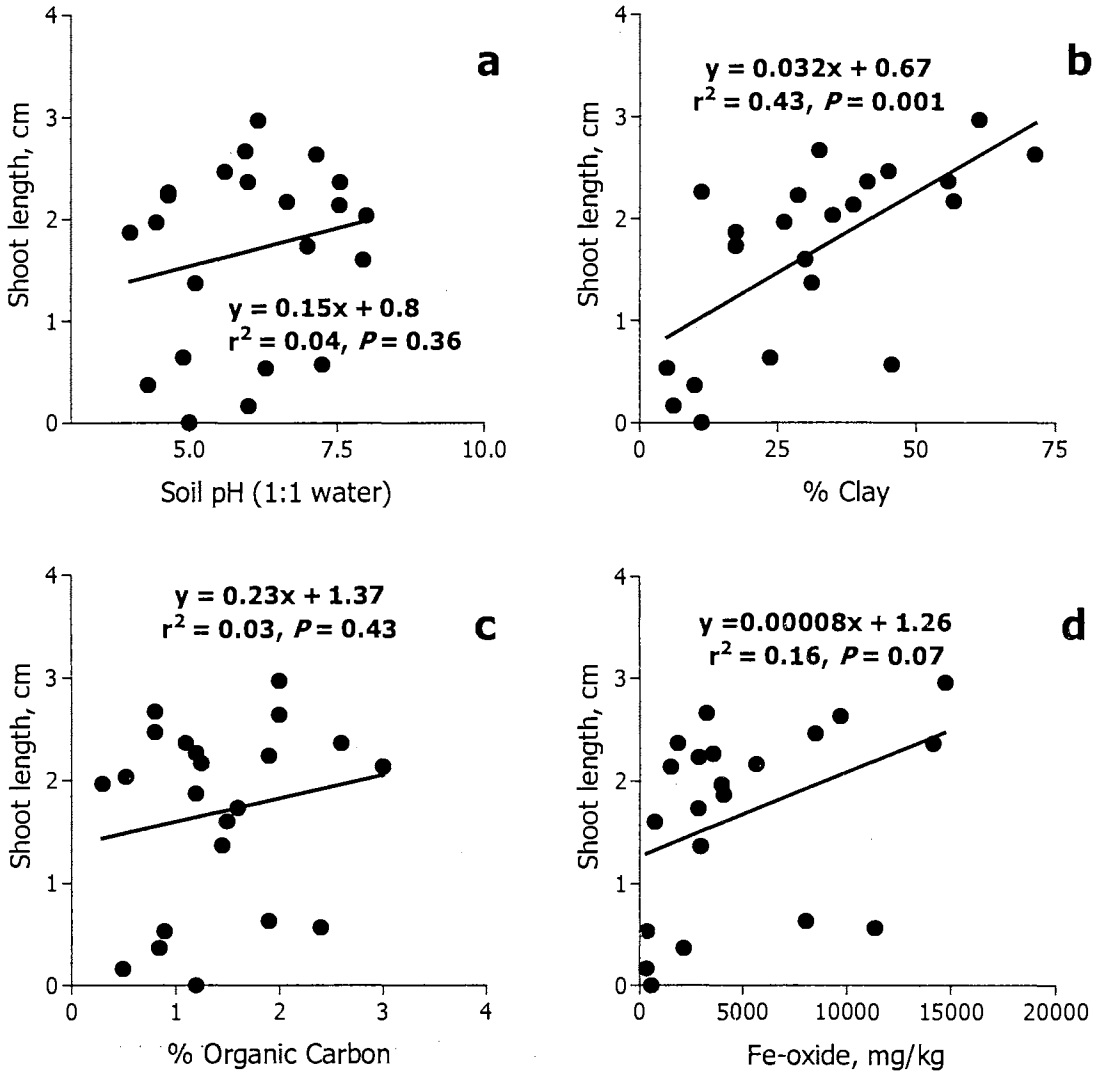

Fig. 4. Shoot length of lettuce grown on 22 arsenic spiked soils with soil properties a) soil pH , b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

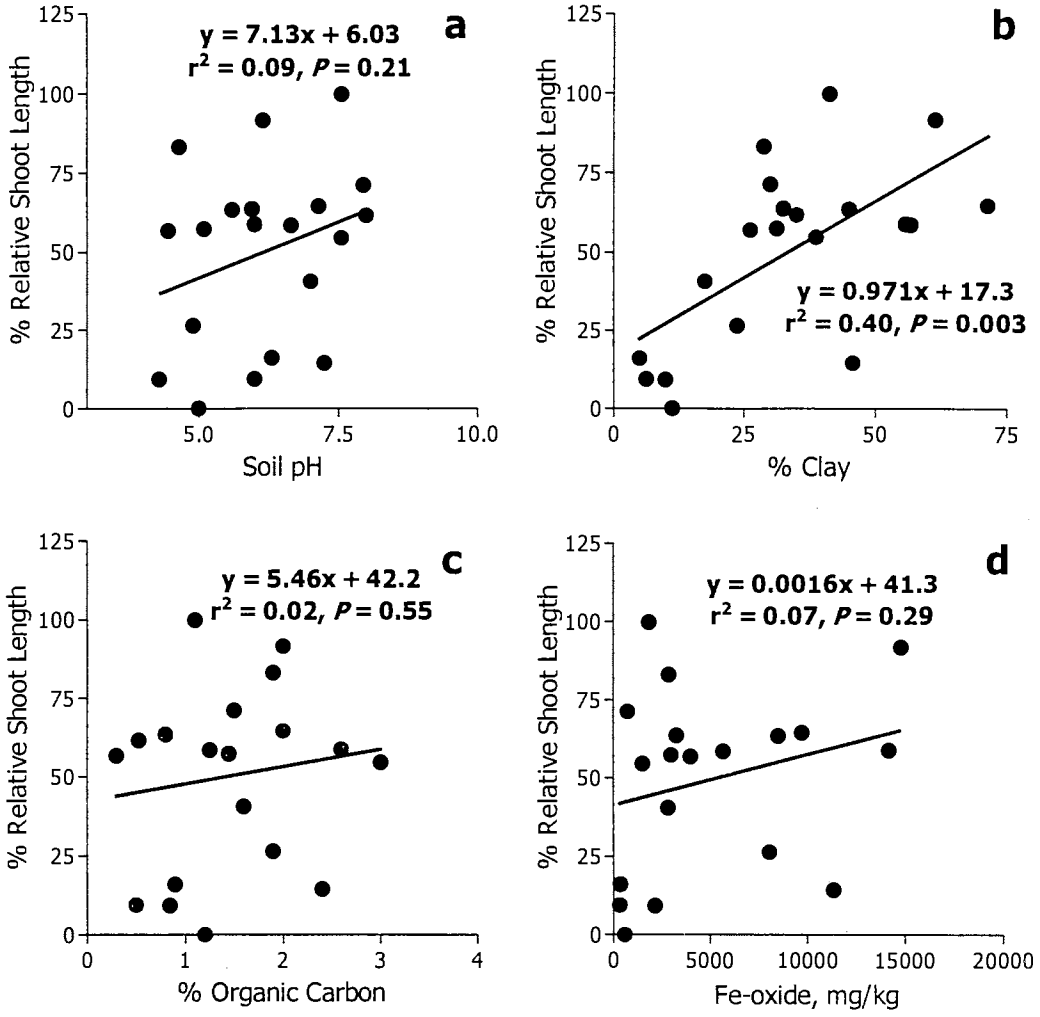

Fig. 5. % Relative Shoot length from lettuce grown on 22 arsenic spiked soils with soil properties a) soil pH, b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

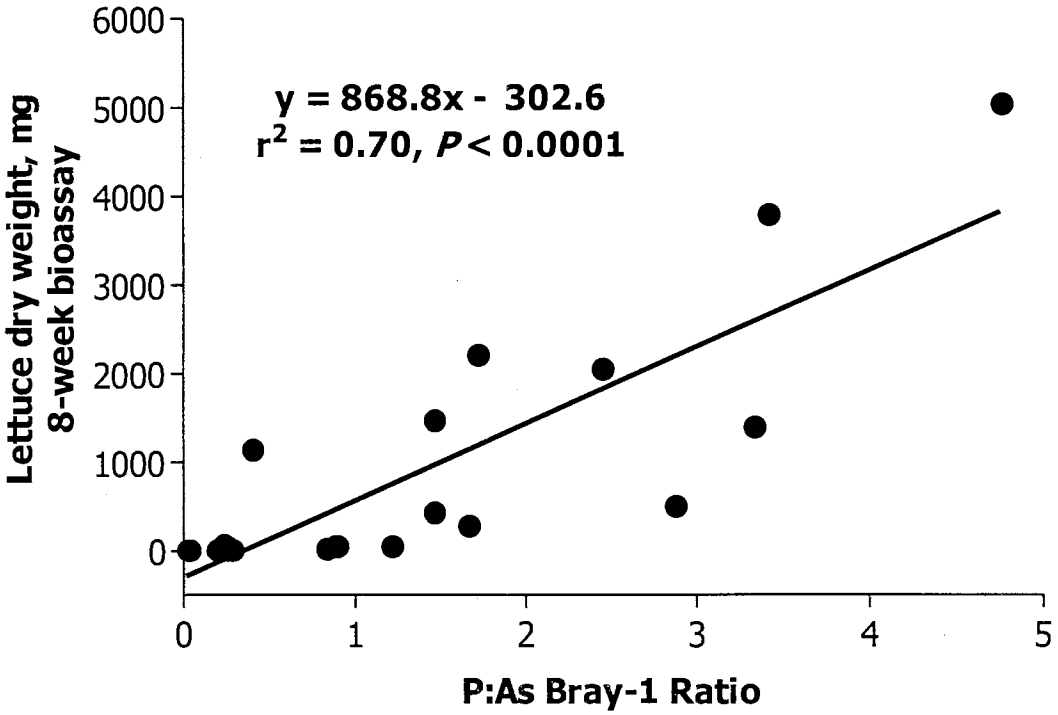

Fig. 6. Phosphorus to Arsenic ratio determined by Bray-1 extraction as it relates to lettuce yield from 8-week bioassay.

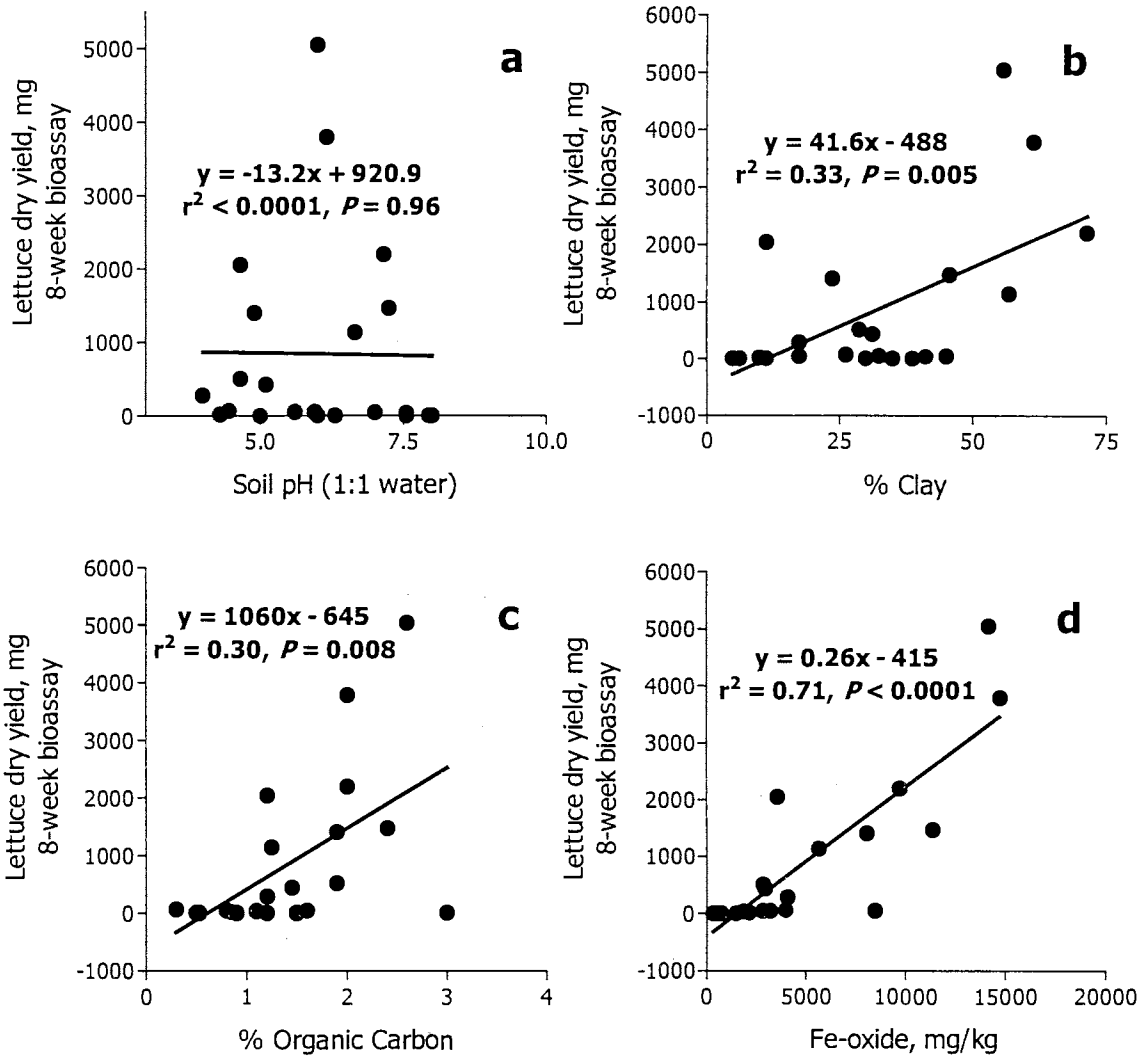

Fig. 7. Lettuce yield dry weight (8-week bioassay) results on 22 arsenic spiked soils with soil properties a) soil pH, b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

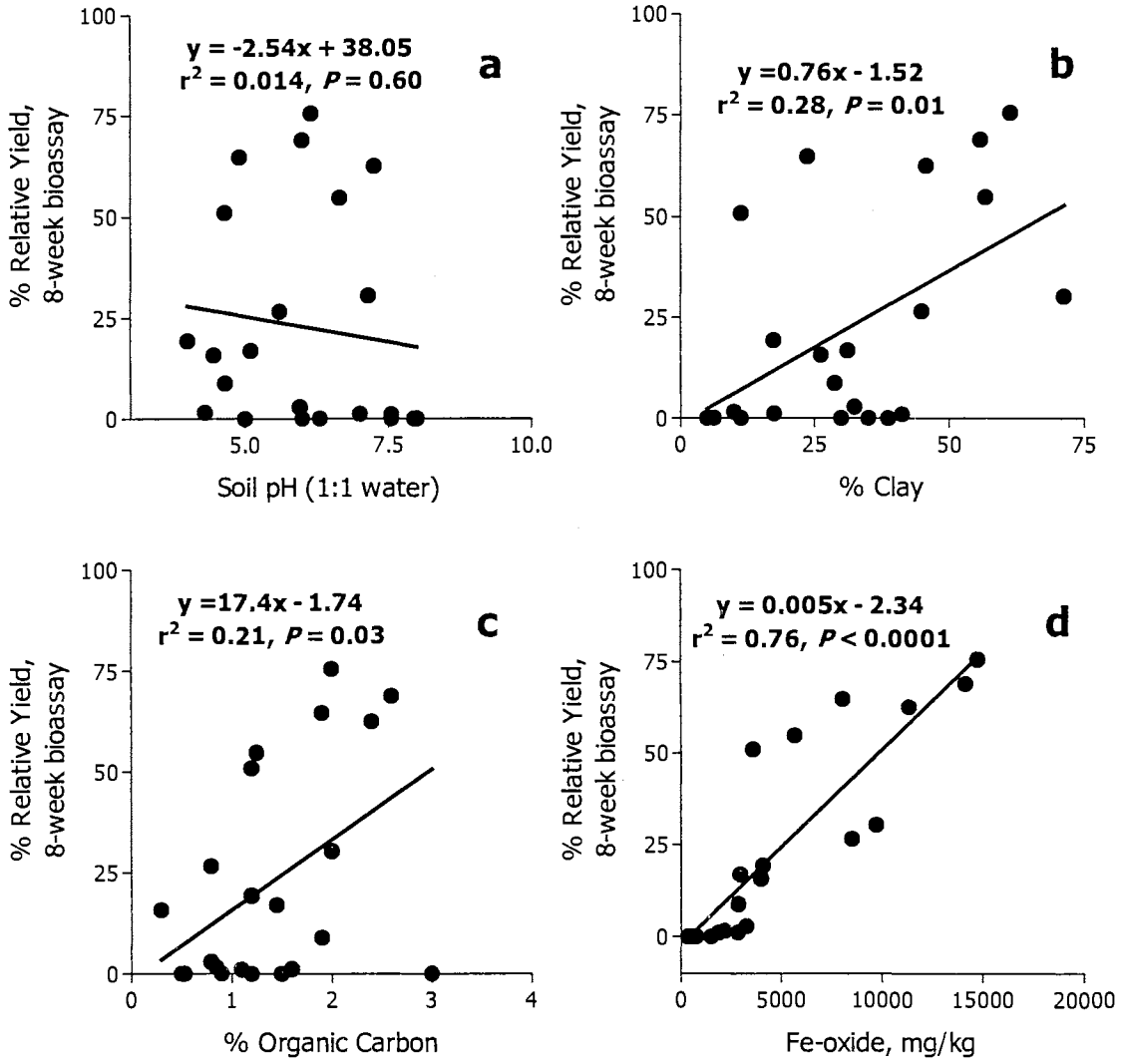

Fig. 8. % Relative yield (arsenic spiked soil yield/control soil yield *100) from lettuce grown on 22 arsenic spiked soils with a) soil pH, b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

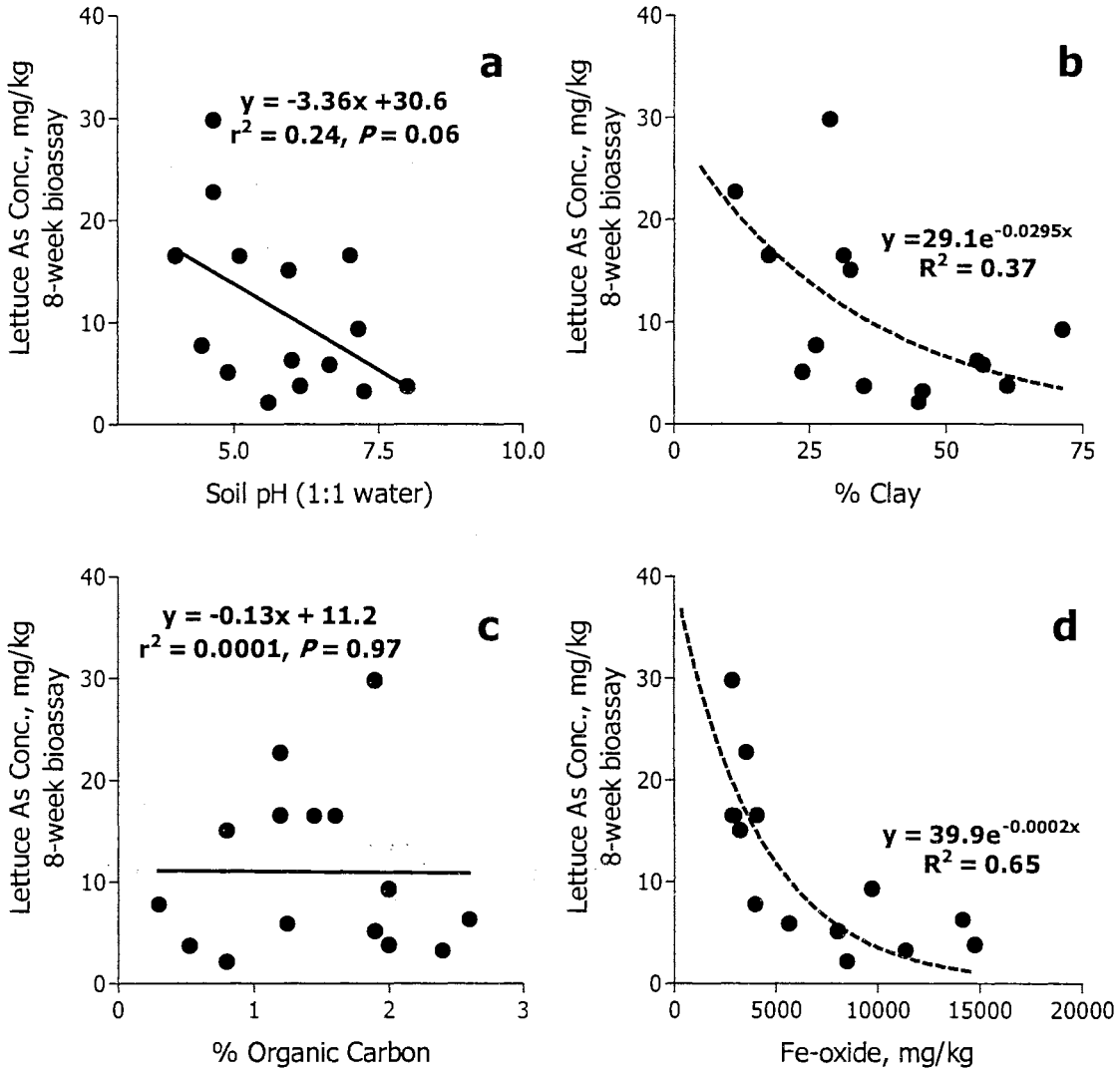

Fig. 9. Lettuce arsenic accumulation (8-week bioassay) from 22 arsenic spiked soils with soil properties a) soil pH, b) % clay, c) % organic carbon, and d) Fe-oxides (ammonium oxalate).

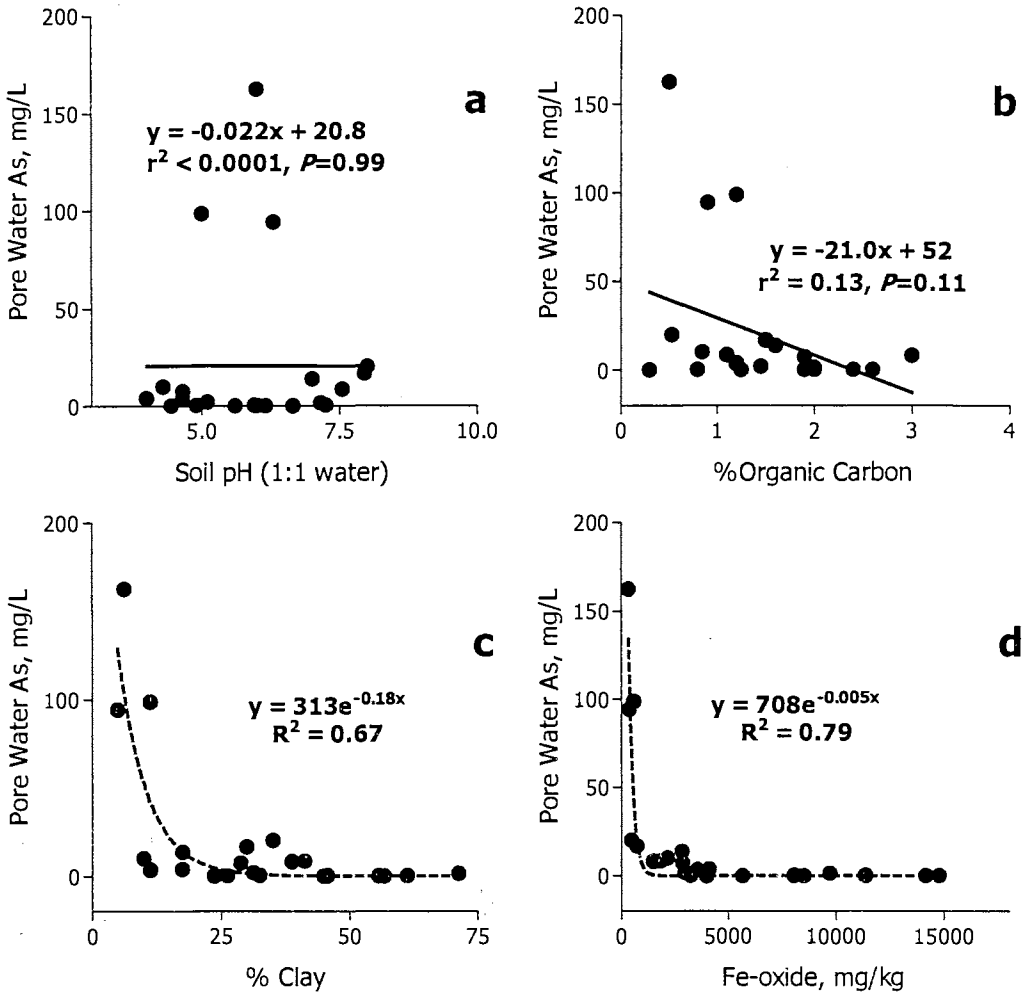

Fig. 10. Pore water extracted arsenic from 22 arsenic spiked soils with soil properties a) soil pH, b) % organic carbon, c) % clay, and d) Fe-oxides (ammonium oxalate).

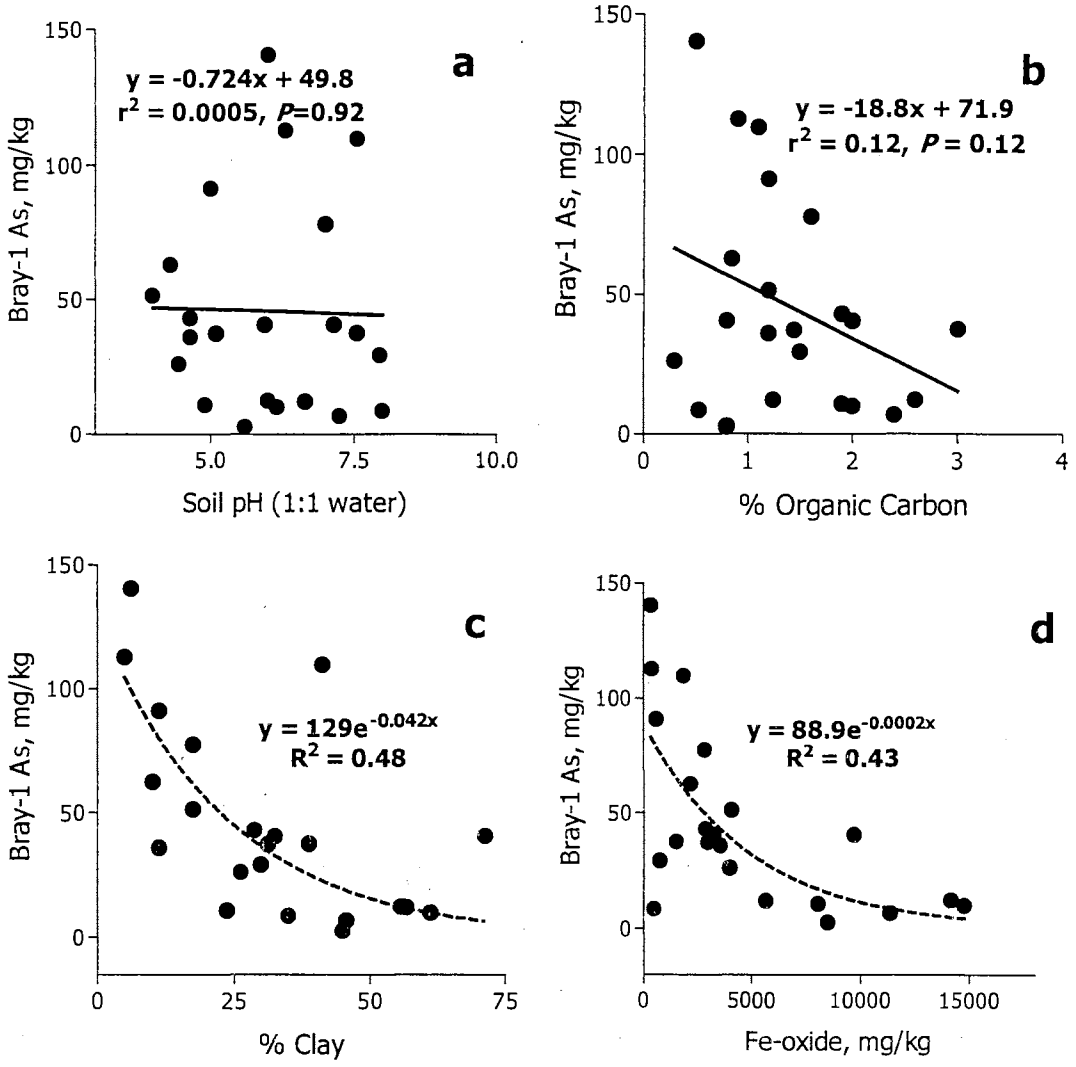

Fig. 11. Bray-1 extracted arsenic from 22 arsenic spiked soils with soil properties a) soil pH, b) % organic carbon, c) % clay, and d) Fe-oxides (ammonium oxalate).

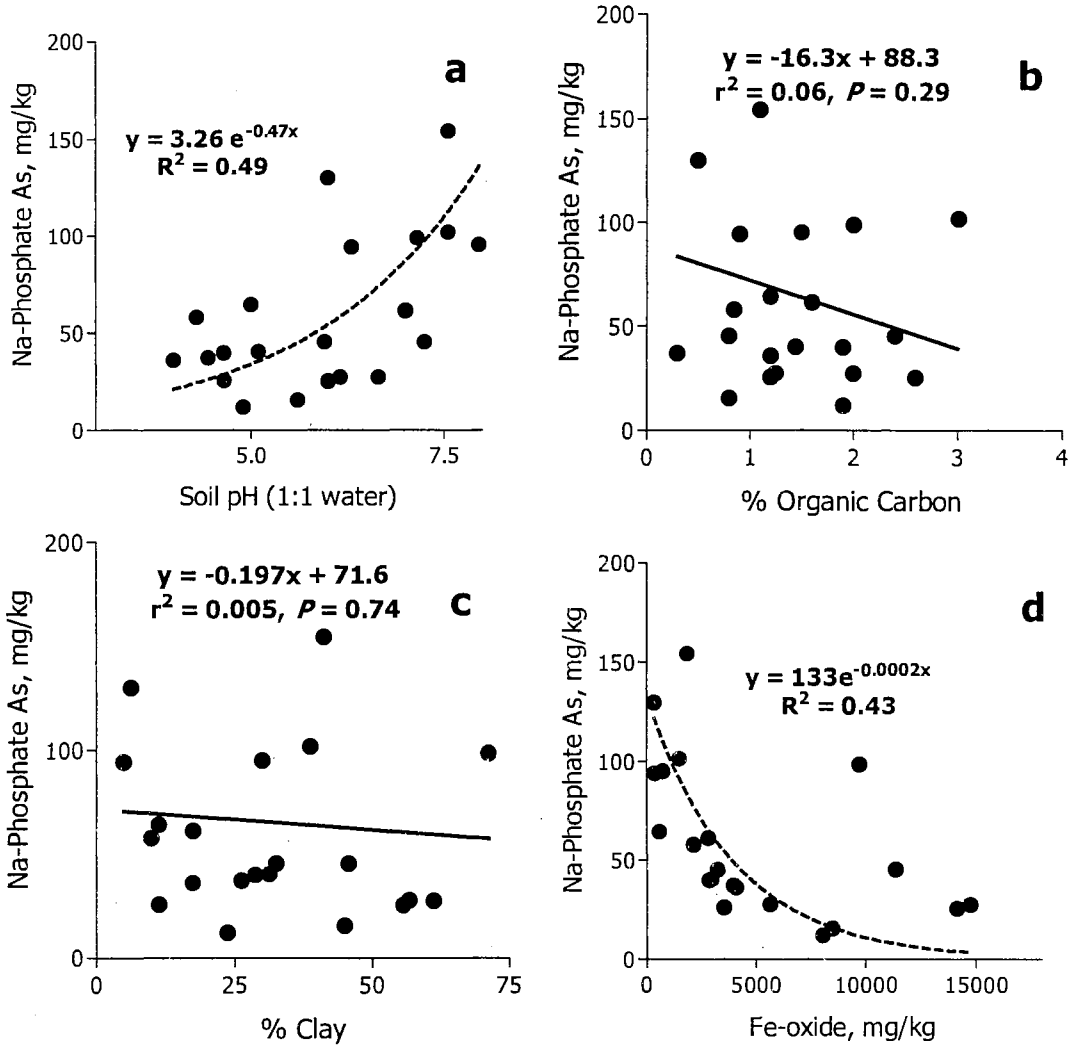

Fig. 12. Na-phosphate extracted arsenic from 22 arsenic spiked soils with soil properties a) soil pH, b) % organic carbon, c) % clay, and d) Fe-oxides (ammonium oxalate).

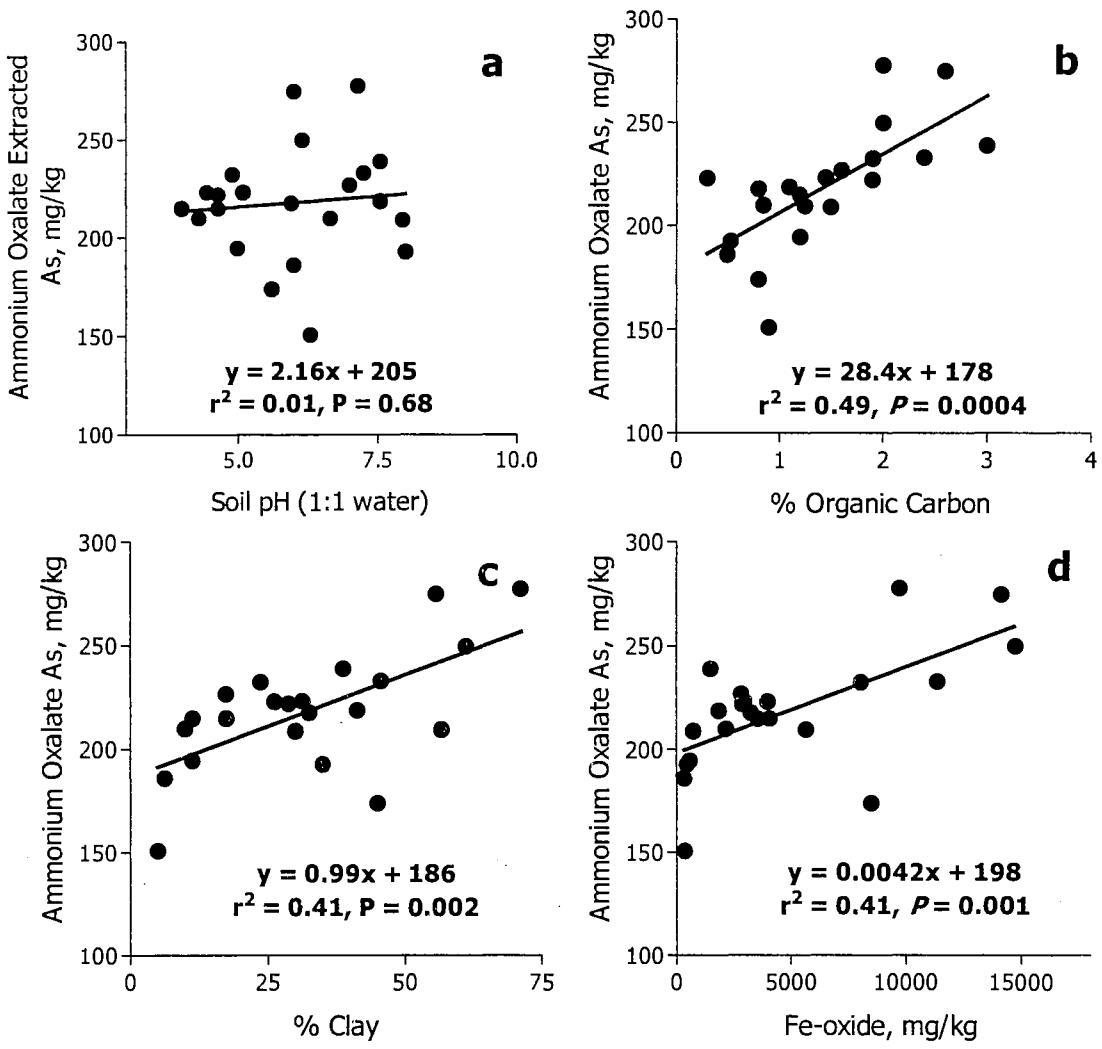

Fig. 13. Ammonium oxalate extracted arsenic from 22 arsenic spiked soils with soil properties a) soil pH, b) % organic carbon, c) % clay, and d) Fe-oxides (ammonium oxalate).

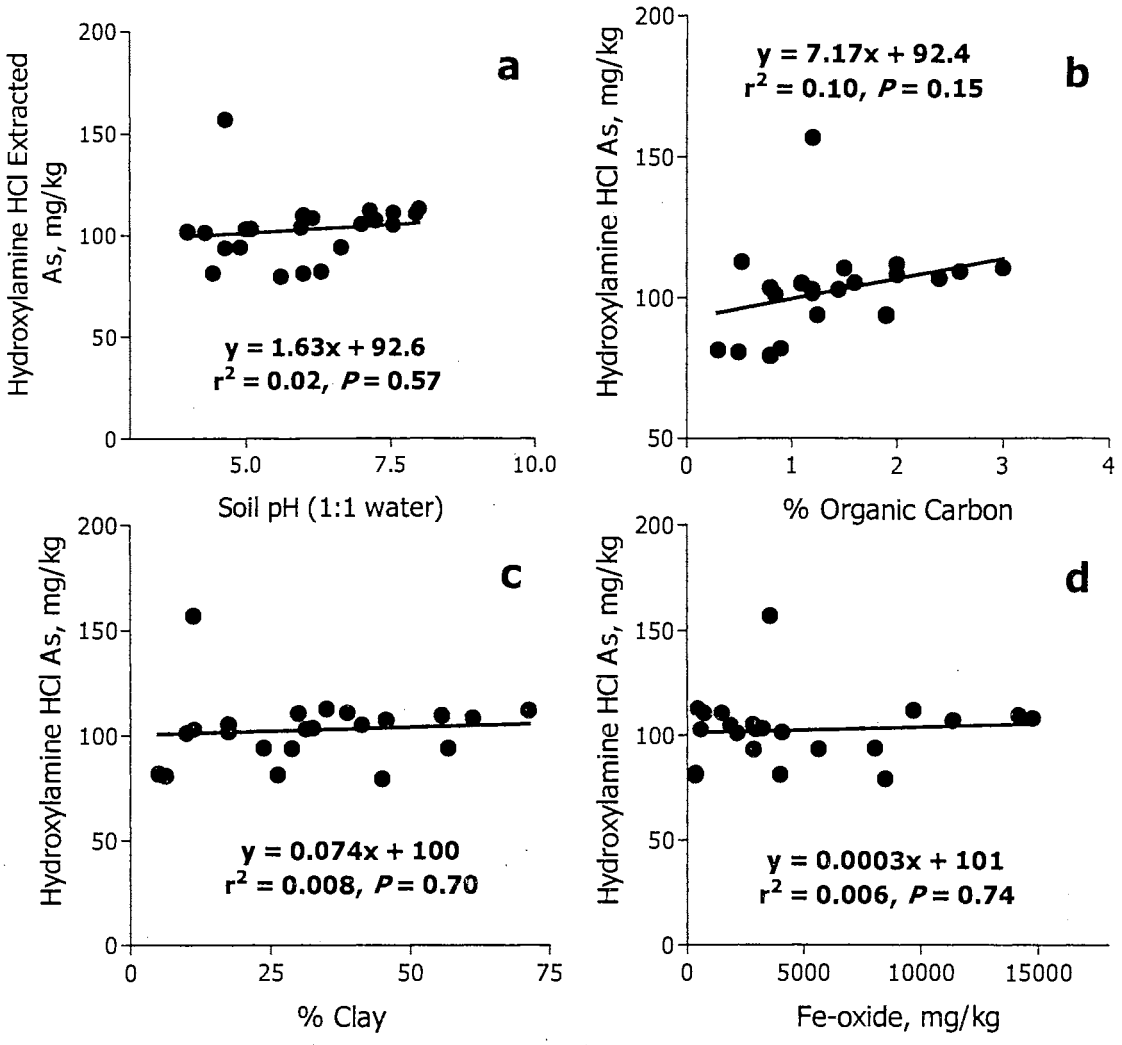

Fig. 14. Hydroxylamine HCl extracted arsenic from 22 arsenic spiked soils with soil properties a) soil pH, b) % organic carbon, c) % clay, and d) Fe-oxides (ammonium oxalate).

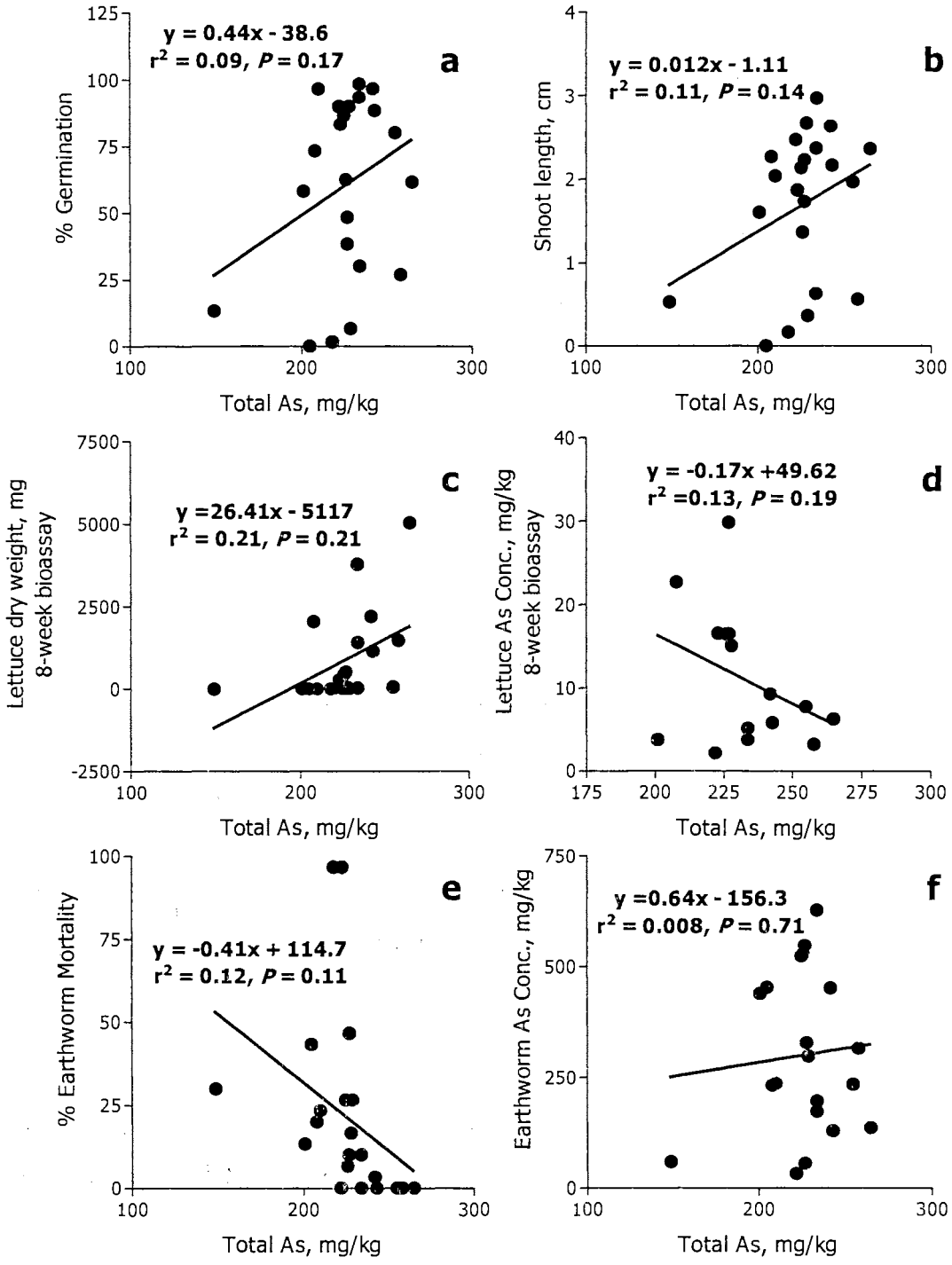

Fig. 15. Total arsenic in soil with a)% seed germination, b) shoot length, c) lettuce yield, d) arsenic in lettuce, e) earthworm mortality, f) earthworm arsenic uptake.

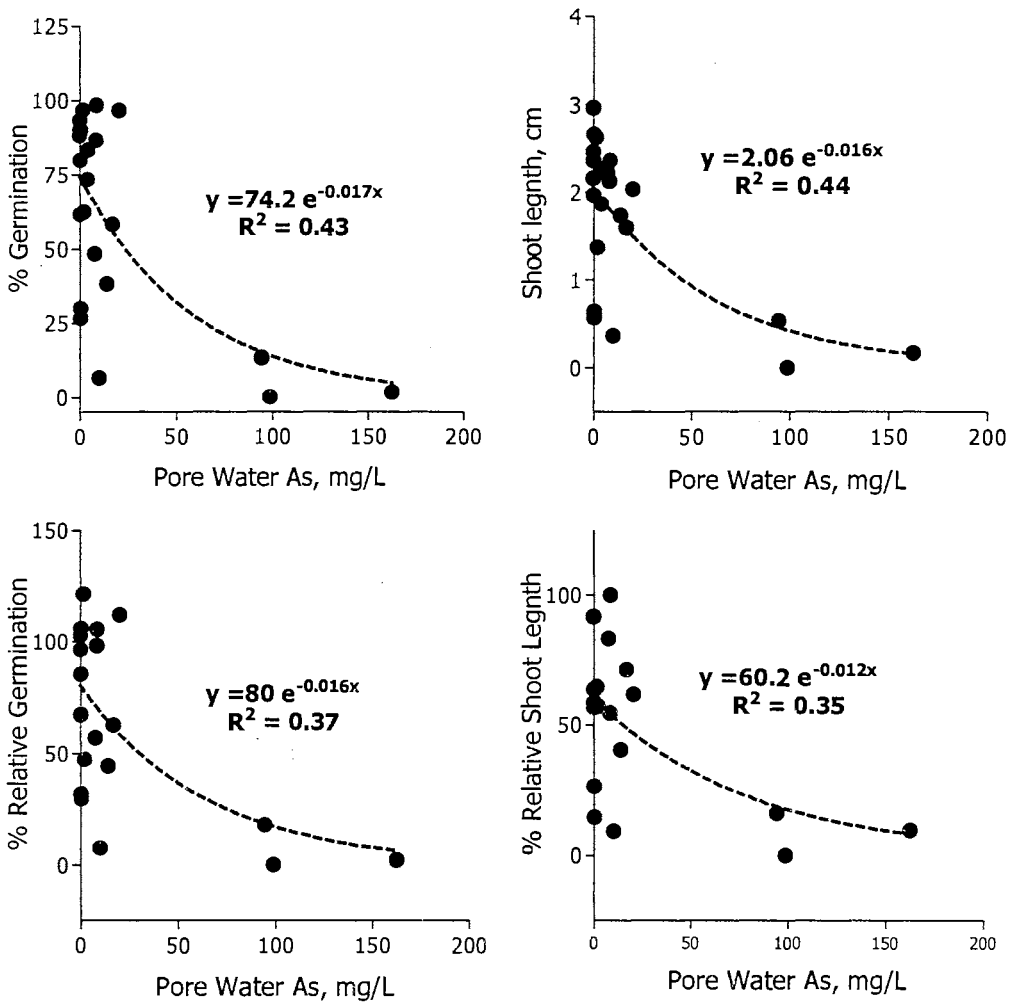

Fig. 16. Pore water arsenic with lettuce endpoints % germination, % relative germination, shoot length, and % relative shoot length.

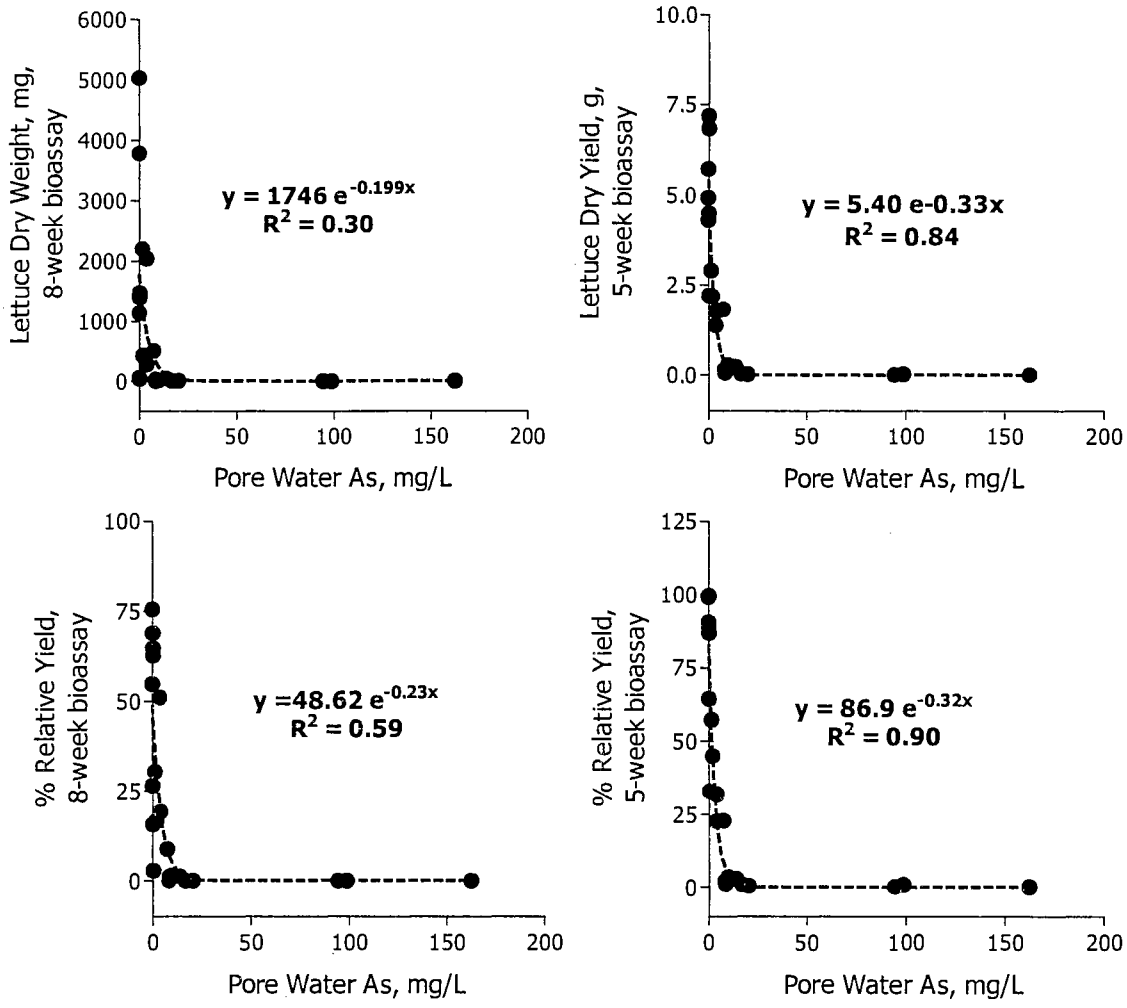

Fig. 17. Pore water arsenic with lettuce yield and % relative yield from 8-week and 5-week bioassay.

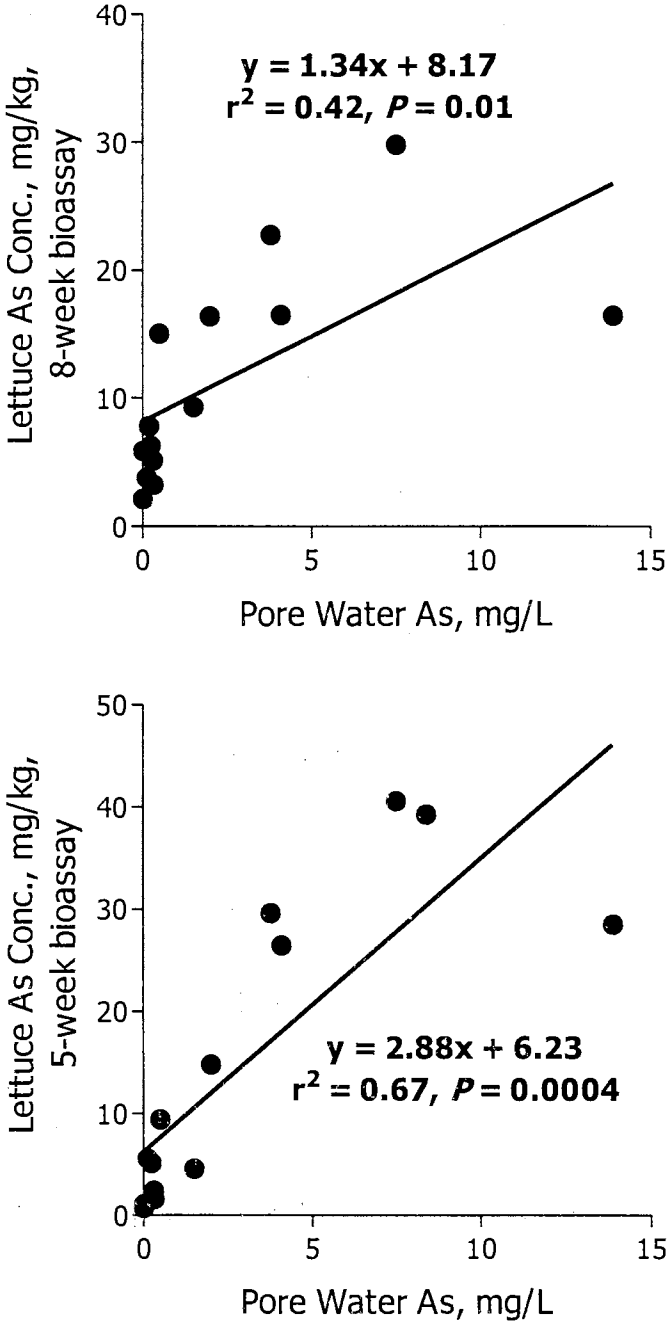

Fig. 18. Pore water arsenic with As accumulated by lettuce from 8-week and 5-week bioassay.

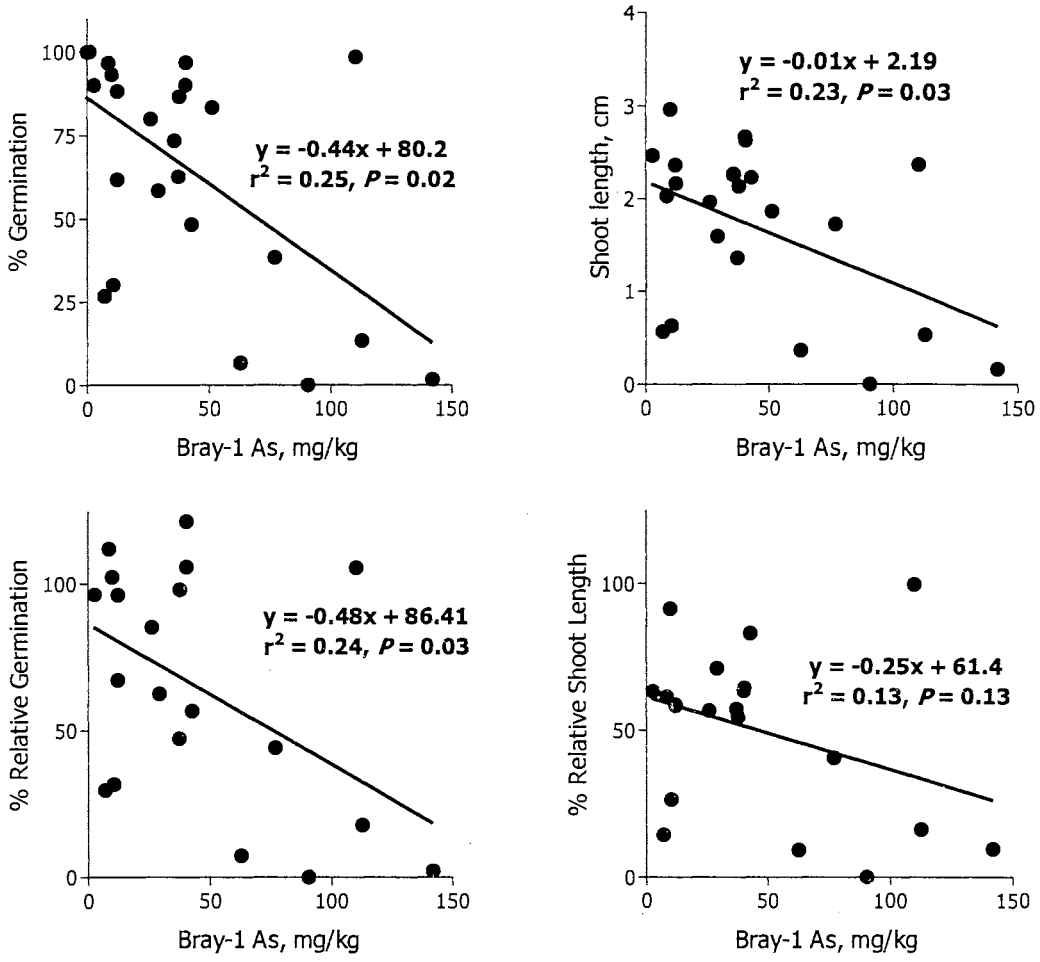

Fig. 19. Bray-1 arsenic with lettuce endpoints % germination, % relative germination, shoot length, and % relative shoot length.

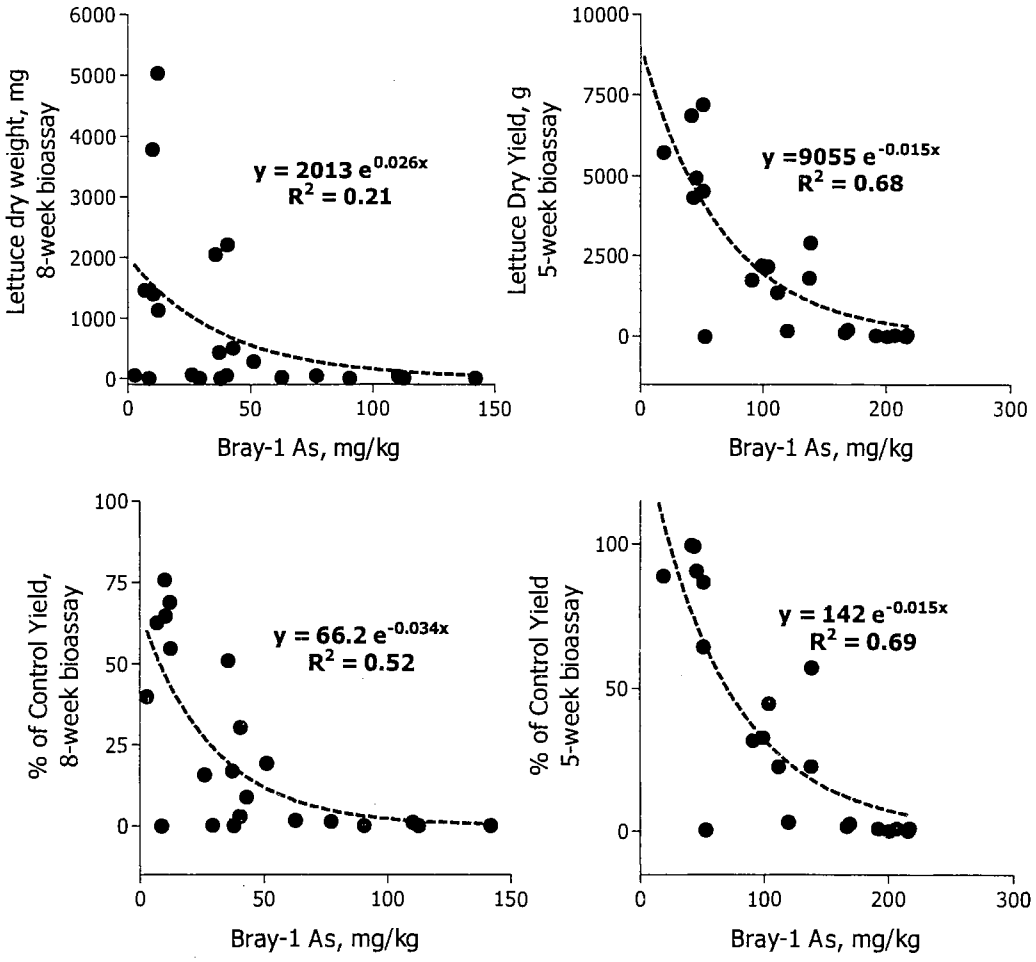

Fig. 20. Bray-1 arsenic with lettuce endpoints lettuce yield and % relative yield from 8-week and 5-week bioassay.

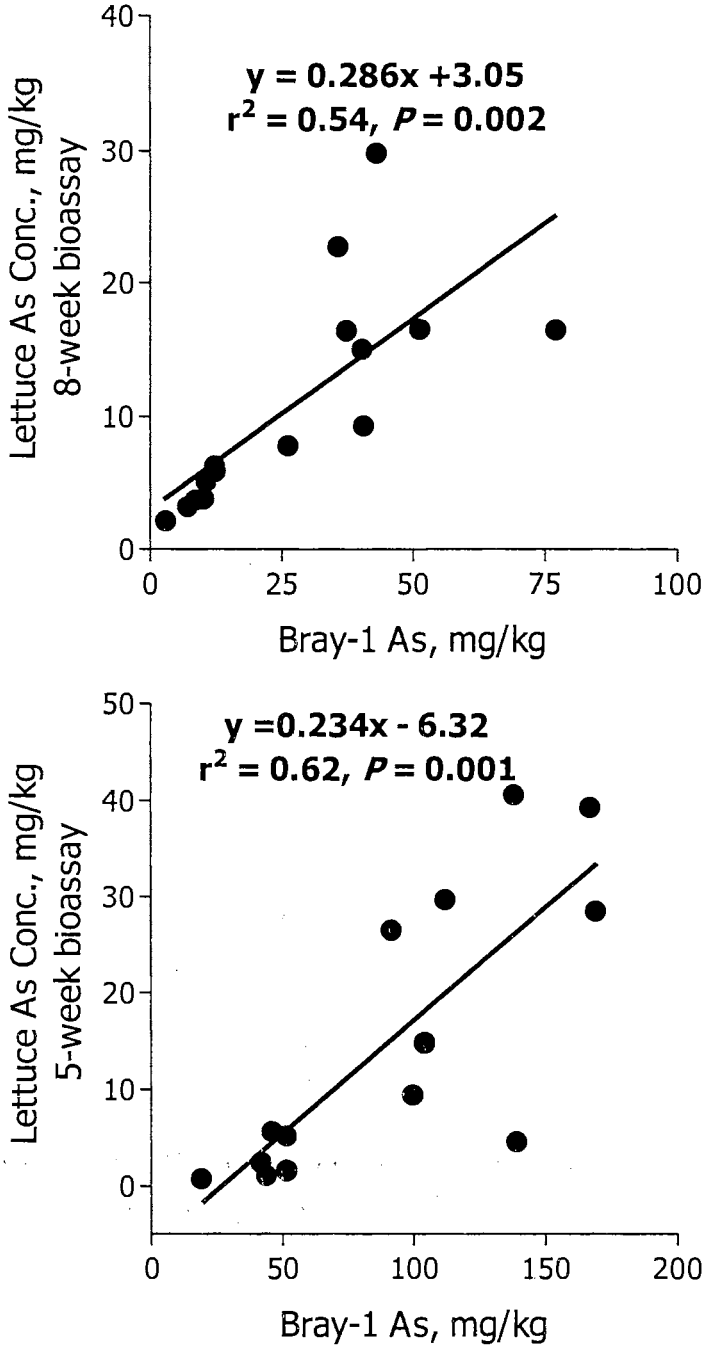

Fig. 21. Bray-1 extracted arsenic and arsenic accumulation by lettuce from 8-week and 5-week bioassay.

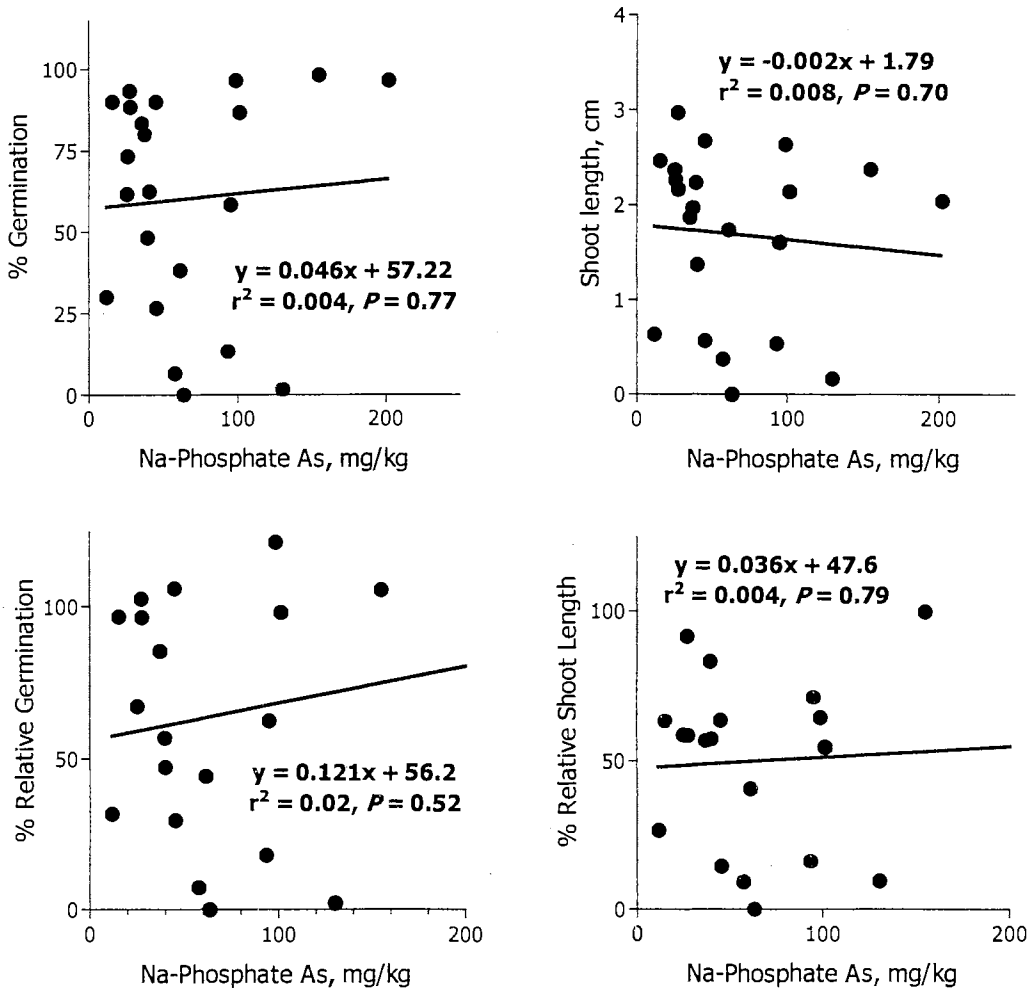

Fig. 22. Na-Phosphate extractable arsenic with lettuce endpoints % germination, % relative germination, shoot length, and % relative shoot length.

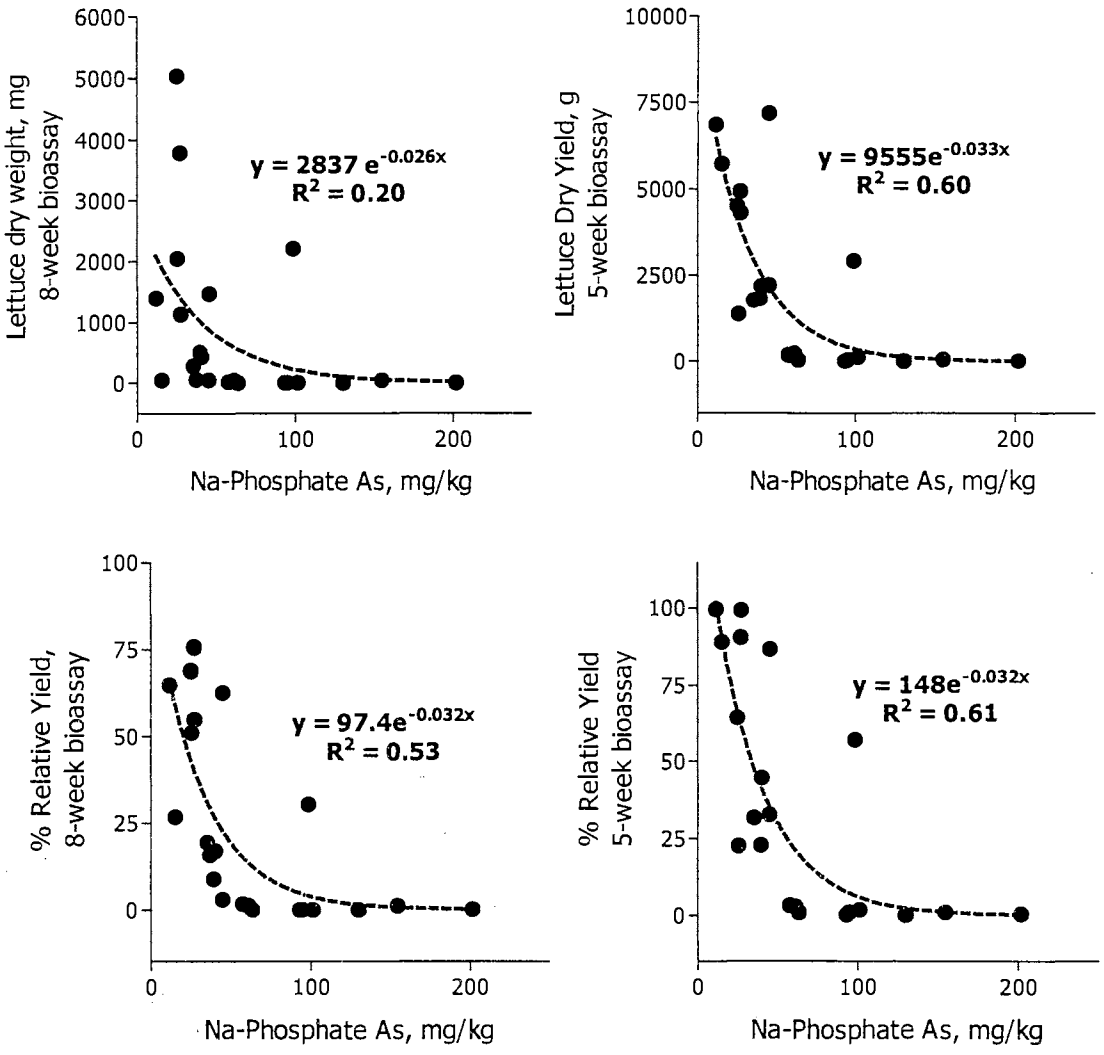

Fig. 23. Na-Phosphate extractable arsenic with lettuce yield and % relative yield for 8-week and 5-week bioassay.

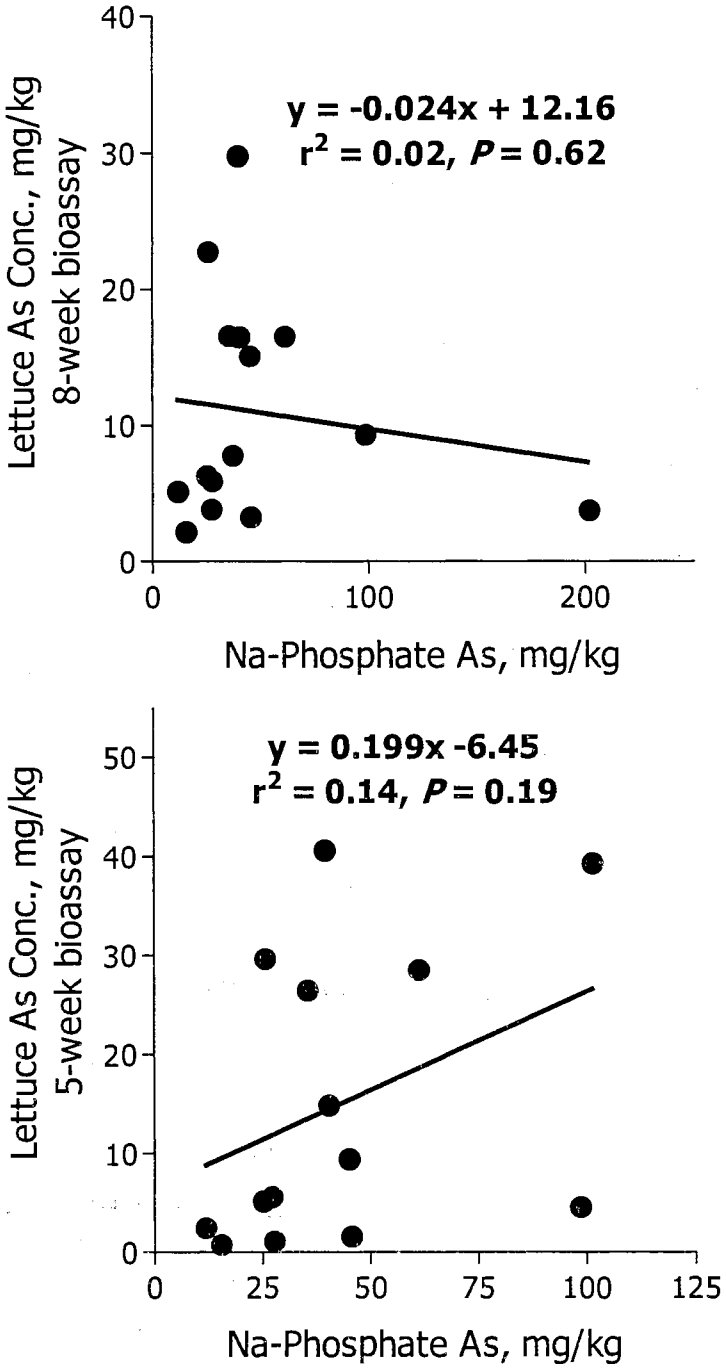

Fig. 24. Na-Phosphate extractable arsenic with arsenic accumulation by lettuce from 8-week and 5-week bioassay.

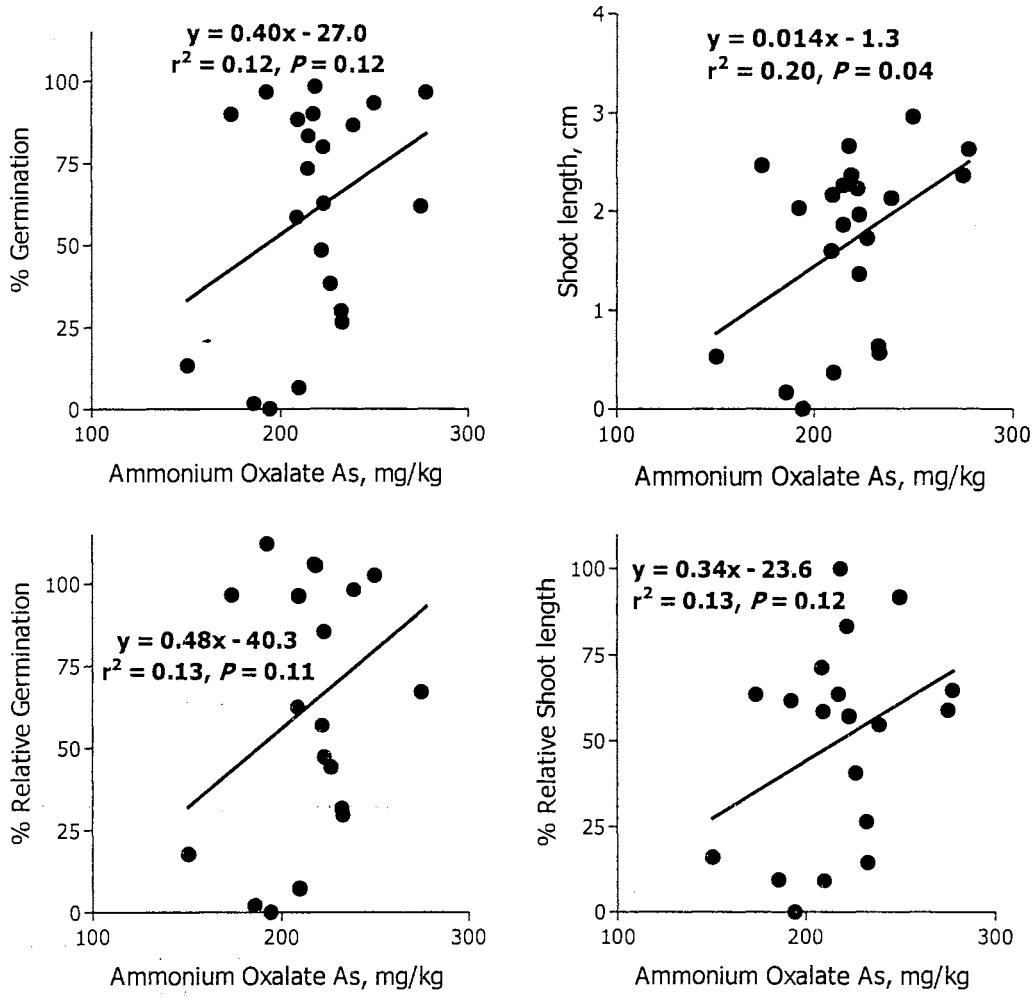

Fig. 25. Ammonium Oxalate extractable arsenic with lettuce endpoints % germination, % relative germination, shoot length, and % relative shoot length.

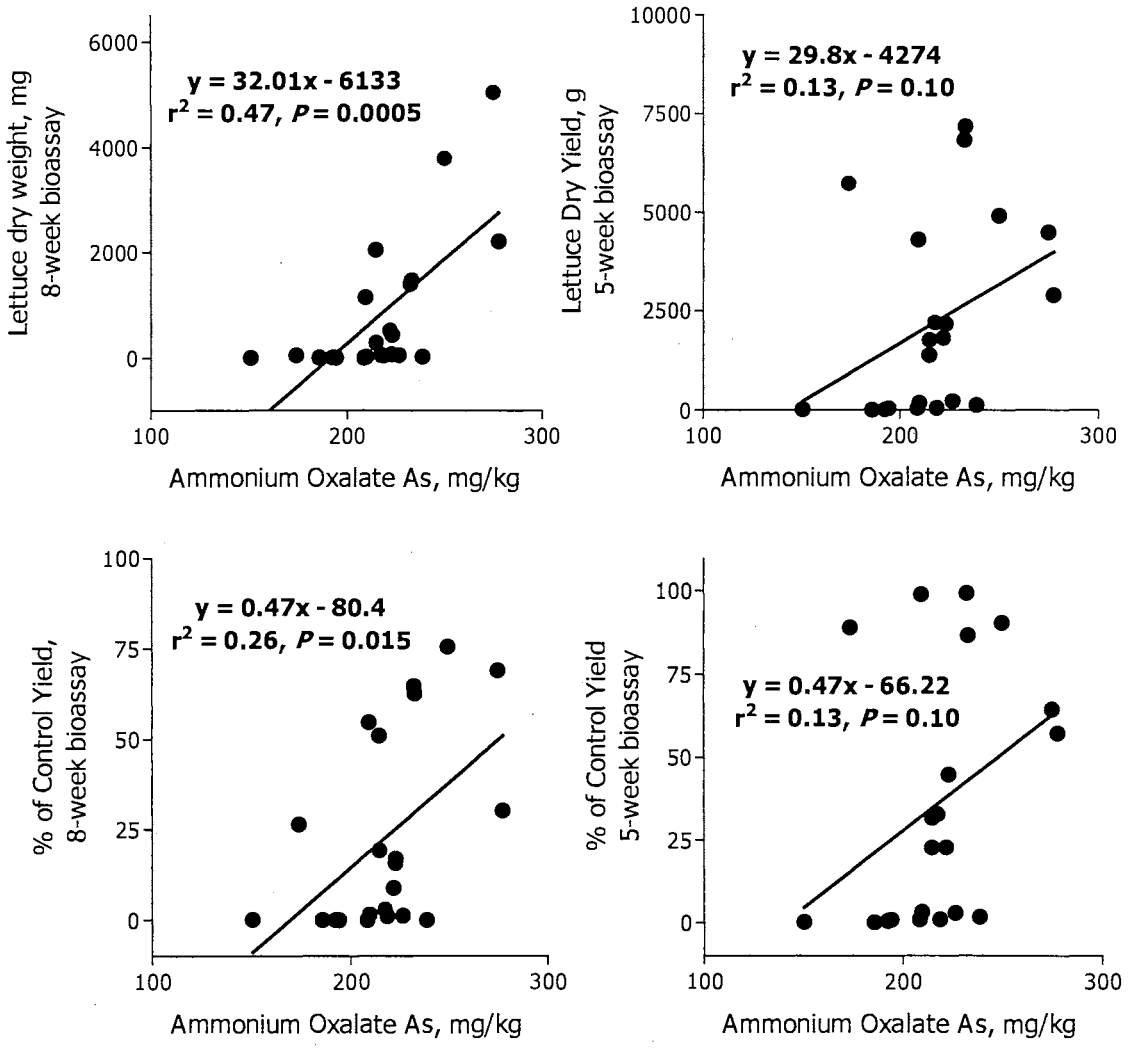

Fig. 26. Ammonium Oxalate extractable arsenic with lettuce yield and % relative yield from 8-week 5-week bioassay.

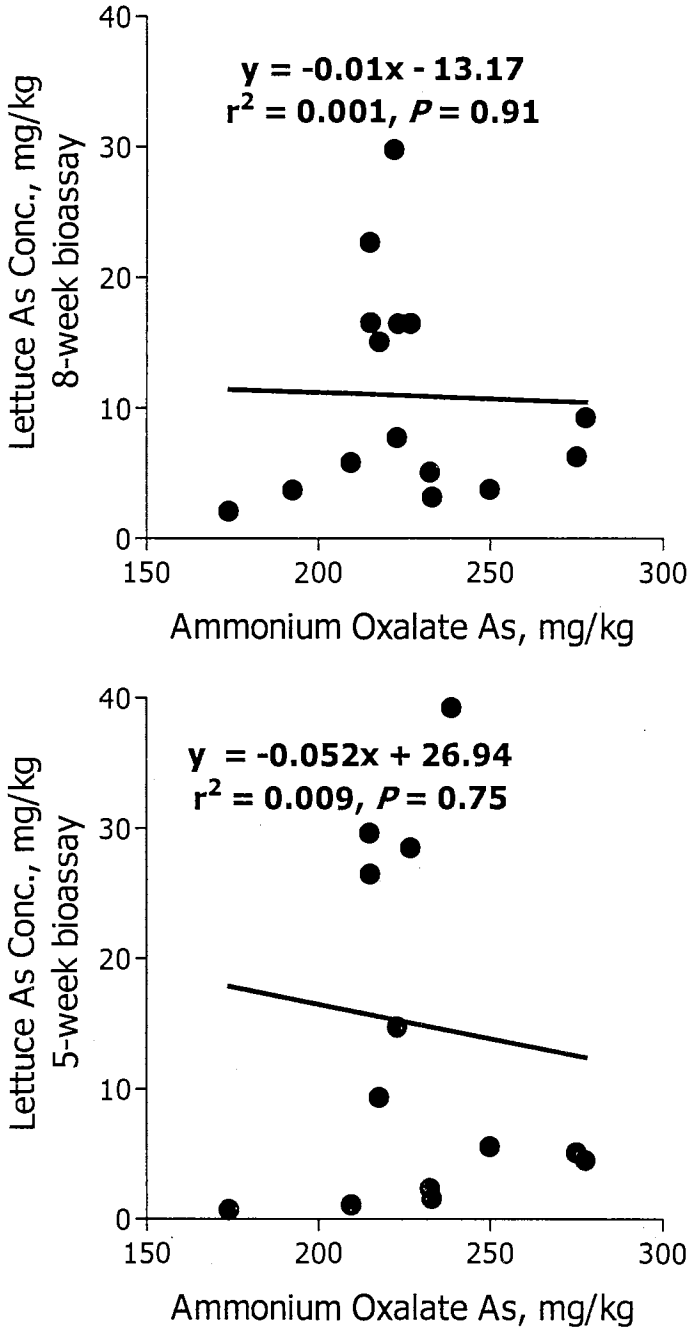

Fig. 27. Ammonium Oxalate extractable arsenic with arsenic accumulated by lettuce from 8-week and 5-week bioassay.

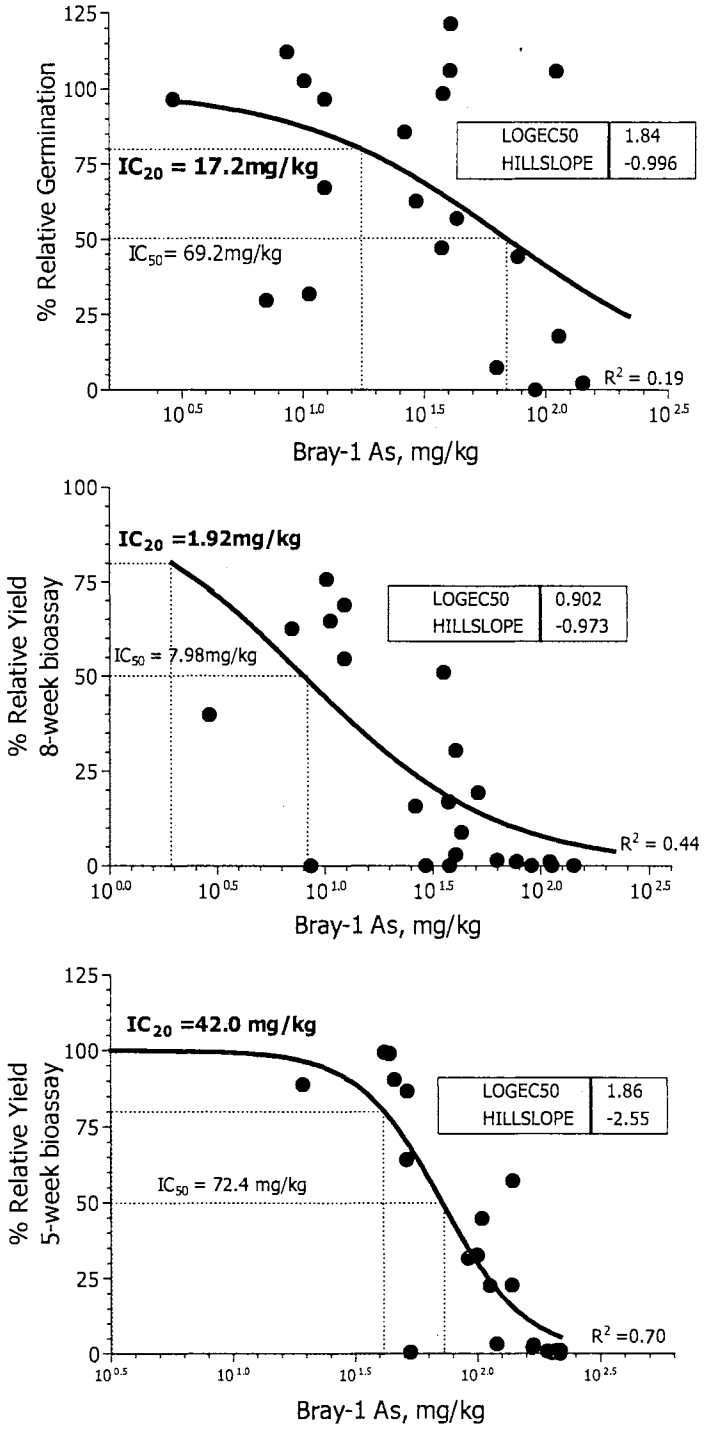

Fig. 28. Concentration-response curve from Bray-1 arsenic and % relative germination and % relative yield for 8-week and 5-week bioassay.

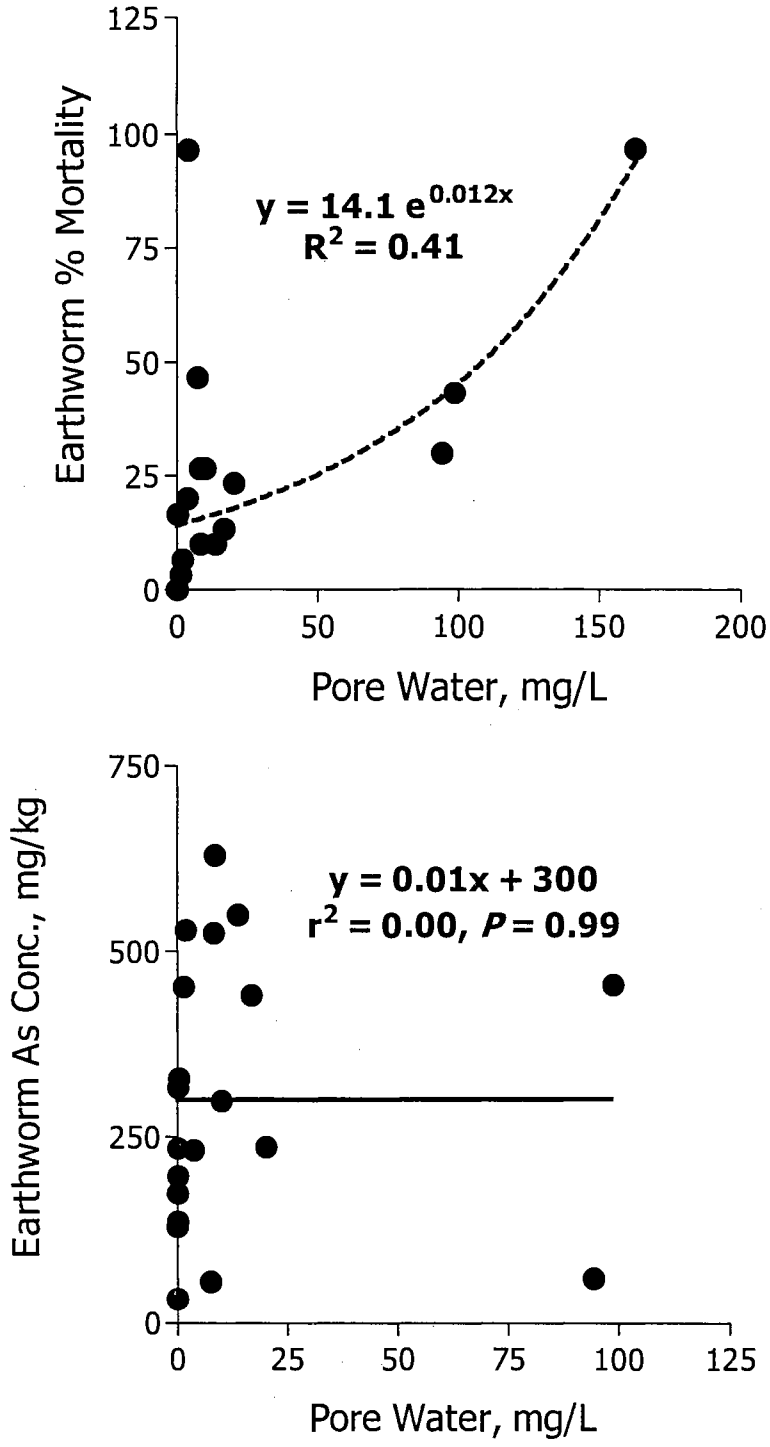

Fig. 29. Earthworm Mortality and arsenic accumulation in a 28-day toxicity test with pore water arsenic.

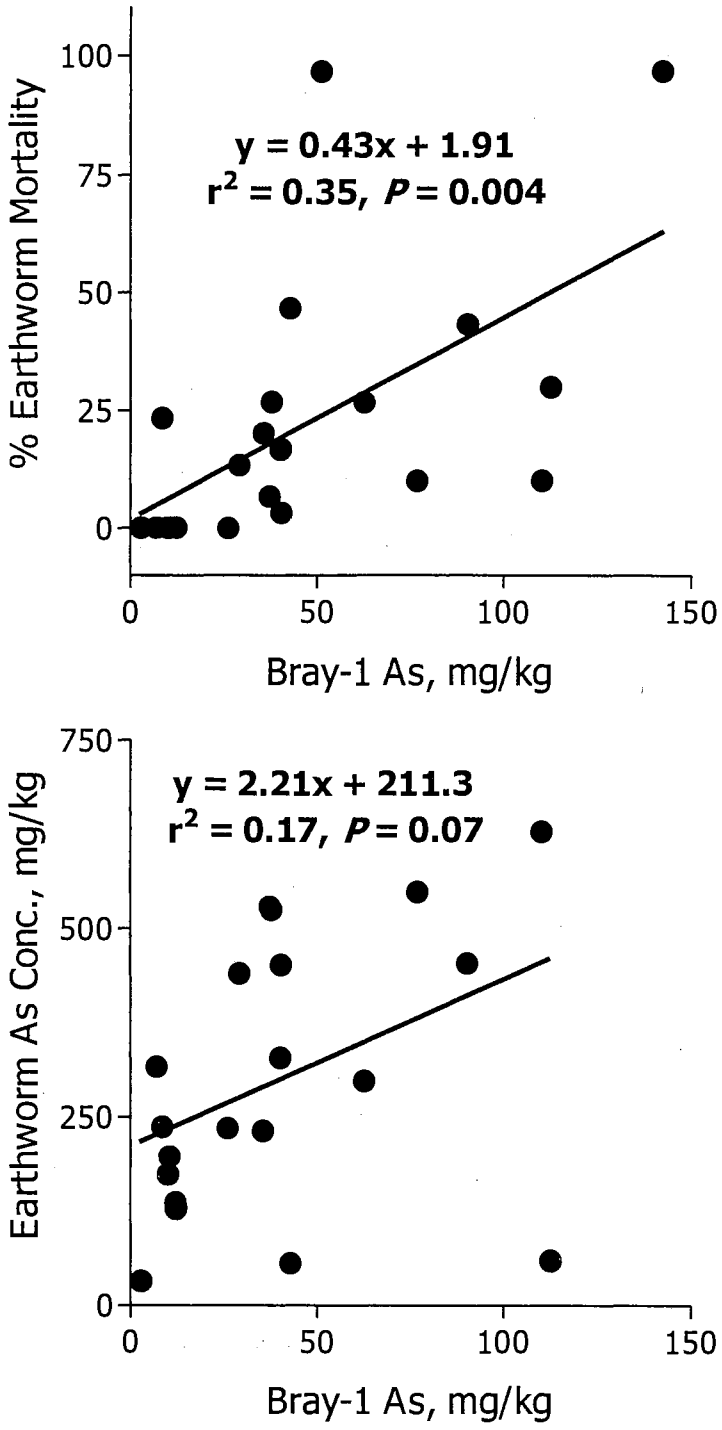

Fig. 30. Earthworm Mortality and arsenic accumulation in a 28-day toxicity test with Bray-1 arsenic.

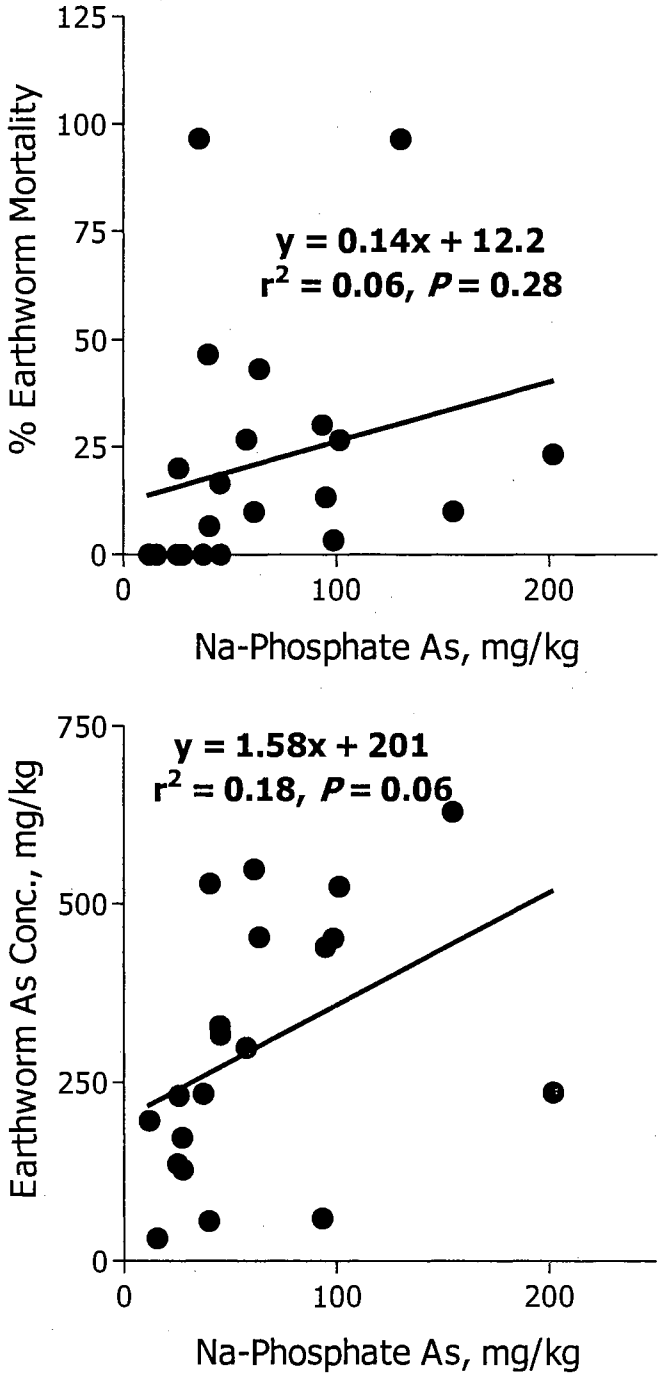

Fig. 31. Earthworm Mortality and arsenic accumulation in a 28-day toxicity test with Na-Phosphate arsenic.

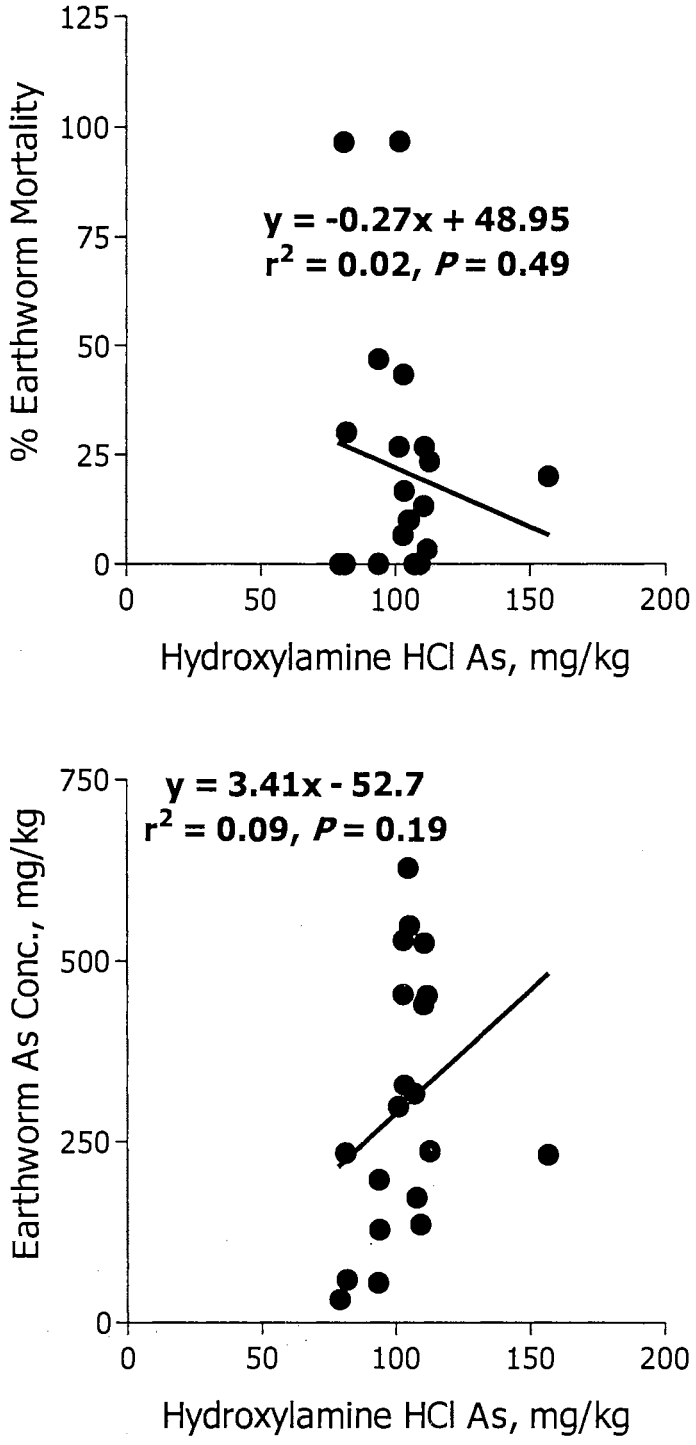

Fig. 32. Earthworm Mortality and arsenic accumulation in a 28-day toxicity test with Hydroxylamine HCl arsenic.

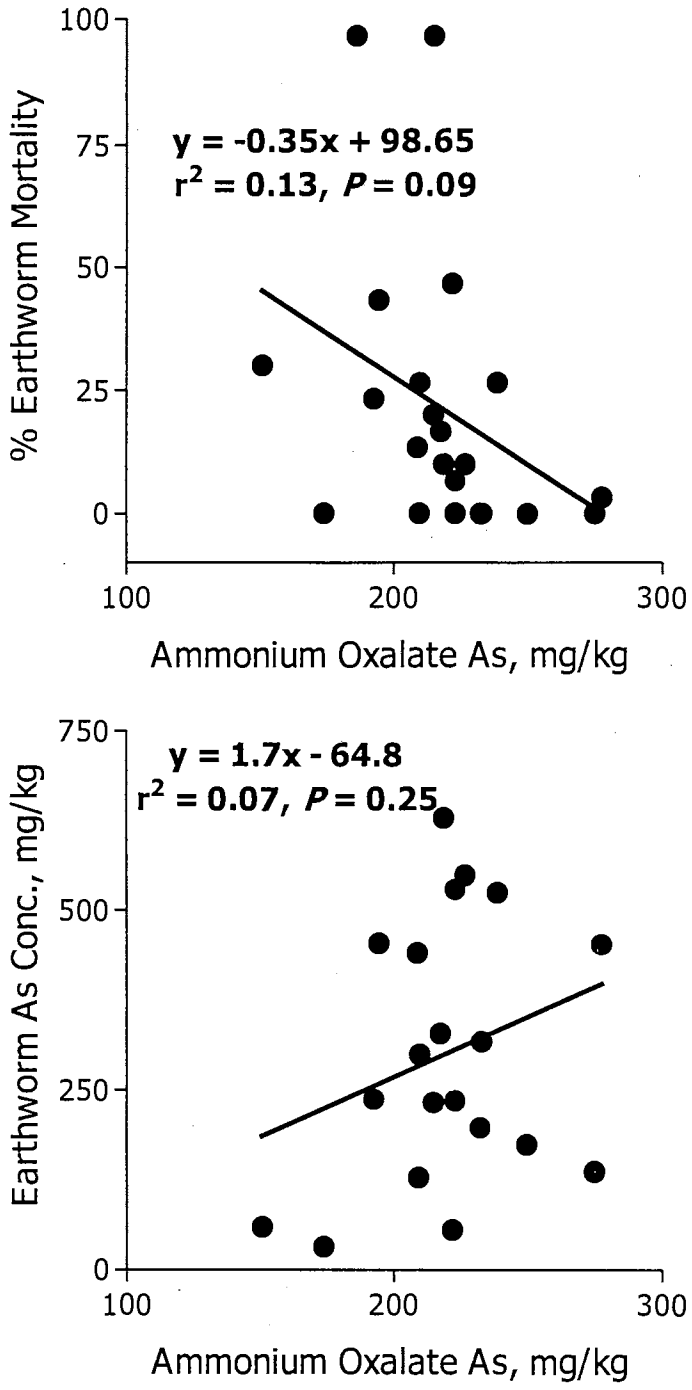

Fig. 33. Earthworm Mortality and arsenic accumulation in a 28-day toxicity test with Ammonium oxalate arsenic.

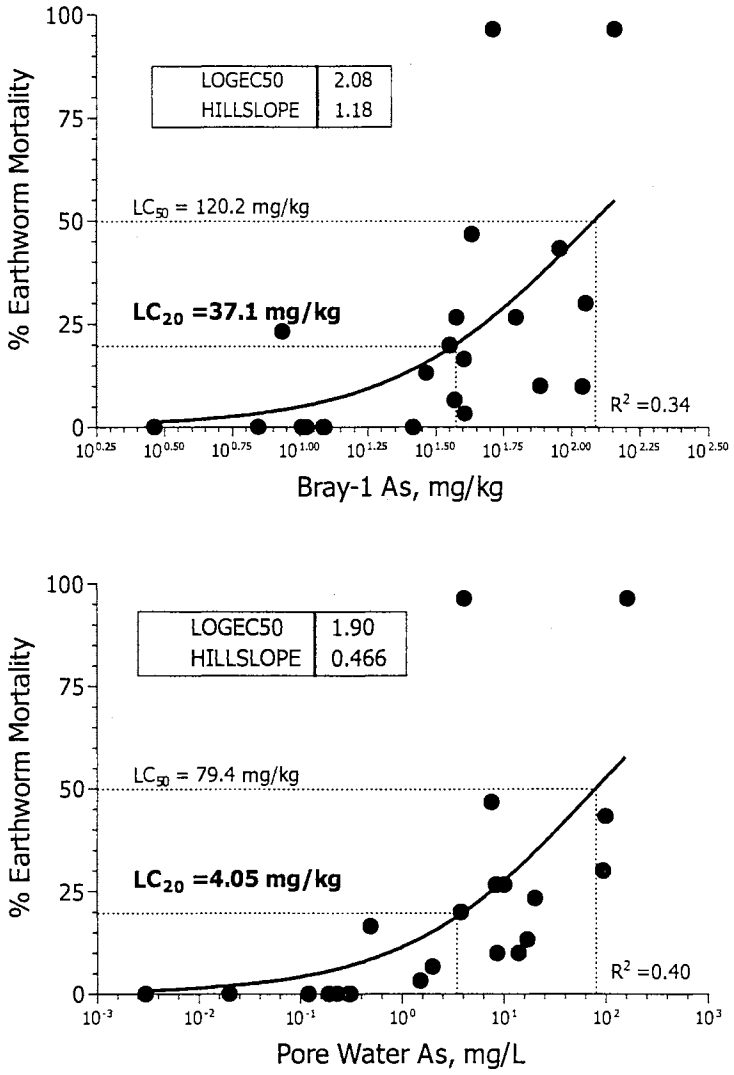

Fig. 34. Concentration-response curve for Bray-1 arsenic and pore water arsenic with earthworm mortality.

CHAPTER II

IRON-BASED REMEDIATION ON ARSENIC CONTAMINATED SOILS: EVALUATION OF INVERTEBRATE AND PHYTO- TOXICITY

ABSTRACT

Arsenic is a ubiquitous element found in many minerals all over the world. In addition to natural deposits, As compounds have been used for hundreds of years as pesticides, herbicides, desiccants, wood preservatives and as a byproduct in mining activities. With the lowering of drinking water standards for As, there is emergent interest in remediating As contaminated soils. For a remediation effort to be successful and useful in multiple applications, the remediation must be able to reduce risk of potential leaching and be ecologically beneficial for soil invertebrates and plants. Re-vegetation of remediated sites is essential for reduction of wind blown particles and soil stabilization. The objective of this study is to evaluate four iron-containing materials: $Fe_2(SO_4)_3$, $FeCl_3$, zero-valence Fe, and Fe-water treatment residual (WTR) for remediation of As (250 mg/kg) contaminated/spiked soils and one slag waste medium. Multiple pathways were investigated including As extractability (leaching potential), plant phytotoxicity and earthworm toxicity. All Fe-treatments used in the soils were effective in reducing the most soluble As (pore water and Bray-1 As) thereby reducing the threat of leaching. The largest decrease in soluble arsenic was in the Fe-WTR treatment. Pore

water As decrease by 98.4% across all media and Bray-1 As decrease by 75.7% across all soils. There were also improvements in seed germination, shoot elongation and shoot yield across all soils and treatments. There is some evidence that Fe-oxide content is principal Fe form for reduction of arsenic solubility and improvements in plant viability. Concentration-response curves establish an IC_{20} = 40.3 mg/kg Bray-1 As for seed germination and IC_{20} = 22.0 mg/kg Bray-1 As for shoot yield. Earthworm arsenic bioaccumulation was decreased in all Fe-treatments in three of the four soils. The sandy Pratt soil had reduced As body burdens in the Fe-Chloride and Fe-WTR treatments. *In situ* treatment of arsenic contaminated soils using Fe-WTR provided the most effective Fe-source to reduce As solubility, improve plant response and reduce bioaccumulation of As by earthwoms.

Keywords – Arsenic Arsenic extractability Iron remediation *In situ* remediation Plant bioassay Phytotoxicity of arsenic Earthworm accumulation of arsenic

INTRODUCTION

Arsenic (As) is in the nitrogen group of elements (N, P, As, Sb, Bi). Although arsenic is described as a metalloid, its behavior is more of a non-metal forming covalent compounds or found in anionic species. Many minerals in nature contain As compounds with approximately 60% being arsenate, 20% sulfides and sulfosalts and the remaining 20% as arsenides, arsenites, oxides and elemental As (O'Neill 1995). Argillaceous sedimentary rock (shales, mudstones, slates) has been found to contain as high as 900 mg/kg As (O'Neill 1995). In addition to natural deposits, As compounds have been used

for hundreds of years as pesticides, herbicides, desiccant for cotton after defoliation and as a wood preservative. Arsenic is also found in significant quantities in phosphate fertilizers with the content depending on the source of the phosphate rock. In the UK, an average content was found to be 7.7 mg As/kg in rock phosphate (O'Neill 1995). Besides natural occurrence of As, mining activities have increased the concentration of As in soils and water. The process of removing metals from ore tends to increase unwanted metals and metalloids, such as Pb and As, in local soil and water. Mine tailings have shown to have as high as 10,000 mg/kg As, although not all is bioavailable or soluble.

There is some similarity between the chemical behaviors of P and As, both form oxyanions (arsenate and phosphate) in the +5 oxidation state in aerobic soils, but phosphate is more stable over a wider range of Eh and pH conditions than arsenate. The distribution of As chemical forms in a solution is pH dependent. It is generally thought that anoxic soil solution i.e., pe+pH<6, the most abundant species of As is As(III) whereas in an oxic soil solution (pe+pH>10), As is mainly present as As(V) species (Sadiq 1997, O'Neill 1995, Masscheleyn et.al. 1991). The more toxic arsenite (AsO_3^{3-}) is readily oxidized to arsenate (AsO_4^{3-}) in air, surface waters and aerobic soil. There is a potential of As leaching into an anaerobic environment and reducing to the more toxic arsenite. In addition, microbial activity can cause methylation, demethylation and/or changes in oxidation state.

In soils, clays have a net negative charge therefore have a preference for positively charged ions from the soil solution, not oxyanions. The oxyanion forms of As can compete with or replace phosphates adsorbed on the positive sites of clay surfaces, although this is thought to be an insignificant amount of the total As adsorption. It is

generally accepted that organicarsenical complexes constitute a minor fraction of the total dissolved As in soil solution and, therefore, for all practical purposes, can be ignored. The basis of the adsorption is due to charge produced by hydration, specific adsorption, changes in cation coordination, isomorphous substitution, crystallinity, etc. Iron oxide/hydroxide surfaces have been found to effectively adsorb As in soils (Manning and Goldberg 1997, Livesey and Huang 1981, Carey et. al. 1996). Fe oxides/hydroxides have a zero charge at pH ranging from 7-10 (mean around 8.5) therefore higher pH favors net negative charge and lower pH enhances net positive charge on these surfaces. Many researchers have reported associations between Al oxide/hydroxide content and As concentrations in soil (Livesey and Huang 1981, Carey et al. 1996). Al oxides and hydroxides, like Fe oxides surfaces, may play a role in As adsorption/chemisorption. In acidic conditions Al oxides and hydroxides have a net positive charge therefore may partake in adsorption in acidic soils but may have a limited role in the near neutral or alkaline soils. Mn oxide surfaces are expected to play a limited role in the adsorption of As in soils with pH>4 because of the net negative charge. Carbonate minerals are unstable in acidic soils, but may play a role in the alkaline soils, particular in calcareous soils (pH 7.5 to 8.5, carbonate content 10 to 1000g/kg $CaCO_3$ equivalent). In calcareous soils the dominant forms of As are controlled by the reactive levels of Ca after consuming the reactive Fe (Sadiq 1997).

The leaching of As from contaminated soils is inhibited by the presence of hydrated Fe and Al oxides, clay and some organic matter. Reducing the amount of leachable As is especially important in areas with shallow water tables and in mining areas and areas with high amount of As in parent materials. The most common valence state in aerobic water is As(V) or arsenate, As(III) prevails in anaerobic soil and

groundwater. In the pH range of 4-10, the predominant As(III) is neutrally charged whereas As(V) is negatively charged (EPA Office of Water, Sadiq, 1997). Due to the negative charge, the ability to remove As(V) is greater than the removal As(III). Several treatment technologies have been developed for removal of As(V) from drinking water. Pre-oxidation of As(III) to As(V) by the use of chlorine, ferric chloride, and potassium permanganate have been employed. Ferric sulfate and alum then can be added to coagulate the As from the water. Other treatments for As removal from water are reverse osmosis and ion exchange filters (Nikolaidis et al.2000). More recently iron fillings or zero-valence iron has been investigated as a filter. Elemental iron oxidizes to form ferrous iron (Fe^{+2}) in an aerobic environment. Then iron (III) will precipitate arsenic as $FeAsO_4$ or FeAsS if sulfate is present in sufficient amounts. Inorganic As is removed from water by forming precipitates, co-precipitates and by adsorption reaction onto the ferric hydroxide solids.

Elemental iron has also been used in the remediation of gold mining waste (Macy 1999). The As in the mining waste pond is oxidized producing As(V) that readily binds with magnetic iron beads. The beads are then removed using magnets and the As removed from the beads allowing the beads to be re-used. The As is reduced by microbes and precipitated with sulfide in the presence of sulfide-reducing bacteria. The final end product is an arsenosulfide that can be removed from the contaminated area.

In general the transfer of As from soil to plant is usually low and not readily translocated to leaves and fruit. Several species of plants (tomatoes, carrots, grapes, cabbage, Sudan grass, cotton) are able to tolerate soils high in As with most of the As concentrated in the roots (Adriano 2002, Wauchope 1981). Arsenates behave much like phosphate in the plant-soil system. Arsenate (AsO_4^{3-}) can be taken up via the

phosphate transport system. Arsenate is thought to replace phosphate in energy transfer phosphorylation reactions (Tamaki and Frankenberger 1992, Bhumbla and Keefer 1994). Arsenic induces phytotoxicity resulting in restricted plant growth, and in essence protects humans and animals from plants with high As content (Smith et al. 1998). The effect of phytotoxicity is influenced by As source, As speciation, and the soil type, with sandy soils being more toxic than clayey soils (Sheppard 1992).

Earthworms have a particularly intimate contact with the soil, ingesting large amount of soil and having a limited barrier between soil solution and organisms. For this reason, and with their importance in terrestrial food webs, earthworms are ideal test organisms for toxicity test in contaminated soils (Conder et al. 2001, Langdon et al. 1999). Studies on earthworms have shown that pH, amount and type of organic matter, and soil type have the greatest affect on the bioavailable fraction of contaminants in a soil (Lanno, et al., 1998; Sample et al., 1999; Wong, et al., 1999; Sijm et al., 2000). Lower pH (acid soils) tends to make heavy metals more available therefore more toxic. Clay soils tend to have higher cation exchange capacity enabling them to hold contaminants and resulting in lower bioavailability. Heavy metals have been found to bioaccumulate in earthworms at different rates in different soils as well as in different genera of earthworm. In addition metals and metalloids may accumulate differently with the presence of other stressors or contaminants. Rida and Bouche (1994) did toxicity test using 186 sites in Mediterranean South France with the native species of earthworms found at those sites. They found that the species *Scherotheca* was particularly sensitive to high levels of Cd, Cu, Pb, and Zn in contrast to other worms. In general, earthworms tend to accumulate more Zn and Cd than other metals (Pb, Ni) found in the soil. *Scherotheca* was found to concentrate even more Cd and Zn than

other earthworm species. Copper was found to accumulate in the earthworms at the same level as found in the soil. In soils that contain sub-lethal As concentrations (40 mg/kg), As bioconcentrates in earthworms (*Lumbricus terrestris*) over time even exceeding soil concentrations in less than 12 days (Meharg et al. 1998). Higher soil As levels, especially when present as Na_2AsO_4, are highly toxic to earthworms causing yellow discoloration, lesions and swelling along the body and death (Langdon et al. 1999, Meharg et al. 1998).

Rida and Bouche (1994) found that body concentrations of heavy metals in the earthworms were a toxic burden and could enter the food chain. This transfer into the food chain can sometimes concentrate into predators. Although the earthworms that were tolerant of the heavy metals may continue to survive, the metals may reach sensitive targets when transferred into the food chain (biomagnification). Callahan et al. (1991) used earthworm to do an on-site assessment of a soil contaminated with organochlorine pesticide (chlorodane) and DDT (plus DDT residues). The result showed that the organochlorine pesticide caused greater mortality that the DDT, but the earthworm body burden of the DDT was at a high enough level to present a potential food chain impact. This study was also able to locate areas of the contaminated site that were at a higher risk (high mortality) and may require more clean up.

Physical removal of a metal or metalloid contaminated soil by excavation and transporting the contaminated soil to hazardous waste landfill is the most commonly used method of soil remediation in the United States. Although soil removal ensures a "clean" site, increasing cost of excavation and transport and limited number of hazardous waste landfill have promoted research and development of other alternatives.

Since metals and metalloids can not be degraded or destroyed alternative technologies are used to manipulate the soil or waste so that the mobility of the contaminants or total content are reduce to an acceptable level (USEPA 1997). Two alternative technologies used are 1) metal removal through pump and treat or soil washing systems and 2) immobilization/stabilization methods (Mulligan et al. 2001). Metal removal can be done either *in situ* or *ex situ,* through soil washing or pump-and-treat technology. This approach can be difficult, time-consuming and incomplete due to the strong reactions between metal cations and soil components. Soil washing with acid solutions increases the removal of the metal cations but can leave the soil sterile and difficult for plant rehabitation. As an alternative, chelating agents or surfactants have been used which readily bind and remove metals. Immobilization of heavy metals is generally achieved through increasing the pH, causing precipitation, and binding the metal cation in the soil. This approach is not effect for As due to its oxyanion structure. Immobilization of As has been tested using ferric chloride and ferric acetate. In an *in situ* pilot scale study, ferrice chloride and ferrice acetate reduced leaching of As contaminated soil over an 11-day period (Stammier et al. 1992).

Several methods are used to determine the effectiveness of remediation. The most common way is through chemical extraction of the contaminant. This type of measurement can indicate the effectiveness of a treatment to reduce soluble forms of the contaminant but it does not give an indication of how living organisms are affected by the remediation. Solvent extraction was used to remediate a soil contaminated with polychlorinated biphenyls (Meier et al. 1997). PCB concentrations were reduced to clean up goal of 2 mg/kg. Acute toxicity test were done on two species of earthworms: *E. fetida* and *L. terrestris* both before and after treatment. No acute toxicity was seen on

the positive control soil. But, neither species survived after a few days on the remediated soil, this is thought to be due to remaining solvent (isopropanol) in the soil. Once the soil was allowed to stand (storage), no acute toxicity (14-day test) was observed. It is thought that this was due to evaporation or biodegradation of the isopropanol solvent. Using a variety of bioassays (higher plants and soil invertebrates) in conjunction with chemical analysis is necessary when assessing the efficiency of remediation and the potential risk to the ecosystem (Phillips et al. 2000, Baud-Grasset et al. 1993, Conder et al. 2001).

Ecological risk assessment can be defined as the evaluation of the potential adverse effect that human activity or exposure to environmental stressors has on the plants and animals of an ecosystem (CENR 1999). Every ecosystem whether, water, soil or sediment, have a variety of conditions and interactions to contemplate. Assessing related risk in a soil or terrestrial ecosystem is challenging. Soils are a varying and complex system composing of at least three phases; solid, air and water. In addition most soil organisms depend on soil abiotic factors, i.e. minerals organic matter, for survival. During an ecological risk assessment, some extractable fraction or total concentration of the contaminant is used in determining the risk associated with a contaminated ecosystem (CENR 1999, USEPA 1994b). The amount of the contaminant that is bioavailable to organisms in the environment can modify the amount and type of clean up that is necessary and the risk associated with the contaminant. In addition, toxicity test can be used to determine if remediation has been successful. Toxicity test are used to expose a test organisms to a contaminated medium e.g., soil, water or sediment, and evaluate the effect of the contaminant on survival, growth, reproduction, or other characteristics of the organisms (endpoint). These test help to determine if the

concentrations of the contaminant are bioavailable and are high enough to cause adverse effects. There are two primary types of ecological risk: 1) risk tolerated by an organism due to a chemical(s) and 2) risk coming from organisms having a toxic burden, thereby contaminating its predators (food chain) (EPA 1994a).

Most soil remediation studies to date have concentration on the reduction of leaching or some other extractable fraction of As in soil. Other studies have look at the As bioavailability to plants or soil invertebrates but none have investigated the effect on multiple pathways. For a remediation effort to be successful and useful in multiple applications, the remediation must be able to reduce risk of leaching and be ecologically beneficial for soil organisms and plants. Re-vegetation of remediated sites is essential for reduction of wind blown particles and soil stabilization. It is known that amorphous Fe-oxide has a high adsorption capacity for As in soils and water (Adriano 2001, Smith et al. 1998). In fact, some water treatment facilities use amorphous Fe-oxides as a coagulant in the drinking water production process. For example ferric chloride can be added to drinking source water to form a gel and flocculate sediment. Alkalinity is adjusted by adding $Ca(OH)_2$ to neutralize the H^+ formed from Fe hydrolysis.

$$FeCl_3 + 3H_2O \xrightarrow{OH^-} Fe(OH)_3 \downarrow flocculant$$

Other Fe-salts, e.g. $FeCl_3$ and $Fe(SO_4)_3$, can be used to form amorphous Fe-oxide by the process:

$$FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 + 3Cl^- + 3H^+$$
$$Fe(SO_4)_3 + 3H_2O \rightarrow Fe(OH)_3 + 3SO_3^- + 3H^+$$

Zero-valence iron (zero-Fe) is fine shavings from industrial uses. In principle, the chemical process that takes place for zero-Fe to form amorphous Fe:

$$2Fe^{o} + O_2 + 4H^{+} \rightarrow 2Fe^{2+}$$
$$2H^{+} + Fe^{2+} + \tfrac{1}{2}O_2 \rightarrow Fe^{3+} + H_2O$$
$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3\,amorphous$$

The initial amorphous FeOOH can be gradually transformed into crystalline iron-oxides (e.g. goethite FeOOH or hematite Fe_2O_3) depending on aging time.

The objective of this study is to evaluate four iron-containing materials for remediation of As contaminated soils and one waste medium. Each remediation treatment is evaluated for reduction of As in multiple pathways. The pathways under consideration are As extractability (leaching potential), plant toxicity, and earthworm toxicity and resulting potential food chain effect.

MATERIALS AND METHODS

Four soils were selected to provide a range of amorphous Fe-oxide content (Table 1). One slag waste soil was also selected to test the ability of iron-based remediation methods on mining waste. Various soil properties of the soils selected for this study are shown in Table 1. Soils were spiked with 250 mg/kg As using reagent grade Na_2AsO_4. The As was dissolved in 1L of de-ionized distilled water and mixed with the soil in large aluminum pans. The soils were thoroughly saturated and mixed well, and then put into a drying oven set at 65-70°C for 24 h. After the initial drying, the soils were saturated again, mixed and dried another two times for a total of 3 wet/dry cycles. After the last drying cycle the soil was homogenized and divided into subsets for iron remediation/treatment. The slag waste had an As content of 370 mg As/kg waste material.

Four iron sources used for remediation of the As contaminated soils were $Fe_2(SO_4)_3$, $FeCl_3$, Peerless-Iron (a zero valence elemental iron) and Fe-water treatment residue, (Fe-WTR sold commercially as Fe-humate). Fe-WTR is a by-product of water treatment facilities that use iron as a coagulant in the drinking water production process

All soils and slag waste were treated on a 20:1 Fe:As molar ratio (66 mmol/kg Fe /kg soil) as shown in Table 2. Iron chloride and $Fe_2(SO_4)_3$ were dissolved in 1L de-ionized distilled water and thoroughly mixed with the soils additional water was added as needed to make slurry. Zero-Fe and Fe-WTR were mixed with dry soil then saturated and mixed again. The saturated soils were place in a constant temperature room set at 33 ± 4°C. The soil-treatments were saturated and mixed thoroughly 2-4 times a week. Sub-samples of each soil-treatment were taken weekly and pore water pH, As and iron content was measured (Appendix B). After 4 weeks of incubation the pore water pH and As content were constant and the incubation was terminated. The pore water pH and electrical conductivity (EC) were measured on each soil-treatment. If the EC exceeded 2 dS/m, the soils was leached by mixing the soil with excess de-ionized water in a bucket, allow the soil to settle (sit un-disturbed for >24 h), then removed the excess water. If the pH was below 4 the soil was gently limed (5-10 g lime/kg soil) with $CaCO_3$ to achieve the target pH of 5.0. The $FeCl_3$ and $Fe_2(SO_4)_3$ treatments produce acid from hydrolysis that caused the pH to decrease greatly. The soils were then oven dried at 70°C and place in sealed containers.

Soil Arsenic Extractability

Soils were extracted with five different soil extractions and wet acid digestion for total metal content (EPA, 3051). All samples were extracted in duplicate and included

reagent blanks and spikes (Appendix B). Statistics were done using SAS for Windows V8 (SAS Institute 2002) and GraphPad Prism 3.03 (GraphPad 1999).

Soil Pore Water

To measure the amount in the soil pore water 40 g of soil was saturated with de-ionized distilled water until a slurry or paste was formed as described by J. Rhoades (1996) when measuring electrical conductivity. Enough water was added to insure at least 5 mL of water available after centrifuging. The soil solution was allowed to sit for 48 h to equilibrate, mixing intermittedly. The soil solution was transferred to tubes and centrifuged at 12,500 RPM for 15 minutes. The supernant was decanted and filtered through 0.45μm syringe filter, acidified and analyzed for As and other elements of interest on inductively coupled plasma atomic emission spectroscopy (ICP).

Bray-1 Extraction

An extracting solution of 0.03M NH_4F and 0.025M HCl is used to determine the weakly adsorbed, weakly soluble and plant available As (Chapter 1). One gram of soil was mixed with 20 mL of Bray-1 solution and shaken on a tabletop reciprocating shaker for 5 minutes. The solution was centrifuged at 7500 RPM for 5 minutes then syringed filtered through 0.45μm filters and analyzed on ICP for phytoavailable As and phosphorus (Kuo 1996).

Sodium Phosphate Extraction

The amount of As associated with water soluble, weakly adsorbed and strongly adsorbed can be measured using this method (Yamamoto 1975). The extractant solution is prepared by mixing 600 mL of 0.1M Na_2HPO_4 with 400 mL of 0.1M NaH_2PO_4. Soil, 1 g, was placed into a 50 mL polystyrene centrifuge tube mixed with 10mL of the

phosphate solution. The tubes were shaken on a tabletop shaker for 8 h then centrifuged for 5 minutes at 10,000 RPM and filtered through 0.45μm filters. Arsenic concentrations were determined using ICP-Hydride generation (ICP-HG).

Modified Hydroxylamine HCl Extraction

Hydroxylamine Hydrochloride solution is used to extract the water soluble, weakly and strongly adsorbed As, Mn-oxide and some amorphous Fe-oxide As (Ross and Wang 1993). The extracting solution, 0.25M $NH_2OH \bullet H_2O$ and 0.25M HCl is modified by adding 0.025M H_3PO_4 (Amacher and Kotuby-Amacher 1994) to prevent re-adsorption of the As. One gram of soil was placed into a 250 mL polystyrene container and mixed with 100 mL of the solution and shaken for 18 h on a tabletop reciprocating shaker. Samples were suction filtered through 0.45μm filters and analyzed for As using ICP-HG (Amacher and Kotuby-Amacher 1994, Ross and Wang 1993).

Modified Acid Ammonium Oxalate Extraction

To measure the amount of As associated with water soluble, weakly adsorbed, strongly adsorbed and As associated with amorphous Fe-oxide. An acid ammonium oxalate method (0.2M ammonium oxalate + 0.2M oxalic acid and 0.1M ascorbic acid) was modified with added 0.025M H_3PO_4 to prevent the re-adsorption of As (Amacher and Kotuby-Amacher 1994). The final pH of the solution was 2.7. One-gram soil was mixed with 50 mL of the modified acid ammonium oxalate solution and shaken on a reciprocating tabletop shaker for 2 h. The soil-solution mixture was centrifuged and filtered through 0.45μm syringe filter. The solution was analyzed for As, iron and other elements of interest on ICP (Loeppert and Inskeep 1996, McKeague and Day, Schwertmann SSSA 1996).

Total Content of Arsenic and other Metals

Total As, Fe and other elements were determined by nitric acid microwave digestion, EPA Method 3051 (USEPA 1994). This analysis was performed to confirm the total amount of As in soil after processing. Certified reference material (CRM020-050, RTC Corporation, Laramie, WY, USA) as well as blanks and spikes were used for quality assurance/quality control (Appendix B).

Plant Bioassay

Plant bioassays were used for evaluation of As remediation treatments to reduce As phytotoxicity. All bioassays were conducted with lettuce, *Latuca sativa var. Paris Island Cos.* The test methods were a 10-day germination test and a 17-day shoot elongation test. Both tests were done with negative controls (no As added control soil and artificial soil) and positive controls (spiked soil or untreated slag). The test methods are described below:

Germination Test

Fifty grams of soil was placed into a deep petri dish and temperature adjusted to 24±2°C (EPA 600/3-88/029, ASTM E 1598). One day prior to planting, water was added to 120% of water-holding capacity (field capacity at $-^1/_3$ bar). Twenty lettuce seeds (*Latuca sativa var. Paris Is.*) were placed into the petri dish and covered with 25g artificial soil (water added to 25% or 6.2 mL/25g). Artificial soil was composed of 69.5% silica sand, 20% kaolin clay 10% 2-mm sieved *Sphagnum* peat moss and approximately 0.5% $CaCO_3$ added to adjust the pH to 7.0. The petri dishes were covered and incubated at 24± 2°C in the dark for 48 hours followed by sequencing 16 h of light and 8 h of dark until termination of the test (Baud-Grasset 1993). Germination

was first determined after 120 hours (5 days) by counting the number of seedlings (leaves or stems) that protruded above the soil surface. After the germination count, soils were watered to 120% of field capacity. Petri dishes were placed on a tray and put into large clear plastic bags to retain moisture and allow the seedlings to grow for another 120 hours (5 days) without the hindrance of a lid. After 10 days, a second seed germination count was taken (EPA 600/3-88/029, ASTM E 1598).

Shoot Elongation Test/Early Seedling Growth

The germination study was continued for a total of 17 days of growth (12 days after more than 50% of control seed germinated) (EPA 600/3-88/029, ASTM E 1598). After counting the total number of plants, each plant was remove and measured from the hypocotyl to the leaf tip. Fresh weight was determined for the shoot portion collected from each plate. The plants were dried at 70°C for 48 h to determine dry matter (EPA 600/3-88/029, ASTM E 1598).

Soil Invertebrate Bioassay

The earthworm, *Eisenia andrei,* was used in a 28-day toxicity bioassay to determine the effect of As and Fe-treatments on ecotoxicity to soil invertebrates. Each bioassay was performed in triplicate for each soil and treatment. Glass jars (473 mL) were used as the environmental chambers for the worm bioassay. The worms were acquired from the Department of Zoology at Oklahoma State University. Soil, 200 g, was placed into each jar and water added to field capacity ($-^1/_3$ bar). The soils were mixed and water added as needed. Earthworms were removed from growth chambers and allowed to depurate for 24 h by placing in a clean environment (no soil) and allowing the worms to eliminate soil from the gut. After depuration, 10 worms were

weighed and placed into each glass jar. Perforated lids were place on jars to allow air exchange and minimize loss of moisture. The jars were placed into a constant temperature chamber with 16 h light, 8 h dark at 20 ± 4° C. The worms were check daily for mortality for the first 5 days, then about every 3 days after that. Mortality was determined if the worms failed to respond when gently poked or prodded. Dead worms were removed, rinsed in de-ionized water and frozen at 4°C. Every seven days, 1 tsp of manure was placed on top of the soil to feed the worms. The horse manure is collected from a local farmer, dried, blended and stored in plastic containers. At the end of the toxicity bioassay (28 days), all worms were removed from the jars and rinsed in de-ionized water. Five, or half of the remaining worms, were depurated for 24 h. The remaining undepurated worms were weighed and frozen at 4°C. After 24 h the depurated worms were washed, weighed and frozen at 4°C until analysis.

One depurated worm and one un-depurated worm from each jar were digested to determine the amount of bioaccumulated As (total of 3 worms per soil and Fe-treatment). Arsenic analysis was determined by wet digestion/dry ashing (Ybanez et al. 1992, Chapter 1) followed by measurement of As by inductively coupled plasma atomic emission spectroscopy with hydride generation (ICP-HG).

Measurement of As by ICP-HG

To determine low level As concentration and to remove potential interferences found on direct analysis of sample digest, ICP-hydride generation was used. The hydride generator is a batch type produced by Thermo Jarrell Ash (TJA) for the IRIS TJA ICP. Reaction rates for hydride generation are controlled by several variables: 1) chemical forms of the As, 2) oxidation state of the hydride-forming element, 3) acid concentration, and 4) concentration of the $NaBH_4$ reducing agent. The least error

occurs when these condition are set so that the reaction goes to completion instantaneously. Inorganic As, arsenate (V) and arsenite (III), can be reduced to arsine gas through the hydride process, although reduction of arsenate is more time consuming. Therefore all samples (10-50 mL) are pre-treated with 40% potassium iodine + 4% ascorbic acid solution (0.5 mL) to reduce all As to arsenite (III), the faster reacting As form (TJA, Dedina and Tsalev, 1995). Hydrochloric acid is used as the sample medium to form hydrides. Samples were mixed with concentrated HCl to a concentration of 3 molar. The efficiency of hydride formation is constant at HCl concentrations above one molar (Dedina and Tsalev 1995). A 0.5% $NaBH_4$ solution with 0.42% NaOH for stability was used as the reducing agent. Acidified sample and base were pumped to the reactor chamber at a rate of 3.25 mL /minute with a 2 minute rinse between samples to eliminate carry over. Arsine gas was separated in a gas-liquid separator via nebulizer directly into the ICP argon plasma.

RESULTS AND DISCUSSIONS

The pH of the soil treatments/additives was monitored weekly during the incubation period for changes in pH and pore water As. By the end of the four-week incubation period, the pH and pore water As had become constant (Appendix B). The zero-valence Peerless iron treatments had the most flux with the overall tendency to increase pH. The pore water As levels during the incubation period stayed consistent in the Bernow and Dennis soils and slag waste material. The Perkins soil had a decrease in pore water As over time, and then stabilized towards the end of the four weeks. The Pratt soil, with the highest level of pore water As, fluctuated throughout the incubation period.

Reduction of Arsenic Extractability

The amendments did not react the same in all materials, this indicates the importance of soil physical and chemical properties on As in soils. In general, the soils with higher initial amounts of clay and Fe-oxide had less of a reduction in extractable As than those with less clay and Fe-oxide content (Table 3).

All treatments reduced pore water As in all the spiked soils (Fig. 1). The slag waste media did not have a significant reduction of pore water As, in fact the zero-Fe treatment increased the pore water As. Pore water As is the fraction that is most water soluble, available and has the greatest potential risk for leaching into groundwater and cause toxicity.

The Bray-1 extractant is commonly used to extract phosphate from soil to determine the amount of phosphates that are plant available. Arsenate, since is chemically similar, can be extracted by Bray-1 to determine the amount of plant available As (Wauchope, 1983; Huang and Fuji, 1996). This fraction has shown good

correlation to plant available As and reductions in yield due to As contamination (Chapter 1). Bray-1 causes the slight dissolution of Fe and Mn oxides and extracts As associated with poorly ordered alumino silicate gels and allophane (Lombi et al., 2000). Overall, Fe-WTR treatment had the greatest reduction of Bray-1 extractable As in the spiked soils (Fig. 2). The greatest reduction resulted in the soils with the least amount of clay content. The other treatments also were successful in reducing the amount of Bray-1 extractable As in the spiked soils. The Fe-chloride and Fe-sulfate treatments had the greatest reduction of Bray-1 extractable As in the slag waste. The slag waste material is a waste product that results from the smelting of ores for lead, which is also high in iron mixed with soil. During the smelting process lime or carbonate is added producing a pH >7.0 waste material. When Fe-chloride or Fe-sulfate is mixed with this type of material, a visibly aggressive reaction between the slag waste and the acidic Fe-solutions results. These conditions along with a temporary reduction of pH, may have allowed enough reduction in pH to allow a reaction between Fe and As. The other treatments did not have this type of reaction with the slag waste.

The Na-phosphate extraction measures, in addition to the previous fraction, the strongly (specifically) adsorbed As. Phosphate competes with arsenate for adsorption sites and is more effective than other anions (nitrate and sulfate) in extracting arsenate from soils (Lombi et al., 2000). All soil treatments had a decrease in Na-phosphate extractable As (Fig. 3). Fe-WTR treatment had the greatest reduction of extractable As, similar to Bray-1 results. The amount of reduction by these treatments depended on the soil and the amount of clay in the soil (Table 3, Fig. 3). None of the treatments reduced Na-phosphate extractable As in the slag waste.

Hydroxylamine HCl acid extraction is much more aggressive releasing some of the As associated with Fe-oxide as well as the water soluble, weakly and strongly adsorbed As. There were not as many significant reductions of extractable As from the treatments for this fraction of As. Fe-WTR additive had the most significant reduction of extractable As from all soils and slag waste (Fig. 4).

Ammonium oxalate extraction is able to release the previous mentioned fraction of arsenic as well as arsenic associated with Fe, Al and Mn-oxides. The results were mixed depending on the soil or medium (Fig. 5). The Bernow soil had no significant change in ammonium oxalate extractable As, where as the Dennis soil had significant reductions in the Fe-chloride and Fe-sulfate treatments. The Perkins and Pratt soils had similar results with Fe-WTR having the greatest amount of reduced extractable As. The slag waste material has either no change or increase amount of extractable As.

Arsenic and Plant Bioassays

The Perkins Fe-sulfate treated soil resulted in lower than anticipated pH and was not conductive or plant or earthworm survival; therefore it was removed from the following sections. There were no significant differences ($P < 0.05$) between germination rates in the treated soils and controls (positive and negative) in Dennis soil or the slag waste material (Fig. 6). Bernow had an increase germination rate in the Fe-WTR treatment over the positive control (spiked soil). Pratt soil had an increased germination rate, as compared to the positive control (40%), for all amendments (77.5-88.3%, Table 4). The Perkins soil had significant increased germination rates, as compared to the spiked soil (33%), for the treatments zero-Fe, Fe-chloride and Fe-WTR (85-88%, Table 4). Extremely high levels of arsenate are known to inhibit seed germination (Wauchoe 1983). Although in this test, overall mean seed germination was

81.3% with a range from 33% to 94% indicating that only a few soils had toxic levels of arsenate to inhibit seed germination (Table 4).

The mean shoot length was 3.9 cm and mean shoot yield (dry weight) was 40.1 mg. Most of the amended soils had an increase in dry weight as compared to the positive controls (Table 5). The slag waste had no significant change in yield or shoot length. Pratt soils had an increase in shoot length across all treatment (Fig. 7). The Perkins soil had increase shoot length across all treatments. The Bernow soils had an increase shoot length in all treatments except zero-Fe. And the Dennis soils had no significant increases or decreases in shoot length. Two soils displayed some As toxicity resulting in low germination and shoot growth. Pratt soil had the lowest amount of Fe-oxide and clay content resulting in high pore water As (282 mg/L) in the positive control soil. The high level of pore water As resulted in poor germination (40%) and retarded growth (3.7mg dry weight, 0.93 cm shoot length). Untreated Perkins soil had the second highest pore water As (18.4 mg/L) that resulted in low germination (33%). Perkins has double the amount of Fe-oxide and clay content than Pratt soil and resulted in better growth in the untreated Perkins soil (10 mg dry yield, 2.07 cm shoot length) although it was well below the average yields and growth rate. The high pore water As may be due to a combination of low clay and low Fe-oxides content (Chapter 1).

Although all soils had relatively the same amount of Fe used for remediation, the amount of Fe-oxide varied greatly depending on the Fe-source (Table 4). In general the most Fe-oxides were found in the Fe-WTR treatment. This may be the reason Fe-WTR had the greatest amount of soluble As reduction and the highest plant responses. The amount of Fe-oxides has more influence on plant viability (% germination and shoot yield) than the total Fe content (Fig. 12).

Relationship Between Arsenic Extractability and Plant Bioassays

The lettuce endpoints (germination, shoot length, and shoot yield) were compared to different fractions of As by correlating endpoints to the various extractions. The hydroxylamine HCl extraction and acid ammonium oxalate did not have any correlation with any lettuce endpoint (Appendix B). Both these extractants are more aggressive and have not been shown to correlate to plant available As from soils (Chapter 1). The predominant concentrations of pore water As was< 1 mg/L, therefore without a good distribution of pore water As the data was removed from further discussion (Appendix B). The remaining extractions, Bray-1 and Na-phosphate, have significant correlation ($P < 0.01$) with plant endpoints (Fig. 8 and 9). These extractions have been shown to correlate to plant endpoints including yield and As accumulation (Chapter 1).

An important step in determining toxicity of a chemical is generating a dose-response curve. A dose-response or concentration-response curve establishes the relationship between exposure to a substance and the incidence and severity of an effect. Concentration-response curves are used to plot the results of this experiment. The equation used to produce these curves (GraphPad 3.0):

$$Y = Bottom + \frac{Top - Bottom}{1 + 10^{(\log IC50 - X) * HillSlope}}$$

Where, Y = Lettuce endpoint or response

X = log Bray-1 or Na-phosphate extracted As mg/kg

Hill Slope = the steepness of the curve

Top and Bottom refer to the curve where Bottom is the Y value at the bottom plateau; Top is the Y value at the top plateau, and LogIC50 is the X value when the response is halfway between Bottom and Top (GraphPad 1999).

The x-axis plots the log concentration of As extracted by Bray-1 and Na-phosphate As and the y-axis plots the response from each test. Concentration-response curves were produce to find an IC_{50} (concentration at which 50% of the samples are inhibited) and IC_{20} (concentration at which 20% of the samples are inhibited). The IC_{20} can be calculated using the equation (Motulsky 1999):

$$IC_{20} = \left(\frac{80}{100-80}\right)^{\frac{1}{H}} * IC_{50}$$ Where H = Hill slope

For % germination the resulting IC_{50} was 44.7 mg/kg Bray-1 As and 49.0 mg/kg Bray-1 As for % relative germination (Fig. 10). The IC_{20} was 40.3 mg/kg and 43.5 mg/kg Bray-1 As respectively. The small differences in these two values indicate that there is little difference on how you express these results. The concentration – response curve for shoot length had IC_{50} of 69.2 and shoot yield was 41.7 mg/kg Bray-1 As, the IC_{20} were 39.7 and 22.0 mg/kg Bray-1 As respectively. Na-phosphate concentration – response curves resulted in an IC_{20} of 26.9 and 30.1 mg/kg Na-phosphate As for germination and % relative germination. The IC_{20} for shoot length and yield was 23.2 and 25.9 mg/kg Na-phosphate (Fig. 11).

Hill slope or slope factor (the steepness of the curve) represents the magnitude of the range of "doses" between non-effected concentration and a lethal or effective concentration. The steeper the curve, the slighter the margin of safety or the more toxic the substance (Motulsky 1999). The Hill slope for Bray-1 As germination and % relative germination was much steeper (H=-13.5 and -11.7 respectively) than for either shoot length (H=-2.39) or shoot yield (H=2.17). This indicates that Bray-1 As has a greater toxic effect on seed germination than on shoot growth.

Remediation Treatments and Earthworm Bioassay

Earthworms are in direct contact with the soil all of their lives and ingest large amount of soil thereby making them an ideal organism for assessing bioavailability and contaminant toxicity to soil organisms. In addition earthworms can tolerate an environment high in heavy metals and toxic compounds because either they do not absorb the metal, excrete it efficiently or accumulate it in a non-toxic form (Ireland, 1983). Earthworm mortality ranged from 0% to 100% with an average of 11.6% mortality (Table 7). One amended soils had 100% mortality Pratt zero-Fe. Since the As spiked soil did not have 100% mortality it can be concluded that the death of the worms was due to multiple factors, not just As concentration. Screening with treatments and clean soils (no As) had 100% mortality in the zero-Fe treatment in Pratt soil. Several heavy metals were analyzed (EPA 3051) in the zero-Fe material, but concentrations were not enough to cause mortality (Appendix B). Mortality may be due to other physio-chemical properties (e.g. change in redox conditions) only prevalent in the sandy soil.

Depurated worms have little (<0.04g) or no soil left in the gut of the earthworm. The As (mg/kg dry weight) that remains is from the earthworm tissue or bioaccumulated As. The undepurated worms will contain soil in the gut as well as arsenic bioaccumulated in the earthworm tissue (mg/kg dry weight). Generally the undepurated worms had greater As concentrations than the depurated worms the difference depending on the soil (Table 8). Earthworms from sandy soils have the highest levels of As concentrations where as earthworms from soils high in indigenous clay and Fe-oxide had the lowest levels of As concentrations. Bernow and Dennis soils, in all treatments, for both depurated and undepurated worms, had decreased As concentrations (Fig. 13

and 13). The Perkins soil had decrease As concentrations in the depurated and undepurated earthworms. The sandy Pratt soil had decreased earthworm As concentration in Fe-chloride and Fe-WTR treatments. The Pratt Fe-sulfate treatment had the same As concentration as spiked soil and the Pratt zero-Fe treated soil had 100% mortality. The slag waste showed mixed results. The undepurated earthworms across all treatments showed a significant ($P<0.05$) decrease in total As (mg/kg dry weight, Fig. 14). The depurated worms from the slag waste had significant decrease in the amount of As accumulated only in the Fe-sulfate and Fe-WTR treatments (Fig. 13). The slag waste zero-Fe and Fe-chloride amendments had no change in As accumulation as compared to the untreated slag waste.

The ranges in As body burdens from treated soils were 29.6 to 364 mg/kg As. The overall averages across all soils and treatments were 147 mg/kg As and 163 mg/kg As for depurated and undepurated worms respectively. At these high concentration there is a potential risk for food chain transfer of the contaminant. Fe-WTR had the greatest reduction (86.5% depurated and 78.9% undepurated) of As concentrations in the earthworm. Overall the As concentrations in Fe-WTR treated soils were 30.6 and 65.4 mg/kg As for depurated and undepurated earthworms respectively. The remaining treatments, zero-Fe, Fe-chloride and Fe-sulfate, had about 30% reduction of As body burdens in depurated worms and 40% in undepurated worms, although in these treatments As body burdens were much greater (150 to 190 mg/kg As, Table 8).

Extractable As was correlated with earthworm mortality and As body burdens. Due to the low mortality there were no significant correlations to extractable As and mortality. Na-phosphate (Fig. 16) and Bray-1 (Fig. 15) had the best correlation to As body burdens in both depurated (R^2 = 0.81 and 0.70 respectively) and undepurated

(R^2= 0.83 and 0.72 respectively) earthworms. Weaker correlations were found in ammonium oxalate extractable As (Fig. 17) with depurated (R^2 = 0.41) and undepurated (R^2 = 0.42) As body burdens. This indicates that several fraction of As (soluble, adsorbed, Fe-oxide associated) are available for earthworm bioaccumulation. The actual amount that is bioaccumulated is dependent on the properties of each soil. Overall, the addition of iron to the soils decreased the amount bioaccumulated by the earthworms.

SUMMARY AND CONCLUSIONS

In situ treatments may be a better alternative for soil remediation than other options. Soil washing and extraction processes may cause more damage to the soil and ecology resulting in new difficulties and longer recovery for the ecosystem. Removing contaminated soil can be expensive, and it is becoming more difficult to find landfills that are equipped for contaminated material. Therefore amendments that not only reduce leaching but also reduce bioavailability of As in soil could be a better approach. Of the four amendments tested Fe-WTR has the best overall performance for reduction of leaching, phytotoxicity and invertebrate bioavailability. The Fe-chloride amendment was next effective in reduction of leaching and bioavailability. The Fe-sulfate and zero-Fe amendments were not as effective in respective to invertebrate toxicity although they were effective in reduction of leaching potential. The Bray-1 and Na-phosphate extractions were the best predictive extractions for plant growth, earthworm mortality and As body burden in the treated soils and slag waste. Concentration-response curves for % seed germination indicate a Bray-1 As IC_{20} = 40.3 mg/kg and IC_{20} = 22.0 mg/kg Bray-1 As for yield. Na-phosphate As concentration–response curves had an IC_{20} of

response curves had an IC_{20} of 26.9 mg/kg As and 25.9 mg/kg As for germination and shoot yield respectively. Nearly every Fe-treatment in every soil was able to reduce the Bray-1 As and Na-phosphate As to below these levels. Use of Bray-1 and Na-phosphate extractants for soils screening may be a useful tool in the ecological risk assessment process.

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Table 1. Select soil properties including pH (1:1 water), % sand, % silt and % clay, % organic carbon and amount of iron and aluminum oxides as measured from modified acid ammonium oxalate extraction.

Soil	pH (1:1 water)	% Sand	% Silt	% Clay	%Organic Carbon	Fe-oxide mmole/kg	Al-oxide mmole/kg
Slag Waste	7.75	na	na	59.1	3.13	60	8.22
Dennis	6.16	17.5	41.9	40.6	0.80	7.07	15.5
Perkins	4.37	60.0	30.0	10.0	0.85	4.64	8.56
Bernow	5.16	56.9	19.4	23.8	0.30	3.66	10.7
Pratt	6.35	90.0	3.75	6.25	0.90	0.99	2.48

Table 2. Weight of iron treatments added (66 m moles/kg) to 1 kg of 250 mg/kg arsenic spiked soil.

Iron Source	% Iron	Amount added to 1 kg soil
Peerless-Fe	100 %	3.69 g
$FeCl_3 \bullet 6H_2O$	20.7 %	17.9 g
$Fe_2(SO_4)_3$ (76.4% pure)	21.3 %	17.3 g
Fe-WTR	2.80 %	132 g

Table 3. % Change in extractable arsenic (pore water, Bray-1, Na-phosphate, hydroxylamine HCl, and ammonium oxalate) from each treated soil.

		% Decrease (increase) in extractable arsenic				
		Pore Water	**Bray-1**	**Na-PO4**	**HaHCL**	**AmmOX**
	Positive Cont	0	0	0	0	0
Slag Waste	zero-Fe	(326)	33.0	(55.4)	3.74	(48.0)
59.1% clay	Fe-chloride	16.6	73.7	(64.2)	3.81	(15.7)
33.5% Fe-oxide	Fe-sulfate	89.2	72.1	(62.3)	6.55	(27.6)
	Fe-WTR	94.6	(22.1)	26.4	26.7	9.28
	Positive Cont	0	0	0	0	0
Dennis	zero-Fe	43.3	55.4	27.3	10.1	(0.4)
45.0% clay	Fe-chloride	62.7	54.4	25.7	10.7	15.1
3.4% Fe-oxide	Fe-sulfate	58.4	59.2	19.1	12.9	12.7
	Fe-WTR	100	31.1	46.0	29.4	(32.0)
	Positive Cont	0	0	0	0	0
Perkins	zero-Fe	46.7	74.3	38.9	(14.8)	(21.9)
10% clay	Fe-chloride	95.2	83.9	57.9	(7.59)	(4.50)
2.2% Fe-oxide	Fe-sulfate	67.9	48.4	60.0	27.2	6.10
	Fe-WTR	100	90.2	82.6	26.6	22.8
	Positive Cont	0.0	0.0	0.0	0.0	0.0
Bernow	zero-Fe	92.0	77.0	38.8	7.43	(1.19)
26.3% clay	Fe-chloride	94.9	84.1	52.5	(3.75)	(2.82)
1.5% Fe-oxide	Fe-sulfate	95.9	78.8	50.9	(8.68)	(3.44)
	Fe-WTR	100	86.4	51.9	15.7	(6.33)
	Positive Cont	0	0	0	0	0
Pratt	zero-Fe	42.8	81.8	75.0	10.6	(49.0)
5.0% clay	Fe-chloride	91.2	78.9	68.5	11.4	(28.2)
1.5% Fe-oxide	Fe-sulfate	71.3	68.7	65.4	54.1	22.8
	Fe-WTR	97.4	95.2	89.7	39.4	27.6

Table 4. Ten day germination test and shoot elongation for controls and treated arsenic spiked soils and corresponding amorphous Fe-oxide levels (ammonium oxalate). Values with the same letter are not significantly different at the $P = 0.05$.

Soil	Treatment	Fe-oxides mmol/kg	% Germ		Avg. shoot length, cm	
Slag Waste	Positive Cont	121	83.3	a	4.84	a
Slag Waste	zero-Fe	216	81.7	a	4.11	a
Slag Waste	Fe-chloride	214	75.0	a	3.92	a
Slag Waste	Fe-sulfate	239	83.3	a	4.35	a
Slag Waste	Fe-WTR	397	86.7	a	4.52	a
Slag Waste	Negative Cont		93.8	a	4.66	a
Dennis	Positive Cont	12.1	81.7	a	4.18	ab
Dennis	zero-Fe	34.3	83.3	a	4.22	ab
Dennis	Fe-chloride	26.5	95.0	a	4.63	b
Dennis	Fe-sulfate	28.1	76.7	a	4.36	ab
Dennis	Fe-WTR	193	83.3	a	4.26	ab
Dennis	Negative Cont	14.1	93.3	a	3.99	a
Perkins	Positive Cont	9.3	33.3	b	2.07	b
Perkins	zero-Fe	55.8	85.0	a	4.43	a
Perkins	Fe-chloride	50.4	85.0	a	4.33	a
Perkins	Fe-WTR	137	88.3	a	4.08	a
Perkins	Negative Cont	9.3	90.0	a	3.84	a
Bernow	Positive Cont	5.4	78.3	b	3.41	a
Bernow	zero-Fe	35.0	86.7	b	3.81	ac
Bernow	Fe-chloride	31.5	85.0	b	4.58	b
Bernow	Fe-sulfate	24.0	83.3	b	4.37	bc
Bernow	Fe-WTR	152	90.0	a	4.31	bc
Bernow	Negative Cont	7.3	85.0	b	4.26	ab
Pratt	Positive Cont	2.7	40.0	b	0.93	b
Pratt	zero-Fe	53.4	80.0	a	4.14	a
Pratt	Fe-chloride	34.4	83.3	a	3.67	a
Pratt	Fe-sulfate	16.0	80.0	a	3.76	a
Pratt	Fe-WTR	110	88.3	a	3.93	a
Pratt	Negative Cont	2.0	77.5	a	3.45	a

Table 5. Shoot dry weight results for controls and treated arsenic spiked soils and corresponding amorphous Fe-oxide levels (ammonium oxalate). Values with the same letter are not significantly different at the $P = 0.05$.

Soil	Treatment	Fe-oxides mmol/kg	Average Yield (dry weight), mg		Average Yield/plant, mg
Slag Waste	Positive Cont	121	47.7	a	2.90
Slag Waste	zero-Fe	216	47.7	a	2.90
Slag Waste	Fe-chloride	214	39.3	a	2.50
Slag Waste	Fe-sulfate	239	46.7	a	2.80
Slag Waste	Fe-WTR	397	39.7	a	2.30
Slag Waste	Negative Cont		47.5	a	2.50
Dennis	Positive Cont	12.1	41.5	b	2.50
Dennis	zero-Fe	34.3	43.7	b	2.70
Dennis	Fe-chloride	26.5	71.0	a	3.70
Dennis	Fe-sulfate	28.1	42.7	b	2.90
Dennis	Fe-WTR	193	57.0	ab	3.40
Dennis	Negative Cont	14.1	41.3	b	2.20
Perkins	Positive Cont	9.3	10.3	c	1.10
Perkins	zero-Fe	55.8	45.3	a	2.70
Perkins	Fe-chloride	50.4	38.0	ab	2.20
Perkins	Fe-WTR	137	45.0	a	2.60
Perkins	Negative Cont	9.3	31.0	b	1.70
Bernow	Positive Cont	5.4	28.3	bc	1.70
Bernow	zero-Fe	35.0	46.7	ab	2.70
Bernow	Fe-chloride	31.5	57.3	ab	3.30
Bernow	Fe-sulfate	24.0	39.7	ac	2.30
Bernow	Fe-WTR	152	45.3	ab	2.50
Bernow	Negative Cont	7.3	39.0	c	2.60
Pratt	Positive Cont	2.7	3.7	c	0.70
Pratt	zero-Fe	53.4	31.7	a	2.00
Pratt	Fe-chloride	34.4	33.3	a	2.00
Pratt	Fe-sulfate	16.0	28.0	a	1.80
Pratt	Fe-WTR	110	43.7	b	2.50
Pratt	Negative Cont	2.0	29.7	ab	2.00

Table 6. Pearson correlation coefficients for soil properties pH, amount of clay, ammonium oxalate extractable Fe and Al and total metals (iron, aluminum, manganese and calcium) for arsenic extractability from all soils/materials and treatments. Only soils (no slag waste material) are included in this data set.

		pH	Clay	Fe	FeTot	Al	AlTot	MnTot	CaTot
Pore Water	r	0.25	**-0.41**	-0.23	**-0.64**	**-0.51**	-0.40	**-0.47**	**-0.51**
	P	*0.24*	***0.05***	*0.28*	***0.003***	***0.01***	*0.08*	***0.04***	***0.02***
Bray-1	r	0.12	**-0.46**	-0.23	**-0.62**	**-0.52**	**-0.51**	-0.43	-0.42
	P	*0.58*	***0.02***	*0.28*	***0.003***	***0.01***	***0.02***	*0.06*	*0.06*
Na-Phosphate	r	0.31	-0.24	-0.21	**-0.51**	**-0.37**	-0.28	-0.26	-0.33
	P	*0.13*	*0.26*	*0.32*	***0.02***	***0.07***	*0.23*	*0.26*	*0.14*
Hydroxylamine HCl	r	0.26	-0.02	010	-0.37	0.2	0.1	-0.21	-0.12
	P	*0.21*	*0.92*	*0.64*	*0.11*	*0.91*	*0.95*	*0.37*	*0.59*
Ammonium Oxalate	r	0.17	-0.39	0.31	**-0.56**	-0.22	**-0.65**	**-0.47**	-0.26
	P	*0.42*	*0.06*	*0.14*	***0.01***	*0.30*	***0.002***	***0.04***	*0.25*

Table 7. Earthworm Mortality from a 28 day toxicity bioassay on 5 soils with four different iron treatments. Values with the same letter are not significantly different at the $P = 0.05$.

Soil	Treatment	28 day % Mortality		Depurated worm weight, mg/worm	Undepurated worm weight, mg/worm
Slag Waste	Positive Cont	3.3	a	217	231
Slag Waste	zero-Fe	0	a	206	191
Slag Waste	Fe-chloride	3.3	a	206	236
Slag Waste	Fe-sulfate	0	a	187	181
Slag Waste	Fe-WTR	10	a	206	222
Artificial Soil	Negative Cont	0	a	153	182
Dennis	Positive Cont	6.7	a	193	212
Dennis	zero-Fe	3.3	a	189	202
Dennis	Fe-chloride	0	a	185	200
Dennis	Fe-sulfate	0	a	182	203
Dennis	Fe-WTR	0	a	200	192
Dennis	Negative Cont	0	a	205	226
Perkins	Positive Cont	22.7	b	205	174
Perkins	zero-Fe	0	a	187	184
Perkins	Fe-chloride	3.3	a	174	177
Perkins	Fe-WTR	0	a	200	212
Perkins	Negative Cont	0	a	245	239
Bernow	Positive Cont	3.3	a	175	174
Bernow	zero-Fe	0	a	166	153
Bernow	Fe-chloride	3.3	a	162	181
Bernow	Fe-sulfate	0	a	198	171
Bernow	Fe-WTR	0	a	180	196
Bernow	Negative Cont	0	a	173	163
Pratt	Positive Cont	70	b	184	143
Pratt	zero-Fe	100	c	0.0	0.0
Pratt	Fe-chloride	0	a	209	189
Pratt	Fe-sulfate	20	a	170	185
Pratt	Fe-WTR	0	a	194	216
Pratt	Negative Cont	0	a	164	173

Table 8. Earthworm arsenic body burdens from depurated and undepurated worms from a 28-day toxicity test and the % decrease of arsenic body burden due to remediation of contaminated soils with Fe-treatments.

Soil	Treatment	Depurated As body burden, mg/kg	Undepurated As body burden, mg/kg	%Decrease As body burden Depurated	%Decrease As body burden Undepurated
Bernow	Positive Cont	303	352	0.0	0.0
Bernow	zero-Fe	128	112	57.8	68.1
Bernow	Fe-chloride	86.0	93.4	71.6	73.4
Bernow	Fe-sulfate	81.7	111	73.0	68.6
Bernow	Fe-WTR	35.0	73.6	88.4	79.1
Bernow	Negative Cont	5.87	2.42		
Dennis	Positive Cont	107	126	0.0	0.0
Dennis	zero-Fe	71.6	86.5	33.3	31.5
Dennis	Fe-chloride	75.1	88.8	30.0	29.7
Dennis	Fe-sulfate	54.2	71.3	49.5	43.5
Dennis	Fe-WTR	23.6	36.3	78.0	71.2
Dennis	Negative Cont	8.28	7.13		
Perkins	Positive Cont	457	467	0.0	0.0
Perkins	zero-Fe	323	342	29.3	26.7
Perkins	Fe-chloride	310	247	32.1	47.1
Perkins	Fe-WTR	29.6	72.4	93.5	84.5
Perkins	Negative Cont	6.87	5.06		
Pratt	Positive Cont	475	521	0.0	0.0
Pratt	zero-Fe		*100% Mortality*		
Pratt	Fe-chloride	314	364	33.8	30.2
Pratt	Fe-sulfate	487	506	(2.6)	3.0
Pratt	Fe-WTR	32.6	74.8	93.1	85.7
Pratt	Negative Cont	4.04	3.72		
Slag waste	Positive Cont	157	268	0.0	0.0
Slag waste	zero-Fe	180	202	(14.6)	24.8
Slag waste	Fe-chloride	175	149	(11.9)	44.6
Slag waste	Fe-sulfate	94.7	106	39.5	60.5
Slag waste	Fe-WTR	32.3	70.0	79.4	73.9
Slag waste	Positive Cont	5.37	3.77		

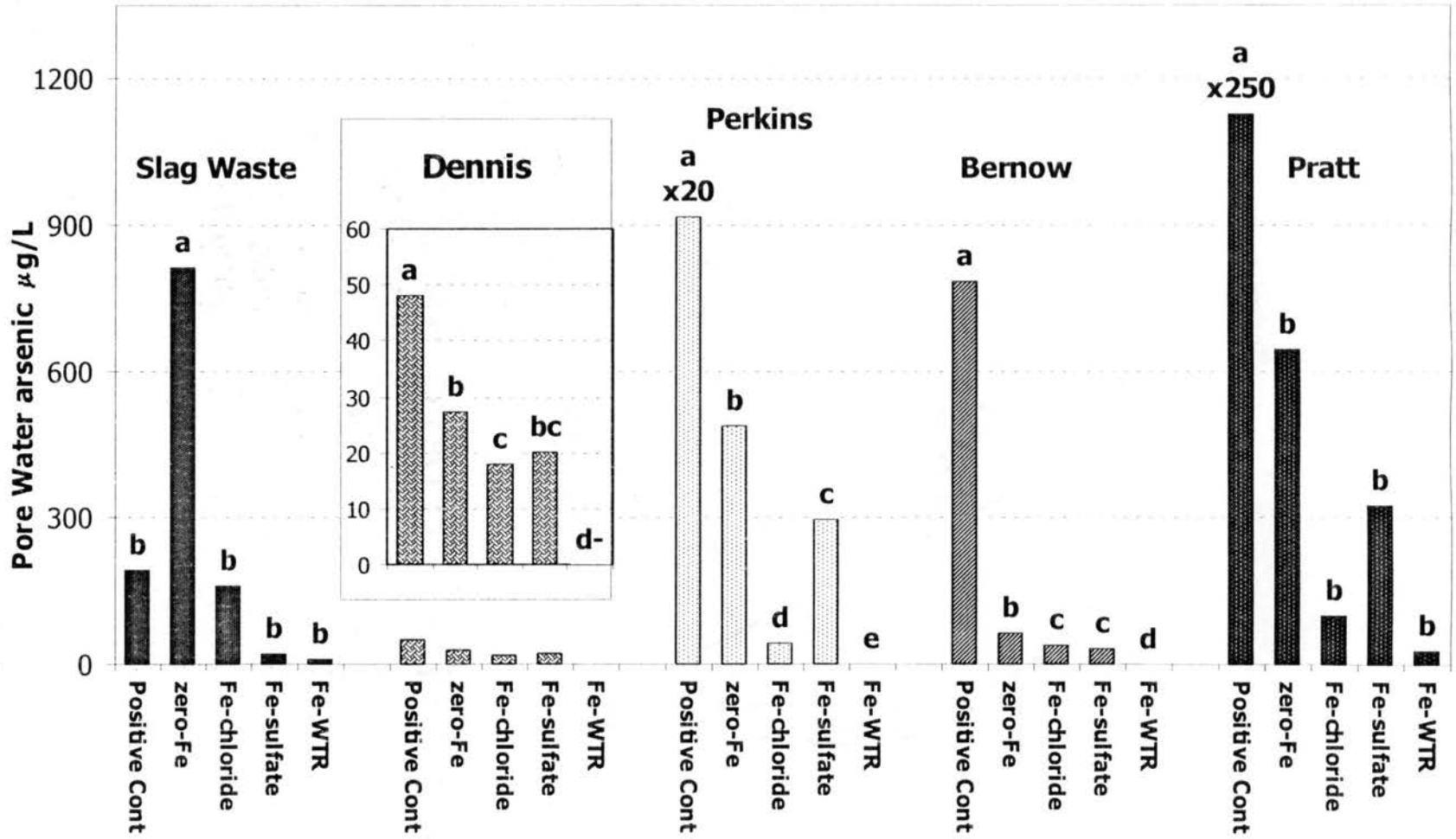

Fig. 1. Pore water arsenic from positive control and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

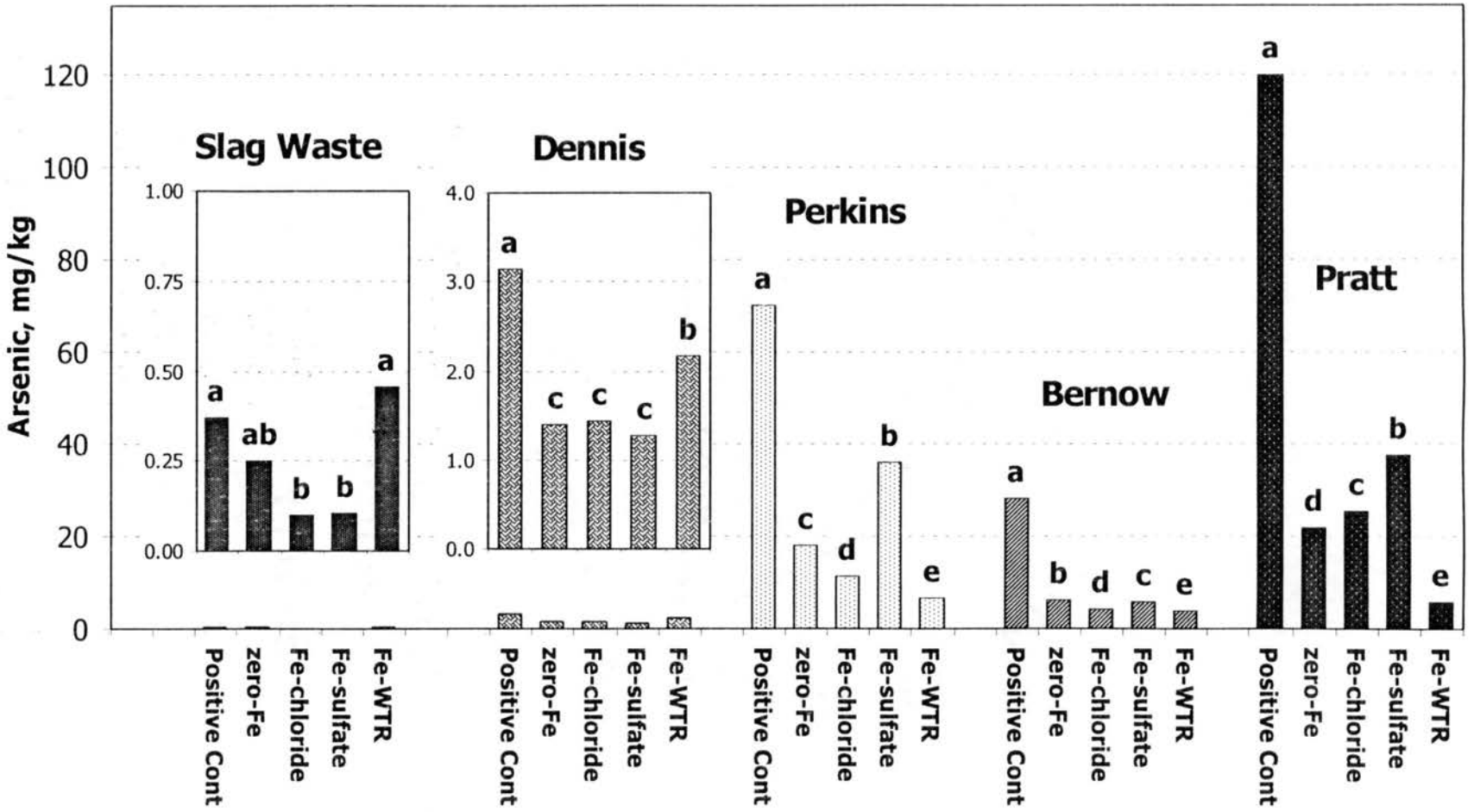

Fig. 2. Bray-1 extractable arsenic from positive control and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

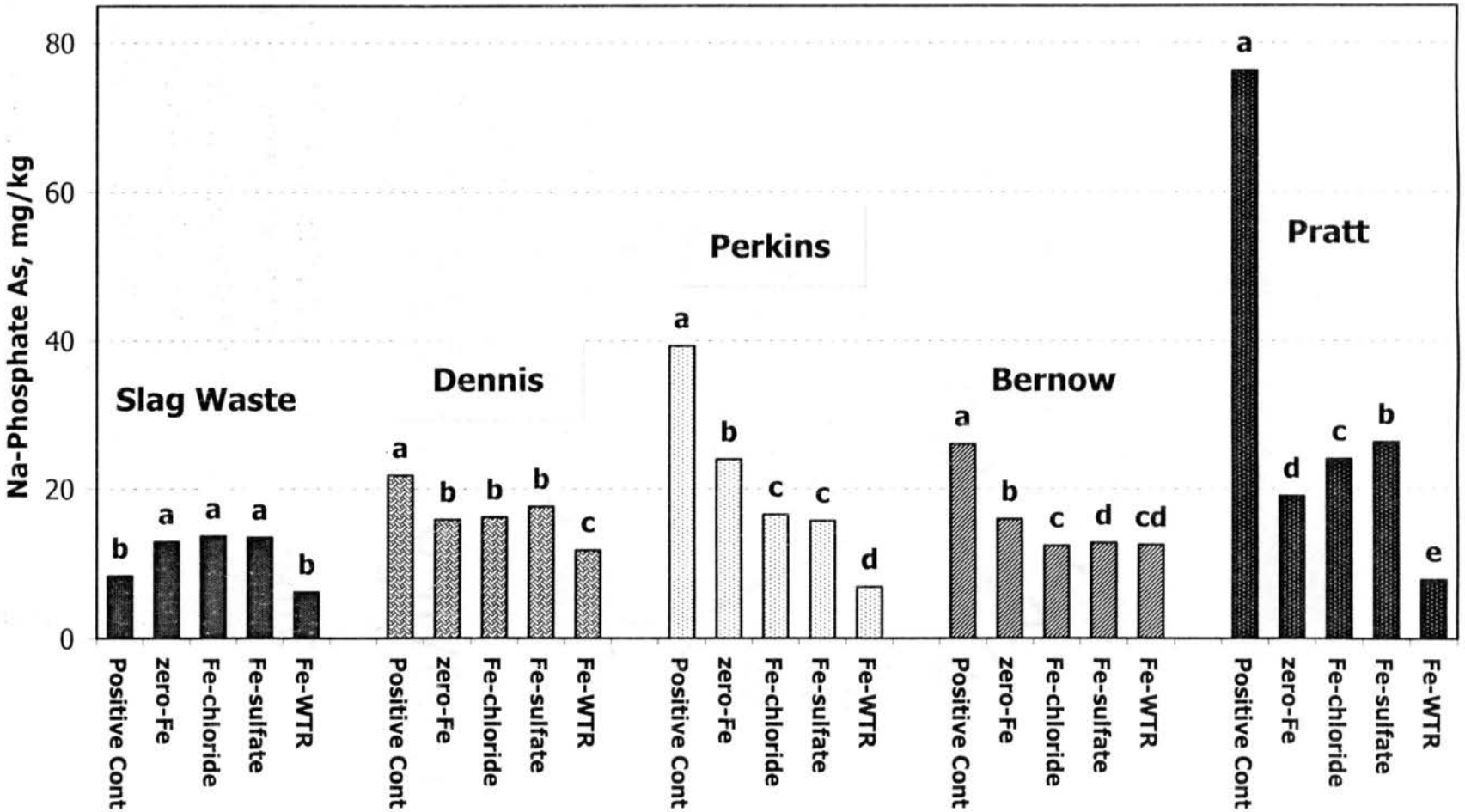

Fig. 3. Na-Phosphate extractable arsenic from positive control and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

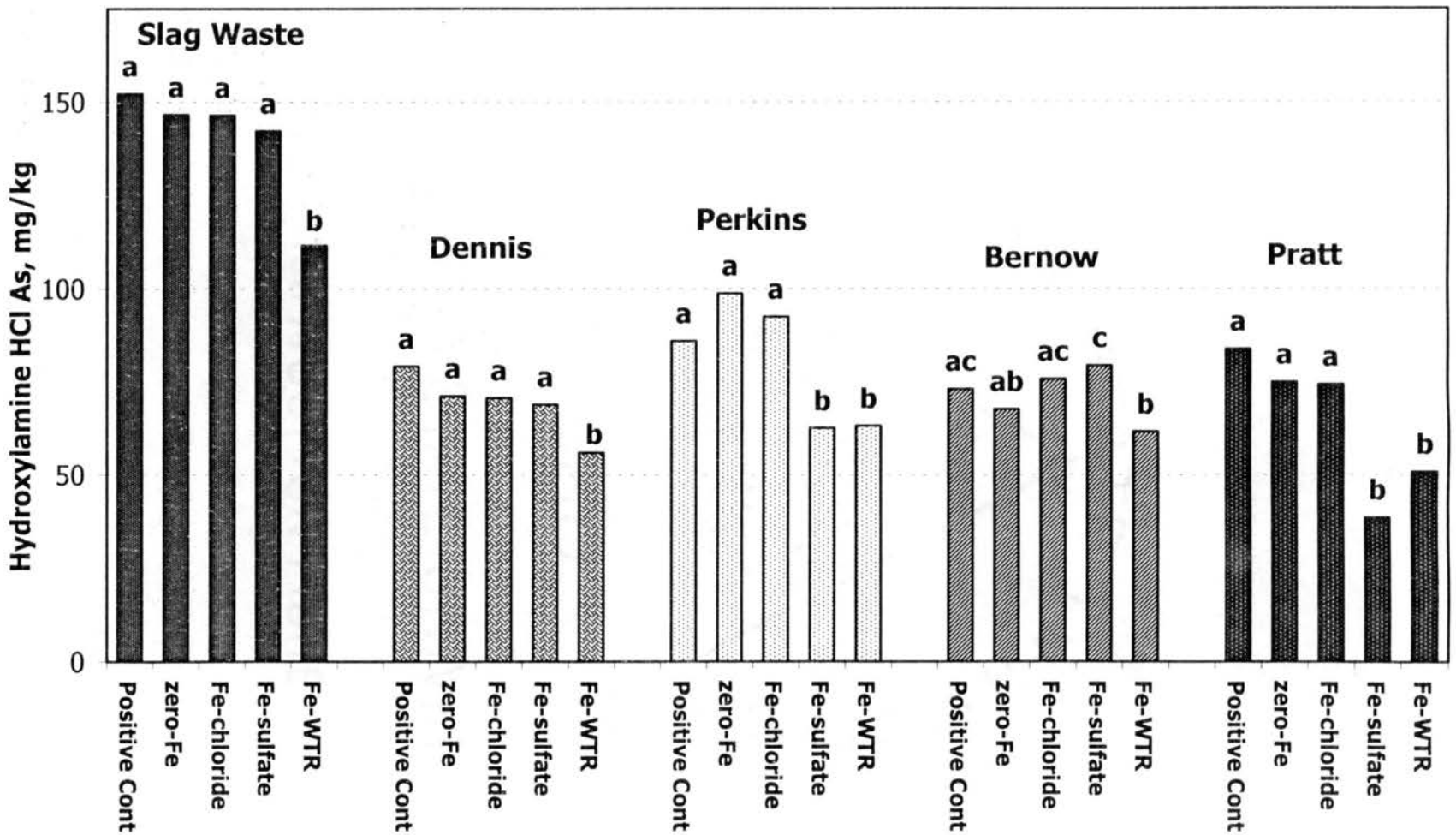

Fig. 4. Modified Hydroxylamine HCl extractable arsenic from positive control and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

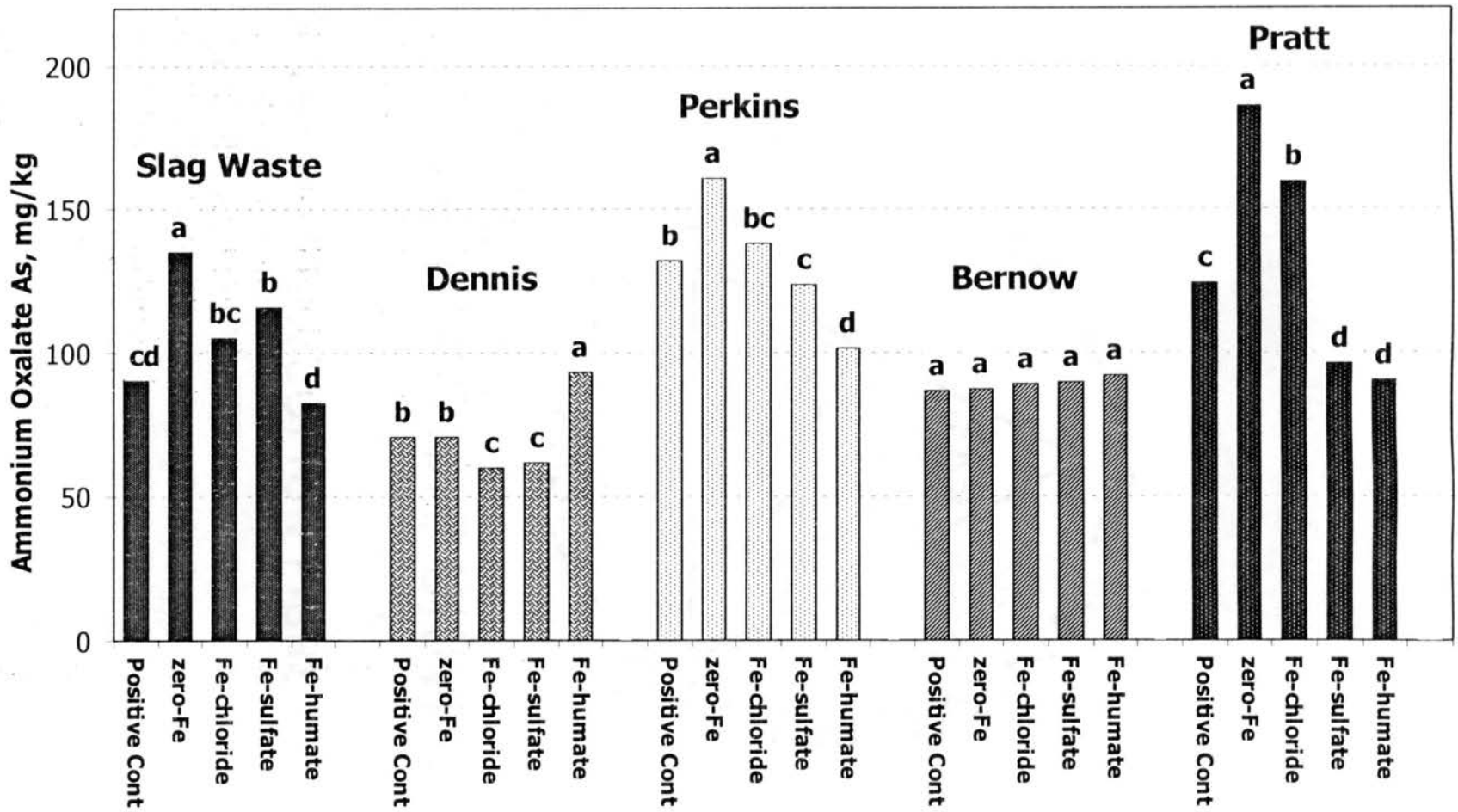

Fig. 5. Modified Ammonium Oxalate extractable arsenic from positive control and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

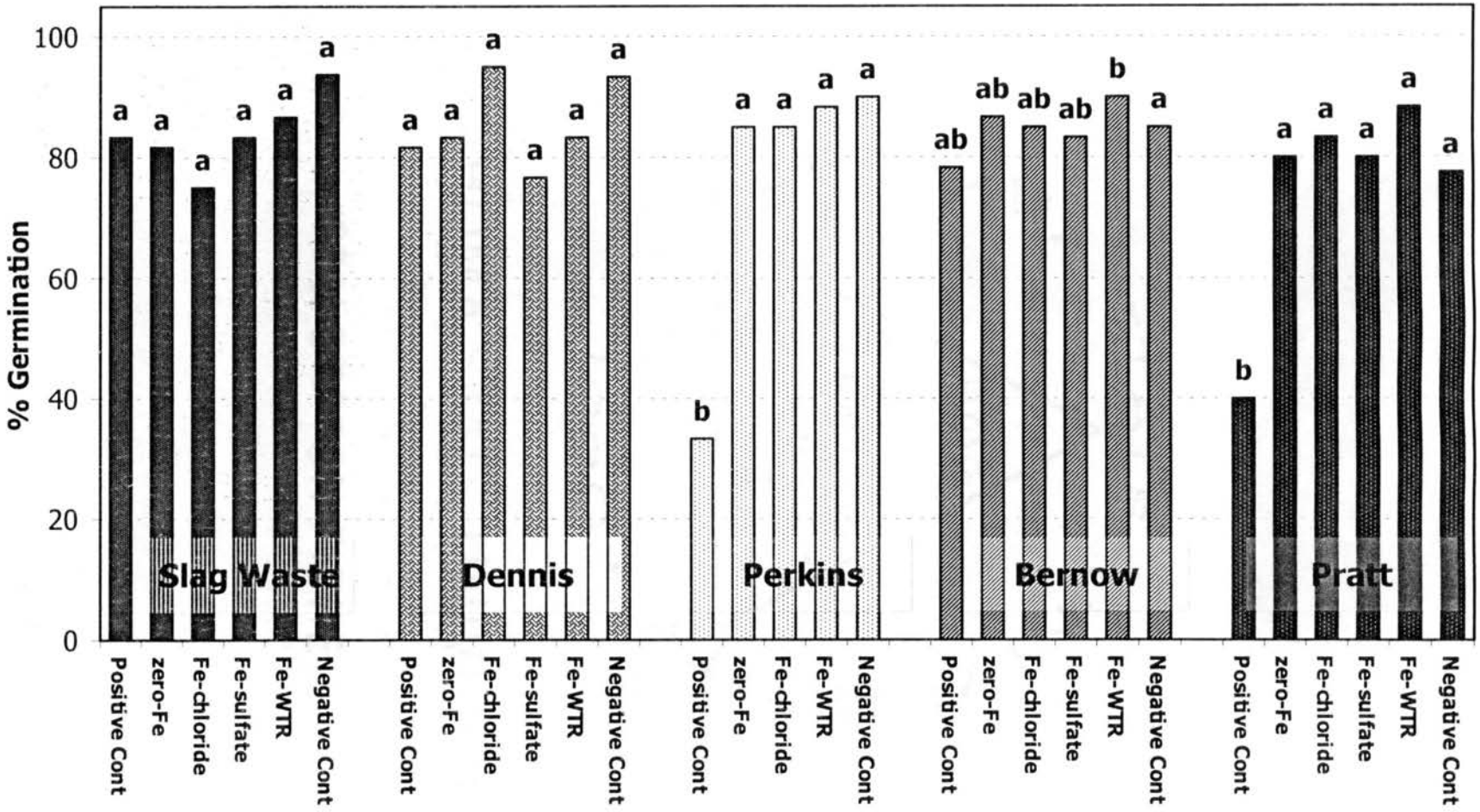

Fig. 6. % Germination from positive and negative controls and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

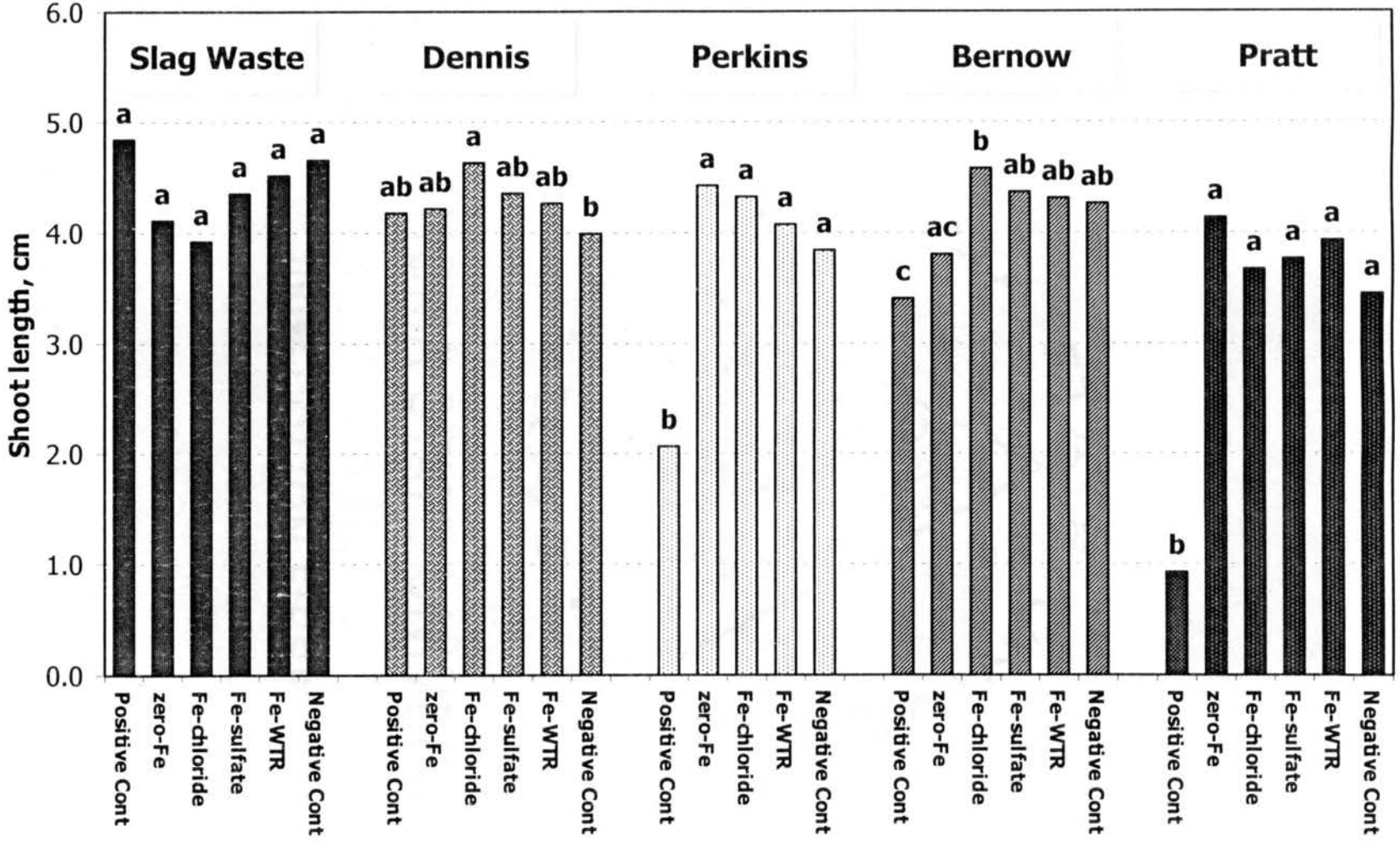

Fig. 7. Shoot length from positive control and treated arsenic spiked soils and mine waste material. Bars with the same letter are not significantly different at the $P = 0.05$.

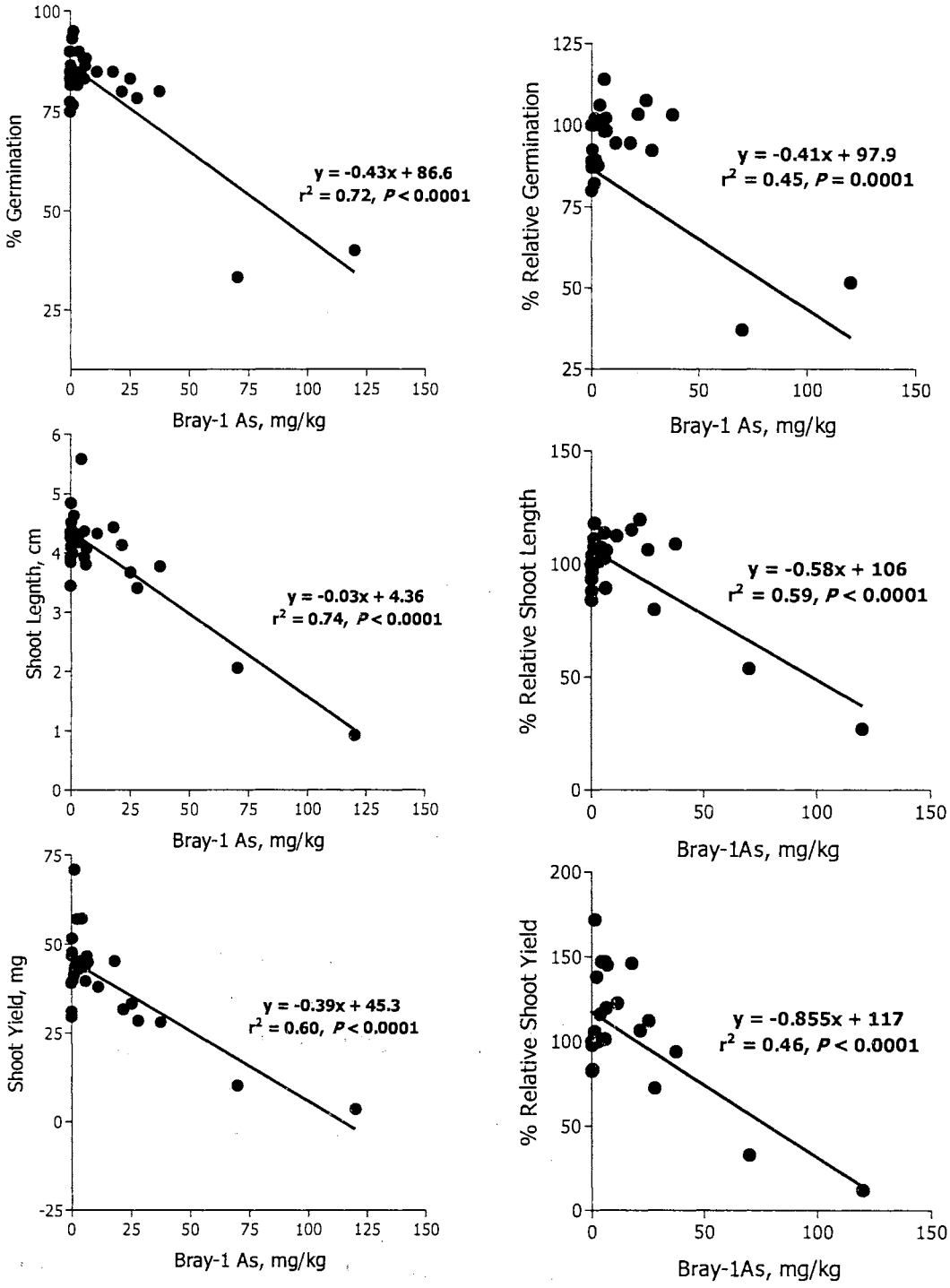

Fig. 8. Bray-1 extracted arsenic with lettuce tests endpoints: % germination, shoot length and shoot yield and the % relative of each endpoint (lettuce response in As spiked & treated soils/lettuce response positive control soils * 100).

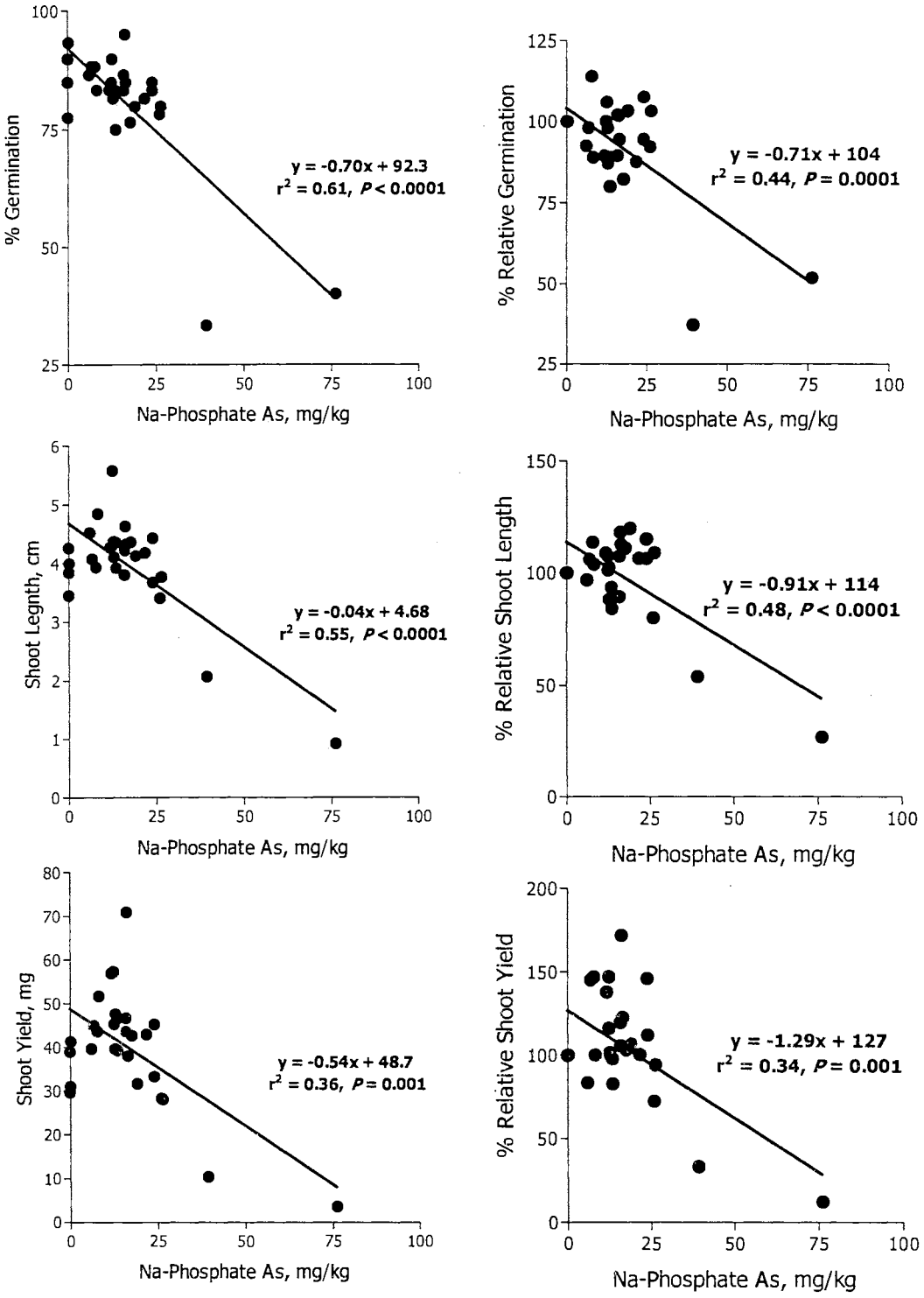

Fig. 9. Na-Phosphate extracted arsenic with lettuce tests endpoints: % germination, shoot length and shoot yield and the % relative of each endpoint (lettuce response in As spiked & treated soils/lettuce response positive control soils * 100).

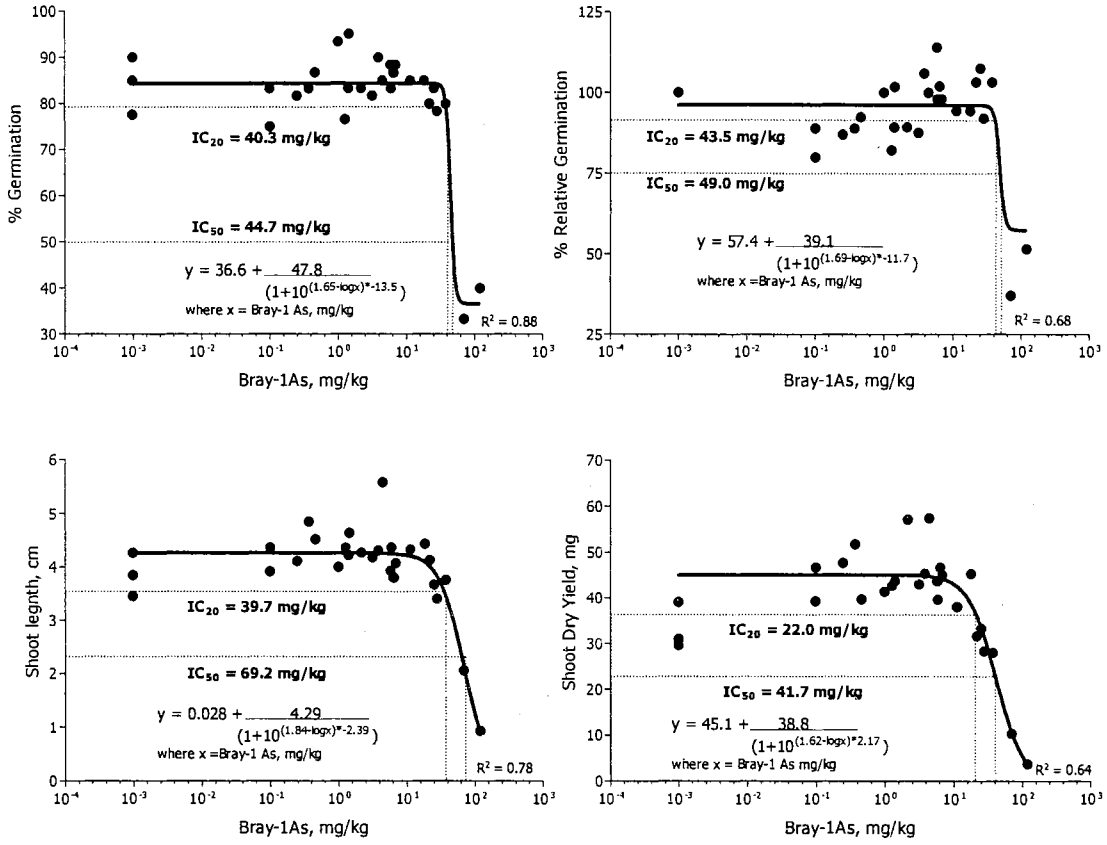

Fig. 10. Concentration response curves for Bray-1 arsenic with plant endpoints; % germination, % relative germination, shoot yield, and % relative shoot yield.

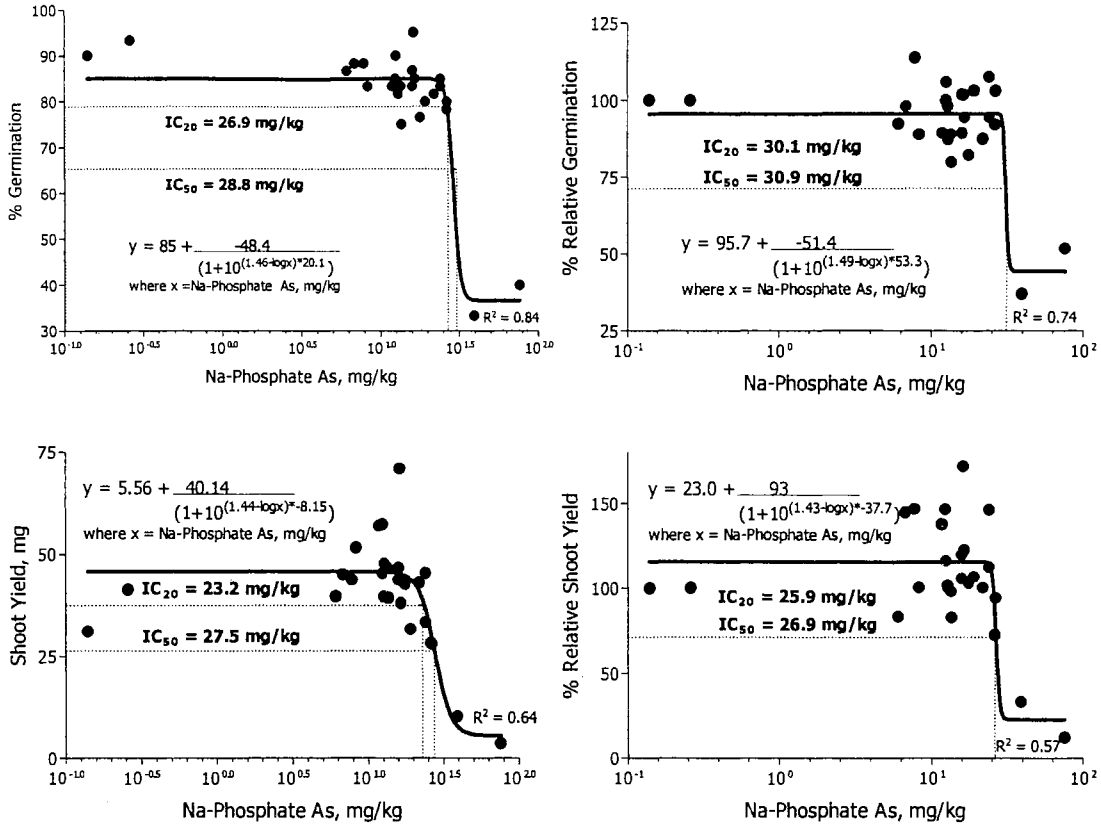

Fig. 11. Concentration response curves for Na-phosphate arsenic with plant endpoints; % germination, % relative germination, shoot yield, and % relative shoot yield.

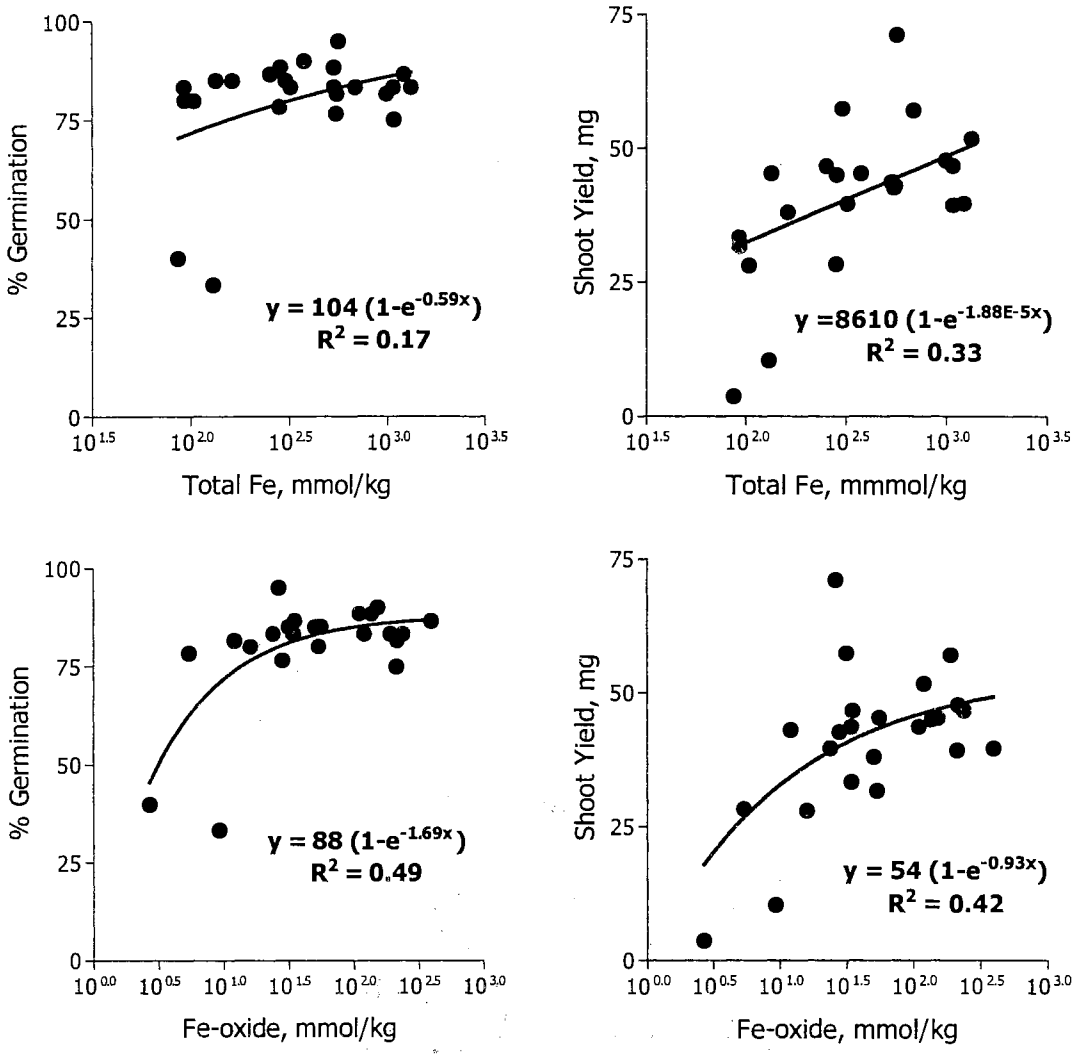

Fig. 12. Total Fe (EPA Method 3051) and Fe-oxide (ammonium oxalate) with plant endpoints % seed germination and shoot yield.

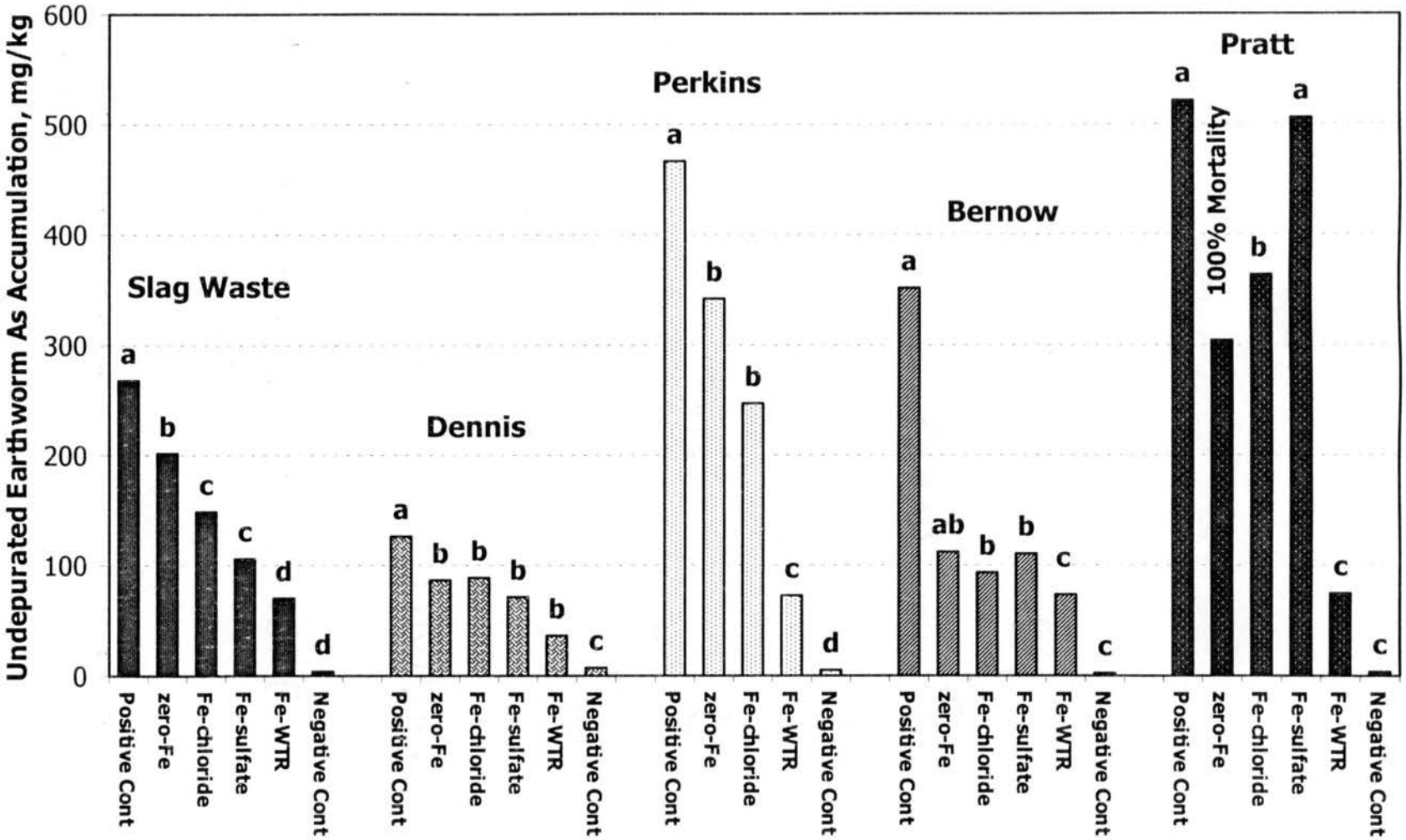

Fig. 14. The earthworm arsenic body burden from positive control and treated arsenic contaminated soils for undepurated worms. Bars with the same letter are not significantly different at the $P = 0.05$.

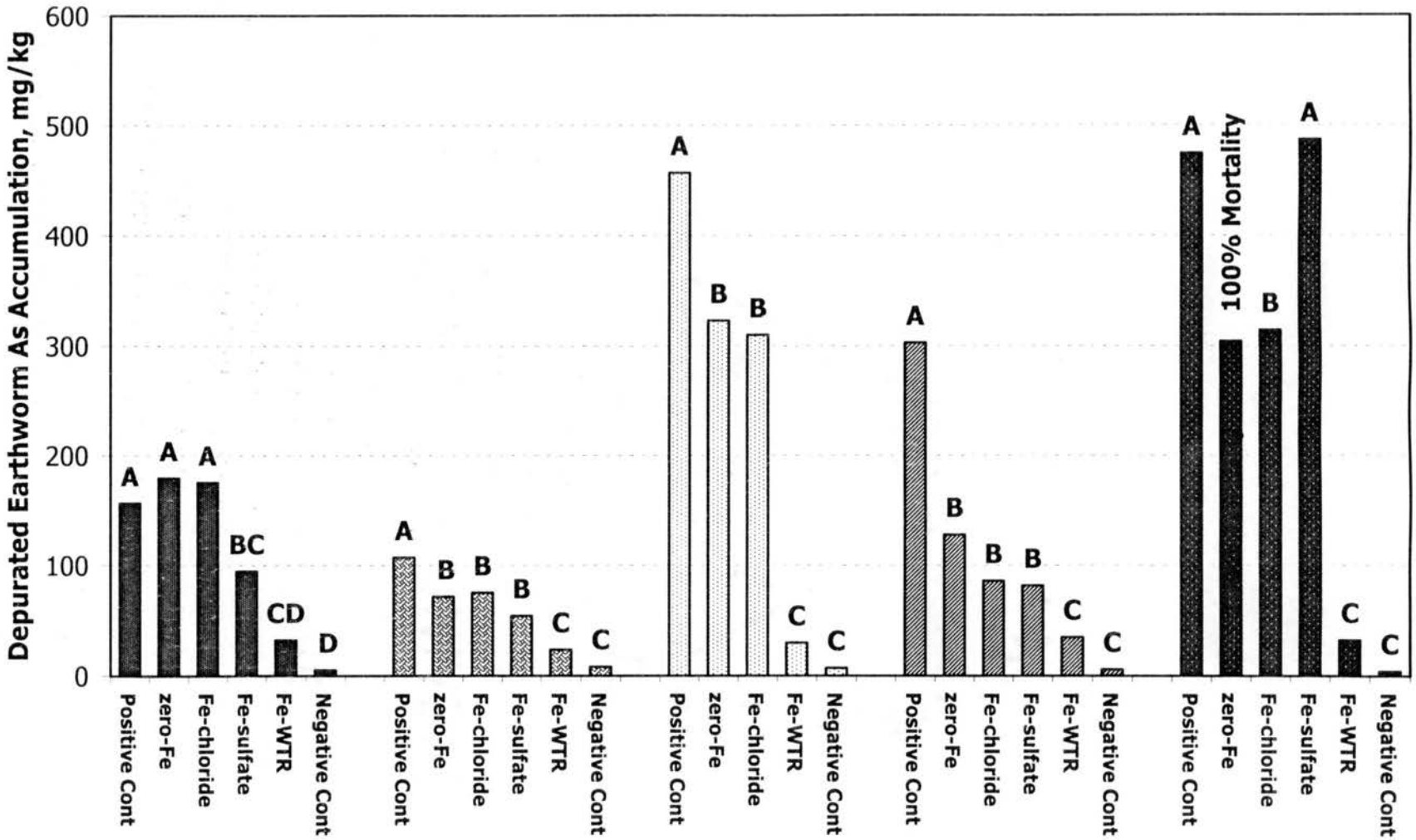

Fig. 13. The earthworm arsenic body burden from positive control and treated arsenic contaminated soils for depurated worms. Bars with the same letter are not significantly different at the $P = 0.05$.

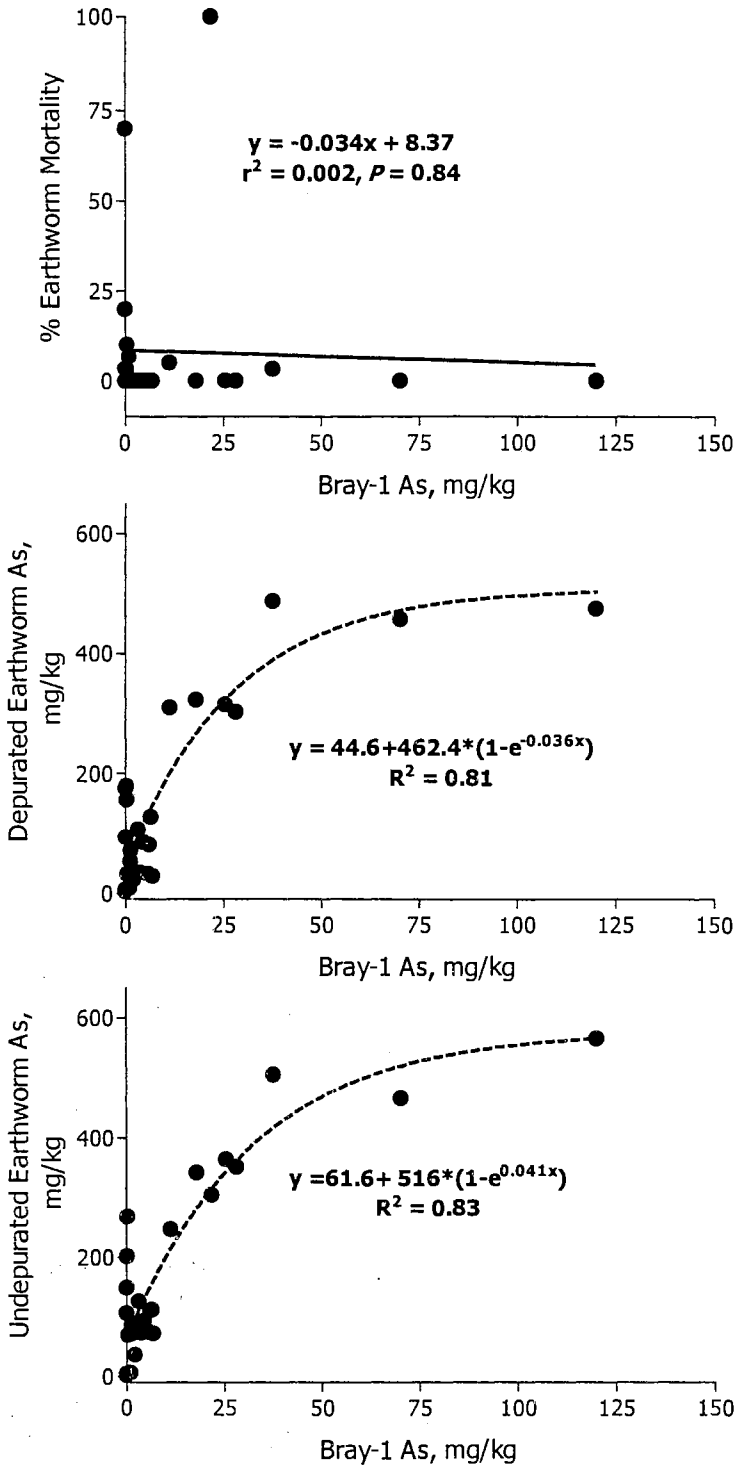

Fig. 15. Bray-1 arsenic with earthworm %mortality and arsenic concentrations (body burdens) in depurated and undepurated worms.

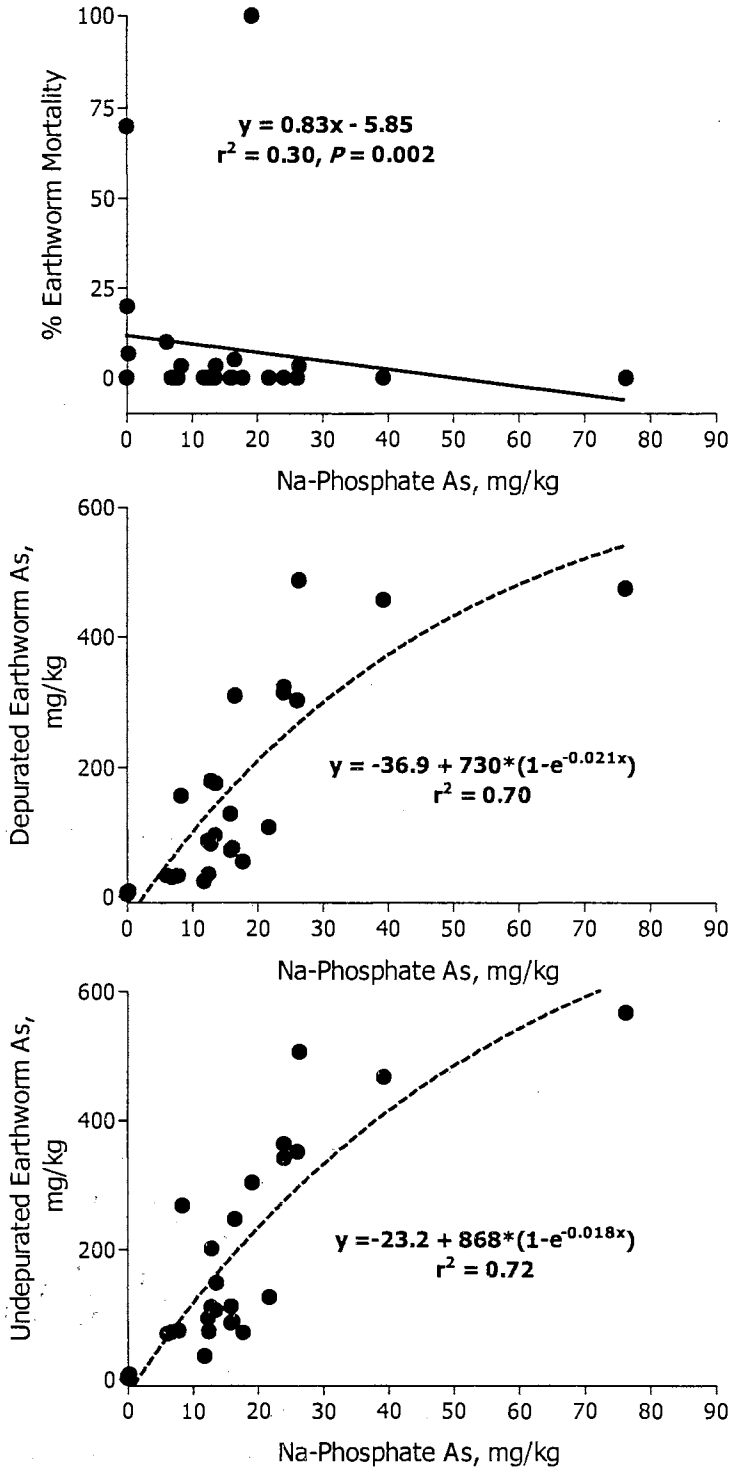

Fig. 16. Na-phosphate arsenic with earthworm %mortality and arsenic concentrations (body burdens) in depurated and undepurated worms.

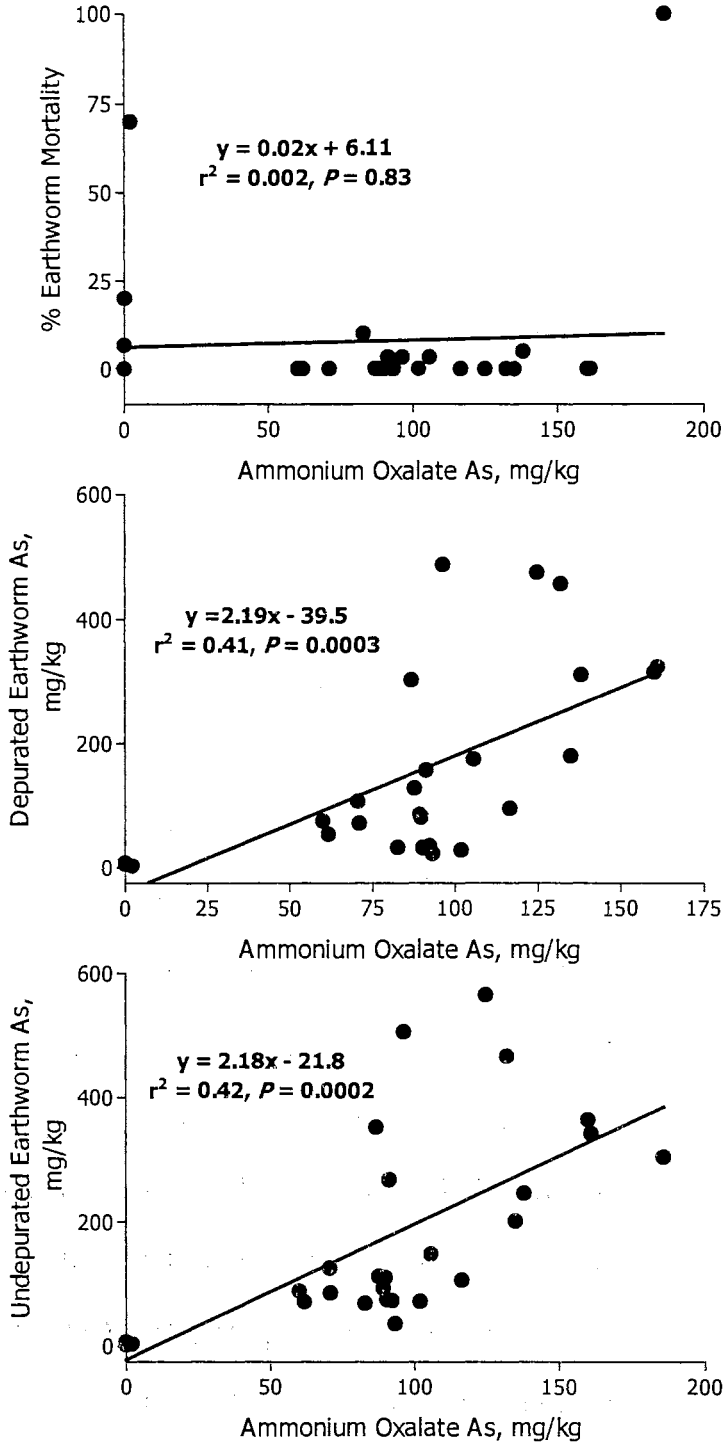

Fig. 17. Ammonium oxalate arsenic with earthworm %mortality and arsenic concentrations (body burdens) in depurated and undepurated worms.

CHAPTER III

ARSENIC BIOACCESSIBILITY FROM IRON-BASED REMEDIATED ARENIC CONTAMINATED SOIL

ABSTRACT

Alternative technologies for remediation of arsenic contaminated soils have gained increasing attention. Immobilization of soil contaminants by treating with Fe-compounds is successful in reducing potential leaching and phytotoxicity to plants. This study investigates the ability of four Fe-compounds: zero-valence Fe, $FeCl_3$, $Fe_2(SO_4)_3$, and Fe-water treatment residuals (Fe-WTR), to reduce gastrointestinal (GI) bioaccessibility in As contaminated soil. Four As-spiked soils, Dennis, Bernow, Perkins, Pratt and one slag waste were treated with the Fe-compounds and incubated for 4 weeks with intermitted wetting/drying to promote reaction between the soil and Fe-compounds. Human GI accessibility was estimated using a modified *in vitro* method (1% pepsin and 0.15M NaCl) and %relative bioavailability (%RBA= GI-As/Total As *100) was determined. All Fe-treatment reduced arsenic %RBA. Dennis soil went from 16.6% (untreated) to 10.3% with zero-Fe, 10.9% with $FeCl_3$, 12.0% with $Fe_2(SO_4)_3$ and 8.6% with Fe-WTR treatment. Bernow followed the same pattern with untreated soil %RBA of 17.3% to 13.9%, 10.2%, 10.5% and 5.1% with zero-Fe, $FeCl_3$, $Fe_2(SO_4)_3$ and Fe-WTR treatments respectively. Perkins soil went from 38.3% (untreated) to 19.2% with zero-Fe, 15.6% with $FeCl_3$, 25.9% with $Fe_2(SO_4)_3$ and 7.1% with Fe-WTR treatment. Pratt soil had the highest %RBA, 83.7%, in the untreated soil to 20.7% with

zero-Fe, 24.3% with $FeCl_3$, 32.5% with $Fe_2(SO_4)_3$ and 6.8% with Fe-WTR treatment. Slag material went from 39.8% (untreated) to 32.5% with $FeCl_3$, 32.8% with $Fe_2(SO_4)_3$ and 24.1% with Fe-WTR treatment. Hazard quotients and human health risk from soil ingestion were reduced with the addition of Fe-compounds to arsenic contaminated soil. Although the Fe-WTR was inconsistent in amorphous Fe content, it had the greatest reduction across all soils and slag and provides the greatest reduction of human exposure to As from contaminated soils.

Keywords— Arsenic Arsenic extractability bioavailable arsenic human health arsenic remediation iron remediation risk assessment

INTRODUCTION

Arsenic is a notorious toxic element found all over the world as an indigenous element in soil. It has been used for decades as herbicides, desiccants and insecticides. More recent industrial used of arsenic include solid-state devices, laser material and bronzing. Mining and smelting activities has traditionally resulted in areas with high amounts of arsenic contamination. These activities are important sources of environmental degradation and have adverse effect on the environment from exposure to heavy metals and metalloids, in particular arsenic. Sites high in soil arsenic poise a risk to human health, phytotoxicity and contamination of soil and water. All soluble inorganic forms of arsenic appear to have toxic effects on humans. Various forms of arsenic have been shown to cause skin cancer and possibly increase the risk of developing lung, bladder, colon and kidney cancer (Hrudey et al. 1996, National Toxicity

Program). The primary routes of exposure to arsenic and arsenic compounds for humans are inhalation, ingestion and dermal contact. Inhaled airborne arsenic has been found to be rapidly absorbed into the systemic circulation through deposition in the respiratory tract (Adriano 2002). Eighty-five to 90% of water-soluble arsenic (III) in lungs can be bioavailable to humans (Hrudey et al. 1996). Ingestion of arsenic can be in the form of water, food or soil. Humans and animals rapidly absorb water-soluble inorganic arsenic. Bioavailability of other As species varies with the chemical form and water solubility (Adriano 2002). Organic arsenic, mainly found in food, is bioavailable depending on water solubility. Arsenobetaine-rich seafood are readily eliminated from the body in an unchanged form and seldom accumulate to toxic levels whereas, arsenosugars found in seaweed are metabolized (Smith et al. 1998). Generally organic arsenic found in seafood, has low toxicity to humans and as a result there is little potential hazards from consumption. Soil bound arsenic, generally, are unlikely to be a threat for human accumulation in uncontaminated areas. In contaminated area, residents nearby have a risk of arsenic ingestion through soil or dust. Children are especially at risk due to incidental ingestion of soil (Basta et al. 2001).

Incidental soil ingestion is due to hand-to-mouth activity and represents a significant direct exposure pathway for non-dietary sources of arsenic in contaminated areas (Chaney and Ryan 1994, Duggan et al. 1985, Wixson and Davies 1994). Arsenic contaminated soils often present an unacceptable risk to human health and must be remediated. The traditional way of remediating a metal or metalloid contaminated soil, have involved physical removal by excavation and transporting the contaminated soil to hazardous waste landfill. Some may believe this is the best way to "clean" the site, but

with the increasing cost of excavation and transportation and the limited number of hazardous waste landfill, other alternatives have become attractive (EPA 1997).

Two alternative technologies being used are metal removal through a pump and treat or soil washing system and immobilization/stabilization methods. Metal removal can be done either *in situ* or *ex situ,* through soil washing or pump-and-treat technology (EPA 1997). This approach can be difficult, time-consuming and incomplete due to the strong reactions between metal cations and soil components. Soil washing with acid solutions increases the removal of the metal cations but can leave the soil sterile and difficult for plant rehabitation and is ineffective for arsenic removal. As an alternative, chelating agents or surfactants that readily bind and remove metals have been used with some success. Immobilization of heavy metals is generally achieved through increasing the pH, causing precipitation, and binding the metal cation in the soil (EPA 1997). This approach is not effect for arsenic due to its oxyanion structure. Immobilization of arsenic has been tested using ferric chloride and ferric acetate. In an *in situ* pilot scale study, ferric chloride and ferric acetate reduced leaching of arsenic contaminated soil over an 11-day period (Stammier et al. 1992). Iron is considered on of the best metallic material for remediation because it's nontoxic and inexpensive (Shokes and Moller 1999).

Current arsenic risk assessment and toxicity study values for oral bioavailability are based on arsenic in drinking water in which arsenic is in a soluble form and has high bioavailability (e.g. 95% to 100%). Use of these values in determining the risk to human health from arsenic contaminated soil does not reflect the actual availability to humans. Bioavailability of arsenic in soils can be defined as the percentage of inorganic arsenic absorbed into the body from soil as compared to that absorbed from drinking

water. Using rabbits and an *in vitro* method, Davis et al. (1992) found that several factors control the accessibility of metals from the soil. These include mineral composition, amount of encapsulation, and rate of dissolution in the gastrointestinal (GI) tract. Arsenic in soil was found to be five times less available than As from Na_2HAsO_4 salt (Rodriguez et. al 1999). Animal models, as human surrogates, have been used to determine contaminant bioavailability via the ingestion pathway. Immature swine, rats, and rabbits have been used to simulate GI bioavailability to humans (Dieter et al. 1993, Rodriguez et. al 1999, Ruby et al. 1999). Determining bioavailability with animal models is expensive and time consuming, requiring specialized facilities and specialized personnel. Chemical *in vitro* laboratory methods do not have the disadvantages associated with animal models and have been successfully used to estimate arsenic bioavailability (Rodriguez et. al 1999, Ruby et al. 1999).

Treatment of arsenic contaminated soils with various Fe-containing compounds has shown to be effective in reducing potential risk of arsenic leaching and reducing arsenic phytotoxicity to plants (Chapter 2). Little information is available for the bioaccessibility of arsenic from contaminated soils treated with Fe-compounds. The objective of this work is to evaluate the effectiveness of four Fe-treatments: zero-valence Fe, $FeCl_3$, $Fe_2(SO_4)_3$ and Fe-water treatment residuals, to reduce soil arsenic accessibility to human GI systems.

MATERIALS AND METHODS

Four soils were selected to provide a range of amorphous Fe-oxide content (Table 1). One slag waste soil was also selected to test the ability of iron-based

remediation methods on mining waste. Various soil properties of the soils selected for this study are shown in Table 1. Soils were spiked with 250 mg/kg arsenic using reagent grade Na_2AsO_4. The arsenic was dissolved in 1L of de-ionized distilled water and mixed with the soil in large aluminum pans. The soils were thoroughly saturated and mixed well, and then put into a drying oven set at 65-70°C for 24 h. After the initial drying, the soils were saturated again, mixed and dried another two times for a total of 3 wet/dry cycles. After the last drying cycle the soil was homogenized and divided into subsets for iron remediation/treatment.

Four iron sources were used for remediation/immobilization/treatment the arsenic contaminated soils. Two iron sources were reagent grade $Fe_2(SO_4)_3$ and $FeCl_3$. The third iron source is Peerless-Iron, a zero valence iron. The last source is Fe-water treatment residue, Fe-WTR (sold commercially as Fe-humate). It is a by-product of water treatment facilities that use iron as a coagulant in the drinking water production process. All soils and slag waste were treated on a 20:1 Fe:As molar ratio as shown in Table 2. Iron chloride and $Fe_2(SO_4)_3$ were dissolved in 1L de-ionized distilled water and thoroughly mixed with the soils additional water was added as needed to make slurry. Peerless-Fe (zero-Fe) and Fe-WTR were mixed with dry soil then saturated and mixed again. The saturated soils were place in a constant temperature room set at 33 ± 4°C. The soil-treatments were saturated and mixed thoroughly 2-4 times a week. Sub-samples of each soil-treatment were taken weekly and pore water pH, arsenic and iron content were measured (Appendix B). After 4 weeks of incubation the pore water pH and arsenic content were stable and the incubation was terminated. The pore water pH and electrical conductivity (EC) were measured on each soil-treatment. If the EC exceeded 2 dS/m, the soil was leached by mixing the soil with excess de-ionized water

in a bucket, allow the soil to settle (sit un-disturbed for >24 h), then removed the excess water. If the pH was below 4 the soil was gently limed (5-10 g lime/kg soil) with $CaCO_3$ to achieve the target pH of 5.0. The $FeCl_3$ and $Fe_2(SO_4)_3$ treatments are very acid forming which caused the pH to decrease greatly. The soils were then oven dried at 70°C and place in sealed containers. Total arsenic, iron and other elements were determined using a nitric acid microwave digestion, EPA Method 3051 (USEPA 1994). This analysis was performed to confirm the total amount of arsenic in soil after processing. Certified reference material (CRM020-050, RTC Corporation, Laramie, WY, USA) as well as blanks and spikes were used for quality assurance/quality control (Appendix B).

In vitro (IVG) Method to Assess Human Bioavailability.

The ability of treatments to reduce As bioavailability to humans was evaluated by using a modified *in vitro* gastrointestinal (IVG) method (Rodriguez et al., 1999). Modifications made to the Rodriguez procedure were elimination of dough-dosing additive. A pepsin solution consisting of 10g/L Pepsin and 8.78g NaCl/L was mixed well then purged with argon gas for 30-60 minutes using an air stone and stir plate for mixing. Decanol was added as needed (~1mL) to control foaming. One gram of test soil was placed into a tall 300mL beaker. Once purged, 150mL of pepsin solution was added to the soil and placed into a water bath set at 37°C. The water bath was equipped with a submersible stir plate. The soil-pepsin solution was mixed with a stir bar while drops of conc. trace metal grade hydrochloric acid was added to reduce the pH to 1.8. Once the pH reached the target, the mixture was held at that pH for one hour while monitoring and adjusting pH as needed. At the end of the hour, a sample of the mixture was removed and centrifuged for 5 minutes at 10,000 RPM. The sample

was filtered through 0.45micon filter and stored at 4°C until analysis with inductive couple plasma-hydride generation.

Measurement of As by ICP-HG

To determine low level arsenic concentration and to remove potential interferences found on direct analysis of sample digest, inductively coupled plasma atomic emission spectroscopy with hydride generation (ICP-HG) was used. The hydride generator is a batch type produced by Thermo Jarrell Ash (TJA) for the IRIS TJA ICP. Reaction rates for hydride generation are controlled by several variables: 1) chemical forms of the arsenic, 2) oxidation state of the hydride-forming element, 3) acid concentration, and 4) concentration of the $NaBH_4$ reducing agent. The least error occurs when these condition are set so that the reaction goes to completion instantaneously. Inorganic arsenic, arsenate (V) and arsenite (III), can be reduced to arsine gas through the hydride process, although reduction of arsenate is more time consuming. Therefore all samples (10-50 mL) are pre-treated with 40% potassium iodine + 4% ascorbic acid solution (0.5 mL) to reduce all As to arsenite (III), the faster reacting As form (TJA, Dedina and Tsalev, 1995). Hydrochloric acid is used as the sample medium to form hydrides. Samples were mixed with concentrated HCl to a concentration of 3 molar. The efficiency of hydride formation is constant at HCl concentrations above one molar (Dedina and Tsalev 1995). A 0.5% $NaBH_4$ solution with 0.42% NaOH for stability was used as the reducing agent. Acidified sample and base were pumped to the reactor chamber at a rate of 3.25 mL /minute with a 2 minute rinse between samples to eliminate carry over. Arsine gas was separated in a gas-liquid separator via nebulizer directly into the ICP argon plasma. Tri-n-butylphosphate was added to samples to reduce foaming (Murer et al. 1992)

RESULTS AND DISCUSSIONS

Remediation Affect on IVG Bioaccessibility

Soil Fe-treatments were successful in reducing the amount of bioaccessible (IVG) arsenic from contaminated soils (Fig. 1). All the amended soils, Dennis, Bernow, Perkins and Pratt, across all treatments had significant ($P < 0.05$) reduction of IVG arsenic availability as compared to the positive controls. The greatest reduction of IVG As was obtained with the Fe-WTR treatment in all soils and slag waste material (Table 3). The largest decrease was in the Pratt soil with > 70% reduction of IVG As in all Fe-treated soils. Fe-WTR had the best success with a reduction of IVG As from 250 mg/kg (positive control) to 28.2 mg/kg IVG As (Table 3). In general, greatest reduction in the arsenic contaminated soil treatments followed the order Fe-WTR> Fe-chloride ≈ zero-Fe > Fe-sulfate. The slag waste material somewhat different trend with reduction followed the order Fe-WTR> Fe-chloride = Fe-sulfate. The zero-Fe amendment has no significant difference from the untreated slag.

The As solubilized by the IVG procedure was greatly influenced by the amount of indigenous clay in the soil. The positive controls that had the lowest amount of IVG As were the soils with the greatest amount of clay (Table 3). Arsenic bioaccessibility followed the order Dennis (40.6% clay) < Bernow (23.8% clay) < Perkins (10.0% clay) < Pratt (6.25% clay). *Geophagia,* the phenomena where animals intentionally ingest non-nutritive soil, may detoxify contaminants by providing adsorption sites. *Geophagia* has been shown to be a positive factor in such issues as: the response to stress and arthritis in rats (Burchfield *et al.*, 1977), reducing the ^{137}Cs content of meat and eggs in

hens (Andersson *et al.*, 1990), and reducing the pH and ammonia level in the intestines of ruminants fed low quality roughage supplemented with molasses (Stephenson *et al.*, 1992). *Geophagia* has also been reported for humans. Early Indians of the American Southwest and Mexico intentionally consumed clays with wild potatoes in order to eliminate the bitter taste and to prevent stomach pains and vomiting associated with eating large quantities of wild potatoes (Johns, 1986). *Geophagia* may have detoxified alkaloids, tannins, and quinones in the pre-evolved potato (Johns, 1986). Arsenic bioaccessibility followed the order Dennis (40.6% clay) < Bernow (23.8% clay) < Perkins (10.0% clay) < Pratt (6.25% clay).

Human Health Risk Assessment

Remediation of arsenic contaminated soils is usually risk-based. Risk is a function of bioavailability. Carcinogenic risk is calculated by the following equation:

$$\text{Risk} = \text{CDI} \times \text{SF}$$

where:

Risk = A unitless probability (e.g., 1 x 10^{-6} meaning 1 in 1,000,000)
CDI = Chronic daily intake, averaged over 70 yr (mg/kg-d)
SF = Cancer slope factor (mg/kg/d)

Furthermore, $$CDI(mg/kg-day) = \frac{CS \bullet IR \bullet CF \bullet EF \bullet ED}{BW \bullet AT}(BIO)$$

Where:

CS = Chemical concentration in soil (mg/kg)
IR = Ingestion Rate (mg soil/day)
CF = Conversion factor (10-6 mg/kg)
EF = Exposure frequency (days/year)
ED = Exposure duration (years)
BW = Body weight (kg)

AT = Averaging time (period over which exposure is averaged – days)

BIO = "Gastro-intestinal bioavailability"

Current risk assessment methodologies for heavy metal/metalloids contaminated soils utilize total metal content for chemical concentrations (CS) in the soil rather than bioavailable concentrations. Bioaccessibility-bioavailability of contaminated soil can be depicted as a multi-step process (Fig. 2). The contaminant goes through dissolution in the gastrointestinal tract followed by contaminant absorption into systematic circulation. Since the rate-limiting step for soil arsenic is contaminant dissolution, the IVG %Relative Bioavailability (%RBA) gives a reasonable measure of bioavailability for human health risk assessment (Rodriguez et. al 1999). The bioavailability (BIO) factor or %RBA can be used to adjust the total content for the amount of contaminant that is bioaccessible at a specific site.

The potential for non-carcinogenic effect (hazard quotient) is evaluated by comparing an exposure levels over a specific period (e.g. life-time) with a reference dose (RfD) derived for a similar exposure period.

$$\text{Hazard Quotient} = \frac{CDI}{RfD}$$

where:

CDI = chronic daily intake averaged over 70 yr (mg/kg-d)

RfD = reference dose (mg/kg-d)

Toxicity data (SF and RfD) for arsenic have been derived from tocxicological studies performed using soluble forms of arsenic and can be accessed through Integrated Risk Information System (IRIS) from USEPA (www.epa.gov/iris). Bioavailability-bioaccessibility data (IVG) can be used to provide more accurate exposure assessments that will result in more reasonable and site-specific risk estimates.

Chronic daily intakes (CDI) were determined for soil As for children (ED= 6y) and adults (ED=50). The ingestion rate (IR) of 200 mg/d for children and 100 mg/g for adults were employed. Exposure frequency (EF) was 260 d/yr (5 days a week) for a child and 208 d/yr (4 days/wk) for an adult, since they tend to spend less time outdoors. Exposure duration (ED) depends on whether the assessment was for a lifetime exposure or residence exposure, 6 years was used for children and 50 years was used for adult. The default body weight (BW) for children is 16 kg and 70 kg for and adult. The averaging time (AT) is determined by multiplying the ED by 365 days or 2190 days for a child and 18250 days for an adult. Relative bioavailability (BIO) was determined by the IVG method (Table 3). The CDI, calculated for each soil and treatment, show a substantial decrease in the potential intake of arsenic with Fe-treatments (Table 4 and 5).

The USEPA has classified arsenic as a human carcinogen and set the oral slope factor to 1.5 (mg/kg)/d for ingestion of inorganic arsenic (IRIS). Risk for each soil and treatment is shown in Table 6. In addition, exposure to inorganic arsenic can cause chronic health hazards such as Blackfoot disease and, skin lesions. The oral Reference dose has been set at 3E-4 mg/kg-d (IRIS), and related Hazard Quotients (HQ) are found in Table 6. Potential for significant effects are noted when non-carcinogen hazard quotients are greater than one. The HQ for children are <1 in all the Dennis and Bernow Fe-treated soils and the Pratt and Perkins Fe-WTR treatment. The HQ for an adult is <1 in all soils and treatments including the positive controls. The Risk factor for children and adults are greater than the generally acceptable level of 1E-6, but the treated soils do show a noticeable decrease in risk.

CONCLUSIONS

The Fe-treatments were successful in reducing the amount of *in vitro* gastric arsenic concentrations. The Fe-WTR treatment had the best overall success with the greatest reduction of % relative bioavailable arsenic. Hazard quotients (HQ) for non-carcinogen effects were at an acceptable levels (<1) for adults in all Fe-treatments. HQ for children was <1 in the high clay soils of Dennis and Bernow with all Fe-treatment. Only the Fe-WTR treatment in Pratt and Perkins has a HQ <1. Although calculated risk was not at acceptable levels (1E-6) there was a substantial decrease due to Fe-treatment. Iron was added in excess amount (20:1 molar ratio) thereby not a limiting factor. This type of remediation may be more appropriate for soils with lower level arsenic contamination (e.g. <100 mg/kg As) and possibly for ecological remediation where there is limited human-soil contact. In addition there is still a need to validate the IVG method for treated soil with the use of an appropriate animal model.

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Table 1. Select soil properties including pH (1:1 water), percent sand, silt and clay, percent organic carbon and amount of iron and aluminum oxides as measured from modified acid ammonium oxalate extraction .

Soil	pH (1:1 water)	% Sand	% Silt	% Clay	%Organic Carbon	Fe-oxide mmole/kg	Al-oxide mmole/kg
Slag Waste	7.75	na	na	59.1	3.13	60	8.22
Dennis	6.16	17.5	41.9	40.6	0.80	7.07	15.5
Perkins	4.37	60	30	10	0.85	4.64	8.56
Bernow	5.16	56.9	19.4	23.8	0.30	3.66	10.7
Pratt	6.35	90	3.75	6.25	0.90	0.99	2.48

Table 2. Weight of iron treatments added (66 m moles/kg) to 1 kg of 250 mg/kg arsenic spiked soil.

Iron Source	% Iron	Amount added to 1 kg soil
Peerless-Fe	100 %	3.69 g
$FeCl_3 \bullet 6H_2O$	20.7 %	17.86 g
$Fe_2(SO_4)_3$ (76.4% pure)	21.3 %	17.29 g
Fe-WTR	2.8 %	131.8 g

Table 3. IVG results from Fe-treatments on arsenic contaminated soils. IVG arsenic represents the amount of arsenic extracted from one gram of soil and the correlating % decrease due to Fe-treatments. The % relative bioavailability (%RBA) is calculate from the total arsenic content.

		IVG As, mg/kg	% Decrease (increase)	Total As mg/kg	% RBA		% Decrease (increase)
	Untreated	160		403	39.8	a	
Slag Waste	zero-Fe	153	4.71	361	42.2	b	(6.19)
59.1% clay	Fe-chloride	121	24.2	374	32.5	c	18.3
60 mmol Fe	Fe-sulfate	120	24.9	366	32.9	c	17.4
	Fe-WTR	78.1	51.2	325	24.1	d	39.5
	As-Spike	40.3		243	16.6	a	
Dennis	zero-Fe	23.1	42.5	225	10.3	b	37.8
40.6% clay	Fe-chloride	25.6	36.4	235	10.9	b	34.3
7.07mmol Fe	Fe-sulfate	25.6	36.4	213	12.1	b	27.3
	Fe-WTR	20.1	50.1	235	8.56	c	48.3
	As-Spike	98.4		257	38.3	a	
Perkins	zero-Fe	48.9	50.3	255	19.2	c	49.8
10% clay	Fe-chloride	41.7	57.6	268	15.6	d	59.3
4.64 mmol Fe	Fe-sulfate	58.9	40.2	228	25.9	b	32.4
	Fe-WTR	16.9	82.8	239	7.08	e	81.5
	As-Spike	47.8		276	17.3	a	
Bernow	zero-Fe	32.6	31.6	234	14.0	b	19.5
23.8% clay	Fe-chloride	26.0	45.5	255	10.2	c	41.1
3.66 mmol Fe	Fe-sulfate	27.9	41.7	266	10.5	c	39.6
	Fe-WTR	11.4	76.2	222	5.13	d	70.4
	As-Spike	250		299	83.7	a	
Pratt	zero-Fe	64.3	74.3	311	20.7	d	75.3
6.25% clay	Fe-chloride	72.1	71.2	297	24.3	c	71.0
0.99 mmol Fe	Fe-sulfate	69.7	72.1	215	32.5	b	61.2
	Fe-WTR	28.2	88.7	414	6.81	e	91.9

Table 4. Chronic daily intakes (CDI) for children exposed to arsenic contaminated soils. IR = 200 mg/d, CF = 1E-6 kg/mg, EF=260 d/y, ED = 6y, BW=16 kg, AT = 2190 d

SOIL	Treatment	CS mg/kg	BIO %RBA	CDI (mg/kg)/d
Slag	Positive Cont	403	39.76	**1.4E-03**
Slag	Zero-Fe	361	42.22	**1.4E-03**
Slag	Fe-chloride	374	32.48	**1.1E-03**
Slag	Fe-sulfate	366	32.85	**1.1E-03**
Slag	Fe-WTR	325	24.05	**7.0E-04**
Dennis	Positive Cont	243	16.56	**3.6E-04**
Dennis	Zero-Fe	225	10.30	**2.1E-04**
Dennis	Fe-chloride	235	10.88	**2.3E-04**
Dennis	Fe-sulfate	213	12.05	**2.3E-04**
Dennis	Fe-WTR	235	8.56	**1.8E-04**
Perkins	Positive Cont	257	38.25	**8.8E-04**
Perkins	Zero-Fe	255	19.20	**4.4E-04**
Perkins	Fe-chloride	268	15.58	**3.7E-04**
Perkins	Fe-sulfate	228	25.86	**5.2E-04**
Perkins	Fe-WTR	239	7.08	**1.5E-04**
Bernow	Positive Cont	276	17.32	**4.3E-04**
Bernow	Zero-Fe	234	13.95	**2.9E-04**
Bernow	Fe-chloride	255	10.20	**2.3E-04**
Bernow	Fe-sulfate	266	10.46	**2.5E-04**
Bernow	Fe-WTR	222	5.13	**1.0E-04**
Pratt	Positive Cont	299	83.69	**2.2E-03**
Pratt	Zero-Fe	311	20.67	**5.7E-04**
Pratt	Fe-chloride	297	24.30	**6.4E-04**
Pratt	Fe-sulfate	215	32.51	**6.2E-04**
Pratt	Fe-WTR	415	6.81	**2.5E-04**

Table 5. Chronic daily intakes (CDI) for adults exposed to arsenic contaminated soils. IR = 100 mg/d, CF = 1E-6 kg/mg, EF=208 d/y, ED = 50y, BW=70 kg, AT = 18250 d

SOIL	Treatment	CS (mg/kg)	BIO (RBA)	CDI (mg/k-d)
Slag	Positive Cont	403	0.40	**1.3E-04**
Slag	Zero-Fe	361	0.42	**1.2E-04**
Slag	Fe-chloride	374	0.32	**9.9E-05**
Slag	Fe-sulfate	366	0.33	**9.8E-05**
Slag	Fe-WTR	325	0.24	**6.4E-05**
Dennis	Positive Cont	243	0.17	**3.3E-05**
Dennis	Zero-Fe	225	0.10	**1.9E-05**
Dennis	Fe-chloride	235	0.11	**2.1E-05**
Dennis	Fe-sulfate	213	0.12	**2.1E-05**
Dennis	Fe-WTR	235	0.09	**1.6E-05**
Perkins	Positive Cont	257	0.38	**8.0E-05**
Perkins	Zero-Fe	255	0.19	**4.0E-05**
Perkins	Fe-chloride	268	0.16	**3.4E-05**
Perkins	Fe-sulfate	228	0.26	**4.8E-05**
Perkins	Fe-WTR	239	0.07	**1.4E-05**
Bernow	Positive Cont	276	0.17	**3.9E-05**
Bernow	Zero-Fe	234	0.14	**2.7E-05**
Bernow	Fe-chloride	255	0.10	**2.1E-05**
Bernow	Fe-sulfate	266	0.10	**2.3E-05**
Bernow	Fe-WTR	222	0.05	**9.3E-06**
Pratt	Positive Cont	299	0.84	**2.0E-04**
Pratt	Zero-Fe	311	0.21	**5.2E-05**
Pratt	Fe-chloride	297	0.24	**5.9E-05**
Pratt	Fe-sulfate	215	0.33	**5.7E-05**
Pratt	Fe-WTR	415	0.07	**2.3E-05**

Table 6. Calculated Hazard Quotient (RfD=3E-4 mg/kg-d) and Risk (SF=1.5 mg/kg-d) for child and adult.

SOIL	Treatment	Child HQ	Adult HQ	Child Risk mg/kg-d	Adult Risk mg/kg-d
Slag	Positive Cont	4.75	0.43	2.14E-03	1.96E-04
Slag	Zero-Fe	4.53	0.41	2.04E-03	1.86E-04
Slag	Fe-chloride	3.60	0.33	1.62E-03	1.48E-04
Slag	Fe-sulfate	3.57	0.33	1.61E-03	1.47E-04
Slag	Fe-WTR	2.32	0.21	1.04E-03	9.54E-05
Dennis	Positive Cont	1.19	0.11	5.38E-04	4.92E-05
Dennis	Zero-Fe	0.69	0.06	3.09E-04	2.83E-05
Dennis	Fe-chloride	0.76	0.07	3.42E-04	3.13E-05
Dennis	Fe-sulfate	0.76	0.07	3.42E-04	3.13E-05
Dennis	Fe-WTR	0.60	0.05	2.68E-04	2.45E-05
Perkins	Positive Cont	2.92	0.27	1.31E-03	1.20E-04
Perkins	Zero-Fe	1.45	0.13	6.53E-04	5.97E-05
Perkins	Fe-chloride	1.24	0.11	5.57E-04	5.10E-05
Perkins	Fe-sulfate	1.75	0.16	7.86E-04	7.19E-05
Perkins	Fe-WTR	0.50	0.05	2.26E-04	2.07E-05
Bernow	Positive Cont	1.42	0.13	6.38E-04	5.83E-05
Bernow	Zero-Fe	0.97	0.09	4.36E-04	3.99E-05
Bernow	Fe-chloride	0.77	0.07	3.48E-04	3.18E-05
Bernow	Fe-sulfate	0.83	0.08	3.72E-04	3.40E-05
Bernow	Fe-WTR	0.34	0.03	1.52E-04	1.39E-05
Pratt	Positive Cont	7.42	0.68	3.34E-03	3.05E-04
Pratt	Zero-Fe	1.91	0.17	8.59E-04	7.85E-05
Pratt	Fe-chloride	2.14	0.20	9.63E-04	8.81E-05
Pratt	Fe-sulfate	2.07	0.19	9.31E-04	8.52E-05
Pratt	Fe-WTR	0.84	0.08	3.77E-04	3.44E-05

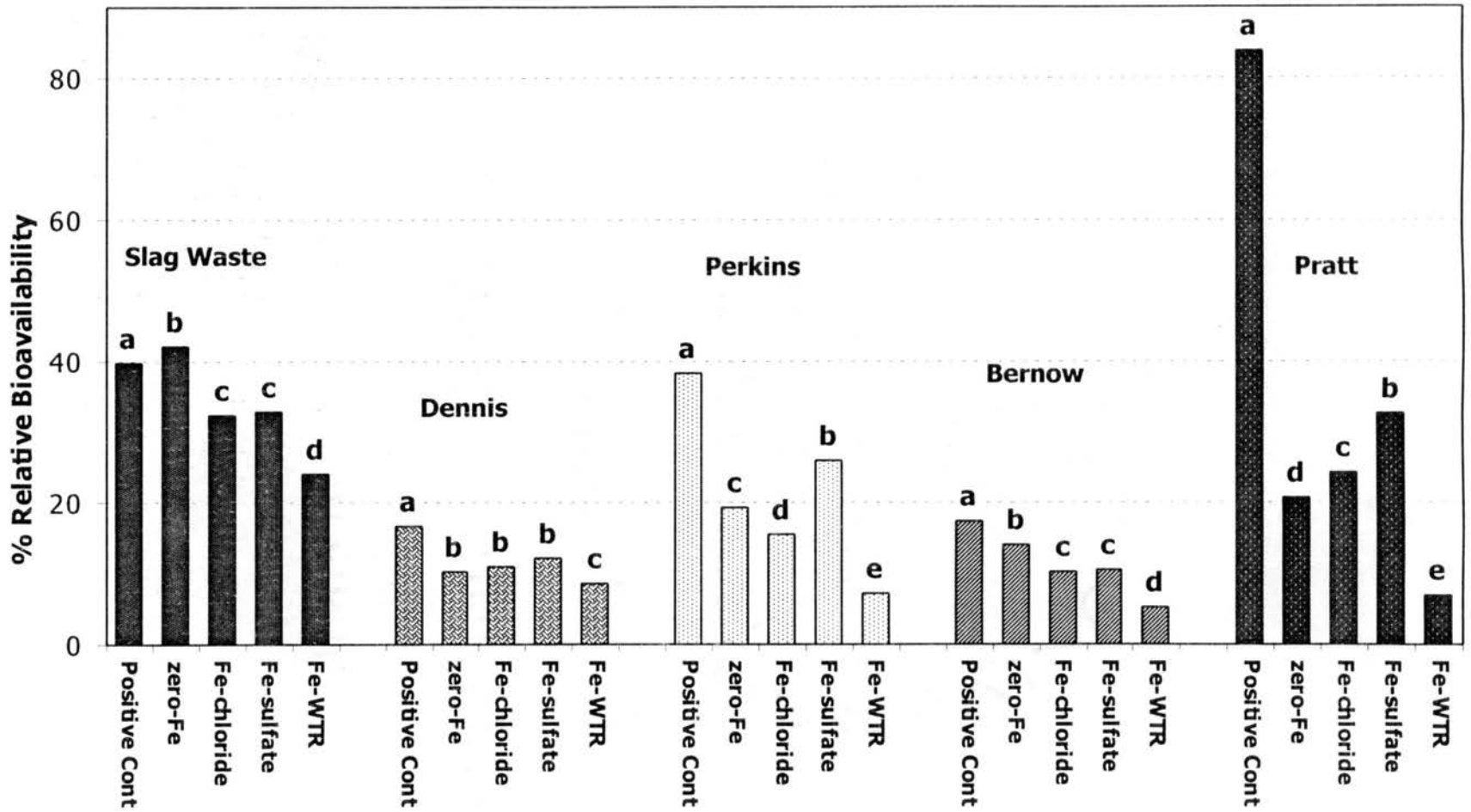

Fig. 1. % Relative bioavailability (%RBA) for four soils and a slag waste remediated with four Fe-treatments. Columns with the same letter designation are not significantly different at $P<0.05$.

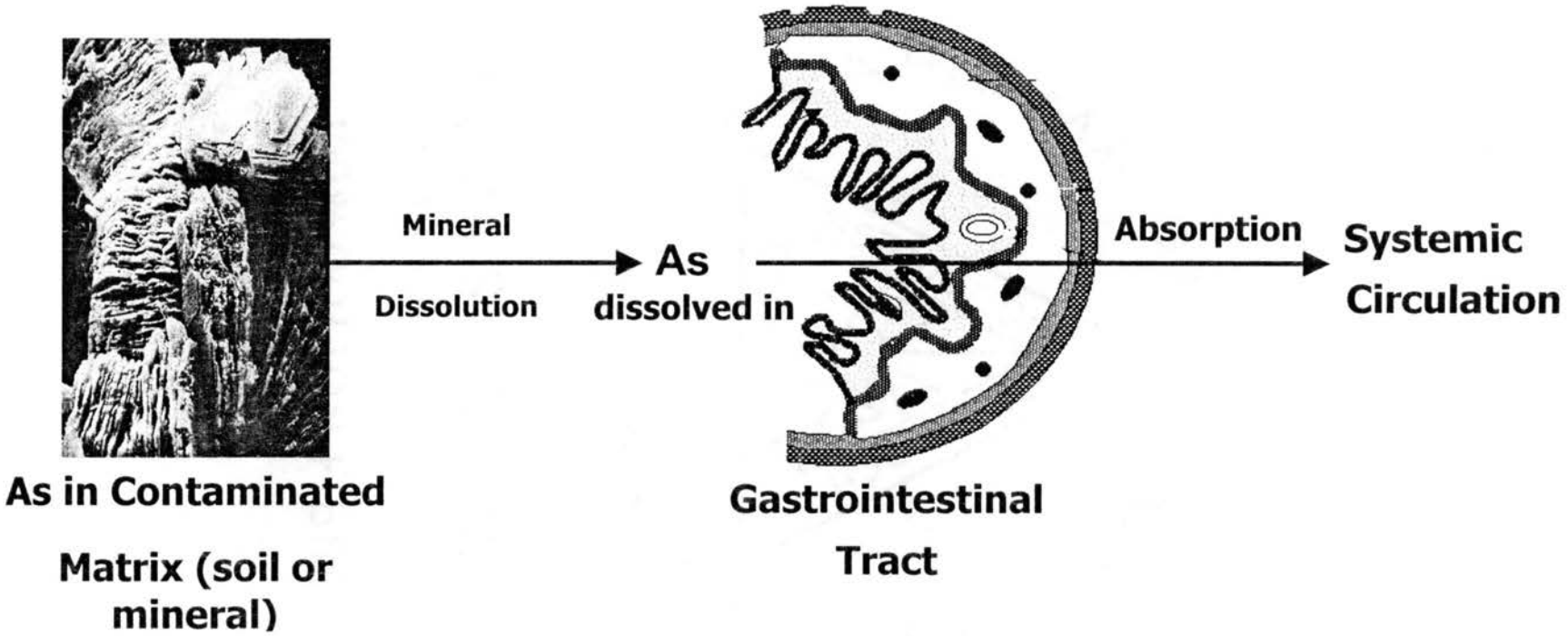

Fig 2. Simplified schematic of contaminant dissolution.

APPENDIX A

Table 1 Results from a 10-day seed germination test and shoot elongation test done on 22 arsenic spiked soils.

Soil	Average % Germ As-soil	Average shoot legnth, cm As-soil	Average %Germ Controls	Average Shoot legnth,cm Controls	Average % Relative germ	Average % Relative shoot length
Bernow B	80.0	2.0	93.8	3.44	85.3	56.79
Cannisteo	86.7	2.1	88.3	3.89	98.1	54.52
Dennis A	30.0	0.6	95.0	2.39	31.6	26.45
Dennis B	90.0	2.5	93.3	3.90	96.4	63.41
Dougherty	0.0	0.0	83.3	1.91	0.0	0.00
Efaw	83.3	1.9				
Hanlon	38.3	1.8	86.7	4.33	44.2	40.51
Haskell	73.3	2.3				
Kirkland	62.5	1.4	88.3	2.39	47.2	57.26
Luton	95.0	2.6	78.3	4.08	121.3	64.56
Mansic A	58.3	1.6	93.3	2.23	62.5	71.18
Mansic B	96.7	2.1	86.3	3.33	112.1	61.54
Osage A	61.7	2.4	91.7	4.03	67.3	58.69
Osage B	93.3	3.0	91.0	3.23	102.6	91.63
Perkins A	6.7	0.4	90.0	3.87	7.4	9.18
Pond Creek A	48.3	2.2	85.0	2.65	56.9	83.11
Pond Creek B	90.0	2.7	85.0	4.17	105.9	63.57
Pratt A	13.3	0.5	75.0	3.26	17.8	16.10
Pratt B	1.7	0.2	78.3	1.77	2.1	9.43
Richfield	96.7	2.4	91.7	2.36	105.5	99.82
Summit A	26.7	0.6	90	4.00	29.6	14.43
Summit B	88.3	2.2	91.7	3.72	96.4	58.44

Table 2. Dry yield from lettuce grown on As spiked soil and control soils during the 8-week bioassay.

Soil	Arsenic Soil Average Yield/pot (g)	Arsenic Soil Average Yield/plant (g)	Arsenic Soil Average Yield/pot (mg)	Control Soil Yield /pot (g)	Control Soil Yield /pot (mg)	% Relative Yield
Bernow B	0.06	0.02	62.7	0.40	397	15.8
Canisteo	0.00	0	0	6.07	6072	0.0
Dennis A	1.40	0.57	1396	2.16	2158	64.7
Dennis B	0.05	0.02	49.3	0.18	184	26.8
Doughtery A	0.00	0	0	1.65	1649	0.0
Efaw	0.28	0.05	280	1.45	1451	19.3
Hanlon A	0.04	0.01	43.0	3.64	3640	1.2
Haskell	2.04	0.51	2042	4.01	4007	51.0
Kirkland A	0.43	0.15	429	2.54	2537	16.9
Luton	2.20	0.72	2196	7.24	7235	30.4
Mansic A	0.00	0	0	2.72	2717	0.0
Mansic B	0.00	0.001	1.33	3.05	3050	0.0
Osage A	5.04	1.52	5037	7.32	7317	68.8
Osage B	3.78	0.79	3783	5.01	5005	75.6
Perkins	0.02	0.03	17.0	1.08	1077	1.6
Pond Creek A	0.51	0.17	505	5.71	5714	8.8
Pond Creek B	0.05	0.01	47.3	1.64	1639	2.9
Pratt A	0.00	0	0	2.65	2654	0.0
Pratt B	0.00	0	0	1.10	1104	0.0
Richfield B	0.03	0.01	33.3	3.23	3227	1.0
Summit A	1.46	0.18	1464	2.34	2340	62.5
Summit B	1.14	0.36	1135	2.07	2073	54.8

Table 3. Lettuce endpoints from 8-week bioassay (mean yield, mean % of control yield and mean arsenic accumulation) grown on 22 arsenic spiked soils.

Soil	Yield, 8-week Bioassay, mg	% Relative Yield	Lettuce As content, mg/kg
Bernow B	62.7	15.8	7.78
Canisteo A	0.0	0.0	n/a
Dennis A	1396	64.7	5.14
Dennis B	49.3	26.8	2.14
Dougherty A	0.0	0.0	n/a
Efaw A	279.7	19.3	16.6
Hanlon A	43.0	1.2	16.5
Haskell	2042	51.0	22.7
Kirkland A	428.7	16.9	16.5
Luton A	2196	30.4	9.30
Mansic A	0.0	0.0	n/a
Mansic B	1.3	0.0	3.74
Osage A	5037	68.8	6.27
Osage B	3783	75.6	3.80
Perkins A	17.0	1.6	n/a
Pond Creek A	505.0	8.8	29.8
Pond Creek B	47.3	2.9	15.1
Pratt A	0.0	0.0	n/a
Pratt B	0.0	0.0	n/a
Richfield B	33.3	1.0	n/a
Summit A	1464	62.5	3.24
Summit B	1135	54.8	5.87

Table 4. Arsenic concentrations in lettuce grown during the 8-week bioassay on arsenic spiked soils. Repetitions, standard reference material, and detection limits are included.

Soil	Rep	Wt. Dry plant, g	As1890* µg/L	As1937 µg/L	DF	As 1890 mg/kg	Average As mg/kg	St Dev %Co Var
Bernow B	1	0.017	13.5	13.4	588	7.9	**7.78**	2.6
	2	0.048	49.5	49.7	208	10.3		*33.7*
	3	0.025	12.7	12.7	400	5.1		
Canisteo A	N/A							
Dennis A	1	0.172	91.1	90.8	58.1	5.30	**5.14**	0.2
	3	0.203	101	101	49.3	4.98		*4.4*
Dennis B	1	0.081	17.4	17.4	123	2.14	**2.14**	
Dougherty A	N/A							
Efaw A	2	0.077	127.5	129	130	16.6	**16.6**	
Hanlon A	1	0.038	77.88	78.33	263	20.5	**16.5**	5.6
	3	0.049	61.28	61.47	204	12.5		*34.2*
Haskell	1	0.183	400	398	54.6	21.8	**22.7**	1.6
	2	0.19	467	469	52.6	24.6		*7.1*
	3	0.21	456	460	47.6	21.7		
Kirkland A	1	0.118	274	272	84.7	23.2	**16.5**	5.9
	2	0.105	129	129	95.2	12.3		*35.9*
	3	0.168	234	231	59.5	13.9		
Luton	2	0.198	155	157	50.5	7.82	**9.30**	2.1
	3	0.192	207	208	52.1	10.8		*22.5*
Mansic A	N/A							
Mansic B	N/A							
Osage A	1	0.186	93.4	93.2	53.8	5.02	**6.27**	1.3
	2	0.175	132	132	57.1	7.56		*20.2*
	3	0.2	125	125	50.0	6.24		
Osage B	1	0.19	59.3	59.4	52.6	3.12	**3.80**	0.85
	2	0.198	94.0	93.5	50.5	4.75		*22.3*
	3	0.208	73.3	73.3	48.1	3.52		
Perkins A	N/A							
Pond Creek A	1	0.018	53.6	53.9	556	29.79	**29.8**	
Pond Creek B	1	0.024	32.5	32.6	417	13.53	**15.1**	2.5
	2	0.041	73.5	74.0	244	17.92		*16.3*
	3	0.056	77.3	77.8	179	13.80		
Pratt A	N/A							

Soil	Rep	Wt. Dry plant, g	As1890* µg/L	As1937 µg/L	DF	As 1890 mg/kg	Average As mg/kg	St Dev %Co Var
Pratt B	N/A							
Richfield B	N/A							
Summit A	1	0.194	53.4	53.0	51.5	2.75	**3.24**	0.66
	2	0.209	62.2	62.2	47.8	2.97		*20.5*
	3	0.085	34.0	33.7	118	4.00		
Summit B	1	0.192	110	110	52.1	5.75	**5.9**	0.2
	3	0.203	122	121	49.3	5.99		*2.8*

	Wt. Dry plant	As1890* µg/L	As1937 µg/L	DF	As mg/kg	% Recovery
Plant SRM	0.194	122.3	122.3	52.8	6.46	80.8
Plant SRM	0.172	116	117.5	59.6	6.91	86.4
Plant SRM	0.201	118.4	118.3	51.0	6.04	75.5

* primary line

Detection limit determined by running the blank 7 times with a correction factor of 3.

Detection Limit		As1890*	As1937
Standard deviation	1	0.36	0.83
Low = Std*5	ppb	1.80	4.15
High = Std*10	ppb	3.59	8.29

* primary line

Table 5. Lettuce yield, % relative yield and As concentration in tissue from 5-week bioassay.

Soil	Lettuce Yield, mg	Lettuce As conc. mg/kg	% Relative Yield
Bernow B	.		
Canisteo A	129	39.3	1.84
Dennis A	6837	2.42	99.5
Dennis B	5725	0.76	89.0
Dougherty A	38.0		0.87
Efaw A	1771	26.5	31.7
Hanlon A	224	28.5	2.78
Haskell	1381	29.6	22.6
Kirkland A	2178	14.8	67.2
Luton A	2901	4.58	57.3
Mansic A	41.3		0.94
Mansic B	17.7		0.49
Osage A	4503	5.16	64.4
Osage B	4924	5.60	90.5
Perkins A	268		3.27
Pond Creek A	1827	40.6	34.2
Pond Creek B	2200	9.42	49.2
Pratt A	14.7		0.24
Pratt B	8.00		0.15
Richfield B	53.7		1.03
Summit A	7179	1.60	86.8
Summit B	4310	1.12	43.8

Table 6 Correlation coeffiecents (r) and *P* values between soil properties pH (1:1 water), % clay, % organic carbon and Fe-oxide (ammonium oxalate extracted) from 22 soils used in arsenic spiking study.

		pH	**%Clay**	**%OC**	**Fe**
pH	r	----	0.43	0.27	-0.02
	P		*0.05*	*0.23*	*0.94*
%Clay	r	0.43	---	0.52	0.73
	P	*0.05*		*0.02*	*<0.001*
%OC	r	0.27	0.52	---	0.52
	P	*0.23*	*0.02*		*0.015*
Fe	r	-0.02	0.73	0.52	---
	P	*0.94*	*<0.001*	*0.015*	

Table 7. Pore water arsenic extracted from 22 arsenic spiked soils.

Soil	Rep	Dry Soil, g	Water Added, mL	Arsenic Conc. mg/L	Average As Conc. mg/L	St Dev. *%Co Var*	DF	As mg/kg	Average As mg/kg
Bernow B	1	40.0	18.4	0.19	**0.19**	0.01	0.46	0.088	0.086
	2	40.0	18.3	0.18		*3.2%*	0.46	0.084	
Canisteo A	1	40.0	28.0	8.46	**8.4**	0.03	0.70	5.92	5.88
	2	40.0	27.7	8.42		*0.3%*	0.69	5.83	
Dennis A	1	40.0	25.7	0.27	**0.30**	0.04	0.64	0.173	0.17
	2	40.0	21.0	0.32		*12.2%*	0.53	0.169	
Dennis B	1	40.0	21.7	*0.003*	**0.00**	0.00	0.54	0.002	0.001
	2	40.0	21.4	*0.002*		*27.4%*	0.53	0.001	
Dougherty A	1	40.0	11.4	100.2	**98.8**	2.02	0.29	28.7	28.2
	2	40.0	11.4	97.4		*2.0%*	0.28	27.7	
Efaw A	1	40.0	17.6	4.00	**4.06**	0.08	0.44	1.76	1.78
	2	40.0	17.5	4.11		*1.9%*	0.44	1.79	
Hanlon A	1	40.0	19.5	13.78	**13.9**	0.13	0.49	6.70	6.71
	2	40.0	19.3	13.96		*0.9%*	0.48	6.72	
Haskell	1	40.0	20.6	3.83	**3.8**	0.02	0.51	1.97	1.96
	2	40.0	20.5	3.80		*0.5%*	0.51	1.94	
Kirkland A	1	40.0	20.8	2.00	**2.01**	0.02	0.52	1.04	1.06
	2	40.0	21.4	2.02		*0.8%*	0.53	1.08	
Luton A	1	40.0	33.3	1.50	**1.5**	0.01	0.83	1.24	1.24
	2	40.1	33.4	1.48		*0.9%*	0.83	1.23	
Mansic A	1	40.0	23.2	16.99	**16.9**	0.20	0.58	9.85	9.81
	2	40.0	23.4	16.71		*1.2%*	0.59	9.78	
Mansic B	1	40.0	15.6	20.77	**20.3**	0.71	0.39	8.09	7.80
	2	40.0	15.2	19.76		*3.5%*	0.38	7.52	
Osage A	1	40.0	25.3	0.23	**0.23**	0.00	0.63	0.143	0.14
	2	40.0	25.1	0.23		*0.1%*	0.63	0.142	
Osage B	1	40.0	26.8	0.12	**0.12**	0.01	0.67	0.078	0.08
	2	40.0	26.6	0.13		*5.9%*	0.66	0.084	
Perkins A	1	40.0	11.3	9.94	**10.1**	0.19	0.28	2.81	2.87
	2	40.0	11.5	10.20		*1.9%*	0.29	2.94	
Pond Creek A	1	40.0	22.6	7.44	**7.49**	0.08	0.56	4.19	4.22
	2	40.0	22.6	7.55		*1.1%*	0.56	4.26	
Pond Creek B	1	40.0	19.5	*0.51*	**0.49**	0.02	0.49	0.247	0.24
	2	40.0	19.4	*0.48*		*4.6%*	0.48	0.231	

Soil	Rep	Dry Soil, g	Water Added, mL	Arsenic Conc. mg/L	Average As Conc. mg/L	St Dev. *%Co Var*	DF	As mg/kg	Average As mg/kg
Pratt A	1	40.0	15.3	91.84	**94.4**	3.56	0.38	35.1	35.9
	2	40.0	15.2	96.87		*3.8%*	0.38	36.7	
Pratt B	1	40.0	12.2	159.70	**162.6**	4.03	0.30	48.6	51.7
	2	40.0	13.3	165.40		*2.5%*	0.33	54.9	
Richfield B	1	40.0	24.6	8.55	**8.6**	0.08	0.61	5.25	5.31
	2	40.0	24.8	8.66		*0.9%*	0.62	5.36	
Summit A	1	40.0	26.8	0.54	**0.31**	0.32	0.67	0.362	0.21
	2	40.0	26.8	0.09		*101.8%*	0.67	0.059	
Summit B	1	40.0	27.5	*0.02*	**0.02**	0.00	0.69	0.012	0.01
	2	40.0	27.6	*0.02*		*0.2%*	0.69	0.012	

Detection limit determined by running the blank 7 times with a correction factor of 3.

Detection Limit		**Arsenic**
Standard deviation		0.034
Low = Std*5	ppb	0.17
High = Std*10	ppb	0.34

Table 8. Bray-1 extractable arsenic from 22 arsenic spiked soils (8-week bioassay).

Soil	Rep	As1972 mg/kg	Average As mg/kg	StDev % CoVar	P 1782 mg/kg	Average P mg/kg	StDev *% CoVar*	As:P ratio
Bernow B	1	25.7	**26.25**	0.72	6.22	**6.28**	0.09	4.2
	2	26.8		*2.75*	6.34		*1.36*	
Canisteo A	1	37.5	**37.56**	0.14	9.56	**9.69**	0.18	3.9
	2	37.7		*0.38*	9.82		*1.88*	
Dennis A	1	10.6	**10.83**	0.37	35.3	**36.2**	1.29	0.3
	2	11.1		*3.40*	37.1		*3.56*	
Dennis B	1	2.79	**2.78**	0.02	2.55	**2.50**	0.07	1.1
	2	2.77		*0.66*	2.45		*2.97*	
Dougherty A	1	88.8	**91.01**	3.11	26.5	**26.8**	0.48	3.4
	2	93.2		*3.42*	27.1		*1.79*	
Efaw A	1	49.1	**51.34**	3.19	83.3	**85.7**	3.46	0.6
	2	53.6		*6.21*	88.2		*4.04*	
Hanlon A	1	75.1	**77.60**	3.48	91.5	**94.4**	4.07	0.8
	2	80.1		*4.48*	97.2		*4.32*	
Haskell	1	34.0	**35.95**	2.81	82.8	**88.2**	7.57	0.4
	2	37.9		*7.83*	93.5		*8.58*	
Kirkland A	1	35.9	**37.26**	1.89	53.4	**54.8**	2.09	0.7
	2	38.6		*5.07*	56.3		*3.80*	
Luton A	1	39.8	**40.58**	1.16	68.2	**69.9**	2.39	0.6
	2	41.4		*2.86*	71.6		*3.42*	
Mansic A	1	26.7	**29.31**	3.75	0.91	**1.10**	0.26	26.8
	2	32.0		*12.79*	1.28		*24.02*	
Mansic B	1	14.6	**8.66**	8.41	0.25	**0.19**	0.07	45.0
	2	2.71		*97.11*	0.14		*38.57*	
Osage A	1	12.1	**12.35**	0.36	58.1	**58.7**	0.93	0.2
	2	12.6		*2.92*	59.4		*1.58*	
Osage B	1	9.75	**10.08**	0.47	34.1	**34.4**	0.52	0.3
	2	10.4		*4.62*	34.8		*1.50*	
Perkins A	1	60.7	**62.68**	2.83	50.9	**52.5**	2.28	1.2
	2	64.7		*4.51*	54.1		*4.34*	
Pond Creek A	1	41.6	**42.91**	1.79	121	**124**	4.17	0.3
	2	44.2		*4.17*	1270		*3.37*	
Pond Creek B	1	39.5	**40.53**	1.48	35.5	**36.2**	0.98	1.1
	2	41.6		*3.66*	36.9		*2.71*	

Soil	Rep	As1972 mg/kg	Average As mg/kg	StDev % CoVar	P 1782 mg/kg	Average P mg/kg	StDev *% CoVar*	As:P ratio
Pratt A	1	119	**112.70**	9.19	23.6	**22.6**	1.35	5.0
	2	106		*8.16*	21.7		*5.97*	
Pratt B	1	145	**141.30**	5.37	32.2	**31.7**	0.74	4.5
	2	138		*3.80*	31.2		*2.32*	
Richfield B	1	105	**109.70**	6.79	27.0	**28.3**	1.77	3.9
	2	115		*6.19*	29.5		*6.28*	
Summit A	1	6.77	**6.84**	0.09	10.2	**10.1**	0.26	0.7
	2	6.90		*1.33*	9.87		*2.60*	
Summit B	1	12.0	**12.14**	0.13	4.92	**4.96**	0.06	2.4
	2	12.2		*1.11*	5.01		*1.24*	

Spiked recoveries for arsenic and phosphate.

Soil	Rep	As1890 mg/L	As1937 mg/L	As1972 mg/L	As 1980 % Recovery	P1782* mg/L	P1859 mg/L	P 1782 % Recovery
Bray-1 spikes	1	4.84	4.75	4.87	96.9	4.65	4.18	94.2
	2	4.96	4.86	5.01	99.3	4.84	4.83	96.5

			As1890	As1937	As1972*	P 1782	P 1859*
Detection Limit	Stdev	1	0.012	0.019	0.024	0.062	0.015
	5* StdDev	Low	**0.062**	**0.095**	**0.118**	**0.309**	**0.074**
	10* StdDev	High	0.123	0.189	0.235	0.618	0.147
Detection Limit	Stdev	2	0.013	0.015	0.040	0.054	0.035
	5* StdDev	Low	**0.067**	**0.077**	**0.198**	**0.272**	**0.174**
	10* StdDev	High	0.133	0.154	0.395	0.543	0.348

Table 9. Na-phosphate extractable (Yamamoto, 1975) arsenic from 22 spiked soils.

		micro g /L		Dilution	As	Avg. As	St Dev
Soil	**Rep**	**As1890**	**As1937****	**Factor**	**mg/kg**	**mg/kg**	***% Co Var***
Bernow B	1	371.8	370.1	100	37.01	**37.26**	0.35
	2	375.4	375.1	100	37.51		*0.95*
Canisteo A	1	99.61	99.83	1000	99.83	**101.7**	2.60
	2	103.5	103.5	1000	103.5		*2.55*
Dennis A	1	12.07	12.04	1000	12.04	**11.90**	0.21
	2	11.81	11.75	1000	11.75		*1.72*
Dennis B	1	177.4	176.0	100	17.6	**15.58**	2.86
	2	134.0	135.5	100	13.55		*18.39*
Dougherty A	1	66.62	67.15	1000	67.15	**64.28**	4.06
	2	60.71	61.41	1000	61.41		*6.31*
Efaw A	1	36.32	36.66	1000	36.66	**36.01**	0.93
	2	35	35.35	1000	35.35		*2.57*
Hanlon A	1	623.7	621.6	100	62.16	**61.39**	1.10
	2	605.2	606.1	100	60.61		*1.79*
Haskell	1	276.3	275.6	100	27.56	**25.78**	2.52
	2	241.2	240	100	24		*9.76*
Kirkland A	1	412.1	411.6	100	41.16	**40.49**	0.95
	2	397.9	398.1	100	39.81		*2.36*
Luton A	1	969.8	968.4	100	96.98	**98.74**	2.49
	2	100.5	100.3	1000	100.5		*2.52*
Mansic A	1	966.7	963.2	100	96.32	**95.15**	1.65
	2	93.6	93.98	1000	93.98		*1.74*
Mansic B	1	2032	2035	100	203.5	**201.6**	2.76
	2	200.8	199.6	1000	199.6		*1.37*
Osage A	1	64.06	63.83	400	25.53	**25.34**	0.27
	2	62.96	62.89	400	25.16		*1.05*
Osage B	1	69.5	69.29	400	27.72	**27.34**	0.54
	2	67.38	67.39	400	26.96		*1.97*
Perkins A	1	147.4	147.7	400	59.08	**57.9**	1.67
	2	141.7	141.8	400	56.72		*2.88*
Pond Creek A	1	98.31	98.58	400	39.43	**39.94**	0.71
	2	100.4	101.1	400	40.44		*1.78*

Soil	Rep	micro g /L As1890	As1937**	Dilution Factor	As mg/kg	Avg. As mg/kg	St Dev % Co Var
Pond Creek B	1	108.3	108.9	400	43.56	**45.36**	2.55
	2	117.7	117.9	400	47.16		*5.61*
Pratt A	1	280.1	279.6	400	111.8	**93.26**	26.28
	2	187.3	186.7	400	74.68		*28.18*
Pratt B	1	314.2	311.5	400	124.6	**129.8**	7.38
	2	338.2	337.6	400	135.0		*5.69*
Richfield B	1	389.1	386.5	400	154.6	**154.3**	0.45
	2	385.7	384.9	400	154.0		*0.29*
Summit A	1	112	111.8	400	44.72	**45.44**	1.02
	2	116	115.4	400	46.16		*2.24*
Summit B	1	69.37	69.17	400	27.67	**27.70**	0.04
	2	69.48	69.32	400	27.73		*0.15*

Detection limit determined by running the blank 7 times with a correction factor of 3.

Detection limit		**As1890**	**As1937**
Standard deviation	1	0.19	0.231
Low = Std*5	ppb	0.95	1.155
High = Std*10	ppb	1.9	2.31
		As1890	**As1937**
Standard deviation	2	0.476	0.525
Low = Std*5	ppb	2.38	2.625
High = Std*10	ppb	4.76	5.25

	Al3082 mg/L	As1890* mg/L	As1937 mg/L	As1972 mg/L	Ca3158 mg/L	Fe2714 mg/L	Mg2802 mg/L	Mn2576 mg/L
Phosphate spike 1	50.2	5.295	5.14	5.18	5.398	56.3	18.98	20.4
% Recovered	*100.4*	*105.9*	*102.8*	*103.6*	*108.0*	*112.5*	*94.9*	*102.1*
Phosphate spike 2	48.7	5.1	5.0	5.0	5.3	55.4	18.82	19.4
% Recovered	*97.4*	*101.6*	*99.6*	*100.4*	*106.4*	*110.8*	*94.1*	*96.9*

Table 10. Hydroxylamine HCl extractable arsenic from 22 arsenic spiked soils.

Soil	Rep	As1890** micro g/L	As1937 micro g/L	As1890 mg/kg	Average As mg/kg	St Dev *% Co Var*
Bernow B	1	342.8	340	85.70	**81.4**	6.13
	2	308.1	305.9	77.03		*7.54*
Canisteo A	1	440.7	439.4	110.18	**111**	0.71
	2	444.7	441.4	111.18		*0.64*
Dennis A	1	380.9	378.4	95.23	**94.0**	1.77
	2	370.9	376.4	92.73		*1.88*
Dennis B	1	330.9	327.9	82.73	**79.4**	4.72
	2	304.2	300.7	76.05		*5.95*
Dougherty A	1	434.9	434.4	108.73	**103**	8.33
	2	387.8	382.3	96.95		*8.10*
Efaw A	1	436.6	431.6	109.15	**102**	10.68
	2	376.2	371.8	94.05		*10.51*
Hanlon A	1	423.3	421.3	105.83	**105**	0.83
	2	418.6	416.2	104.65		*0.79*
Haskell	1	831.2	830.7	207.80	**157**	72.11
	2	423.3	418.1	105.83		*45.98*
Kirkland A	1	407.7	403.4	101.93	**103**	1.47
	2	416	410.9	104.00		*1.43*
Luton A	1	464.3	462.3	116.08	**112**	6.08
	2	429.9	429.2	107.48		*5.44*
Mansic A	1	407.6	401.7	101.90	**111**	12.14
	2	476.3	473.4	119.08		*10.99*
Mansic B	1	453.4	451.6	113.35	**113**	0.92
	2	448.2	443.2	112.05		*0.82*
Osage A	1	437.5	435.8	109.38	**109**	0.04
	2	437.3	435.2	109.33		*0.03*
Osage B	1	437.5	438.1	109.38	**108**	1.79
	2	427.4	425.3	106.85		*1.65*
Perkins A	1	430.6	428.8	107.65	**101**	9.21
	2	378.5	375	94.63		*9.11*
Pond Creek A	1	368.4	364.3	92.10	**93.6**	2.09
	2	380.2	376.2	95.05		*2.23*

Soil	Rep	As1890** micro g/L	As1937 micro g/L	As1890 mg/kg	Average As mg/kg	St Dev % Co Var
Pond Creek B	1	394.9	389.3	98.73	**103**	6.65
	2	432.5	428.7	108.13		*6.43*
Pratt A	1	341.3	337.7	85.33	**81.8**	4.93
	2	313.4	313.2	78.35		*6.03*
Pratt B	1	336.9	333.7	84.23	**80.7**	5.00
	2	308.6	305.8	77.15		*6.20*
Richfield B	1	415	409.1	103.75	**105**	1.82
	2	425.3	421.3	106.33		*1.73*
Summit A	1	431.3	428.2	107.83	**107**	1.04
	2	425.4	420.4	106.35		*0.97*
Summit B	1	382.4	380.7	95.60	**93.9**	2.46
	2	368.5	367.7	92.13		*2.62*

Spiked recoveries of arsenic.

Soil	Spike	Rep	As1890**	As1937	% Recovery As 1937	% Recovery As 1890
Pratt B	As Spike 50	1	390.1	387.7	101.0	**100.8**
Canisteo A	As Spike 50	1	478.1	472.1	96.5	**97.4**
Efaw A	As Spike 50	1	486.4	481.2	99.9	**100.0**
Pratt B	As Spike 100	2	415.3	412.2	101.6	**101.6**
Pratt B	As Spike 100	2	408.3	404.7	99.7	**99.9**
Efaw A	As Spike 100	2	486.9	481.4	102.0	**102.2**

			As1890 mg/L	As1937 mg/L	Fe2714mg/L	Al3082mg/L	Mn2576 mg/L	Mg2802 mg/L
Detection Limit	St Dev		0.0111	0.035	0.066	0.0755	0.0007	0.0022
7/8/02	5*Stdev	low	0.0556	0.175	0.33	0.3775	0.0035	0.011
	10*StDev	high	0.1112	0.35	0.66	0.755	0.007	0.022

Table 11. Ammonium Oxalate + 0.025M H3PO4 extractable arsenic from 22 arsenic spiked soils.

Soil	Rep	As1890** (mg/L)	As1972 (mg/L)	Total As (mg/kg)	Average As (mg/kg)	St Dev *%Co Var*
Bernow B	1	5.56	5.54	222.4	223.0	0.74
	2	5.59	5.52	223.5		*0.33*
Canisteo A	1	5.71	5.65	228.4	**239.0**	14.88
	2	6.24	6.21	249.4		*6.23*
Dennis A	1	5.92	5.89	237.0	**232.5**	6.34
	2	5.70	5.69	228		*2.73*
Dennis B	1	4.42	4.41	177.0	**173.9**	4.27
	2	4.27	4.23	170.9		*2.46*
Dougherty A	1	4.97	4.96	198.9	**194.5**	6.28
	2	4.75	4.71	190.0		*3.23*
Efaw A	1	5.34	5.34	213.5	**215**	2.09
	2	5.41	5.37	216.5		*0.97*
Hanlon A	1	5.72	5.62	228.7	**226.8**	2.72
	2	5.62	5.52	224.8		*1.20*
Haskell	1	5.53	5.54	221.2	**214.8**	8.94
	2	5.21	5.22	208.5		*4.16*
Kirkland A	1	5.54	5.45	221.4	**223.2**	2.49
	2	5.62	5.53	224.9		*1.12*
Luton A	1	6.34	6.31	253.5	**277.6**	34.14
	2	7.54	7.49	301.8		*12.30*
Mansic A	1	4.86	4.80	194.5	**208.8**	20.25
	2	5.58	5.54	223.2		*9.70*
Mansic B	1	5.07	4.96	202.6	**192.6**	14.17
	2	4.56	4.56	182.7		*7.36*
Osage A	1	6.30	6.16	252	**275.1**	32.61
	2	7.45	7.27	298.1		*11.86*
Osage B	1	5.84	5.79	233.4	**249.9**	23.28
	2	6.66	6.58	266.4		*9.31*
Perkins A	1	5.17	5.20	206.9	**209.9**	4.30
	2	5.32	5.34	213.0		*2.05*
Pond Creek A	1	5.32	5.24	212.9	**222.0**	12.81
	2	5.78	5.69	231.0		*5.77*
Pond Creek B	1	5.50	5.44	219.9	**217.6**	3.28
	2	5.38	5.31	215.2		*1.51*

Soil	Rep	As1890** (mg/L)	As1972 (mg/L)	Total As (mg/kg)	Average As (mg/kg)	St Dev *%Co Var*
Pratt A	1	4.01	3.95	160.2	**150.8**	13.29
	2	3.54	3.49	141.4		*8.82*
Pratt B	1	4.17	4.17	166.6	**186.1**	27.55
	2	5.14	5.14	205.6		*14.80*
Richfield B	1	5.64	5.61	225.6	**218.7**	9.73
	2	5.30	5.28	211.8		*4.45*
Summit A	1	5.64	5.64	225.6	**233.0**	10.52
	2	6.01	5.98	240.5		*4.51*
Summit B	1	5.37	5.33	214.8	**209.5**	7.41
	2	5.11	5.09	204.3		*3.54*

Arsenic spiked recoveries.

Soil	Rep	As1890* mg/L	As1972 mg/L	As1890 % Recovery
Summit B - As Spike	2	14.55	14.59	94.4
Luton A - As Spike	1	16.04	16.08	97.0
Kirkland A - As Spike	2	15.28	15.39	96.6

Detection limit determined by running the blank 7 times with a correction factor of 3.

		Al3082 mg/L	As1890* mg/L	As1937 mg/L	As1972 mg/L	Fe2714 mg/L	Mg2802 mg/L	Mn2576 mg/L
St Dev	1	0.0311	0.03242	0.0199	0.0429	0.0287	0.0026	0.0004
5*St Dev	Low	**0.1555**	**0.1621**	**0.1**	**0.2145**	**0.144**	**0.013**	**0.002**
10*St Dev	High	0.311	0.3242	0.199	0.429	0.287	0.026	0.004

Table 12. Ammonium Oxalate +0.025 M H3PO4 extractable iron and aluminum

Soil	Rep	Fe-oxide	Average Fe-	Al-oxide	Average Al-
Bernow B	1	3957	**4001**	1199	**1177**
	2	4044		1154	
Canisteo A	1	1440	**1509**	1580	**1680**
	2	1577		1779	
Dennis A	1	8184	**8074**	1745	**1676**
	2	7964		1606	
Dennis B	1	7124	**8514**	1881	**2083**
	2	9904		2286	
Dougherty A	1	646	**594**	300	**291**
	2	542		282	
Efaw A	1	4024	**4102**	1201	**1211**
	2	4180		1221	
Hanlon A	1	2879	**2854**	841	**832**
	2	2829		824	
Haskell	1	4256	**3574**	899	**863**
	2	2891		827	
Kirkland A	1	2948	**2977**	1420	**1416**
	2	3006		1412	
Luton A	1	8904	**9730**	2872	**3109**
	2	10556		3346	
Mansic A	1	751	**752**	801	**839.4**
	2	753		878	
Mansic B	1	485	**477**	593	**569.6**
	2	468		546	
Osage A	1	12648	**14168**	2404	**2712**
	2	15688		3020	
Osage B	1	14292	**14766**	2706	**2736**
	2	15240		2766	
Perkins A	1	2161	**2181**	769	**759**
	2	2202		749	
Pond Creek A	1	2810	**2891**	1246	**1329**
	2	2973		1411	
Pond Creek B	1	3266	**3265**	1381	**1394**
	2	3264		1408	
Pratt A	1	368	**382**	264	**250**
	2	396		236	
Pratt B	1	354	**349**	259	**240**
	2	344		221	
Richfield B	1	1936	**1870**	1441	**1420**
	2	1804		1399	
Summit A	1	11004	**11376**	4068	**4208**
	2	11748		4348	
Summit B	1	5732	**5668**	2715	**2693**
	2	5604		2671	

Table 13. Total arsenic content of 22 spiked soils as determined by USEPA method 3051.

Soil	Rep	Total As mg/kg	Average As, mg/kg	St Dev *CoVar*
Bernow B	1	256	**255**	0.43
	2	255		*0.17*
Canisteo A	1	226	**225**	1.26
	2	224		*0.56*
Dennis A	1	230	**234**	5.37
	2	238		*2.30*
Dennis B	1	229	**222**	9.80
	2	216		*4.41*
Dougherty A	1	213	**205**	12.48
	2	196		*6.10*
Efaw A	1	223	**223**	0.15
	2	223		*0.07*
Hanlon A	1	222	**228**	7.79
	2	233		*3.42*
Haskell	1	198	**208**	14.02
	2	218		*6.75*
Kirkland A	1	226	**226**	0.26
	2	226		*0.12*
Luton A	1	240	**242**	2.43
	2	243		*1.01*
Mansic A	1	204	**202**	3.22
	2	199		*1.60*
Mansic B	1	214	**210**	4.48
	2	207		*2.13*
Osage A	1	262	**265**	4.44
	2	268		*1.68*
Osage B	1	238	**234**	5.69
	2	230		*2.43*
Perkins A	1	221	**229**	9.64
	2	235		*4.22*
Pond Creek A	1	233	**227**	8.55
	2	221		*3.77*
Pond Creek B	1	231	**228**	4.37
	2	225		*1.91*
Pratt A	1	153	**149**	6.43
	2	144		*4.32*
Pratt B	1	207	**218**	16.01
	2	230		*7.33*
Richfield B	1	233	**234**	1.15
	2	235		*0.49*
Summit A	1	259	**257**	2.50
	2	255		*0.97*
Summit B	1	238	**243**	6.94
	2	248		*2.85*

Table 14. Earthworm Mortality and arsenic accumulation from a 28-toxicity test in 22 arsenic spiked soils (Bradham, 2002).

Soil	Average Earthworm % Mortality	Average Earthworm As Accum, mg/kg
Bernow B	0.00	234.5
Canisteo A	26.67	524.6
Dennis A	0.00	197.3
Dennis B	0.00	32.25
Dougherty A	43.33	453.6
Efaw A	96.67	n/a
Hanlon A	10.00	548.7
Haskell	20.00	232.2
Kirkland A	6.67	528.6
Luton A	3.33	451.7
Mansic A	13.33	439.6
Mansic B	23.33	236.9
Osage A	0.00	136.1
Osage B	0.00	173.5
Perkins A	26.67	299.0
Pond Creek A	46.67	55.80
Pond Creek B	16.67	328.8
Pratt A	30.00	59.78
Pratt B	96.67	n/a
Richfield B	6.67	628.5
Summit A	0.00	316.7
Summit B	0.00	128.8

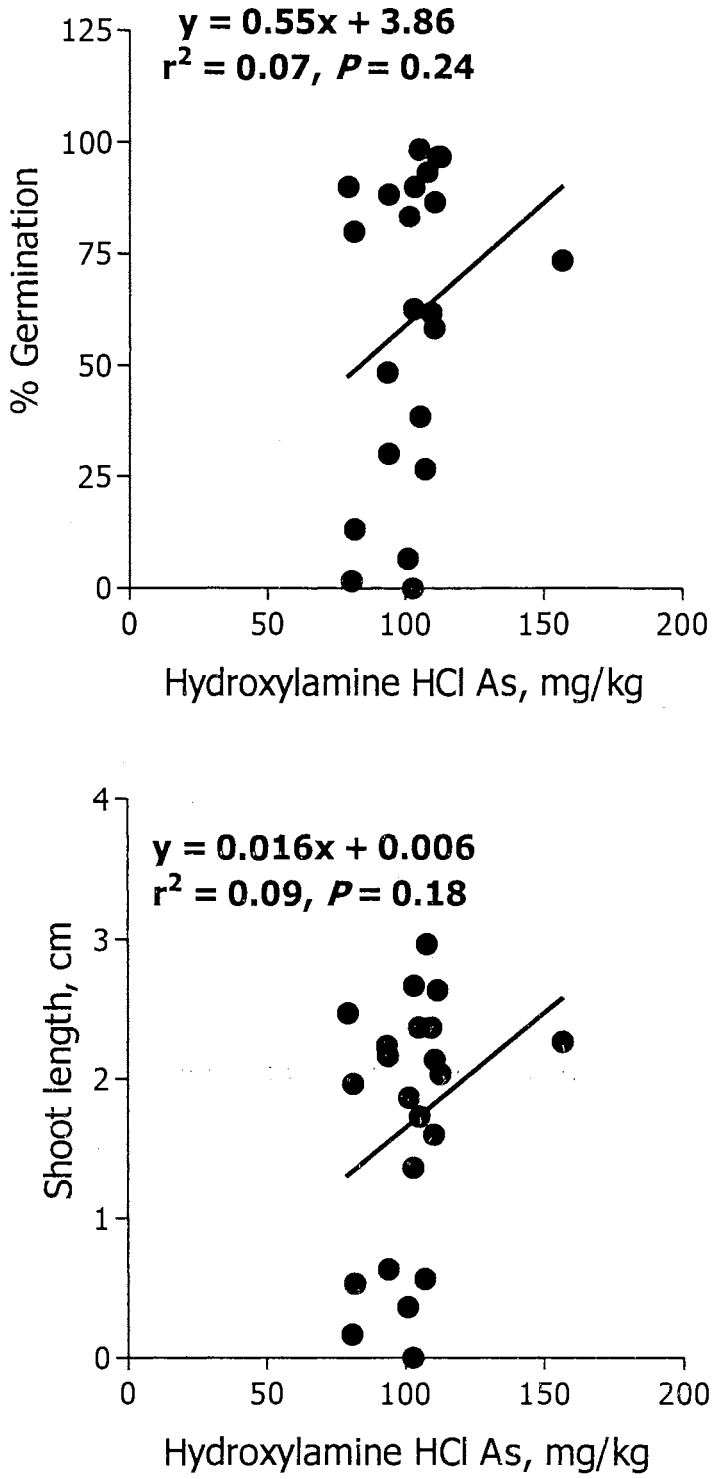

Fig. 1. Hydroxylamine HCl arsenic with % seed germination and shoot elongation

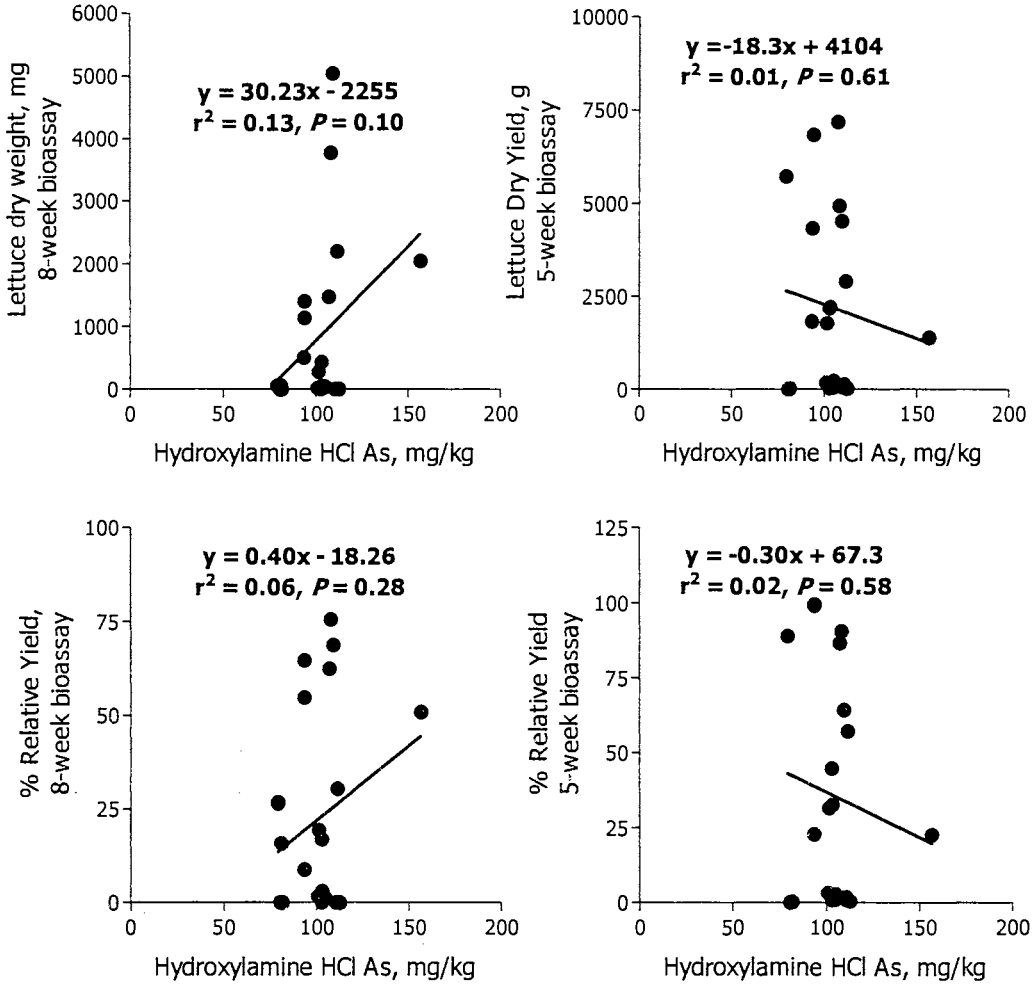

Fig 2. Hydroxylamine HCl extracted As with Lettuce endpoints Yield and % Relative yield.

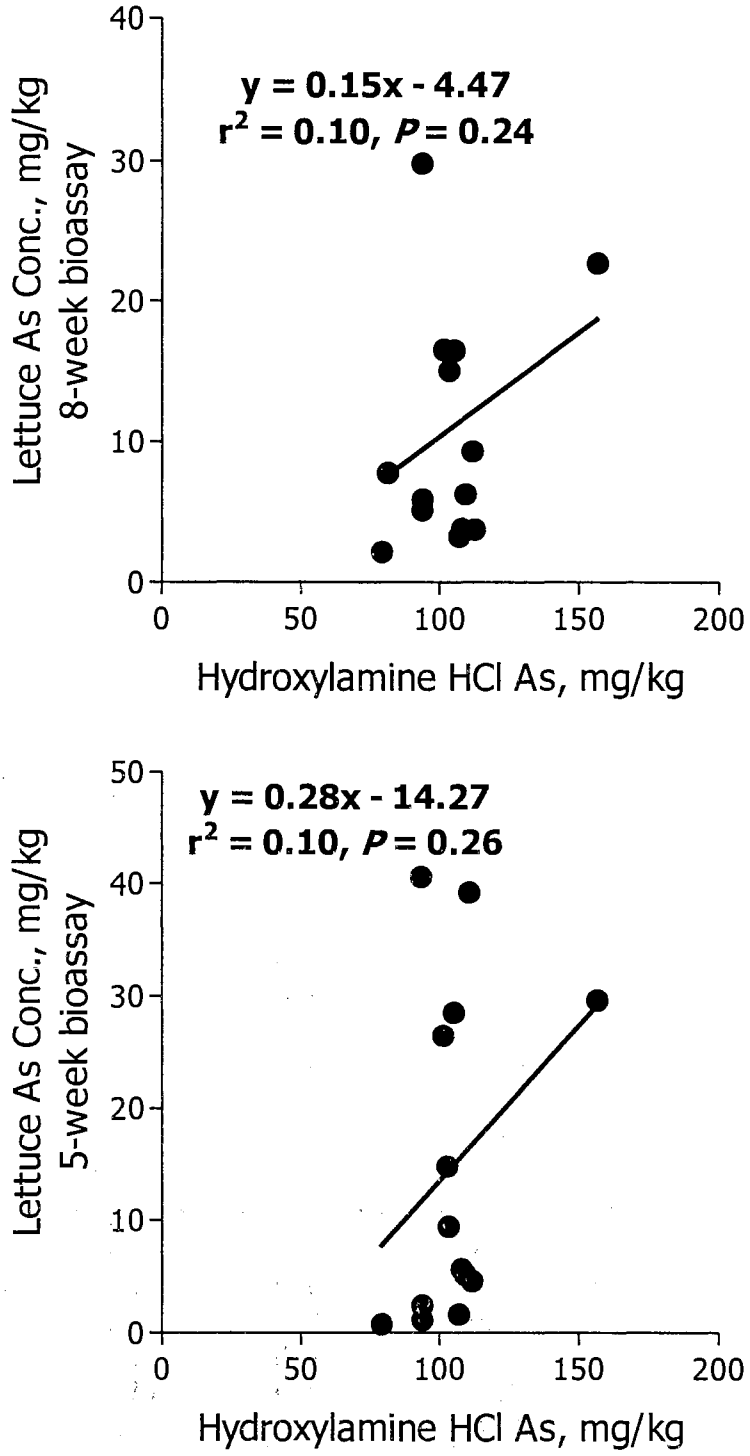

Fig 3. Hydroxylamine HCl extracted arsenic with lettuce tissue arsenic concentration from a 8-week and 5-week bioassay.

APPENDIX B

Table 1. Pore water pH and arsenic concentrations from each week during the incubation phase of treatment.

			zero-Fe pH		
	week 0	week 1	week 2	week 3	week 4
Bernow	4.65	5.00	5.22	4.44	5.37
Dennis	6.41	6.30	6.36	6.20	6.61
Perkins	4.42	5.21	5.80	5.25	6.54
Pratt	7.00	7.31	7.57	7.09	7.89
Slag Waste	7.73	7.78	7.92	7.98	7.78

		zero-Fe As concentration (mg/L)		
	week 1	week 2	week 3	week 4
Bernow	0.07	0.10	0.03	0.03
Dennis	0.02	0.02	0.01	0.02
Perkins	0.42	0.58	0.25	0.22
Pratt	0.96	0.68	0.46	1.42
Slag Waste	7.73	7.78	7.92	7.98

			FeCl3 pH		
	week 0	week 1	week 2	week 3	week 4
Bernow	4.65	2.31	2.32	2.39	2.28
Dennis	6.41	2.87	2.92	3.02	3.01
Perkins	4.42	2.15	2.10	6.20	2.34
Pratt	7.00	2.16	2.24	2.73	2.42
Slag Waste	7.73	7.00	7.14	6.98	7.20

		FeCl3 As concentration (mg/L)		
	week 1	week 2	week 3	week 4
Bernow	0.48	0.47	0.39	0.40
Dennis	0.03	0.02	0.01	0.02
Perkins	4.87	5.54	6.70	2.73
Pratt	5.24	7.74	4.28	8.73
Slag Waste	0.02	0.02	0.01	0.02

			Fe2(SO4)3 pH		
	week 0	week 1	week 2	week 3	week 4
Bernow	4.65	2.95	3.00	3.07	3.02
Dennis	6.41	3.37	3.43	3.41	3.41
Perkins	4.42	2.31	2.63	2.57	2.47
Pratt	7.00	2.13	2.21	2.39	2.39
Slag Waste	7.73	7.79	7.79	7.72	7.86

		Fe2(SO4)3 As concentration (mg/L)		
	week 1	week 2	week 3	week 4
Bernow	0.38	0.46	0.69	1.77
Dennis	0.05	0.13	0.11	3.26
Perkins	4.92	5.02	5.49	7.12
Pratt	53.39	85.30	33.15	13.37
Slag Waste	0.02	0.03	0.02	0.04

			Fe-WTR pH		
	week 0	week 1	week 2	week 3	week 4
Bernow	4.65	5.25	5.23	5.33	5.17
Dennis	6.41	5.82	5.85	5.91	5.96
Perkins	4.42	5.23	5.28	5.37	4.43
Pratt	7.00	5.64	5.61	5.77	5.41
Slag Waste	7.73	7.87	7.78	7.75	7.63

		Fe-WTR As concentration (mg/L)		
	week 1	week 2	week 3	week 4
Bernow	0.03	0.06	0.05	0.03
Dennis	0.06	0.09	0.05	0.07
Perkins	0.13	0.10	0.93	0.07
Pratt	0.20	0.28	0.07	0.06
Slag Waste	0.06	0.05	0.05	0.06

Table 2. Pore water arsenic from 4 soils and slag waste treated with zero-Fe, $FeCl_3$, $Fe_2(SO_4)_3$ or Fe-water treatment residuals (Fe-WTR).

Soil	Treatment	Rep	As1890 mg/L	Average As, mg/L
Bernow	Spike	1	0.7775	0.787
Bernow	Spike	2	0.7972	
Bernow	Zero-Fe	1	0.0658	0.063
Bernow	Zero-Fe	2	0.0599	
Bernow	$FeCl_3$	1	0.0421	0.040
Bernow	$FeCl_3$	2	0.0376	
Bernow	$Fe_2(SO_4)_3$	1	0.0384	0.032
Bernow	$Fe_2(SO_4)_3$	2	0.0263	
Bernow	Fe-WTR	1	0	0.00
Bernow	Fe-WTR	2	0	
Bernow	Control	1	0.0049	0.007
Bernow	Control	2	0.0080	
Dennis	Spike	1	0.0513	0.048
Dennis	Spike	2	0.0453	
Dennis	Zero-Fe	1	0.0266	0.027
Dennis	Zero-Fe	2	0.0282	
Dennis	$FeCl_3$	1	0.0210	0.018
Dennis	$FeCl_3$	2	0.0150	
Dennis	$Fe_2(SO_4)_3$	1	0.0171	0.020
Dennis	$Fe_2(SO_4)_3$	2	0.0231	
Dennis	Fe-WTR	1		0.00
Dennis	Fe-WTR	2	0	
Dennis	Control	1	0.0117	0.014
Dennis	Control	2	0.0168	
Perkins	Spike	1	18.28	18.4
Perkins	Spike	2	18.50	
Perkins	Zero-Fe	1	0.4898	0.490
Perkins	Zero-Fe	2		
Perkins	$FeCl_3$	1		0.045
Perkins	$FeCl_3$	2	0.0445	
Perkins	$Fe_2(SO_4)_3$	1	0.3505	0.295
Perkins	$Fe_2(SO_4)_3$	2	0.2403	
Perkins	Fe-WTR	1	0	0.00
Perkins	Fe-WTR	2	0	
Perkins	Control	1	0	0.00
Perkins	Control	2	0	

Soil	Treatment	Rep	As1890 mg/L	Average As, mg/L
Pratt	Spike	1	275.9	283
Pratt	Spike	2	289.1	
Pratt	Zero-Fe	1	0.6202	0.647
Pratt	Zero-Fe	2	0.6735	
Pratt	$FeCl_3$	1	0.0984	0.099
Pratt	$FeCl_3$	2	0.1005	
Pratt	$Fe_2(SO_4)_3$	1	0.3041	0.324
Pratt	$Fe_2(SO_4)_3$	2	0.3445	
Pratt	Fe-WTR	1	0.0129	0.030
Pratt	Fe-WTR	2	0.0463	
Pratt	Control	1	0.0263	0.027
Pratt	Control	2	0.0272	
Slag	Untreat	1	0.1919	0.191
Slag	Untreat	2	0.1905	
Slag	Zero-Fe	1	1.098	0.815
Slag	Zero-Fe	2	0.5322	
Slag	$FeCl_3$	1	0.1973	0.159
Slag	$FeCl_3$	2	0.1215	
Slag	$Fe_2(SO_4)_3$	1	0.0174	0.021
Slag	$Fe_2(SO_4)_3$	2	0.0238	
Slag	Fe-WTR	1	0.0143	0.010
Slag	Fe-WTR	2	0.0062	

Detection Limit			Low DL	High DL
St Dev	1	0.01044	0.0522	0.1044

Table 3. Bray-1 extracted arsenic from 4 soils and slag waste treated with zero-Fe, $FeCl_3$, $Fe_2(SO_4)_3$ or Fe-water treatment residuals (Fe-WTR).

Soil	Treatment	Rep	As conc mg/kg	Average As, mg/kg	SD *CoV*
Bernow	Spike	1	28.28	**28.2**	0.19
Bernow	Spike	2	28.01		*0.7*
Bernow	Zero-Fe	1	6.478	**6.47**	0.01
Bernow	Zero-Fe	2	6.459		*0.2*
Bernow	$FeCl_3$	1	4.475	**4.47**	0.01
Bernow	$FeCl_3$	2	4.456		*0.3*
Bernow	$Fe_2(SO_4)_3$	1	5.863	**5.96**	0.14
Bernow	$Fe_2(SO_4)_3$	2	6.059		*2.3*
Bernow	Fe-WTR	1	3.841	**3.83**	0.01
Bernow	Fe-WTR	2	3.822		*0.4*
Bernow	Control	1	*0.039*	***0.07***	0.05
Bernow	Control	2	*0.103*		*63.7*
Dennis	Spike	1	3.093	**3.15**	0.08
Dennis	Spike	2	3.201		*2.4*
Dennis	Zero-Fe	1	1.382	**1.40**	0.03
Dennis	Zero-Fe	2	1.426		*2.2*
Dennis	$FeCl_3$	1	1.439	**1.44**	0.00
Dennis	$FeCl_3$	2	1.432		*0.3*
Dennis	$Fe_2(SO_4)_3$	1	1.185	**1.28**	0.14
Dennis	$Fe_2(SO_4)_3$	2	1.382		*10.9*
Dennis	Fe-WTR	1	2.18	**2.17**	0.02
Dennis	Fe-WTR	2	2.155		*0.8*
Dennis	Control	1	0	**0.00**	0.00
Dennis	Control	2	0		*0.0*
Perkins	Spike	1	72.01	**70.2**	2.54
Perkins	Spike	2	68.42		*3.6*
Perkins	Zero-Fe	1	18.1	**18.1**	0.06
Perkins	Zero-Fe	2	18.02		*0.3*
Perkins	$FeCl_3$	1	11.19	**11.3**	0.16
Perkins	$FeCl_3$	2	11.42		*1.4*
Perkins	$Fe_2(SO_4)_3$	1	37.47	**36.2**	1.77
Perkins	$Fe_2(SO_4)_3$	2	34.97		*4.9*
Perkins	Fe-WTR	1	6.82	**6.86**	0.06
Perkins	Fe-WTR	2	6.902		*0.8*
Perkins	Control	1	*0.292*	**0.28**	0.02
Perkins	Control	2	*0.266*		*6.6*

Soil	Treatment	Rep	As conc mg/kg	Average As, mg/kg	SD *CoV*
Pratt	Spike	1	121.2	**120**	1.48
Pratt	Spike	2	119.1		*1.2*
Pratt	Zero-Fe	1	21.72	**21.9**	0.18
Pratt	Zero-Fe	2	21.97		*0.8*
Pratt	$FeCl_3$	1	26.19	**25.4**	1.11
Pratt	$FeCl_3$	2	24.62		*4.4*
Pratt	$Fe_2(SO_4)_3$	1	37.04	**37.6**	0.83
Pratt	$Fe_2(SO_4)_3$	2	38.21		*2.2*
Pratt	Fe-WTR	1	5.66	**5.81**	0.21
Pratt	Fe-WTR	2	5.952		*3.6*
Pratt	Control	1	*0.152*	**0.10**	0.08
Pratt	Control	2	*0.044*		*77.9*
SLAG	Untreat	1	0.501	**0.37**	0.18
SLAG	Untreat	2	0.247		*48.0*
SLAG	Zero-Fe	1	*0.266*	**0.25**	0.02
SLAG	Zero-Fe	2	*0.235*		*8.8*
SLAG	$FeCl_3$	1	*0.089*	**0.10**	0.01
SLAG	$FeCl_3$	2	*0.108*		*13.6*
SLAG	$Fe_2(SO_4)_3$	1	*0.101*	**0.10**	0.00
SLAG	$Fe_2(SO_4)_3$	2	*0.108*		*4.7*
SLAG	Fe-WTR	1	0.488	**0.46**	0.04
SLAG	Fe-WTR	2	0.425		*9.8*

		As1972* mg/L	As 1972 % Recovery
Bray-1	1	5.040	100.8
Bray-1	2	4.813	96.3

			As1972*
Detection Limit	Stdev	1	0.024
	5* StdDev	Low	**0.118**
	10* StdDev	High	0.235
Detection Limit	Stdev	2	0.040
	5* StdDev	Low	**0.198**
	10* StdDev	High	0.395

Table 4. Na-phosphate extracted arsenic from 4 soils and slag waste treated with zero-Fe, $FeCl_3$, $Fe_2(SO_4)_3$ or Fe-water treatment residuals (Fe-WTR).

Soil	Treatment	Rep	As1890* mg/L	Average As mg/kg	StDev *Co Var*
Bernow	Spike	1	26.11	**26.1**	0.08
Bernow	Spike	2	26.00		*0.3*
Bernow	Zero-Fe	1	16.08	**15.9**	0.19
Bernow	Zero-Fe	2	15.81		*1.2*
Bernow	$FeCl_3$	1	12.49	**12.4**	0.15
Bernow	$FeCl_3$	2	12.27		*1.2*
Bernow	$Fe_2(SO_4)_3$	1	12.76	**12.8**	0.04
Bernow	$Fe_2(SO_4)_3$	2	12.81		*0.3*
Bernow	Fe-WTR	1	12.49	**12.5**	0.04
Bernow	Fe-WTR	2	12.55		*0.4*
Bernow	Control	1	0	**0.00**	0.00
Bernow	Control	2	0		
Dennis	Spike	1	23.62	**21.8**	1.56
Dennis	Spike	3	20.98		*7.2*
Dennis	Spike	4	20.87		
Dennis	Zero-Fe	1	15.33	**15.9**	0.75
Dennis	Zero-Fe	2	16.39		*4.8*
Dennis	$FeCl_3$	1	16.40	**16.2**	0.26
Dennis	$FeCl_3$	2	16.03		*1.6*
Dennis	$Fe_2(SO_4)_3$	1	16.62	**17.7**	1.47
Dennis	$Fe_2(SO_4)_3$	2	18.69		*8.3*
Dennis	Fe-WTR	1	11.05	**11.8**	1.05
Dennis	Fe-WTR	2	12.53		*8.9*
Dennis	Control	1	0.26	***0.26***	0.00
Dennis	Control	2	0.26		*0.9*
Perkins	Spike	1	36.99	**39.3**	2.28
Perkins	Spike	3	39.38		*5.8*
Perkins	Spike	4	41.55		
Perkins	Zero-Fe	1	24.41	**24.0**	0.58
Perkins	Zero-Fe	2	23.60		*2.4*
Perkins	$FeCl_3$	1	16.80	**16.6**	0.36
Perkins	$FeCl_3$	2	16.30		*2.2*
Perkins	$Fe_2(SO_4)_3$	3	15.61	**15.7**	0.18
Perkins	$Fe_2(SO_4)_3$	4	15.87		*1.2*
Perkins	Fe-WTR	1	6.79	**6.83**	0.06
Perkins	Fe-WTR	2	6.87		*0.8*
Perkins	Control	1	0.15	***0.14***	0.01
Perkins	Control	2	0.13		*8.0*

Soil	Treatment	Rep	As1890* mg/L	Average As mg/kg	StDev *Co Var*
Pratt	Spike	3	78.17	**76.3**	2.67
Pratt	Spike	4	74.39		*3.5*
Pratt	Zero-Fe	1	19.91	**19.1**	1.16
Pratt	Zero-Fe	2	18.28		*6.1*
Pratt	$FeCl_3$	3	25.08	**24.1**	1.46
Pratt	$FeCl_3$	4	23.02		*6.1*
Pratt	$Fe_2(SO_4)_3$	1	27.17	**26.4**	1.54
Pratt	$Fe_2(SO_4)_3$	2	25.03		*5.8*
Pratt	$Fe_2(SO_4)_3$	3	28.12		
Pratt	$Fe_2(SO_4)_3$	4	25.11		
Pratt	Fe-WTR	1	6.54	**7.84**	1.05
Pratt	Fe-WTR	2	8.56		*13.4*
Pratt	Fe-WTR	3	8.81		
Pratt	Fe-WTR	4	7.44		
Pratt	Control	1	0	**0.00**	0.00
Pratt	Control	2	0		
SLAG	Untreat	1	9.34	**8.31**	1.46
SLAG	Untreat	2	7.28		*17.6*
SLAG	Zero-Fe	1	12.56	**12.9**	0.51
SLAG	Zero-Fe	2	13.27		*3.9*
SLAG	$FeCl_3$	1	13.04	**13.6**	0.85
SLAG	$FeCl_3$	2	14.24		*6.2*
SLAG	$Fe_2(SO_4)_3$	1	13.06	**13.5**	0.60
SLAG	$Fe_2(SO_4)_3$	2	13.91		*4.4*
SLAG	Fe-WTR	1	5.21	**6.12**	1.29
SLAG	Fe-WTR	2	7.02		*21.0*

		As1890* mg/L	As1937 mg/L
Phosphate spike	1	5.295	5.139
% Recovered		*105.9*	*102.8*
Phosphate spike	2	5.1	5.0
% Recovered		*101.6*	*99.6*

			As1890* mg/L	As1937 mg/L
Detect Limit	StDev	7/1/02	0.01134	0.0188
	Low	Stdev*5	**0.057**	**0.094**
	High	Stdev*10	0.11	0.19

Table 5. Hydroxyamine HCl extracted arsenic from 4 soils and slag waste treated with zero-Fe, $FeCl_3$, $Fe_2(SO_4)_3$ or Fe-water treatment residuals (Fe-WTR).

Soil	Treatment	Rep	As1890 mg/L	Dilution Factor	As mg/kg	Average As, mg/kg	SD *CoV*
Bernow	Spike	1	0.6945	100	69.5	**73.1**	5.16
Bernow	Spike	2	0.7675	100	76.7		*7.06*
Bernow	Zero-Fe	1	0.6809	100	68.1	**67.7**	0.60
Bernow	Zero-Fe	2	0.6724	100	67.2		*0.89*
Bernow	FeCl3	1	0.7151	100	71.5	**75.8**	6.12
Bernow	FeCl3	2	0.8017	100	80.2		*8.07*
Bernow	Fe2(SO4)3	1	0.7859	100	78.6	**79.4**	1.20
Bernow	Fe2(SO4)3	2	0.803	100	80.3		*1.51*
Bernow	Fe-WTR	1	0.6169	100	61.7	**61.6**	0.11
Bernow	Fe-WTR	2	0.6154	100	61.5		*0.18*
Bernow	Control	1	*0.0212*	100	*2.12*	**3.7**	
Bernow	Control	2	0.0748	100	7.5		
Dennis	Spike	1	0.7974	100	79.7	**79.2**	0.77
Dennis	Spike	2	0.7865	100	78.7		*0.97*
Dennis	Zero-Fe	1	0.69	100	69.0	**71.2**	3.15
Dennis	Zero-Fe	2	0.7345	100	73.4		*4.42*
Dennis	FeCl3	1	0.7073	100	70.7	**70.7**	0.04
Dennis	FeCl3	2	0.7067	100	70.7		*0.06*
Dennis	Fe2(SO4)3	1	0.6089	100	60.9	**69.0**	8.75
Dennis	Fe2(SO4)3	2	0.7327	100	73.3		*12.69*
	Fe2(SO4)3	1	0.6503	100	65.0		
	Fe2(SO4)3	2	0.7665	100	76.6		
Dennis	Fe-WTR	1	0.5781	100	57.8	**55.9**	2.72
Dennis	Fe-WTR	2	0.5396	100	54.0		*4.87*
Dennis	Control	1	*0.0211*	100	*2.1*	***2.4***	
Dennis	Control	2	*0.026*	100	*2.6*		
Perkins	Spike	1	0.8029	100	80.3	**86.0**	8.05
Perkins	Spike	2	0.9167	100	91.7		*9.36*
Perkins	Zero-Fe	1	1.1191	100	111.9	**98.7**	18.68
Perkins	Zero-Fe	2	0.8549	100	85.5		*18.92*
Perkins	FeCl3	1	0.9034	100	90.3	**92.5**	3.06
Perkins	FeCl3	2	0.9466	100	94.7		*3.31*
Perkins	Fe2(SO4)3	1	0.5876	100	58.8	**62.6**	5.37
Perkins	Fe2(SO4)3	2	0.6636	100	66.4		*8.58*
Perkins	Fe-WTR	1	0.6726	100	67.3	**63.1**	5.84
Perkins	Fe-WTR	2	0.59	100	59.0		*9.25*
Perkins	Control	1	*0.026*	100	*2.6*	***1.8***	
Perkins	Control	2	*0.0094*	100	*0.9*		

Pratt	Spike	2	0.8256	100	82.6	**83.9**	4.97
	Spike	1	0.8523	100	85.2		*5.93*
Pratt	Zero-Fe	1	0.7222	100	72.2	**75.0**	3.93
	Zero-Fe	2	0.7778	100	77.8		*5.24*
Pratt	FeCl3	1	0.6649	100	66.5	**74.4**	11.12
	FeCl3	2	0.8221	100	82.2		*14.95*
	Fe2(SO4)3	1	0.3758	100	37.6	**38.5**	1.28
Pratt	Fe2(SO4)3	2	0.3938	100	39.4		*3.32*
Pratt	Fe-WTR	1	0.5713	100	57.1	**50.8**	8.93
Pratt	Fe-WTR	2	0.4449	100	44.5		*17.58*
Pratt	Control	1	*0.0142*	100	*1.4*	***1.4***	
Pratt	Control	2	*0.0139*	100	*1.4*		
SLAG	Untreat	1	1.4384	100	143.8	**152**	11.82
SLAG	Untreat	2	1.6056	100	160.6		*7.77*
SLAG	Zero-Fe	1	1.4802	100	148.0	**147**	2.14
SLAG	Zero-Fe	2	1.4499	100	145.0		*1.46*
SLAG	FeCl3	1	1.4124	100	141.2	**146**	7.29
SLAG	FeCl3	2	1.5155	100	151.6		*4.98*
SLAG	Fe2(SO4)3	1	1.4345	100	143.5	**142**	1.73
SLAG	Fe2(SO4)3	2	1.41	100	141.0		*1.22*
SLAG	Fe-WTR	1	1.111	100	111.1	**112**	0.75
SLAG	Fe-WTR	2	1.1216	100	112.2		*0.67*

			As1890 mg/L	As1937 mg/L	**As1890 % Recovery**
Hydroxylamine HCl spike		1	4.9017	4.833	**98.0**
Hydroxylamine HCl spike		2	4.9507	4.903	**99.0**
Perkins spike	Fe(SO4)	1	1.6948	1.732	**110.7**
Pratt spike	FeCl3	1	2.0129	2.07	**105.8**
Pratt - 1	FeCl3 Spike	+ As=2	2.7044	2.672	**98.5**
Perkins - 1	zero-Fe Spike	+ As=2	3.0927	3.128	**93.9**

			As1890 mg/L	As1937 mg/L
Detection Limit	St Dev		0.0139	0.0229
	5*Stdev	low	0.0694	0.1145
	10*StDev	high	0.1388	0.229

			As1890 mg/L	As1937 mg/L
Detection Limit	St Dev		0.0111	0.035
7/8/02	5*Stdev	low	0.0556	0.175
	10*StDev	high	0.1112	0.35

Table 6. Ammonium oxalate extracted arsenic from 4 soils and slag waste treated with zero-Fe, $FeCl_3$, $Fe_2(SO_4)_3$ or Fe-water treatment residuals (Fe-WTR).

Soil	Treatment	Rep	Al3082 mg/L	As1890* mg/L	Fe2714 mg/L	DF	Al mg/kg	Average Al mg/kg	Fe mg/kg	Average Fe mg/kg	As mg/kg	Average As mg/kg	StDev *Co Var*
Bernow	Spike	3	8.853	1.0679	3.768	80	708.2	699	301.4	**307**	85.4	**86.9**	2.1
Bernow	Spike	4	8.624	1.1046	3.898	80	689.9		311.8		88.4		*2.4*
Bernow	Zero-Fe	1	5.744	0.91227	19.66	100	574.4	546	1966	**1955**	91.2	**87.9**	4.7
Bernow	Zero-Fe	2	5.166	0.84638	19.44	100	516.6		1944		84.6		*5.3*
Bernow	$FeCl_3$	1	7.809	0.91432	17.9	100	780.9	546	1790	**1763**	91.4	**89.3**	2.9
Bernow	$FeCl_3$	2	7.313	0.87261	17.36	100	731.3		1736		87.3		*3.3*
Bernow	$Fe_2(SO_4)_3$	1	7.862	0.83598	12.5	100	786.2	756	1250	**1341**	83.6	**89.9**	8.9
Bernow	$Fe_2(SO_4)_3$	2	9.009	0.96176	14.32	100	900.9		1432		96.2		*9.9*
Bernow	Fe-WTR	1	9.575	0.91978	86.63	100	957.5	844	8663	**8542**	92.0	**92.4**	4.6
Bernow	Fe-WTR	2	9.613	0.98499	84.21	100	961.3		8421		98.5		*5.0*
Bernow	Fe-WTR	1	17.96	1.7343	170.4	50	898	959	8520		86.7		
Bernow	Fe-WTR	2	17.4	1.8079	161.3	50	870		8065		*90.4*		
Bernow	Control	1	5.501	0	3.881	100	550.1	576	388.1	**407**	0.0	**0.0**	0.0
Bernow	Control	2	6.012	0	4.267	100	601.2		426.7		0.0		
Dennis	Spike	1	9.154	0.69967	6.921	100	915.4	895	692.1	**678**	70.0	**70.7**	1.1
Dennis	Spike	2	8.75	0.71485	6.638	100	875		663.8		71.5		*1.5*
Dennis	Zero-Fe	1	8.025	0.71532	20.55	100	802.5	819	2055	**1920**	71.5	**71.0**	0.8
Dennis	Zero-Fe	2	8.346	0.70446	17.84	100	834.6		1784		70.4		*1.1*
Dennis	$FeCl_3$	1	8.341	0.62032	15.56	100	834.1	807	1556	**1482**	62.0	**60.0**	2.8
Dennis	$FeCl_3$	2	7.793	0.58056	14.08	100	779.3		1408		58.1		*4.7*
Dennis	$Fe_2(SO_4)_3$	1	7.687	0.64374	16.76	100	768.7	746	1676	**1569**	64.4	**61.7**	3.7
Dennis	$Fe_2(SO_4)_3$	2	7.226	0.59078	14.61	100	722.6		1461		59.1		*6.1*

Soil	Treatment	Rep	Al3082 mg/L	As1890* mg/L	Fe2714 mg/L	DF	Al mg/kg	Average Al mg/kg	Fe mg/kg	Average Fe mg/kg	As mg/kg	Average As mg/kg	StDev *Co Var*
Dennis	Fe-WTR	1	12.96	0.9732	109.9	100	1296	1273	10990	**10795**	97.3	**93.4**	3.4
Dennis	Fe-WTR	2	12.49	0.92519	106	100	1249		10600		92.5		*3.6*
Dennis	Fe-WTR	1	25.61	1.8493	214.2	50	1281		10710		92.5		
Dennis	Fe-WTR	2	24.62	1.8247	212.1	50	1231		10605		91.2		
Dennis	Control	1	8.077	0	7.495	100	807.7	836	749.5	**790**	0.0	**0.0**	0.0
Dennis	Control	2	8.633	0	8.31	100	863.3		831		0.0		
Perkins	Spike	1	4.961	1.3337	4.652	100	496.1	488	465.2	**517**	133.4	**132**	3.1
Perkins	Spike	1	9.652	2.5714	13.04	50	482.6		652		128.6		
Perkins	Spike	2	4.859	1.3444	4.346	100	485.9		434.6		134.4		*2.4*
Perkins	Zero-Fe	1	4.135	1.5045	29.23	100	413.5	485	2923	**3119**	150.5	**161**	10.1
Perkins	Zero-Fe	3	6.517	2.028	38.91	80	521.4		3112.8		162.2		*6.3*
Perkins	Zero-Fe	4	6.498	2.1329	41.5	80	519.8		3320		170.6		
Perkins	$FeCl_3$	1	5.803	1.3838	27.98	100	580.3	577	2798	**2817**	138.4	**138**	0.4
Perkins	$FeCl_3$	2	5.736	1.37777	28.35	100	573.6		2835		137.8		*0.3*
Perkins	$Fe_2(SO_4)_3$	1	4.514	1.3147	17.03	100	451.4	420	1703	**1592**	131.5	**124**	10.5
Perkins	$Fe_2(SO_4)_3$	2	3.883	1.1666	14.8	100	388.3		1480		116.7		*8.4*
Perkins	Fe-WTR	1	6.731	1.007	72.11	100	673.1	684	7211	**7673**	100.7	**102**	1.8
Perkins	Fe-WTR	2	6.955	1.0328	81.35	100	695.5		8135		103.3		*1.8*
Perkins	Control	1	4.401	0.00247	4.429	100	440.1	462	442.9	**519**	0.2	**0.2**	0.1
Perkins	Control	2	4.835	0.00165	5.947	100	483.5		594.7		0.2		*28.1*
Pratt	Spike	1	1.237	1.2332	1.144	100	123.7	120	114.4	**150**	123.3	**125**	10.1
Pratt	Spike	1	2.335	2.2709	4.937	50	116.8		246.85		113.5		
Pratt	Spike	2	1.162	1.2461	1.066	100	116.2		106.6		124.6		*8.1*
Pratt	Spike	4	1.527	1.7256	1.635	80	122.2		130.8		138.0		
Pratt	Zero-Fe	1	1.827	2.0292	31.91	100	182.7	170	3191	**2983**	202.9	**186**	15.0

Soil	Treatment	Rep	Al3082 mg/L	As1890* mg/L	Fe2714 mg/L	DF	Al mg/kg	Average Al mg/kg	Fe mg/kg	Average Fe mg/kg	As mg/kg	Average As mg/kg	StDev *Co Var*
Pratt	Zero-Fe	1	3.389	3.4861	58.62	50	169.5		2931		174.3		
Pratt	Zero-Fe	2	1.562	1.8091	28.26	100	156.2		2826		180.9		*8.1*
Pratt	$FeCl_3$	1	1.091	1.707	20.86	100	109.1	102	2086	**1921**	170.7	**160**	15.0
Pratt	$FeCl_3$	2	0.9551	1.4945	17.56	100	95.51		1756		149.5		*9.4*
Pratt	$Fe_2(SO_4)_3$	1	1.698	1.8398	16.55	50	84.9	138	827.5	**892**	92.0	**96.4**	
Pratt	$Fe_2(SO_4)_3$	2	1.811	1.9625	18	50	90.55		900		98.1		
Pratt	$Fe_2(SO_4)_3$	2	2.397	0.99085	9.477	100	239.7		947.7		99.1		
Pratt	Fe-WTR	1	4.495	0.88972	61.13	100	449.5	456	6113	**6170**	89.0	**90.4**	2.0
Pratt	Fe-WTR	2	4.631	0.91767	62.26	100	463.1		6226		91.8		*2.2*
Pratt	Control	1	1.274	0.0188	1.115	100	127.4	134	111.5	**111**	1.9	**2.2**	0.4
Pratt	Control	2	1.401	0.02445	1.094	100	140.1		109.4		2.4		*18.5*
SLAG	Untreat	1	4.641	0.9511	69.35	100	464.1	448	6935	**6744**	95.1	**91.3**	4.0
SLAG	Untreat	1	9.127	1.7441	136.8	50	456.4		6840		87.2		
SLAG	Untreat	2	4.232	0.91568	64.58	100	423.2		6458		91.6		*4.3*
SLAG	Zero-Fe	1	6.918	1.4641	121.1	100	691.8	690	12110	**12090**	146.4	**135**	11.9
SLAG	Zero-Fe	3	8.578	1.5339	148.9	80	686.2		11912		122.7		*8.8*
SLAG	Zero-Fe	4	8.635	1.7038	153.1	80	690.8		12248		136.3		
SLAG	$FeCl_3$	1	5.938	1.0442	108.8	100	593.8	611	10880	**11940**	104.4	**106**	1.8
SLAG	$FeCl_3$	2	6.271	1.0692	130	100	627.1		13000		106.9		*1.7*
SLAG	$Fe_2(SO_4)_3$	1	7.114	1.1979	137.2	100	711.4	693	13720	**13370**	119.8	**117**	4.6
SLAG	$Fe_2(SO_4)_3$	2	6.751	1.1324	130.2	100	675.1		13020		113.2		*4.0*
SLAG	Fe-WTR	1	12.6	0.88424	234.9	100	1260	1170	23490	**22215**	88.4	**82.8**	7.9
SLAG	Fe-WTR	2	10.8	0.77212	209.4	100	1080		20940		77.2		*9.6*

		As1890* mg/L	As1937 mg/L	Fe2714 mg/L	As1890 %Recover	As1937 %Recover	Fe2714 %Recover
Matrix spike	1	2.1823	2.219	4.604	109.1	111.0	115.1
	2	2.0134	2.037	4.326	100.7	101.9	108.2
	3	2	1.974	4.27	100.0	98.7	106.8
Pratt FeCl3 spike + 2ppm	1	3.4508	3.452	29.86	93.09	92.67	96.76
SLAG Fe(SO4) spike + 2ppm	2	3.0517	2.974	127.4	97.42	95.02	90.87

			Al3082 mg/L	As1890* mg/L	As1937 mg/L	Fe2714 mg/L
Detection Limit	St Dev	1	0.0311	0.03242	0.0199	0.0287
	5*St Dev	Low	**0.1555**	**0.1621**	**0.1**	**0.144**
	10*St Dev	High	0.311	0.3242	0.199	0.287
Detection Limit	St Dev	2	0.0945	0.0761	0.012	0.031
	5*St Dev	Low	**0.4725**	**0.3805**	**0.06**	**0.155**
	10*St Dev	High	0.945	0.761	0.1196	0.31
Detection Limit	St Dev	3	0.0328	0.01294	0.019	0.2302
	5*St Dev	Low	**0.164**	**0.0647**	**0.095**	**1.151**
	10*St Dev	High	0.328	0.1294	0.19	2.302

Table 7. Total Metal content (EPA 3051) from <0.25 mm fraction of soil and slag waste.

Soil	Treatment	As mg/kg	Fe mg/kg	Al mg/kg	Mn mg/kg	Ca mg/kg	Mg mg/kg	P mg/kg	Mo mg/kg	Zn mg/kg
Slag Waste	Positive Cont	409	80950	15245	788	61975	11273	17.6	26.5	15740
Slag Waste	zero-Fe	361	67475	18403	694	56775	11793	17.8	20.9	11530
Slag Waste	Fe-chloride	374	72125	17155	672	50750	11110	17.7	19.9	12010
Slag Waste	Fe-sulfate	366	73425	16938	680	52550	11440	17.9	20.6	12195
Slag Waste	Fe-WTR	325	90300	14528	846	50725	8760	38.2	24.9	11425
Dennis	Positive Cont	243	31700	43733	560	1220	3837	160.4	5.0	55.3
Dennis	zero-Fe	225	31873	34360	512	1161	3241	138.6	4.8	60.5
Dennis	Fe-chloride	235	33053	40165	443	3055	3524	154.8	4.9	59.6
Dennis	Fe-sulfate	213	32243	37323	502	3236	3157	146.6	5.0	54.7
Dennis	Fe-WTR	235	49243	32920	653	3529	2989	731.0	6.8	56.9
Perkins	Positive Cont	257	7828	12180	85.9	805	1232	197.4	1.9	29.6
Perkins	zero-Fe	255	10693	12185	109	1271	1227	196.0	1.9	31.3
Perkins	Fe-chloride	268	11923	9028	73.6	1305	1278	196.8	1.8	32.6
Perkins	Fe-sulfate	228	9758	9730	43.9	143	1007	153.6	1.8	24.6
Perkins	Fe-WTR	239	23653	11183	206	2100	1050	13.1	4.5	24.1
Bernow	Positive Cont	276	16115	30823	46.2	1064	2048	95.0	4.1	36.8
Bernow	zero-Fe	234	16170	23665	54.1	1371	1624	79.2	4.1	32.9
Bernow	Fe-chloride	255	18710	27718	56.3	1803	1893	94.7	4.4	36.5
Bernow	Fe-sulfate	266	19403	28490	60.4	2432	1950	94.6	4.2	37.1
Bernow	Fe-WTR	222	29675	21073	168	2202	1448	584	5.8	29.4
Pratt	Positive Cont	299	5003	7153	80.3	973	1403	2.9	1.9	27.9
Pratt	zero-Fe	311	8203	5540	92.9	853	1154	2.5	2.4	28.5
Pratt	Fe-chloride	297	7105	5608	32.0	1231	1062	2.5	1.8	25.9
Pratt	Fe-sulfate	215	6695	4660	30.5	1475	875	2.3	1.7	30.8
Pratt	Fe-WTR	414	35998	10183	321	4330	1589	24.9	7.2	30.2

Table 8. Earthworm toxicity test on arsenic spiked soils and slag waste remediated with four different iron amendments, results including arsenic content, % mortality and worm weight.

Soil	Treatment	Depurated worm, As mg/kg	Undepurated worm, As mg/kg	28 day % Mortality	Depurated worm weight, mg/worm	Undepurated worm weight, mg/worm	Average worm weight mg/worm
Bernow	As-Spike	302.9	351.8	3.3	174.5	174.2	174.3
Bernow	zero-Fe	128.0	112.1	0	165.9	153.0	159.4
Bernow	FeCl3	86.0	93.4	3.3	161.7	181.3	171.5
Bernow	Fe2(SO4)3	81.7	110.6	0	197.5	170.6	184.0
Bernow	Fe-WTR	35.0	73.6	0	179.7	195.8	187.8
Bernow	Control	5.9	2.4	0	173.4	163.3	168.3
Dennis	As-Spike	107.3	126.2	6.7	192.7	212.2	202.5
Dennis	zero-Fe	71.6	86.5	3.3	188.8	201.5	195.2
Dennis	FeCl3	75.1	88.8	0	185.4	200.3	192.9
Dennis	Fe2(SO4)3	54.2	71.3	0	181.6	203.3	192.4
Dennis	Fe-WTR	23.6	36.3	0	200.3	192.0	196.1
Dennis	Control	8.3	7.1	0	205.1	226.1	215.6
Perkins	As-Spike	456.9	466.7	22.7	204.7	174.3	189.5
Perkins	zero-Fe	290.9	342.0	0	186.8	184.3	185.5
Perkins	FeCl3	321.1	247.0	3.3	173.5	176.5	175.0
Perkins	Fe2(SO4)3		*39.4*	100	0.0	0.0	0.0
Perkins	Fe-WTR	29.6	72.4	0	199.7	211.5	205.6
Perkins	Control	6.9	5.1	0	245.4	239.1	242.3
Pratt	As-Spike	474.8	566.6	70	184.2	142.5	163.3
Pratt	zero-Fe		*304.3*	100	0.0	0.0	0.0
Pratt	FeCl3	314.4	363.8	0	208.5	189.4	199.0
Pratt	Fe2(SO4)3	487.0	506.0	20	169.7	184.6	177.2
Pratt	Fe-WTR	32.6	74.8	0	193.7	216.4	205.1
Pratt	Control	4.0	4.0	0	163.7	173.4	168.6

Soil	Treatment	Depurated worm, As mg/kg	Undepurated worm, As mg/kg	28 day % Mortality	Depurated worm weight, mg/worm	Undepurated worm weight, mg/worm	Average worm weight mg/worm
Slag Waste	Untreated	156.6	268.1	3.3	217.2	231.2	224.2
Slag Waste	zero-Fe	179.5	201.7	0	206.0	190.7	198.4
Slag Waste	FeCl3	175.3	148.5	3.3	205.8	236.3	221.1
Slag Waste	Fe2(SO4)3	94.7	106.0	0	186.8	181.2	184.0
Slag Waste	Fe-WTR	32.3	70.0	10	206.1	222.0	214.1
Artificial Soil	Control	5.4	6.6	0	152.8	181.5	167.1

Table 9. Earthworm arsenic body burdens and respective decrease (increase) as compared to positive control (arsenic spiked) from Fe-remediated arsenic contaminated soils and one slag material.

Soil	Treatment	Average As body burden, mg/kg	% Decrease (increase) due to treatment	Average As body burden, mg/kg	% Decrease (increase) due to treatment
Bernow	Positive Control	303		352	
Bernow	Fe^{o}	128	57.8	112	68.1
Bernow	$FeCl_3$	86.0	71.6	93.4	73.4
Bernow	$Fe_2(SO_4)_3$	81.7	73.0	111	68.6
Bernow	Fe-WTR	35.0	88.4	73.6	79.1
Bernow	Negative Control	5.87		2.42	
Dennis	Positive Control	107		126	
Dennis	Fe^{o}	71.6	33.3	86.5	31.5
Dennis	$FeCl_3$	75.1	30.0	88.8	29.7
Dennis	$Fe_2(SO_4)_3$	54.2	49.5	71.3	43.5
Dennis	Fe-WTR	23.6	78.0	36.3	71.2
Dennis	Negative Control	8.28		7.13	
Perkins	Positive Control	457		467	
Perkins	Fe^{o}	323	29.3	342	26.7
Perkins	$FeCl_3$	310	32.1	247	47.1
Perkins	$Fe_2(SO_4)_3$	*39.4*		*39.4*	
Perkins	Fe-WTR	29.6	93.5	72.4	84.5
Perkins	Negative Control	6.87		5.06	
Pratt	Positive Control	475		521	
Pratt	Fe^{o}	*304*		*304*	
Pratt	$FeCl_3$	314	33.8	364	30.2
Pratt	$Fe_2(SO_4)_3$	487	(2.6)	506	3.0
Pratt	Fe-WTR	32.6	93.1	74.8	85.7
Pratt	Negative Control	4.04		3.72	
Slag waste	Positive Control	157		268	
Slag waste	Fe^{o}	180	(14.6)	202	24.8
Slag waste	$FeCl_3$	175	(11.9)	149	44.6
Slag waste	$Fe_2(SO_4)_3$	94.7	39.5	106	60.5
Slag waste	Fe-WTR	32.3	79.4	70.0	73.9
Slag waste	Control-Artificial	5.37		3.77	

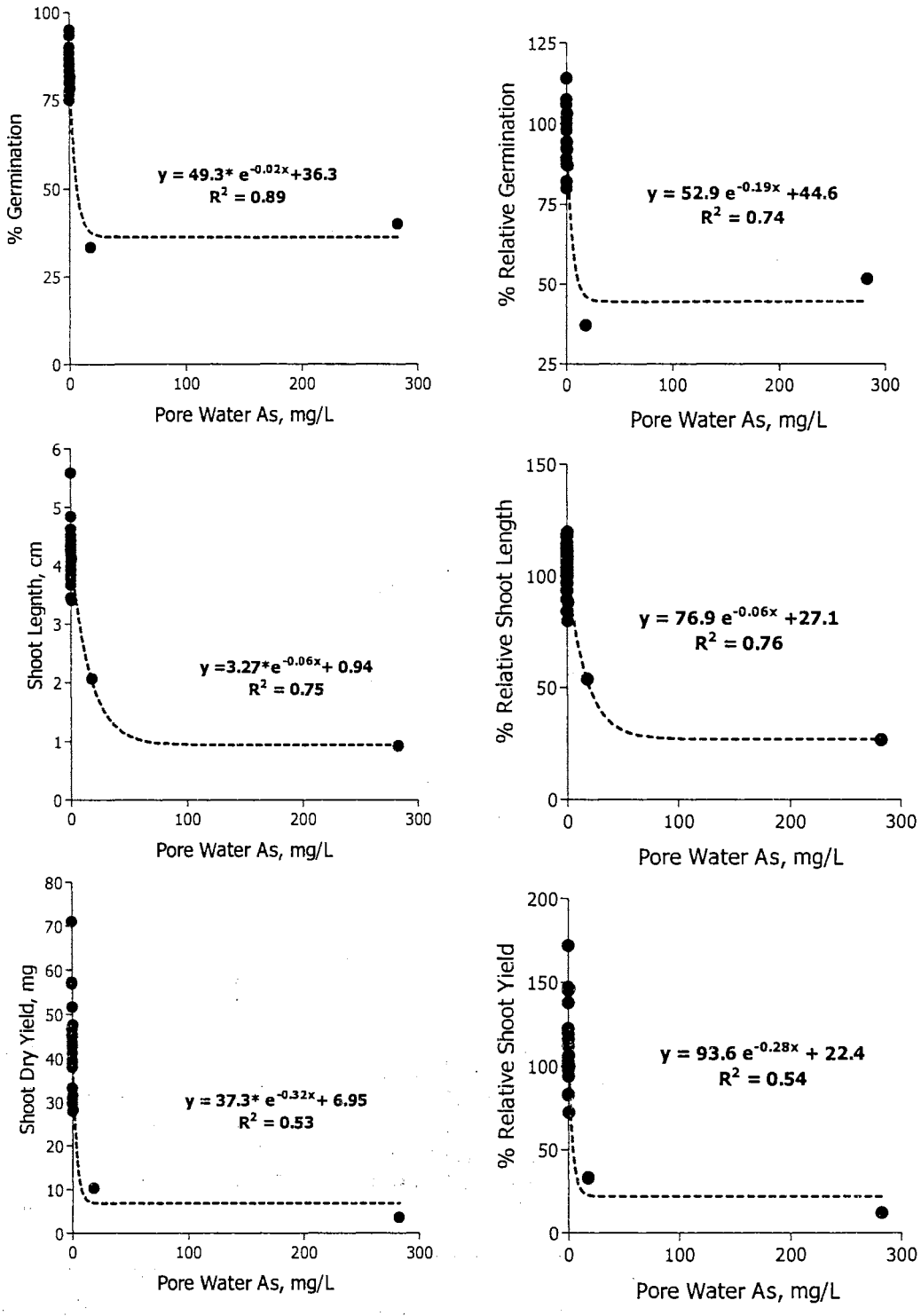

Fig. 1 Pore water arsenic levels with lettuce endpoints germination, shoot lenght and shoot yield and % relative for each parameter.

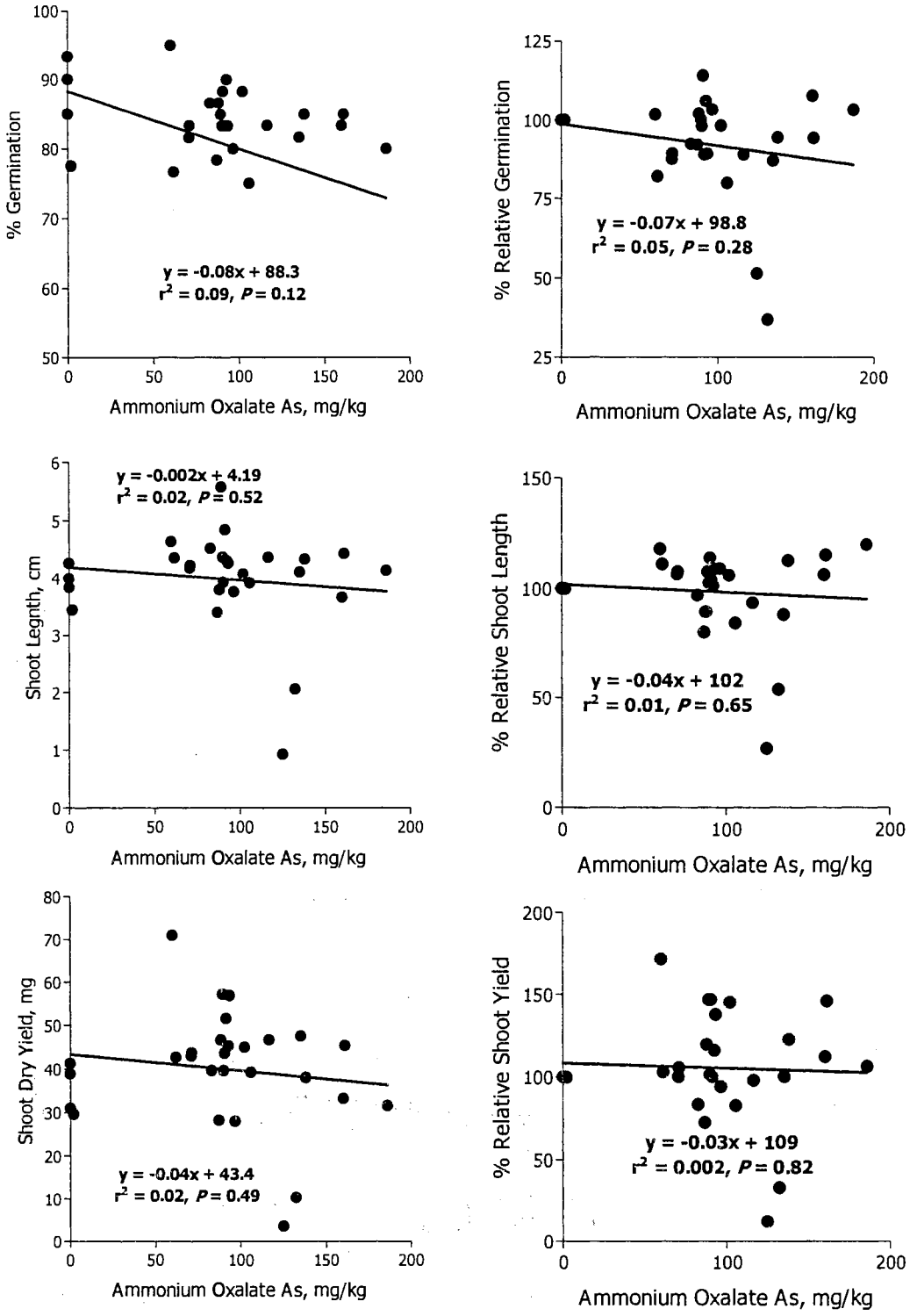

Fig. 2 Ammonium Oxalate extracted arsenic with lettuce tests endpoints: % germination, shoot length and shoot dry weight and %relative for each response.

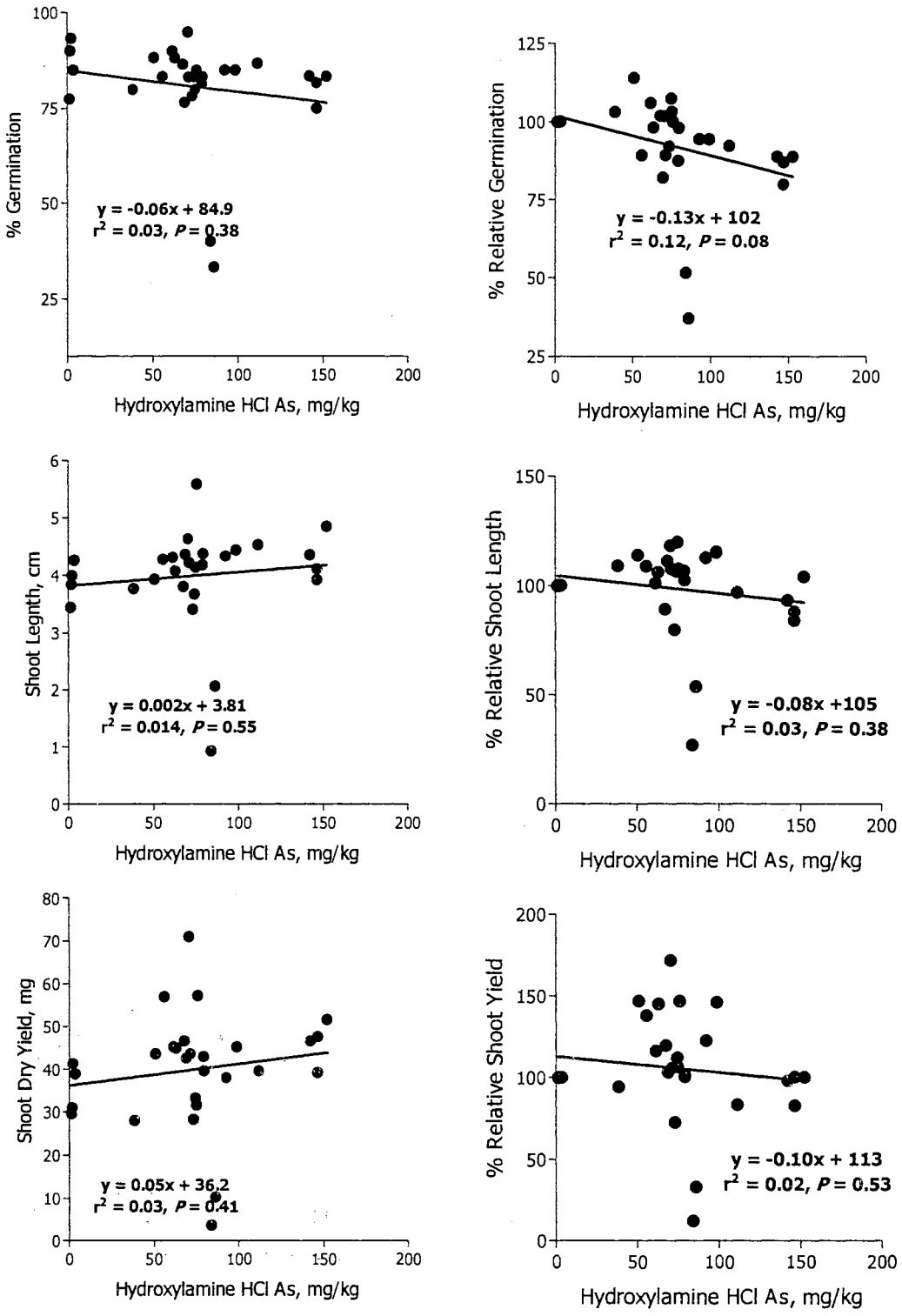

Fig. 3. Hydroxylamine HCl extracted arsenic with lettuce tests endpoints: % germination, shoot length and shoot dry weight and % relative for each response.

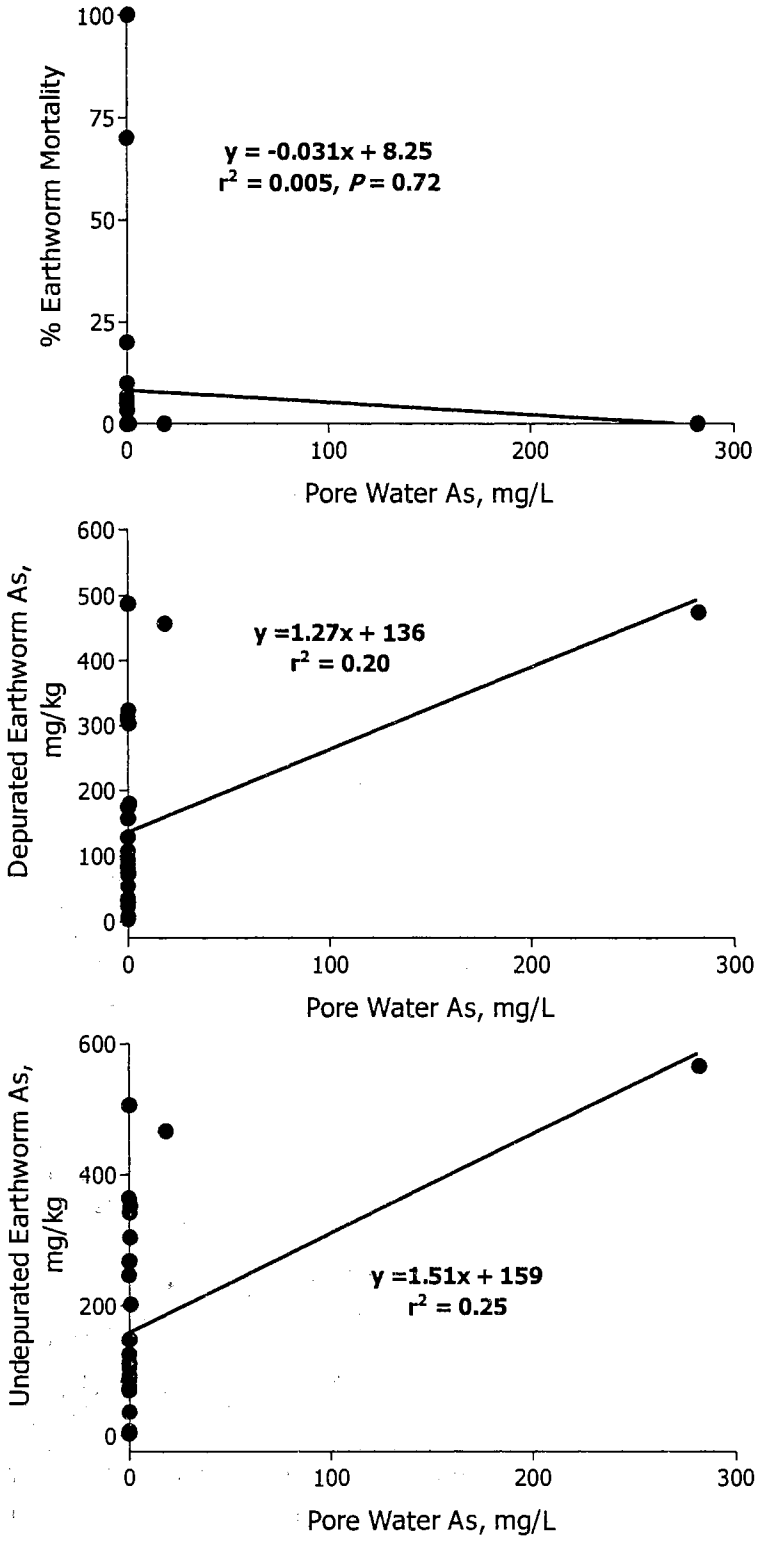

Fig. 4. Pore water arsenic with earthworm mortality and arsenic body burdens (depurated and undepurated) from a 28-day toxicity test on arsenic remediated soils.

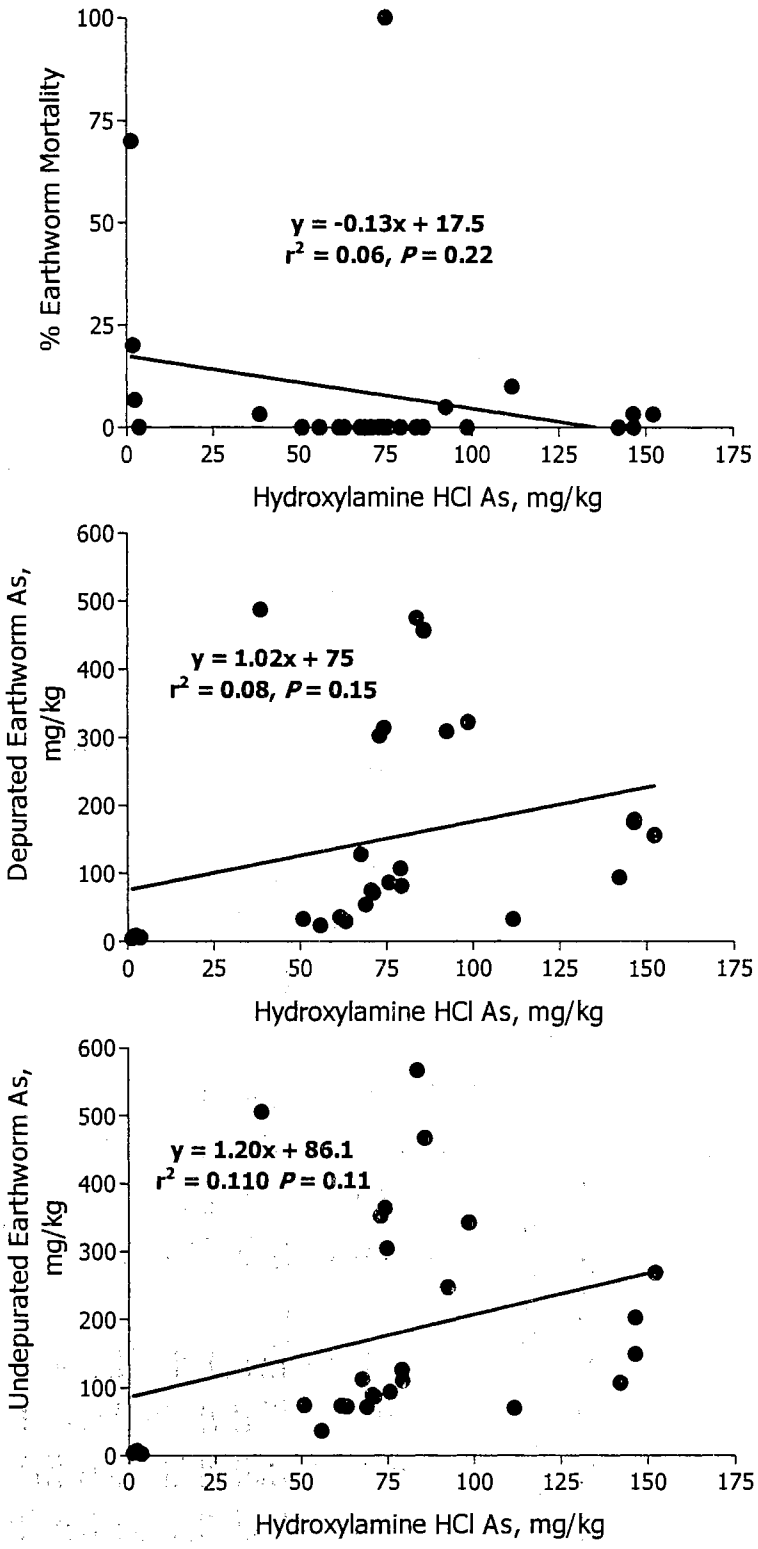

Fig. 5. Hydroxylamine HCl arsenic with earthworm % mortality and arsenic concentrations (body burdens) in depurated and undepurated worms.

VITA 2

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Doctor of Philosophy

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