THE SORPTION OF ROXARSONE, AN ORGANOARSENICAL ANIMAL FEED ADDITIVE

by

Brenda L. Brown

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Madeline E. Schreiber, Chair
J. Donald Rimstidt
Gregory Mullins

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Madeline E. Schreiber, Advisor

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ABSTRACT

The organoarsenical roxarsone is added to poultry feed to increase weight gain. Studies have shown that roxarsone does not accumulate in poultry tissue but is excreted, resulting in elevated arsenic concentrations (~40 mg/kg) in poultry litter. However, there is little understanding of the fate of roxarsone once it is introduced into agricultural watersheds.

Using batch experiments, I investigated the sorption characteristics of roxarsone to Ap and Bt soils of the Frederick series, common in the Shenandoah Valley of Virginia, an area of intense poultry production. Results demonstrate that roxarsone sorbs strongly to Bt soils, but only showed moderate to low sorption onto Ap soils. Sorption to the Ap soils demonstrated stronger pH dependence than did sorption to the Bt soils. Removing organic matter (OM) from Ap soils significantly changed the sorption characteristics, suggesting that OM may be coating mineral surfaces in these soils.

Results of this study have implications for roxarsone transport in agricultural watersheds. For soils that have had years of poultry litter application, there will be both sorption and subsequent leaching of roxarsone. In the OM-rich Ap horizon, OM controls sorption. Because roxarsone is loosely bound to OM, it would be rapidly leached into water after a recharge event or field irrigation. Once roxarsone reaches the Bt soils, it is strongly sorbed into iron oxides or clays, decreasing the potential for leaching. However, competition from phosphate or organic acids for sorption sites on mineral surfaces may affect roxarsone retention in the Bt soils.

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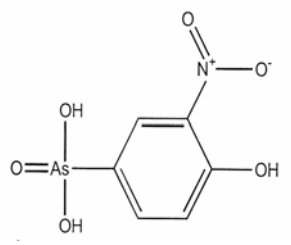

Figure 1. Roxarsone

The organoarsenical roxarsone (3-nitro-4-hydroxyphenyl-arsonic acid) (Figure 1) is added to poultry feed at a concentration of 22.7 to 45.5 grams per ton as a growth stimulant. When combined with anticoccidial drugs, roxarsone also aids in the control of coccidiosis, a debilitating disease affecting chickens. In fact, dozens of organic arsenicals were tested during the 1930's in order to find a treatment for this disease, of which roxarsone showed the most promise (Anderson, 1998). However, it was discovered that the drug's greatest potential was in its use as a growth stimulant for poultry and swine. No longer primarily used for the control of coccidiosis, roxarsone is now used for growth stimulation, improved feed conversion, better feathering, increased egg production, and pigmentation (Anderson, 1998). In Virginia, poultry production units (one unit represents a broiler complex comprised of a group of farms served by a single feed mill) typically use a combination of roxarsone, anticoccidials, and antibiotics to optimize growth rate (Chapman and Johnson, 2002). Poultry manure is mixed with pine bedding material to make poultry litter, which is often applied to soil as a fertilizer. In the Shenandoah Valley of Virginia, there are approximately 364,000 tons of poultry litter produced each year, with most of the litter being applied as fertilizer on cropland (Mullins, 2000).

1.1a Background on roxarsone

Organoarsenic chemistry began in 1760, when L. C. Cadet de Gassicourt synthesized the first organometallic compound in a laboratory. This compound was called "Cadet's fuming arsenical liquid" and was a mixture whose main component was $((CH_3)_2As)_2O$. In 1907, Robert Koch discovered the first medicinal use of an organoarsenical, p-arsanilic acid (atoxyl). This compound was used to treat sleeping sickness. In 1945, Morehouse and Mayfield discovered that the organoarsenical roxarsone (3-nitro-4-hydroxyphenyl arsonic

acid) could be used to promote growth and control coccidiosis in chickens (Calvert, 1975). Roxarsone, the most commonly used of the organoarsenic feed additives, contains the least amount of arsenic (28.5%) at each recommended use level in the animal feed (Alpharma, 1999). Arsenic is detected at low levels in tissues of animals fed arsenicals (Wershaw et al., 1999; Calvert, 1975). However, studies have shown that the arsenic level rapidly decreases to below the F.D.A. limit of 0.5 parts per million (ppm) when the arsenicals are removed from the feed for the required 5-day period before slaughter (Anderson and Chamblee, 2001). Consequently, arsenic in edible animal tissue does not appear to be a potential problem for the consumer.

1.1b Previous work on environmental fate of roxarsone

During the 42-day growth period for administering roxarsone, each broiler excretes about 150 milligrams of the compound (Anderson and Chamblee, 2001) resulting in a total arsenic concentration of 30 to 50 milligrams per kilogram (mg/kg) in poultry litter (Garbarino at al., 2002). When poultry litter is used as fertilizer, it is tilled into the fields at a rate of one to two metric tons per hectare (Garbarino et al., 2002). Wershaw et al. (1999) conclude that approximately 1 x 10^6 kilograms per year of roxarsone and its degradation products are added annually to the environment from the use of poultry litter as fertilizer.

In an early study of the distribution of arsenic from poultry litter, Morrison (1969) compared fields that had poultry litter containing 15 to 30 mg/kg arsenic applied at a rate of 4 to 6 tons per acre per year for 20 years to fields that had no history of poultry litter applications. The soil, alfalfa crop, and clover crop in litter-treated fields contained 1.83 mg/kg, 0.12 mg/kg, and 0.15 mg/kg of arsenic, respectively. The soil, alfalfa crop, and clover crop in non-treated fields contained 2.65 mg/kg, 0.1 mg/kg, and 0.17 mg/kg of arsenic, respectively. Although these data suggest that the roxarsone did not provide a significant input of arsenic to the soil or crops, the drainage water from the litter-treated fields contained 0.29 mg/L of arsenic, which is significantly higher than the current arsenic drinking water standard of 50 μg/L (0.05 mg/L)

Recent studies have documented that roxarsone can be easily leached from poultry litter. Hancock et al. (2002) collected fresh poultry manure and found concentrations of total arsenic up to 27 mg/kg; yet older, composted manure had less than 2 mg/kg total arsenic. The authors also found that although the initial form of arsenic from poultry waste was organic arsenic, the arsenic in soil pore water in sediments collected beside an agricultural field amended with poultry litter was mostly inorganic.

In studies by Garbarino et al. (2002) and Rutherford et al. (2003), arsenic speciation of soil extracts indicated that soils amended with poultry litter have 5 to 10 times more extractable arsenic than soils not amended with poultry litter. Results also indicate that arsenate is the dominant species leaching from the litter, suggesting that transformation reactions may play a very important role in controlling the fate of roxarsone. Experiments in which broiler litter was mixed with deionized water demonstrated that about 70 percent of the total available arsenic was water-extractable. A speciation analysis showed that the majority of the water-extractable arsenic was roxarsone with arsenite, dimethylarsinate (DMA), and unknowns as minor components. These results suggest that roxarsone may be chemically or biologically transformed to inorganic forms (see section below on *Transformation Potential*).

1.2 Arsenic compounds in the environment

Many different arsenic compounds have been identified in the environment and have been classified into three major groups: inorganic, organic, and arsine (Masscheleyn et al., 1991).

1.2a Inorganic arsenic

Inorganic arsenic is a constituent of many minerals and is frequently associated with sulfur, such as arsenopyrite (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3). Iron and manganese sulfide ores in sedimentary shale, bituminous coal and rock phosphates are also typically high in arsenic (Tamaki and Frankenberger, 1992).

Once released from mineral sources, arsenic forms a variety of inorganic

and organic compounds in soils, sediments, and natural waters, but the majority is in the inorganic form of arsenate [As(V)] or arsenite [As(III)] (Smith et al., 1998). Arsenic chemical forms in water are negatively charged oxyanions ($HAsO_4^{2-}$, $H_2AsO_4^-$, $H_2AsO_3^-$, and $HAsO_3^{2-}$) (Sadiq, 1997). These compounds may change valency states depending on the pH and redox conditions (Smith et al., 1998). In oxic soil (Eh>200 mV; pH 5-8), arsenic is usually present in the +5 oxidation state; however, in reducing conditions, arsenic in the +3 oxidation state is the predominant form (Tamaki and Frankenberger, 1992). Both As(V) and As(III) may undergo chemical and/or microbial oxidation-reduction and methylation in soils (Gao et al., 1994).

1.2b Organic arsenic

Microbial methylation of the inorganic arsenic oxyanions (see section below on *Microbial Transformations*) forms methylarsenic compounds, such as monomethylarsonics, di- and trimethylarsines. Further transformation of these organoarsenic compounds may lead to the formation of arsine gas (NRCC 1978).

Naturally occurring organoarsenic compounds are widely distributed in the environment, and are found in the atmosphere, aquatic systems, soil and sediments, and biological tissue (Cullen and Reimer, 1989). There are approximately twenty known naturally occurring organoarsenicals (Andreae, 1986). In the literature, there is little evidence for other than methyl species of naturally occurring organoarsenic compounds. The As-C bonds are formed by living organisms presumably within the metabolic pathways of the cell (Cullen and Reimer, 1989).

Synthetic organoarsenic compounds, including alkyl- and aryl-arsenicals, are used commercially, most often as biocides in agriculture and forestry and as animal feed additives. The more commonly used compounds for biocides are sodium or ammonium salts of methanearsonic acid (MMA), monosodium methylarsonate (MSMA), disodium methylarsonate (DSMA), and monoammonium methanearsonate (MAMA). The methanoarsonates differ from the inorganic orthoarsenate due to a methyl substitution of one of the hydroxyl

groups linked to the arsenic atom. Replacement of another hydroxyl by a second methyl group results in the formation of dimethylarsinic or cacodylic acid (Hiltbold, 1975). In a study by Bednar et al. (2002a), it was noted that, in the 1990's, more than 3000 metric tons per year of monosodium methylarsonate (MSMA), disodium methylarsonate (DSMA) [sodium salts of monomethylarsonic acid (MMA)], and dimethylarsinic acid (DMA) were applied to cotton fields in the United States. This translates into more than 1000 metric tons of arsenic introduced into the environment each year from the use of MSMA and DSMA alone.

The organoarsenic compounds synthesized as animal feed additives include arsanilic acid, roxarsone, carbarsone (p-ureidobenzenearsonic acid), and nitarsone (4-nitrophenyl arsonic acid). Arsanilic acid and roxarsone are used for increased weight gain and improved feed efficiency in chickens and swine and for the control of swine dysentery, while carbarsone and nitarsone are used as antihistomonads in turkeys (Andreae, 1986).

1.2c Arsenic toxicity

Arsenic pollution is a concern because of its toxicological effects on biota. Plants and microorganisms have been reported to be sensitive to a range of arsenic concentrations in the soil, with relatively high concentrations decreasing soil microbial activity and inducing phytotoxic responses in plants (Smith et al., 1998). Overall, arsenic toxicity is dependent on the species, route of administration, physical and chemical form of the compound, and the dose. As(III) is considered more toxic than As(V) because it reacts with sulfydryl groups of cysteine in proteins, inactivating many enzymes (Tamaki and Frankenberger, 1992).

Humans may be exposed to arsenic from a variety of sources in the environment, including food, drinking water, and air. Arsenic residues may be present in meat when medicated feed is not withdrawn five days before the animals are slaughtered (Smith et al., 1998). In the United States, the mean daily arsenic intakes for adults range from 20 and 30 μg, according to the FDA Total Diet Study for market baskets (Borum and Abernathy, 1994). Typical

arsenic concentrations are reported to be about 100 μg/L in human blood and around 15 μg/L in urine (Fowler, 1977). Arsenic undergoes methylation in the liver and leads to the formation of monomethylarsonic and dimethylarsinic acids. These two methylated arsenicals differ in toxicity when compared to inorganic species by one and two orders of magnitude, respectively. The organic forms of arsenic are excreted in the urine faster than the inorganic forms (Buchet and Lison, 2000).

The NRCC (1978) estimated that 5 to 15% of arsenic ingested by humans is absorbed, with arsenic compounds distributed in the kidney, liver, lungs, spleen and the gastrointestinal tract wall within twenty-four hours of absorption. Reported effects on these organs following chronic exposure to arsenic include skin lesions, nephritis, cirrhosis, diabetes, hearing loss, and electromyographic abnormalities. Cardiovascular effects of inorganic arsenic include arrhythmias, hypertension, heart and brain ischaemia, and peripheral artery disorders such as Blackfoot disease leading to gangrene (ATSDR, 1993). Effects of acute and chronic arsenic poisoning in humans vary depending on the age, sex, dose and duration of exposure and the chemical form and oxidation state of the arsenic compound (Fowler, 1977; NRCC, 1978). In cases of acute poisoning, As(III) is considered more toxic than As(V). However, with chronic exposure to lower doses, such as in drinking water, reduction of As(V) makes the distinction less important (Smith et al., 1998). Initiation of cancer appears to be the most common long-term effect of chronic exposure to inorganic arsenic. Although most animal experiments have not demonstrated a direct relationship between arsenic and carcinogenesis (NAS, 1977), epidemiological studies have shown a causal relationship between environmental, occupational and medicinal exposure of humans to inorganic arsenic and cancer of the skin and lungs (NRC, 1999).

1.3 Arsenic cycling

According to the global biogeochemical arsenic cycle of MacKenzie et al. (1979), there is a net gain of arsenic for land (6.6×10^{10} g/yr.) and oceans (5.66×10^{10} g/yr.) while there is a net loss of arsenic from sediments (1.129×10^{11} g/yr.). This

loss is mostly attributed to anthropogenic activities such as mining, burning of fossil fuels, and roasting of arsenic-bearing ores. Within agroecosystems, the primary inputs of arsenic into the system are organoarsenical herbicides, pesticides, and defoliants, with minor inputs from fertilizers, irrigation water, and oxidation of arsine. The major transfer mechanisms in agricultural systems include transformation to methylarsines, soil erosion, plant uptake and translocation, and harvesting of crops.

Once released into the atmosphere, the residence time for arsenic is estimated to be about nine days (Tamaki and Frankenberger, 1992). Even though anthropogenic activities have strongly modified arsenic transfer rates overall, arsenic is not accumulated to a significant extent by biota on a global scale (Woolson, 1983).

1.4 Biogeochemical controls on arsenic release and transport

1.4a Redox effects on arsenic speciation

Redox conditions strongly influence the oxidation state of inorganic arsenic. The ratio of As(III) to As(V) is largely determined by the Eh; however, the Eh is not a function of a single redox couple but a combination of factors, such as the ferric/ferrous redox pair, pH, microbial population, and moisture content (Gao et al., 1994). In oxic soils, arsenate is the predominant arsenic species, but arsenite is found at Eh<300 mV over a pH range of 4 to 8 (Sadiq, 1997). As the Eh decreases due to water saturation or anoxic conditions, the arsenite content increases while arsenate decreases. There is no clear correlation between redox potential and organic arsenic species due to the occurrence of microbial transformations (Woolson and Kearney, 1973).

The influence of redox potential and pH on the speciation and solubility of arsenic in a contaminated soil was investigated by Masscheleyn et al. (1991). Solubility in soils was greatly affected by alterations in the oxidation state of arsenic caused by the redox potential and pH. Arsenic solubility was low and a major portion was present as As(V) at higher soil redox levels (500 to 200 mV or

pH of 10-18). At an alkaline soil pH, the reduction of As(V) to As(III) released arsenic into soil solution. Arsenic solubility, under moderately reduced soil conditions (0-100 mV, pH of 7-9), was controlled by the dissolution of iron oxyhydroxides. It was suggested that arsenic was coprecipitated with Fe oxyhydroxides and released upon their solubilization. The soluble arsenic content increased by 13 fold upon a reduction from 500 mV to –200 mV.

1.4b Dissolution/precipitation controls on arsenic transport

Dissolution of arsenic-bearing minerals, such as arsenopyrite and arsenic-rich metal oxides, is the dominant source of arsenic to natural waters. Arsenopyrite (FeAsS) and arsenian pyrite (FeS_2 containing arsenic in trace to atom percent levels) are quantitatively the most important As-bearing sulfides reported from mineralized areas (Foster, 1999). Dissolution of arsenic-rich iron and manganese oxides is another dominant source of arsenic to hydrogeologic systems (Smedley and Kinniburgh, 2002).

The main precipitation reactions controlling arsenic transport are the formation of arsenic-bearing sulfides in reducing environments (Moore et al., 1988). As-bearing precipitates often form in close association with sulfide minerals, probably because the free energy of formation of these phases is exceeded near the site of sulfide dissolution (Foster, 2003). During the formation of these phases, arsenic typically substitutes for a structural oxyanion such as phosphate, sulfate, or carbonate (Foster, 2003).

1.4c Sorption/desorption

Physical and chemical properties that influence sorption-desorption processes strongly control arsenic concentration in sediment and water. Arsenic has a high affinity for mineral surfaces, such as Fe-oxides, Al-oxides, Mn-oxides and clays. However, the reactivity of these surfaces may vary considerably depending on soil solution composition, pH, and charge density (Smith et al., 1998).

The presence of competitive anions also controls arsenic sorption to mineral surfaces. Due to the chemical similarity between P and As, phosphate is

known to displace sorbed arsenic from soils (e.g., Woolson et al., 1973). Xu et al. (1988) also found that sulfate and fulvic acids may decrease arsenic sorption and increase the mobility and leaching potential of arsenic at pH<7. Other anions such as molybdate, silica, and carbonate have also been shown to compete with arsenate for sorption sites on mineral surfaces (Stollenwerk, 2003).

i. Sorption of roxarsone and organoarsenicals

The sorption of roxarsone is influenced by the fact that it is an organometallic compound and thus exhibits characteristics of both an organic and metallic molecule. Rutherford et al. (2002) examined the sorption characteristics of roxarsone on common soil components to determine where it is sequestered in soils. In this study, they examined the effects of varying pH and ionic strength, and sorption onto natural soil components such as iron oxide, peat, aluminum oxide, and silica. Results showed that roxarsone sorption onto soils decreased as the pH increased (pH 2 to pH 8). The effect of ionic strength on roxarsone sorption to natural organic matter was also determined. Increased sorption occurred as the background concentration of calcium chloride was increased from 0 to 0.004 M; sorption remained constant from 0.004 M to 0.02 M. When roxarsone and arsenate sorption characteristics to different soil components were compared, roxarsone had lower sorption to iron oxide than arsenate. The authors suggested that this was due to the larger size of the roxarsone molecule, preventing it from packing as efficiently as the arsenate ion to a metal hydroxide surface. Similar results were found for sorption to aluminum oxide. Roxarsone had a higher sorption rate than arsenate onto organic matter, which was assumed to be a result of the organic portion of the roxarsone molecule aiding in the sorption to peat. However, at low concentrations of roxarsone and arsenate, both are less strongly sorbed by organic matter than by iron oxides. The study also indicated that the arsenic sorbed by organic matter is readily mobilized by a rain event, as opposed to the arsenic sorbed by iron oxide, which is sequestered in the metal hydroxides.

Useful information on roxarsone sorption can also be deduced from studies on sorption of other synthetic organoarsenicals, including cacodylic acid, DMSA, and MSMA. Wauchope (1975) found that adsorption of the following compounds by sixteen soils increased in the following order: phosphate < Na cacodylate < DSMA = arsenate. Sorption was correlated with soil clay content. Subsoils have been found to be more sorptive for MSMA than surface soils, probably due to their greater clay and iron oxides contents. Dickens and Hiltbold (1967) compared several clay minerals as to their sorption of DSMA. Kaolinite and limonite were considerably more effective than the 2:1 type clays of vermiculite and montmorillonite over a range of solution concentrations. This was attributed to the affinity of methanearsonate for mineral surfaces with exposed hydroxyl groups. After four years of repeated applications of MSMA, monoammonium methanearsonate (MAMA) and DSMA to turf, Johnson and Hiltbold (1969) determined that approximately 90% of the arsenic in the soils was associated with the clay fraction.

Woolson and Kearney (1973), in an 8-week period after the application of cacodylic acid to soils, observed declining amounts of water-soluble cacodylic acid and increasing amounts in a less soluble fraction associated with aluminum.

ii. Sorption of inorganic arsenic

Because previous studies have documented that the potential for biotransformation of roxarsone to inorganic arsenic exists (see section below on Microbial Transformations), it is important to discuss the sorption characteristics of inorganic arsenic. The capacity for arsenic sorption by soils depends on arsenic speciation, sorbent properties and solution phase conditions such as pH and existence of competitive ions like phosphate. The inorganic arsenic species arsenate and arsenite are the dominant chemical forms in most soils and water (Gao et al., 1994). The minerals that sorb arsenic most effectively include clays, iron oxide, aluminum oxide and hydroxide, manganese oxide, carbonate minerals, and organic matter. Sadiq (1997) states that iron oxides/hydroxides are most frequently involved in the sorption of arsenic in both acidic and alkaline

soils. In acidic soils, the surfaces of aluminum oxides/hydroxides, clays, manganese oxides and biogenic particles may sorb arsenic. The carbonate minerals sorb arsenic in calcareous soils.

iii. Sorption to metal oxides

Metal (Fe, Al, and Mn) oxides, upon exposure to water, complete their coordination shells with OH groups (Hingston et al., 1972). These OH groups can bind or release H^+, depending on pH, and a surface charge develops (Stollenwerk, 2003). The adsorption properties of the metal oxides are due to the presence of the OH_2^+, OH, and O^- surface functional groups (Sposito, 1984).

Two mechanisms for adsorption of solutes by a solid surface are outer-sphere and inner-sphere surface complexation. Outer-sphere, or non-specific adsorption (physisorption), involves weak, electrostatic, attractive forces between the charged surface and the sorbing, oppositely charged arsenic ion. Inner-sphere, or specific adsorption (chemisorption), describes the formation of chemical bonds between the surface and sorbing arsenic (Stollenwerk, 2003). Inner-sphere, complex bonds can involve one bond, called monodentate, or two bonds, called bidentate. The bidentate adsorbed complex is stronger than the monodentate complex and is more difficult to break (Foster, 1999). Inner-sphere complexes are formed when arsenic adsorbs by ligand exchange with OH and OH_2^+ surface functional groups (Stollenwerk, 2003). The mechanism of arsenic adsorption to mineral oxides has been addressed in studies using a variety of chemical and spectroscopic tools. Most of theses studies have documented that As(III) and As(V) form inner-sphere complexes on goethite and other Fe oxides (e.g., Manning et al., 1998; O'Reilly, 2001).

One of the most important controls on arsenic adsorption to Fe oxides is pH, as pH affects not only the speciation of arsenic but also the extent of protonation/deprotonation of mineral surfaces (Stollenwerk, 2003). The pH dependence is controlled by the isoelectric point, or pzc, which is defined as the solution pH at which the net charge on a particle is zero. The pzc for hematite (natural), goethite (synthetic), goethite (natural) and amorphous iron oxide are 7,

8.5, 7.9, and 7, respectively (Kosmulski, 2001). At pH values above the pzc, the solid surfaces are negatively charged, and oxyanions such as arsenate will exhibit weak sorption.

Because the pzc of most manganese oxides are low (2.5-6.4; Oscarson et al., 1983); the Mn oxides generally carry a net negative surface charge in soils with pH>6 and are expected to have a limited role in the sorption of arsenic in soils with pH>6. However, ligand exchange of arsenic ligands has been shown to occur at Mn surfaces (Sadiq, 1997).

Sorption of arsenic on aluminum oxide and hydroxide surfaces can also partially be explained by surface charge distribution. The Al-oxides carry a net positive charge in acidic soils (pH>6). Like Fe-oxide surfaces, arsenic may undergo specific sorption on Al-oxide surfaces (Sadiq, 1988). The sorption of arsenate onto α-Al_2O_3 (corundum) was investigated by Halter and Pfeifer (2001). The adsorption constants of arsenic onto α-Al_2O_3 resemble those onto clay minerals, indicating that aluminum sites in corundum are a good proxy for the octahedrally coordinated Al sites in other minerals. Comparison with the stability of arsenic surface complexes with iron suggests that surface complexes with aluminum are more stable.

iv. Sorption to clay minerals

Clay particles are usually negatively charged silicate minerals and consequently would not be expected to adsorb arsenic oxyanions from soil solutions. The point of zero charges (pzc) for phyllosilicates are typically below pH 5 due to permanent structural negative charge. Yet arsenic sorption to clay minerals has been reported by several investigators to occur. Manning and Goldberg (1997a) studied the adsorption of arsenite at the clay mineral-water interface. Four materials were used: kaolinite, illite, montmorillonite, and amorphous aluminum hydroxide. Their results indicate that at low pH there was low arsenite adsorption; maximum adsorption of arsenite occurred at pH 7.5 and pH 9.5. The authors proposed that the sites of oxyanion adsorption on phyllosilicates are ΞAl-OH and $\Xi Al\text{-}OH^{2+}$ functional groups at exposed crystal edges. Lin and Puls

(2000) investigated the adsorption of As(III) and As(V) on three types of clay minerals: 1:1 layer clays (halloysite and kaolinite), 2:1 layer clays (illite and illite/montmorillonite) and a 2:1:1 layer clay (chlorite). The halloysite and the chlorite had much greater adsorption (25-35 fold) than the other clay minerals. The clay minerals exhibited lower adsorption of As(III) than As(V), which was also affected by pH. Adsorption of As(III) increased at pH 7.5 while As(V) adsorption decreased at pH 7.5. The authors suggest that positive charge on the clay surface could be created from edge defects, such as protonation of broken Al-OH bonds exposed at particle edges, leading to anion adsorption at these sites.

v. Sorption to dissolved and solid phase organic matter

Organic compounds such as humic acids contain surface functional groups that can adsorb oxyanions (Stollenwerk, 2003). A study by Thanabalasingam and Pickering (1986) showed that As(V) and As(III) sorption by humic acids varies with pH, adsorbate concentration and ash content of the substrate. The maximum uptake occurred at pH 5.5. The higher values are from the humic acids with higher ash and calcium contents. Uptake decreased when ash content was reduced, indicating that Ca and polyvalent cations can be involved in arsenic retention. The retention was subject to competition from other anions, particularly phosphates, and to a lesser extent carbonates and sulfates. It is suggested that the major anion retention sites on organic matter are amino groups. If that were true, then any other organic component containing such groups would also adsorb arsenic.

The presence of fulvic acid reduces arsenic sorption by competing for binding sites (Xu et al., 1991). Grafe et al. (2001) investigated the sorption of As(V) and As(III) on goethite in the presence of peat humic acid, fulvic acid, and citric acid. Both peat humic acid and fulvic acid decreased As(V) sorption by 27% between pH 6-9 and by 17% between pH 3-8 respectively, while citric acid had no effect. Between pH 3 and 8, all three organic acids decreased As(III) sorption. The different pH regions in which the organic acids decreased As(V)

sorption suggests that more than one functional group on these organic polymers may be responsible for binding to the goethite.

Organic matter tends to bind most metals more strongly than do particles of lithogenic or authigenic origin. However, because of the similar types of overall electrical charges on soil organic matter and As oxyanions [at pH>3, organic substances begin to turn into negative polyelectrolytes (Tate and Theng, 1980)], limited interaction between these two substances is expected (Sadiq, 1997). Lin et al. (2002) studied the effect of organic materials on the sorption of arsenate by calcareous soils. They found that as organic substances are introduced into the soils by fertilizer application, the organic anions (such as humic, fulvic, and citric acids) compete with the arsenic anions for sorption sites in soils. Therefore, the presence of organic anions decreases arsenic sorption.

Redman et al. (2002) did a preliminary study of the effect of natural organic matter on arsenic sorption by metal oxides and the association of arsenate with natural organic matter as a function of calcium concentration. It was observed that the presence of natural organic matter significantly decreased arsenic sorption onto metal oxides. There was also a low correlation between increasing calcium ions and increasing arsenate association with natural organic matter. This may be due to the formation of calcium arsenates, including arsenate apatite, which precipitate under alkaline conditions (Bothe and Brown, 1999).

Xu et al. (1991) studied the effects of pH and natural organic materials on the mobility of arsenic in the environment. They concluded that under reducing conditions, the predominance of As(III) over As(V) and the release of arsenic from iron hydroxides would lead to increased mobility and aqueous concentrations with decreasing pH. Under oxidizing conditions, a slight reduction of pH would decrease mobility due to increased adsorption and (potential) coprecipitation with iron. However, a large pH reduction (to 4 or lower) would increase mobility even under oxidizing conditions. The fulvic acid was typically present as an anion and could compete with arsenic oxyanions for the positively charged sites on the solid surface. Minor decreases in As(V) adsorption were

noted at fulvic acid concentrations of 10 mg/L or lower. However, large decreases were seen as the concentration increased to 25 mg/L in the pH range of 4 to 8.

Kalbitz and Wennrich (1998) studied the influence of dissolved organic matter on heavy metal and arsenic mobility. The arsenic was mobilized to a low extent only. There was a weak correlation between dissolved organic matter and arsenic concentration, but its effect on arsenic mobilization was not clarified.

vi. Sorption to carbonate minerals

Carbonate minerals may be involved in arsenic sorption, but only in alkaline soils because they are unstable in acidic soils. Carbonate surfaces have a positive charge in soils with pH>9 and thus play an important role in As sorption in alkaline soils (Sadiq, 1997). Goldberg and Glaubig (1988) investigated arsenate sorption on a calcareous, montmorillonitic soil as a function of solution pH (2-11). The dominant inorganic constituents of the soil were montmorillonite, kaolinite, and calcite. Reference minerals for these constituents were then used for As(V) sorption studies over a range of pH values. For the two clay minerals, maximum arsenate sorption was observed near pH 5. For calcite, maximum arsenate sorption peaked between pH 10 and 12. The authors believe that ligand exchange or chemisorption is responsible for As sorption on carbonate surfaces in soils. Van Der Hoek et al. (1994) compared the leaching of As from an acidic and alkaline fly ash over a wide range of pH, with the sorption of arsenate on hematite, portlandite and mullite. They found that for alkaline fly ash it is likely that a Ca-phase controls the leaching of arsenate. Portlandite, which is formed upon hydration of lime, strongly sorbed arsenate at high pH (>12).

1.4d Microbial biotransformations

Microorganisms vary in their ability to methylate inorganic arsenic compounds present in the soil (NRCC, 1978). Bacteria, fungi, and yeast form volatile methylated derivatives of arsenic under both aerobic and anaerobic conditions. Bacteria only produce dimethylarsine, while fungi synthesize trimethylarsine.

Dimethylarsine is an oxidation product of trimethylarsine and both compounds may undergo demethylation by soil bacteria. MacKenzie et al. (1979) estimated that up to 2.1 x 10^{10} g/yr. of arsenic is lost to the atmosphere from the land surface. The continental vapor flux is about eight times that of the continental dust flux, indicating that the biogenic contribution may play a significant role in the cycling of arsenic (MacKenzie et al., 1979). It has not been established whether plants can release volatile arsenic as appears to be the case for selenium (Tamaki and Frankenberger, 1992).

i. Transformation potential of roxarsone

Although field studies suggest that roxarsone is transformed, either by chemical or biological reactions, to other organic and inorganic forms of arsenic, few studies have closely examined the potential biodegradation pathways. In a study on the environmental reaction mechanisms of roxarsone, Wershaw et al. (1999) found that most of the roxarsone was excreted unchanged. However, the researchers detected 3-amino-4-hydroxyphenylarsonic acid in the urine of hens fed roxarsone, but that was not substantiated by recent work using electrospray ionization mass spectrometric analysis by Garbarino et al. (2002).

The proposed transformation pathways are oxidative, methylation /demethylation, and photodegradation. Wershaw et al. (1999) noted that microbial biodegradation of aromatic compounds occurs in the following manner: N- and O- demethylation, hydroxylation, and deamination followed by ring fission, chain shortening, and oxidative removal of substituents. Carboxylic acid groups are formed on the cleaved ends of rings after the oxidative ring fission occurs. The authors hypothesize that if roxarsone were to undergo such a reaction sequence, arsonoalkyl acids would result. The arsonoalkyl acids could be converted to alkylarsines, which are stable in anaerobic environments. In aerobic environments, methylarsines are rapidly oxidized to AsO_4^{3-}. Wershaw et al. (1999) also listed possible environmental reaction mechanisms of roxarsone as reduction of nitro group, oxidative aromatic ring fission, and rupture of C-As

bond. The environmental-degradation products of roxarsone would be 3-amino-4-hydroxyphenylarsonic acid, methylarsines, and AsO_4^{3-}.

Demethylation of organoarsenicals in both natural waters and soil has been observed and is probably widespread among many bacteria. Bacteria were able to demethylate methanearsonic acid, which is transformed to arsenate and carbon dioxide (Tamaki and Frankenberger, 1992). Garbarino et al. (2002) found arsenite in bed sediment from a drainage ditch adjacent to an agricultural field where roxarsone had been applied.. The authors hypothesized that in an anoxic environment, such as the drainage ditch, bacteria can reduce arsenate to arsenite and methylate arsenate to dimethylarsinate.

Bednar et al. (2002b) studied photodegradation of roxarsone in poultry litter leachate. They determined that arsenite could be cleaved from roxarsone at pH 4-8 and that the degradation rate increases with increasing pH. The rate also increases when nitrate and natural organic matter is present (a common occurrence in poultry litter leachate). The arsenite is rapidly oxidized to arsenate by additional photochemical reactions.

Garbarino et al. (2003) examined the degradation of roxarsone during poultry litter composting. Results indicated that roxarsone was stable in fresh dry litter. However, when water was added to litter and the mixture was allowed to compost, the speciation of arsenic shifted from roxarsone to primarily arsenate in about 30 days. Increasing the amount of water increased the amount of degradation. Experiments also indicated that the degradation process was biotic, since the rate of degradation was directly proportional to the incubation temperature; heat sterilization eliminated the degradation

1.5 Summary of background information on roxarsone biogeochemistry

Roxarsone, a synthetic organoarsenical, is a poultry feed additive used primarily as a growth stimulant. Although this drug received FDA approval because less than 0.5 ppm is retained in chicken tissue, little work has been done on determining the fate of roxarsone once it is introduced into agricultural watersheds through poultry litter application. Initial studies indicate that the

approximately 1 x 10^6 kilograms per year of roxarsone is introduced into the environment. The study by Garbarino et al. (2003) suggests that roxarsone is transformed, either by chemical or biological reactions, to other organic as well as inorganic forms of arsenic. The proposed transformation pathways of roxarsone by microbes are oxidative, methylation /demethylation, and photodegradation. Products of roxarsone transformation include a variety of organic (arsonoalkyl acids) and inorganic arsenic species. Once introduced into the soil and subsurface environment, sorption of roxarsone, inorganic and other organic arsenic compounds can potentially occur onto metal oxides, clay minerals, organic matter, and carbonate minerals, with sorption by metal oxides being the most important sink for arsenic in soils.

1.6 Research Objectives

Recent studies by Redman et al. (2002) and Garbarino et al. (2003) showed interesting patterns of roxarsone sorption, but to date, no quantitative studies on Roxarsone sorption have been conducted. Sorption characteristics (e.g. distribution coefficients) are necessary for constructing predictive-type models of arsenic cycling. Thus, the main objective of this study was to conduct a quantitative study of roxarsone sorption to the Frederick series soils (taxonomic class: fine, mixed, semiactive, mesic Typic Paleudults), the main soil type in the Shenandoah Valley of Virginia where over 148 million poultry are raised every year (VADEQ, 1998). A second objective was to examine what soil components (metal oxides, organic matter, clay minerals) are responsible for the sorption. The final objective was to use the data to evaluate probable mechanisms of roxarsone sorption.

FIELD STUDY AREA

2.1 Muddy Creek site

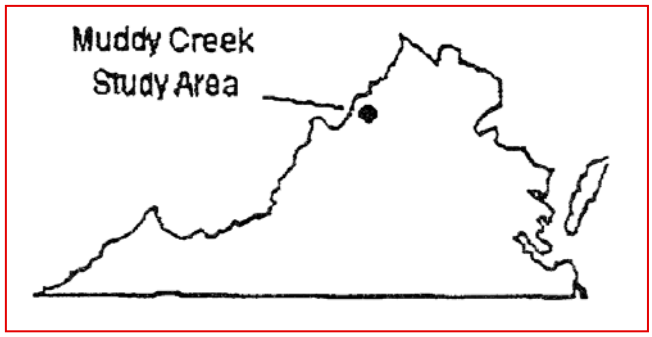

Figure 2. Muddy Creek Subcatchment

The study site (Figure 2) is located along Route 771 in the Muddy Creek subcatchment, which is drained by the Muddy Creek tributary (approximately 1.5-km in length). This small (1.2-km^2) watershed is approximately 20-km northwest of Harrisonburg, Rockingham County, Virginia, USA. Muddy Creek, which drains a larger 20,025-acre watershed, flows south to the Dry River, which discharges to the North River. The North River flows to the South Fork of the Shenandoah River, which flows to the Potomac River and then to the Chesapeake Bay.

Ninety-eight percent of Virginia's concentrated poultry operations are located in the Chesapeake Bay watershed (VADEQ, 1998). The study site includes two farms, which contain cornfields, cattle pastures, and a poultry barn. Agriculture is the primary land use in the catchment and roughly 20% of the area is forested (Hyer, 2000). Dry poultry manure and liquid dairy cattle manure are applied on the cornfields at the annual rate of about 4500 kg/ha and 57 m^3/ha, respectively, as a fertilizer and method of waste disposal (Hyer et al., 2001).

Figure 2 is an aerial view of the Muddy Creek tributary subcatchment. Soil samples were collected from the cornfield marked by a white circle. Dr. Greg Mullins (Department of Crop, Soil and Environmental Sciences, Virginia Tech) collected the control soil samples at a site approximately one mile west of Mt. Crawford on route 727 in Rockingham County.

2.2 Geology

The Muddy Creek drainage basin is in the southern part of the Singers Glen quadrangle and mostly includes Cambrian and Ordovician rocks of the

Shenandoah Valley of the Valley and Ridge province. The Conococheague Limestone (Lower Ordovician and Upper Cambrian) underlies the subcatchment. The bedrock is composed of interbedded limestone, dolostone, and sandstone. Irregular bedrock weathering has resulted in a clay-rich soil (dominated by kaolinite and illite) variable in thickness between 0 and 20 m (Hyer et al., 2001). The depth to groundwater varies from 2 to 20 m below the surface.

2.3 Soils

The soils of the Muddy Creek subcatchment have been characterized as well-drained, silt loam of the Frederick series with low organic matter content (Hockman et al., 1982). The soils from the control group have also been classified as belonging to the Frederick series, which consists of very deep, well-drained soils formed in residuum derived mainly from dolomite limestone with interbeds of sandstone and siltstone (Mullins, written correspondence, 2002). These soils have been subdivided into several horizons: Ap, Bt1, Bt2, Bt3, and Bt4. We obtained samples from the three top horizons. The Ap layer, from 0 to 22 cm, is a brown silt loam; weak fine and medium granular structure; friable, slightly sticky, slightly plastic; 5% coarse angular chert gravel; slightly acid; abrupt smooth boundary. The Bt1 layer, from 22 to 50 cm, is a light brown, silty clay loam; weak fine and medium subangular blocky structure; friable, sticky and slightly plastic; common distinct patchy clay films on faces of peds; 2% medium angular chert gravel; strongly acid; clear wavy boundary. The Bt2 layer, from 50 to 83 cm, is a yellowish red silty clay; moderate fine and medium angular blocky structure; friable, sticky, moderately plastic; many distinct clay films on faces of peds; 5% angular chert gravel; strongly acid; clear wavy boundary. The soils from the Muddy Creek site are comparable to the Ap and Bt1 layers of the Frederick series control group.

2.4 Previous work at site

The USGS National Water-Quality Assessment (NAWQA) program conducted a groundwater study of the Muddy Creek watershed from 1993 to 1995. Nitrate

was found to be present in 93% of the samples taken, nitrate-N concentrations above the MCL of 10 mg/L were detected in 7% of the groundwater samples, and 86% of the groundwater samples contained one or more pesticides or degradation products (Ferrari and Ator, 2002).

A poultry waste management study conducted by the Virginia Department of Environmental Quality (1998) showed that out of 1309 contract growers raising poultry in Virginia, 567 were located in Rockingham County in the Shenandoah Valley. Fifty to sixty percent of wells surveyed in the Shenandoah Valley, the region with the highest concentration of poultry operations in Virginia, had nitrate-N levels of 10 mg/L or higher. In addition, 356 miles of streams are impaired due to non-point source agricultural (crop and animal) pollution. The major poultry production drainage areas in the Shenandoah Valley are the North River in Augusta County, the North Fork of the Shenandoah River in Rockingham County and the South Fork of the Shenandoah River in Page County.

In 1999, the Muddy Creek TMDL Establishment Workgroup released results of a study of the fecal coliform levels in Muddy Creek. Poultry litter, accumulated on the land and available for runoff during wet weather, represents a potentially significant source of fecal coliform loading to Muddy Creek and its tributaries. Total poultry numbers and the resulting litter production for the Muddy Creek watershed are 50,098 chickens/751 tons; 508,325 broilers/3966 tons; 351,336 turkeys/17,565 tons (Muddy Creek TMDL Workgroup, 1999).

Hyer et al. (2001) studied the processes controlling the transport of atrazine and other agricultural chemicals in the Muddy Creek watershed. The streamwater, overland flow, soil water, groundwater, and rainfall were analyzed during the summer. Atrazine was present in all the components examined and was greatest in samples with elevated dissolved organic carbon and potassium concentrations. These results suggest that sediment-associated atrazine transport was enabled by the sorption of atrazine to mobile manure colloids. Thus colloid transport may be an important mechanism to consider when analyzing the fate of agricultural chemicals in the Muddy Creek watershed.

MATERIALS AND METHODS

3.1 Reagents

Deionized water (Nanopure) was used for preparation of samples and standards. All reagents were of analytical grade or better.

3.2 Soil sample collection and preparation

Two soil samples were collected at the Muddy Creek site: a well-drained sandy loam (0-15 cm. depth), and a clay loam (30-45 cm. depth). These layers are approximately equivalent to the Ap (control) and Bt1 (control) layers of the Frederick series soils used for comparison. Poultry litter was also collected from the site. The Muddy Creek soils were excavated with a shovel and placed in plastic bags. Soils and poultry litter were transported in an ice cooler to the lab, where they were refrigerated until analysis. Greg Mullins of the Crop and Soil Environmental Sciences Department at Virginia Tech obtained the Frederick series control soils (Ap, Bt1, and Bt2). These soils were collected in 5-gallon buckets and refrigerated until analysis. Chemical characteristics of the soils are presented in Table 1a and 1b.

Particle size analyses for the soils are presented in Table 1c. The surface area for the Ap soils was calculated using BET. Results indicate that the specific surface area for the Ap-MC soil is 5.17 m^2/g and the Ap-Control soil is 6.33 m^2/g. Details of the BET analysis are provided in Appendix F. Soils were sieved to less than 2 mm for all sorption experiments. For experiments requiring removal of organic matter from soils, a method using 30% H_2O_2 and subsequent heating was used (modified from Jackson, 1960). See Appendix D for method details.

Table 1a. Chemical Analysis* of Muddy Creek (MC) and Control Soils

Soil type	Na %	Fe %	Al %	Ca %	Mg %	K %	Pb mg/kg	Zn mg/kg	Mn mg/kg	Cu mg/kg
Ap-control	0.015	1.04	1.16	0.10	0.08	0.07	18.4	28.3	708	11.9
Ap-MC	0.024	1.56	1.38	0.55	0.18	0.15	25.1	108	436	86.7
Bt1-control	0.063	1.63	1.70	0.09	0.10	0.09	11.9	27.7	172	7.9
Bt1-MC	0.022	3.11	2.47	0.10	0.17	0.17	22.1	46.7	118	52.0
Bt2-control	0.011	3.18	1.98	0.09	0.08	0.07	15.9	21.8	91	13.3

Notes: *Aqua regia digestion and ICP-MS analysis for trace elements performed by Activation Laboratories Ltd. Control soils collected one mile west of Mount Crawford, VA on route 727. Muddy Creek (MC) soils collected at farm approximately one mile northwest of Stultz Mill, VA on route 771.

Table 1b. Chemical Analysis* Muddy Creek (MC) and Control Soils

Soil type	pH	OM %	P mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	Zn mg/kg	Mn mg/kg	Cu mg/kg
Ap-control	6.1	1.4	9	71	418	53	1.7	7.7	1.0
Ap-MC	6.8	2.8	298	152	1283	179	14.4	8.9	3.8
Bt1-control	5.9	0.7	1	20	336	92	0.4	2.2	0.2
Bt1-MC	6.3	0.9	30	109	494	150	1.4	3.8	0.9
Bt2-control	5.5	0.6	1	22	416	109	0.3	1.3	0.2

Notes: *P, K, Ca, Mg, Cu, Mn, and Zn extracted using the Mehlich 1 Procedure (EPA Method 365.2). Analysis was conducted by ICP-AES or colorimetric procedures by the Virginia Cooperative Extension Soil Testing Laboratory. Organic matter (OM) analyzed using the Walkley-Black Method.

Table 1c. Particle size analysis of Muddy Creek soils

Soil type	%Sand	%Silt	%Clay	Textural class
Ap-Control	27.5	64.6	7.9	Silt Loam
Ap-MC	52.2	40.9	6.9	Loam/Sandy Loam
Bt1-Control	15.7	68.0	16.3	Silty Loam
Bt1-MC	22.0	44.4	33.6	Clay Loam
Bt2-Control	14.2	45.0	40.7	Silty Clay

Notes: Particle size analysis conducted by W.T. Price of the Virginia Tech Soils Laboratory.

3.3 Digestion and analytical methods

EPA Method 3052 (*Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices*) was used to determine the total As concentration in the soil samples and poultry litter. Homogenization of the sample was achieved by oven-drying (at 95ºC) the soils and poultry litter, and then crushing the samples with an agate pestle and mortar and mixing with a spoon. The sample (0.5 g) was digested in 9 mL of concentrated nitric acid for 20 min using an ETHOS PLUS Microwave (Milestone Laboratory System). The temperature was ramped to 180 ± 5ºC in ~ 10 min and maintained at this temperature for 10 min. After cooling, samples were filtered, diluted to volume.

Arsenic was analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) or graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman background correction. During initial method development, it was determined that both of these methods yielded full recovery of arsenic from roxarsone (data not shown). Detection limits for ICP-AES ranged from 15 to 25 μg/L. The detection limit for the GFAAS was approximately 3 μg/L. All analyses were conducted on filtered (0.2 μm) samples preserved with nitric acid.

3.4 Determination of roxarsone adsorption by soils

ASTM Method D 4319 – 93 (Re-approved 2001) (*Standard Test Method for Distribution Ratios by the Short-Term Batch Method*) was used for the sorption tests. Six sets of sorption experiments were conducted. The first set was the initial equilibrium sorption experiment, used to determine when roxarsone sorption reached equilibrium with the Ap-MC and the Bt1-MC soils. These were run for 3, 6, 9, and 12 days to see when equilibrium was reached at different roxarsone concentrations (10, 50, 100, 200 μg/L for Ap; 100, 200, 500, and 1000 μg/L for Bt). An additional experiment was conducted over a 3-day period to determine the amount of arsenic leaching from the soils with no added roxarsone.

The second set, designed to construct an adsorption isotherm, was performed on the Muddy Creek and control soils by varying the amount of roxarsone (0, 100, 200, 500, and 1000 μg/L). Samples were run in triplicate.

The third set, designed to construct an adsorption edge, was conducted on the Muddy Creek and control soils with 200 μg/L roxarsone and varying values of pH (3, 5, 7, 9, and 11). Samples were run in triplicate.

The fourth, fifth, and sixth sets were to determine the adsorption isotherms for kaolin, goethite and the Ap horizon soils with the organic matter removed, respectively. Samples were run in triplicate.

For all of the experiments above, a 6.25 g of homogenized soil sample was placed into an acid-washed centrifuge tube. Twenty-five mL of 0.01 M NaCl or $NaNO_3$ was added to the tube. The pH was adjusted by addition of NaOH or HCl. After spiking with roxarsone, the sample was mixed and placed on a wrist shaker for 24 hours of the 3-d period (for all experiments other than the initial equilibrium). During the latter two days of the contact period, the mixture was allowed to stand and settle. The sample was centrifuged for 30 min (3500 x g) at 25ºC. The supernatant was filtered with a 0.45 μm filter followed by a 0.2 μm filter and then preserved with HNO_3 (pH<2). See the appendixes for a more in-depth description of the experiments.

3.5 Calculation of C* and K_d

At low concentrations of arsenic, there appears to be a direct, linear relationship between the concentration of the solute, C solution, and the amount of a solute sorbed onto the soil, C* sorbed (see Results, Figure 5). As a result, the adsorption isotherm of C as a function of C* will plot as a straight line on graph paper. The linear sorption isotherm (Fetter, 1999) is described by the equation $C^* = K_dC$, where

C* = mass of solute sorbed per dry weight of solid (mg/kg)

C = concentration of solute in solution in equilibrium with the mass of solute sorbed onto the solid (mg/L)

K_d = coefficient (L/kg)

The coefficient K_d is called the distribution, or partition, coefficient. It is equal to the slope of the linear sorption isotherm.

C (or C solution) and C* (or C* sorbed) were calculated as follows:

C solution = concentration of As in final solution (μg/L)

C* sorbed = concentration of As sorbed onto the soils (μg/L) * volume of the solution added to the soil sample (L) / weight of the soil sample (g). [Note: The concentration of As sorbed onto the soils was found by subtracting the final concentration of As in solution (C solution) from the initial concentration of As added to the soil (C added)].

RESULTS

4.1 Sorption experiments

Several sorption experiments were performed. The initial experiments were conducted to 1) determine the time required for roxarsone sorption to reach equilibrium with the Ap and Bt horizons of the Frederick Series soils and 2) determine how much arsenic leached from the soils. The adsorption isotherm experiments were conducted to determine the sorption characteristics of roxarsone to the different soils and horizons. The adsorption edge experiments were conducted to evaluate whether the sorption of roxarsone to the different soils was pH dependent. The kaolinite and goethite sorption experiments were designed to examine the individual ability of these minerals to sorb roxarsone. Finally, the experiments involving the removal of organic matter from soil collected from the Ap horizon was performed in order to determine how organic matter affected roxarsone sorption.

4.1a Initial equilibrium experiments

Two equilibrium experiments were conducted using the two types of soils collected from the Muddy Creek site, the Ap-MC and Bt1-MC soils, to determine the time required for sorption to reach equilibrium. Figure 3 shows the Ap-MC

soil results. Figure 4 shows the Bt1-MC soil results. (For statistical analysis, see Appendix C.1a for Ap-MC soil and C.1b for Bt1-MC soil.)

Another objective of these initial experiments was to determine how much arsenic leaches out of the soils over the experimental period. Results indicate that after 3 days of reaction of the soils with 0.01 M NaCl (no added roxarsone), there was 27.0 μg/L of arsenic leached from the Ap-MC soils and 13.9 μg/L from the Ap-Control soils. No detectable arsenic was leached from the Bt soils.

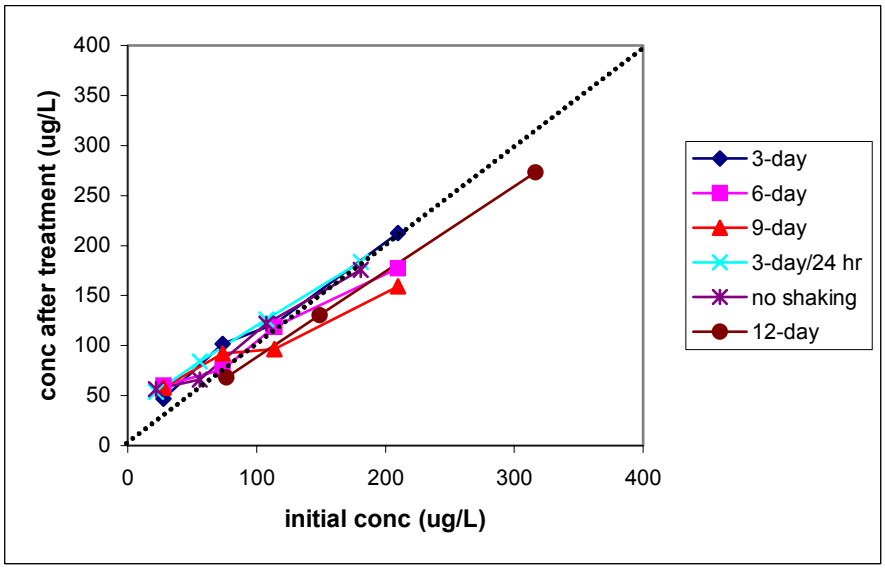

Figure 3. Comparison of 3-, 6-, 9-, 12-day and 3-day/24 hr Shaking and No Shaking Equilibrium Sorption Experiments of Ap-MC Soils. Dashed line represents the 1:1 (no sorption) line. Data that fall above the line (post-treatment arsenic > added arsenic) indicate an additional source of arsenic (leached from soils). Data that fall below the line (post-treatment arsenic < added arsenic) indicate a loss of arsenic (i.e., sorption to the soils).

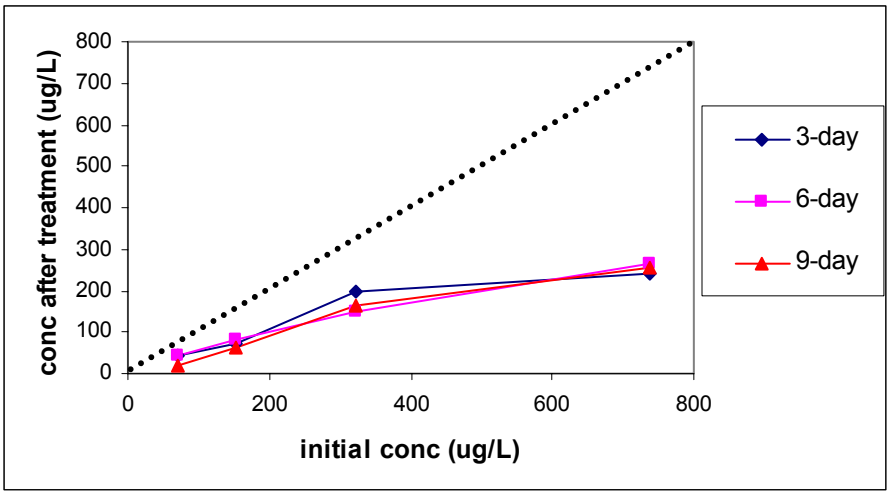

Figure 4. Comparison of 3-, 6-, and 9-day Equilibrium Sorption Experiments of Bt1-MC Soils. Dashed line represents the 1:1 (no sorption) line. Data that fall above the line (post-treatment arsenic > added arsenic) indicate an additional source of arsenic (leached from soils). Data that fall below the line (post-treatment arsenic < added arsenic) indicate a loss of arsenic (i.e., sorption to the soils).

4.1b Adsorption isotherm

The adsorption isotherm in Figure 5 shows the linear relationship between the concentration of roxarsone in solution (μg/L) and the concentration of roxarsone that has been sorbed to the (solid) soil (μg/g). A simple regression model, the F test, was used to determine if the variance between soils was significant. (see Appendix C.2a). If the tail probability (p value, or Pr>F) associated with the F ratio in the F distribution is smaller than 0.05 (α-value), then the variance estimates are different and the results are significant. When comparing Bt1-MC to Bt1-Control, Bt1-MC to Bt2-Control, and Bt1-Control to Bt2-Control, the Pr>F was 0.314, 0.827, and 0.984 respectively. Because all values are greater than 0.05, there is no significant difference in the slopes (i.e. K_d values) and an average of their slopes was determined and designated as Bt combined. The Ap soils were then compared to this slope. When comparing Ap-MC to Bt combined and Ap-Control to Bt combined, the Pr>F was 0.0001 and 0.0002, respectively, indicating a significant difference in the slopes. When comparing Ap-MC to Ap-

Control, the Pr>F was 0.1846, surprisingly indicating that there was no significant difference between their slopes.

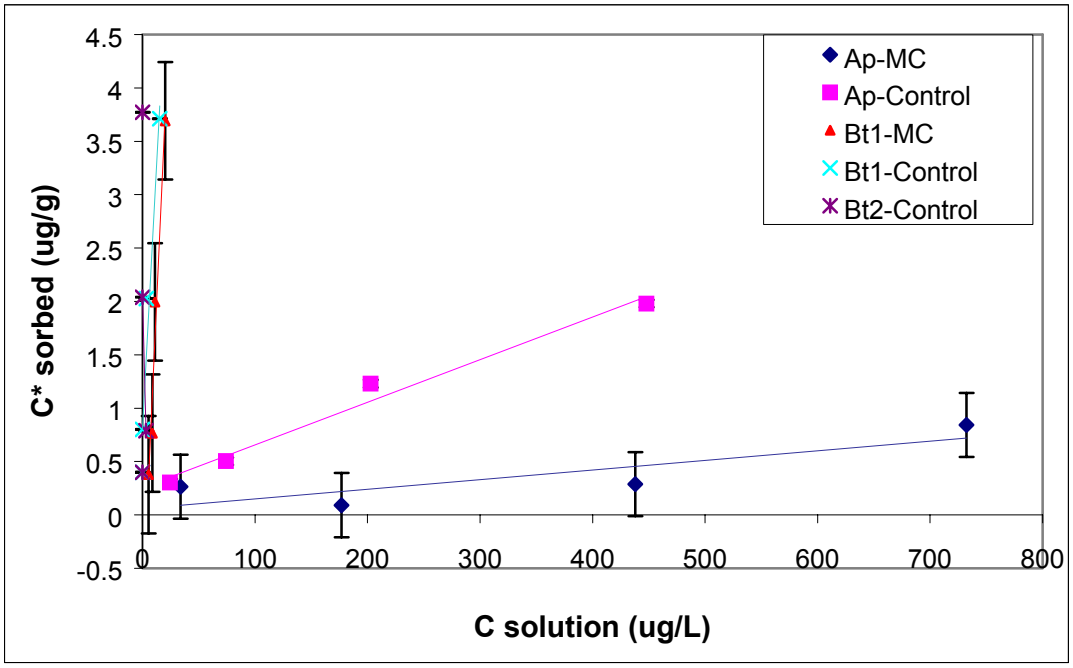

Figure 5. Linear Isotherm of Roxarsone Adsorption. Error bars represent standard deviation of triplicate experiments. Solid lines represent linear regressions (not through origin) of the averaged data. The regression is not forced through zero since the initial concentration of arsenic in the soils is not zero in all cases.

4.1c pH envelope

The pH envelope, or edge, for roxarsone sorption to the different soils is presented in Figure 6. The pH envelope shows the relationship between pH and the concentration of roxarsone that has been sorbed onto the soil (µg/g). Characterization of roxarsone adsorption as a function of pH was carried out at a low concentration (200 µg/L), which resulted in low arsenic loading on the soils.

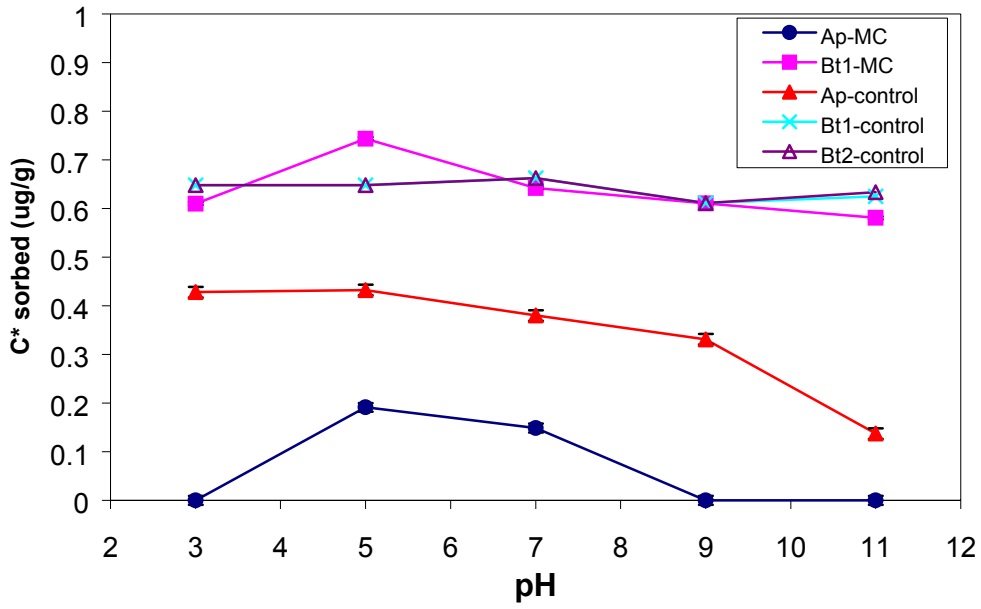

Figure 6. pH Dependence of Roxarsone Sorption. Error bars represent the standard deviation of triplicate experiments.

4.1d Kaolinite and goethite isotherms

Because iron oxides and clays may play significant roles in roxarsone sorption, it was decided to examine the adsorption isotherms of these minerals individually. Kaolinite and illite are the dominant clays in the Muddy Creek soil, and the color of the soil indicates that goethite and hematite are likely present in the soil. Due to ease of accessibility, kaolinite and goethite were chosen to represent the iron oxides and clays. The adsorption isotherms in Figure 7 show the relationship between the concentration of roxarsone in solution (µg/L) and the concentration of roxarsone that has been sorbed (µg/g) onto the kaolinite and goethite.

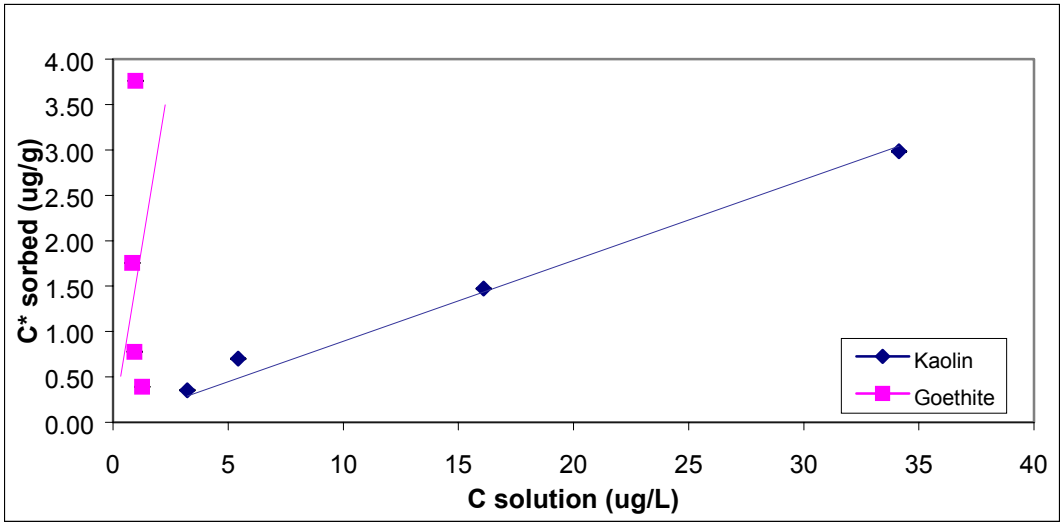

Figure 7. Kaolinite and Goethite Adsorption Isotherms. Error bars represent standard deviation of triplicate experiments. The lines represent linear regression through the origin (because the original concentration of arsenic in the minerals is assumed to be negligible) for the averaged data.

4.1e Treated Ap soils (O.M. removed) isotherms

Figure 8 shows a comparison of the sorption capabilities by Ap-MC soils before and after organic matter had been removed. An F-test analysis was performed (see Appendix C.6a) to determine if the variance in slopes (K_d values) was significant. Comparing Ap-MC regression lines before and after the H_2O_2 treatment (see Appendix D for description of the method), the Pr>F was <0.0001 and is statistically significant.

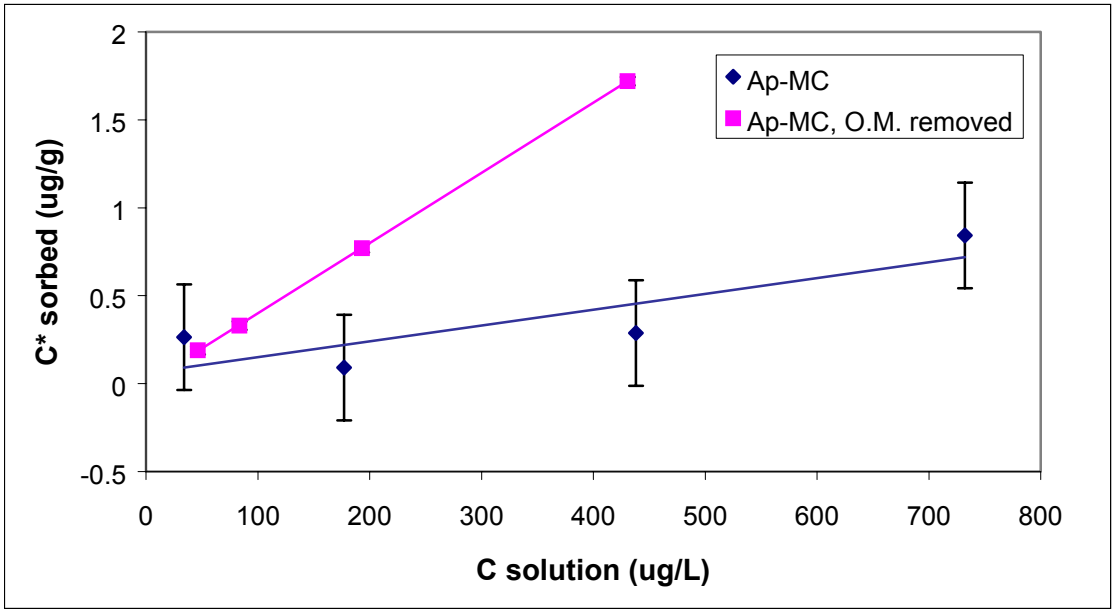

Figure 8. Linear Isotherms of Ap-MC Soils: Roxarsone Adsorption Before and After Removal of Organic Matter. Treatment with hydrogen peroxide reduced the OM from 2.8% to 0.3%. Solid lines represent linear regressions not through origin (because the initial concentration of arsenic in the soil is not zero) of the averaged data. Error bars represent standard deviation of triplicate experiments.

A comparison of the sorption capabilities by Ap-Control soils before and after organic matter has been removed is shown in Figure 9. Once again, an F-test analysis was performed (see Appendix C.6a) to determine if the variance in slopes (K_d values) is significant. Comparing Ap-Control regression lines before and after the H_2O_2 treatment (see Appendix D for description of the method), the Pr>F was 0.0031 and thus the difference in slopes is statistically significant.

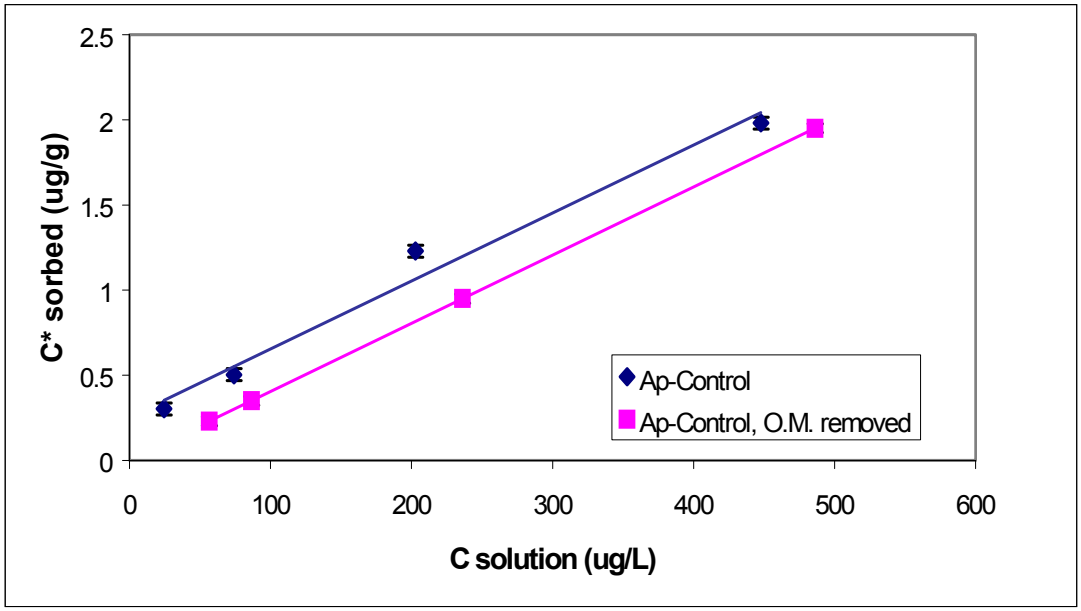

Figure 9. Linear Isotherms of Ap-Control Soils: Roxarsone Adsorption Before and After Removal of Organic Matter. Treatment with hydrogen peroxide reduced the OM from 1.4% to 0.4%. Solid lines represent linear regressions not through origin (because the initial concentration of arsenic in the soil is not zero) of the averaged data. Error bars represent standard deviation of triplicate experiments.

4.1f Calculation of distribution coefficients (K_d)

Distribution coefficients (K_d) were calculated to describe the linear adsorption of roxarsone to the Muddy Creek and control soils (Tables 2a, 2b). The K_d values for the Ap soils after the organic matter was removed by a H_2O_2 treatment are shown in the third column.

The K_d values were calculated using two linear regression methods: one where the regression line was forced through zero (Table 2a) and the other where the line was not forced through zero (Table 2b). These two methods were used to determine if there was any statistically significant difference when the regression line was forced through zero (implying that the initial concentration is zero) and when not forced through zero (implying that the initial concentration is greater than zero). There is no significant difference between the two assumptions. The resulting values of K_d are within the error estimate of each

other, suggesting that leaching of low concentrations of arsenic from the soils does not significantly affect the K_d value.

Table 2a. Calculated Kd values (linear regression forced through zero)

Soil Type	K_d (L/g)	K_d (L/g) after O.M. Removal
Ap-control	0.005 ±0.002	0.003 ±0.0003
Ap-MC	0.001 ±0.001	0.004 ±0.0003
Bt1-control	0.260 ±0.077	NA
Bt1-MC	0.165 ±0.048	NA
Bt2-control	0.251 ±0.385	NA

Notes: ± denote the standard error, an estimated standard deviation of K_d
Values are based on the averaged data of triplicate experiments.
The linear regression has been forced through zero.
NA = not applicable
Statistical analysis provided in Appendix C.2

Table 2b. Calculated Kd values (linear regression not forced through zero)

Soil Type	K_d (L/g)	K_d (L/g) after O.M. Removal
Ap-control	0.004 ±0.0004	0.004 ±0.000001
Ap-MC	0.001 ±0.0004	0.004 ±0.000015
Bt1-control	0.200 ±0.039	NA
Bt1-MC	0.231 ±0.031	NA
Bt2-control	-0.410 ±0.622	NA

Notes: ± denote the standard error, an estimated standard deviation of K_d.
Values are based on the averaged data of triplicate experiments.
The linear regression has not been forced through zero.
NA = not applicable
Statistical analysis provided in Appendix C.2a

DISCUSSION

An examination of the physical and chemical characteristics of the Muddy Creek and control soils (Table 1a and 1b) shows that the amended soils (Ap-MC and Bt1-MC) have higher levels of P, K, Ca, Zn, Mg, Pb, Cu, and Fe than the control soils. The most significant increase was in the phosphorus content (Table 1b), with 30 times the amount extracted from the Muddy Creek soils than from the control soils. The Ap-MC soil (2.8% OM) has twice the amount of organic matter than the Ap-Control soil (1.4% OM), which is expected since manure has been added to the soil (Table 1b). There is no significant difference in organic matter between the Bt layers (0.6-0.9% OM).

5.1 Sorption kinetics

The initial adsorption experiments were performed on soils from the field site that has been fertilized with poultry litter containing roxarsone for many years. The graph of initial vs. final roxarsone concentration after reaction with the Ap-MC soil shows little sorption of roxarsone during the first three days, then showed a gradual increase during the 3- to 6-day period, and the most sorption during the 6- to 9-day period, with a slight decrease in sorption during the 9- to 12-day period. The data points that fall above the 1:1 line indicate that there was more leaching of arsenic from the soils than sorption onto the soils (Ap-MC leached 27 μg/L of arsenic over 3 days) (Appendix C.2 Tables 6-10: No rox). However, a statistical comparison of the regression slopes for the 3-. 6-, 9-, and 12-day experiments for the Ap-MC soils demonstrates that there is no statistically significant difference between the 3-day and the 12-day experiment, and only a slight statistically significant difference between the 6-day and 9-day experiments (Appendix C.1a). It was decided that this slight improvement in sorption at such low amounts did not warrant adding an additional six days to each experiment.

The Bt1-MC soil (Figure 4) shows a stronger pattern of sorption, with all data points falling beneath the 1:1 no sorption line. The initial control experiment showed no detectable arsenic leaching from the Bt1-MC soil. When comparing the slope of the regressions for the 3-, 6-, and 9-day experiments (Appendix

C.1b), there is no statistically significant difference between their slopes (for the 3-day experiment: slope of regression line=0.293 ±0.083; for the 6-day experiment: slope of regression line=0.324 ±0.027; and for the 9-day experiment: slope of regression line=0.323 ±0.068). Based on this information, the 3-day sorption period was chosen for the experiments.

5.2 Roxarsone sorption to Bt soils

The linear isotherms of roxarsone adsorption (Figure 5) show several interesting patterns. First, roxarsone sorbs strongly to the Bt soils (K_d for Bt1-MC, Bt1-Control, Bt2-Control are 0.165, 0.259, 0.252 L/g, respectively). One possible explanation for this strong sorption is that the roxarsone is adsorbing to iron oxides. The goethite adsorption isotherm (Figure 7) exhibits rapid and efficient adsorption of roxarsone. The most likely explanation for this pattern is that As(V) in the roxarsone structure is involved in a ligand exchange reaction by which an inner-sphere bidentate surface complex is formed (Grossl et al., 1997). The Bt soils have high Fe content (1.63%, 3.11%, and 3.18% for Bt1-control, Bt1-MC, and Bt2-control, respectively). The Bt soils have a bright orange/red color, indicating the presence of hematite (Rimstidt, private correspondence). The Ap soils also have significant amounts of Fe present (Ap-MC 1.56%; Ap-Control 1.04%), but are brown in color. The difference in color is probably due to the presence of goethite in the Ap soils (Rimstidt, private correspondence). Orange coatings on soil particles were observed during collection of the Muddy Creek soils. Rutherford et al. (2003), in a study of the fate of roxarsone in poultry litter, found that strongly bound arsenic positively correlated with Fe in amended fields, which suggests sorption or coprecipitation of As and Fe in the soil column.

Another possible explanation for the strong sorption of roxarsone to Bt soils is that the roxarsone is adsorbed to clay minerals. As shown in Table 1c, the Bt soils are rich in clay. The linear adsorption isotherm for roxarsone to kaolinite (Figure 7) shows moderate adsorption, which is similar to the results of Lin and Puls (2000). These authors attributed the moderate As(V) adsorption to exposed hydroxyl groups at particle edges of the clay mineral. Evidence for

inner-sphere surface complexation (chemisorption) of As(V) and As(III) on kaolinite was suggested by Foster (2003).

5.3 Roxarsone sorption to Ap soils

The Ap soils only exhibited moderate to low sorption capacity (K_d for Ap-MC and Ap-Control are 0.001 and 0.005 L/g, respectively) in comparison to sorption to Bt soils (Table 2a and 2b). There are several possible explanations for these patterns.

First, the Ap-MC soils have a high concentration of phosphorus (P in Ap-MC soil is 298 mg/kg, compared to 9 mg/kg in the Ap-control soil) due to poultry litter application. Qafoku et al. (1999) found that phosphate can compete with arsenate for all available adsorption sites, non-specific and specific, thus increasing arsenic mobility. Peryea and Kammereck (1997) also discovered that phosphate appears to out-compete arsenic for sorption sites, possibly because of higher sorption affinities for phosphate or because of simple mass-action effects. The low sorption by the Ap-MC soils could thus be due to the high amount of phosphate in the Ap-MC soil. Measurement of phosphate concentrations in the supernatant extracted from the Muddy Creek soils shows that there is little phosphate coming off the Bt1-MC soil (0.13 mg/L) and even less from the Ap-MC soil (<0.01 mg/L). Yet, the Ap-MC soil had 30 times the phosphorus (Table 1b) than the Bt1-MC soil. Although the speciation of the phosphorus was not determined and, consequently, the amount of phosphate in the soils is not known, it is interesting to note that the available phosphate from the soils is so low. This hints that the phosphate may be tightly sorbed to the available sites in the soils.

A second possible explanation for the roxarsone-Ap sorption patterns is that organic ligands added to the soils via manure application complex Fe and Al, which decreases sorption of oxyanions, such as As(V), to these metal oxides (Leytem et al., 2002). These ligands can also compete for sorption sites, effectively coating the mineral surfaces.

The difference in sorption capacities between the Ap-MC and the Ap-control soils could be attributed to the difference in particle size between these soils (Table 1c). The Ap-MC is a sandy loam whereas the Ap-Control is a silt loam. The smaller grain size of the Ap-Control provides more surface area, which provides more sorption sites. The specific surface area for the Ap-MC soil is 5.17 m^2/g and the Ap-Control soil is 6.33 m^2/g (Appendix F: Table 22). Consequently, more sorption sites are available in the Ap-Control soil. In addition, the Ap-Control soil does not have a history of poultry litter (manure) application, so competition from organic ligands, or coating by organic matter, is unlikely.

However, the sorption differences between the Ap-MC and Ap-Control also suggest that organic matter is adsorbing roxarsone. This is suggested by results of the sequential extraction procedure performed on the soils (see Appendix E). Although not completely successful in extracting the total arsenic from the soils, the extraction results do help explain some of the observed sorption patterns. The iron-oxide-bound arsenic concentration (Method II) was 0.212 mg/kg for the Ap-MC soil and 0.256 mg/kg for the Ap-Control soil. These soils had the about the same amount of total iron (1.56% vs. 1.04%). These results indicate that thus a difference in Fe(III) oxide concentration is unlikely to cause the observed differences in Ap sorption patterns.

A more likely explanation for the roxarsone sorption patterns to the Ap soils involves adsorption to organic matter. The organic matter-bound arsenic concentration (Method II) was 7.242 mg/kg for the Ap-MC soil and 5.642 mg/kg for the Ap-Control soil. Further evidence that organic matter controls roxarsone sorption in Ap soils was yielded from a comparison of the Ap isotherms before and after organic matter (OM) removal, shown in Figures 8 and 9. After OM removal, K_d values for Ap-MC increased from 0.001 to 0.004 L/g, while values for the Ap-Control decreased from 0.005 to 0.004 L/g. These changes were very significant for the Ap-MC soils (Pr>F value: < 0.0001) and significant for Ap-Control soils (Pr>F value: 0.0031). Calculation of the F-test was done by SAS (see Appendix C.6a). Both soils after treatment had a K_d value of 0.004 L/g.

Organic matter may affect sorption of roxarsone via several mechanisms. In a study of phosphorus adsorption, organic matter functions by either sorbing P or blocking the site associated with P sorption (Leytem et al., 2002). Thus, it is possible that organic matter may have been blocking roxarsone adsorption in the Ap-MC soil but not blocking it in the Ap-Control soil, perhaps due to the difference in OM content. Ran et al. (2002) examined the effect of organic matter heterogeneity on equilibrium sorption and desorption of hydrophobic organic contaminants (HOCs) by peat and sediments. Peat and sediments may contain different types of condensed OM, and the pore filling in the condensed OM domain may be an important mechanism for the sorption of HOCs. About 95 to 99% of the OM surface area is formed by micropores (<2-0.5 nm), so the molecular diffusion in OM and intraparticle micropores may be very slow and sensitive to the solute size, shape, and hydrophobicity. Because roxarsone is a large molecule, it may be too big to fit into these micropores. This could be a possible reason that roxarsone sorption was at its lowest in the soil that contained the highest percentage of organic matter.

The Ap-MC soil showed an increase in sorption after the OM was removed (Figure 8), yet Ap-Control soil showed a slight decrease in sorption after the OM was removed (Figure 9). This could be explained by the relative amount of organic matter present in each soil. The Ap-MC soils, containing 2.8% OM, have twice the amount of organic matter than the Ap-Control soils, containing 1.4% OM. It is likely that the organic matter may be coating the mineral surfaces in the Ap-MC soils, thus blocking the sorption sites of the iron oxides and clays. When the OM is removed in the Ap-MC soils, the sorption sites of the iron oxides and clays are now exposed and available for bonding, and sorption increases significantly. As a consequence, the sorption is more than likely dominated by OM. There may not be enough organic matter in the Ap-Control soils to coat the mineral surfaces, and, consequently, the OM does not play a significant role in sorption. The lack of significant change in sorption before and after the removal of organic matter supports the idea that clays and iron oxides are responsible for the sorption by the Ap-Control soil. Another conclusion that can be reached as a

result of removing the organic matter from the Ap soils is that phosphate is not out-competing roxarsone for sorption sites. Since the sorption after OM removal is the same for both Ap soils, this indicates that phosphate is not blocking sorption in the Ap-MC soil. The phosphate has not been removed (only the organic matter has been), so if phosphate was out-competing roxarsone for the sorption sites on the mineral surfaces, the sorption of roxarsone would still be low.

Taking all the data into consideration, the two most important controls on the sorption of roxarsone to the Ap soils are: 1) particle size, with the smaller particles having more surface area and consequently, more sorption sites; and 2) partitioning of roxarsone into organic matter that is coating (blocking) mineral surfaces.

5.4 pH dependence of roxarsone sorption

The pH envelopes (or edges) for roxarsone sorption to the different soils are presented in Figure 6. This graph shows the relative positions of the individual adsorption edges. Characterization of the roxarsone adsorption as a function of pH was carried out at low concentration (200 μg/L), which resulted in low arsenic loading on the soils. The sorption of roxarsone by the Bt1- and Bt2-Control soils does not appear to be pH dependent, because sorption remains high over the entire range of pH (3-11) tested. The Bt1-MC soil also exhibits strong sorption over this pH range, with a slight tailing at pH>9. Sorption on these soils is most likely occurring on clay minerals and iron oxides, both of which typically show pH dependence, with a decrease in sorption occurring above pH>9, above the pzc for most clays and iron oxides (Manning and Goldberg, 1997a; Manning et al., 1998; Manning and Goldberg, 1997b). However, at low arsenic loading, this pH dependence is not expected because there is enough variation in the surface charge so that there are enough available sorption sites. This was documented by Luxton (2002) in his study of adsorption of arsenite on goethite. At low concentrations (0.05 and 0.10 mM), arsenite adsorption on goethite displayed no significant pH dependency. Grafe et al. (2002), in a study of adsorption of

arsenate and arsenite on ferrihydrite, the sorption of arsenate on ferrihydrite showed no pH dependence (no tailing).

In contrast to the Bt soils, the sorption patterns of the Ap soils do show pH dependence. The Ap-Control soil shows a pattern of decreasing sorption with increasing pH. This is similar to the patterns of arsenic adsorption to humic acids observed by Thanabalasingam and Pickering (1986). Their results showed that at higher pH, humic acids dissolve, which would lead to a rapid decrease in sorption. They also noted that increasing the pH promotes dissociation of the weak acid functional groups present in the substrates, which would then inhibit interaction with the arsenic oxyanions if bonding were essentially electrostatic.

The Ap-MC soil exhibits a sorption pattern in which no sorption occurs at pH 3 and 9-11, and sorption peaks at pH 5. Thanabalasingam and Pickering (1986) also found that sorption of arsenate to humic acids declined at pH<5, due to increased protonation of both adsorbent and adsorbate. So at low pH, everything, including the roxarsone and the organic matter, is protonated. The consequent neutral charge for roxarsone and it's slight hydrophobicity results in an absence of sorption.

5.5 Implications for roxarsone transport in agricultural watersheds

The sorption characteristics exhibited by soils from Muddy Creek may have implications for roxarsone transport in agricultural watersheds. For soils that have had years of poultry litter application, there may be both sorption and subsequent leaching of roxarsone within the soil system. The topsoil, or Ap horizon, would have high organic matter content due to manure additions. In this soil layer, OM would probably dominate sorption. Results of this study suggest that roxarsone is loosely bound to OM, and would be rapidly released into water via overland flow or shallow percolation through the soil and may be seen in surface water after a rain event or field irrigation. This is supported by the study of Rutherford et al. (2003), who examined the leaching of arsenic to water from poultry litter after it had been applied to soil. Analysis of poultry litter showed that 75% of the arsenic was readily soluble in water. Extraction of soils showed that

weakly bound arsenic mobilized by water correlates positively with C, P, Cu, and Zn in amended fields and appeared to come primarily from the litter. Once the roxarsone reaches the Bt horizon, it is sorbed very strongly into either iron oxides or clays, and that will be less prone to be released into percolating water. However, competition between roxarsone and phosphate or organic acids may affect sorption onto iron oxides in the Bt horizon. A schematic of the controls on transport of roxarsone through the Ap and Bt horizons is depicted in Figure 10.

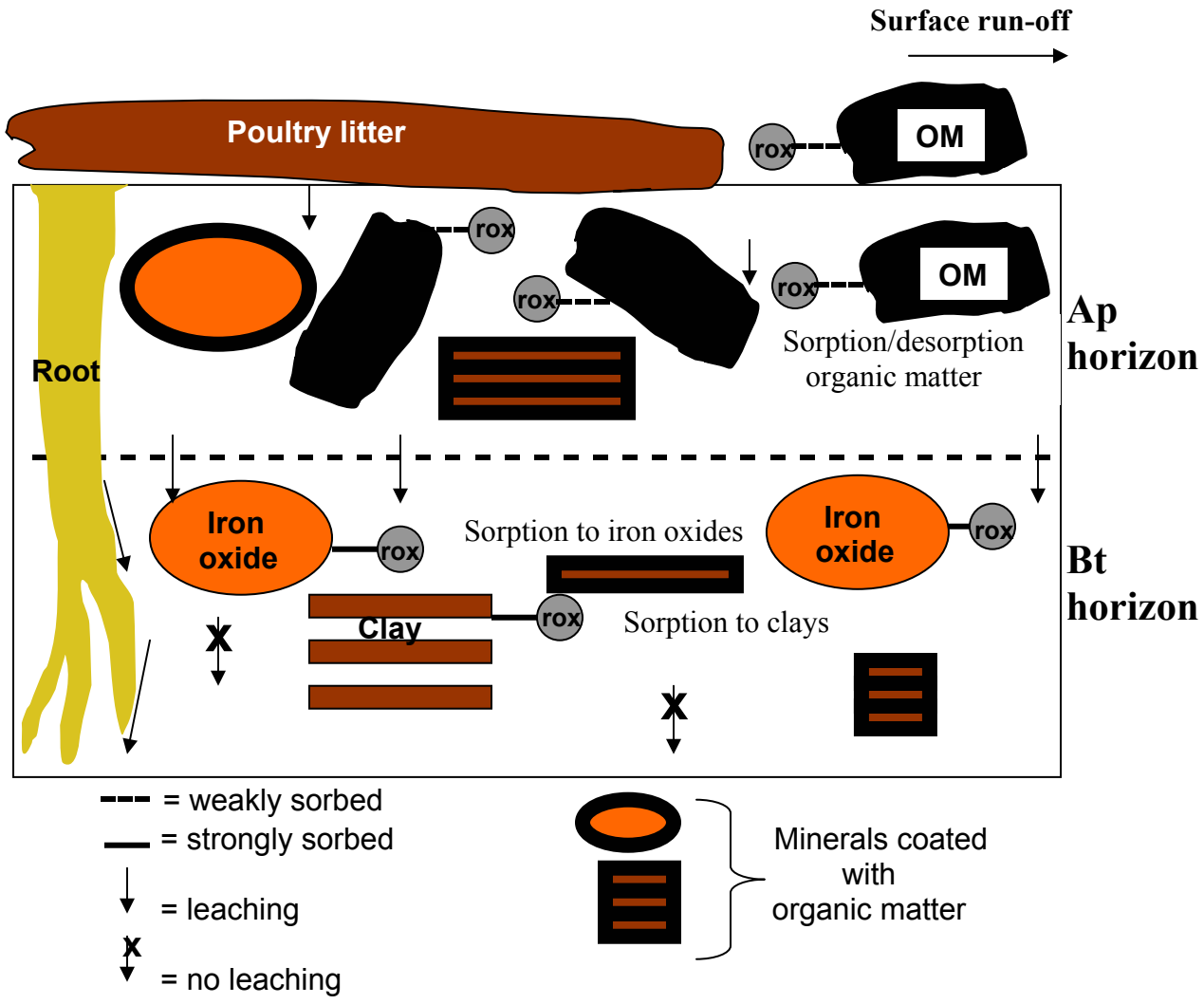

Figure 10. Schematic of roxarsone transport through the Ap and Bt soils

5.6 Suggestions for future research

There are several areas of research that could be pursued in the future. Our research has not determined whether the organic portion or the As(V) portion of the roxarsone molecule is responsible for binding to mineral surfaces. If the As(V) portion could be blocked in some manner, any sorption observed would be by the organic part of the molecule.

Another area of research is in the area of X-ray absorption spectroscopy (XAS). Extended X-ray absorption fine structure (EXAFS) spectroscopy would give an average As-Fe interatomic distance, which would be indicative of the type of inner-sphere mechanism (for bonding) taking place.

An improved method of sequential extraction of roxarsone/As(V) would provide valuable information on where the arsenic is being sequestered. Leaching or sorption would occur, depending on whether the arsenic is tightly or loosely bound, as our research has indicated.

CONCLUSION

Roxarsone, an organoarsenic feed additive, is introduced to agricultural watersheds through application of poultry litter as fertilizer. However, little work has been done to evaluate the fate of roxarsone once it has been introduced into the environment. The mobilization of arsenic derived from roxarsone in the litter may result in arsenic leaching into the soil, surface water, and groundwater.

In the Shenandoah Valley of Virginia, over 364,000 tons of poultry litter are produced each year and applied to cropland as fertilizer. The potential for sorption of roxarsone by the Frederick series soils, the main soil type present in the Shenandoah Valley, was investigated through use of batch experiments. Adsorption isotherms and a pH envelope for roxarsone sorption to the different soil horizons were determined.

From the initial sorption equilibrium experiments, it was determined that 3 days was sufficient for roxarsone to reach equilibrium with the Ap and Bt

horizons of the Frederick Series soils from the Muddy Creek (MC) and control sites. The adsorption isotherm experiments were conducted to determine the sorption characteristics of roxarsone to the soils. Roxarsone sorbs strongly to the Bt soils. The most likely explanation for this pattern is that the roxarsone is adsorbing to iron oxides or clays. The Ap soils only exhibited moderate to low sorption. Results of experiments conducted when organic matter was removed from soils indicate that the organic matter may be coating the mineral surfaces in the Ap-MC soils, thus blocking the sorption sites of the iron oxides and clays. The two most important controls exerted on the Ap soils ability to sorb Roxarsone appear to be particle size and partitioning of roxarsone onto organic matter, which can coat (block) of mineral surfaces.

The pH adsorption edge experiments were conducted to evaluate whether the sorption of roxarsone to the different soils was pH dependent. The sorption of roxarsone by the Bt1- and Bt2-Control soils does not appear to be pH dependent, because sorption remains high over the entire range of pH (3-11) tested. The Bt1-MC soil also exhibits strong sorption over this pH range, with a slight tailing at pH>9. Sorption on these soils is most likely occurring on the clay minerals and iron oxides, both of which typically show pH dependence, with a decrease of sorption occurring above pH>9. The sorption capabilities of the Ap soils do show pH dependence, especially the Ap-MC soil, with sorption probably occurring onto the organic matter.

The sorption characteristics exhibited by soils from Muddy Creek may have implications for roxarsone transport in agricultural watersheds. For soils that have had years of poultry litter application, there may be both sorption and subsequent leaching of roxarsone within the soil system. The topsoil, or Ap horizon, would have high organic matter content due to the manure. In this soil layer, OM would probably dominate sorption. Results of this study suggest that roxarsone is loosely bound to OM, and would be rapidly leached into the water via overland flow or shallow percolation through the soil and may be seen in surface water after a rain event or field irrigation. Once the roxarsone reaches the Bt horizon, it is sorbed very strongly into either iron oxides or clays, and that

will be less prone to be released into water. However, competition between roxarsone and phosphate or organic acids may affect sorption onto iron oxides in the Bt horizon.

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APPENDICES

APPENDIX A: Comparison of sample preparation methods

A.1 Dry ashing

For the dry ashing procedure, we used the following method. Put 50-mL aqueous sample in Pyrex beaker. Add 5 mL 40% $MgNO_3$ and stir with glass rod. Place in sand bath (on hot plate) and evaporate until only white ash remains. Cover with watch glass and heat in muffle furnace overnight (12-14 hrs) at 450°C for aqueous samples. [For high organic content solid samples such as poultry litter, place 5-g solid sample in beaker. Add 5 mL 50% HNO3, 1 mL 20% $Mg(NO_3)_2$ and 25 mL nanopure water. Place in sand bath and evaporate until only ash remains. Cover with watch glass and heat at 150°C for 1 hr, 300°C for 30 minutes, then overnight (12-14 hrs) at 450°C.] Allow to cool and add 1 mL nanopure water to wet ash. Add 3.5 mL 6 N HCl and stir with glass rod until all ash is dissolved. Transfer by pipette to 25 mL Erlenmeyer flask and rinse beaker with nanopure water until filled to 25 mL. Filter through Whatman #1 filter paper if necessary. The ICP-AES or GFAAS is used to analyze sample for total arsenic content.

A.2 Microwave digestion

For the microwave digestion procedure, we used EPA Method 3015 “Microwave Assisted Acid Digestion of Aqueous Samples and Extracts”. ETHOS PLUS labstation microwave (1.6 kW) by Milestone was used to digest the samples. Add 5 mL concentrated nitric acid to 45-mL aqueous sample. Heat the mixture in the microwave for 10 minutes at 160°C and then for 10 minutes at 170°C. The ICP-AES or GFAAS is used to analyze sample for total arsenic content.

A.3 Ultraviolet photolysis

For the ultraviolet photolysis procedure, we used the method used by H. Hasegawa et al. 1999. (Note: we used a 1000 W high-pressure xenon lamp (Oriel model #66021) instead of a 400 W high-pressure mercury lamp.) Each 5-mL aqueous sample is acidified to pH 2 with 1 M HCl and then place in a quartz reaction vessel. The sample is irradiated for 30, 60, 90, or 120 minutes to determine when digestion is complete. It is completed after 30 minutes. Cooling 25°C waters circulate about the quartz reaction vessel during irradiation. The ICP-AES or GFAAS is used to analyze sample for total arsenic content.

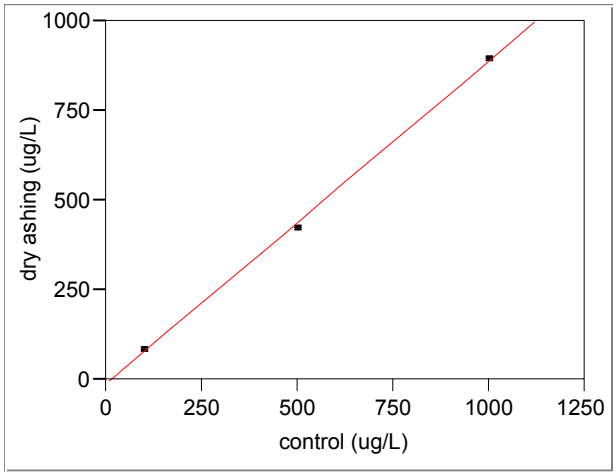
A.4 Results

The results indicate that there is no significant difference between dry ashing and microwave digestion methods (dry ashing: R^2=0.995, P-value=0.018, MW: R^2=0.995, P-value=0.014). However, the ultraviolet digestion method yielded results that were 40% higher than the actual amount of Roxarsone present, so this method was discarded and digestion was not done for the 500 and 1000 μg/L concentrations by this method.

Comparison of Dry Ashing, Microwave, and Ultraviolet Digestion Methods

Roxarsone conc. (μg/L)	Dry ashing (μg/L)	Microwave (μg/L)	Ultraviolet (μg/L)
100	85	102	140
500	425	407	---
1000	895	818	---

Bivariate Fit of Dry Ashing by Control



Linear Fit

Dry ashing = -12.54098 + 0.9016393 control

Summary of Fit

RSquare	0.999197
RSquare Adj	0.998395
Root Mean Square Error	16.29643
Mean of Response	468.3333
Observations (or Sum Wgts)	3

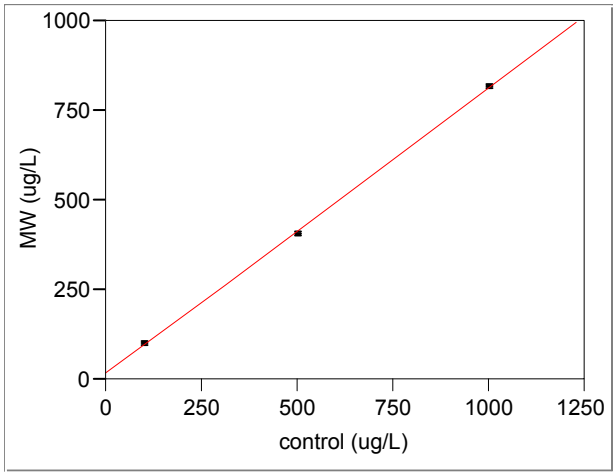
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	330601.09	330601	1244.856
Error	1	265.57	266	Prob > F
C. Total	2	330866.67		0.0180

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-12.54098	16.56143	-0.76	0.5874
control	0.9016393	0.025555	35.28	0.0180

Bivariate Fit of MW by Control



Linear Fit

MW = 17.459016 + 0.7966393 control

Summary of Fit

RSquare	0.99955
RSquare Adj	0.999101
Root Mean Square Error	10.77375
Mean of Response	442.3333
Observations (or Sum Wgts)	3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	258084.59	258085	2223.453
Error	1	116.07	116	Prob > F
C. Total	2	258200.67		0.0135

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	17.459016	10.94895	1.59	0.3566
control	0.7966393	0.016895	47.15	0.0135

APPENDIX B: Sorption methods

To determine the amount of time needed for the mixture to reach equilibrium, an initial sorption experiment was run, following the sorption method ASTM D4319 (93). Weigh out 6.25-g soil into centrifuge tube. Add 25-mL solution containing 10, 50, 100 or 200 μg/L roxarsone in nanopure water (this is a 1:4 soil to solution ratio as required). Mix three samples for each of the four different roxarsone concentrations solutions (12 samples). Place the 12 samples on a wrist shaker for 6 hours. Allow settling for two days. Remove one sample of each of the four different concentrations (10, 50, 100, 200 μg/L roxarsone). Centrifuge for 30 minutes at 3500-rpm (25°C). Use 30-mL syringe to remove supernatant from centrifuge tube. Filter the supernatant twice – first through 0.45 μm and then through 0.20 μm filter. Add one drop concentrated HNO_3 to preserve sample. These are the 3-day samples. Shake the remaining 8 samples on the wrist shaker for 6 hours. Allow settling for two days. Remove one sample of each of the four different concentrations (10, 50, 100, 200 μg/L roxarsone). Centrifuge, filter, and preserve as above. These are the 6-day samples. Shake the remaining 4 samples on the wrist shaker for 6 hours. Allow settling for two days. Remove one sample of each of the four different concentrations (10, 50, 100, 200 μg/L roxarsone). Centrifuge, filter, and preserve as above. These are the 9-day samples. GFAAS is used to analyze samples for total arsenic content.

As a modified ASTM D4319 (93) procedure, we decided to run two additional sets of experiments. Weigh out 6.25-g soil into (eight) centrifuge tubes. Add 25-mL solution containing 10, 50, 100 or 200 μg/L roxarsone in nanopure water (two sets). Place one set of each of the four different concentrations (10, 50, 100, 200 μg/L roxarsone) aside. These are the control samples that will not be shaken. Place the other set of (four) samples on the wrist shaker. Shake for 24 hours. Allow settling for 2 days. Centrifuge, filter, and preserve all eight samples as above. GFAAS is used to analyze samples for total arsenic content.

For the sorption experiments, we used a modified ASTM D4319 (93) Method. Weigh out 6.25 g (homogenized) soil into centrifuge tube. Add 25 mL solution (nanopure water, 100, 200, 500, or 1000 ug/L roxarsone) and shake well. Place on wrist shaker for 24 hrs. Allow to settle for 2 days. Centrifuge for 30 minutes at 3500-rpm (25°C). Use 30-mL syringe to remove supernatant from centrifuge tube. Filter the supernatant twice – first through 0.45 μm and then through 0.20 μm filter. Add one drop concentrated HNO_3 to preserve sample. GFAAS is used to analyze sample for total arsenic content.

APPENDIX C: Sorption data

C.1 Initial sorption equilibrium experiments

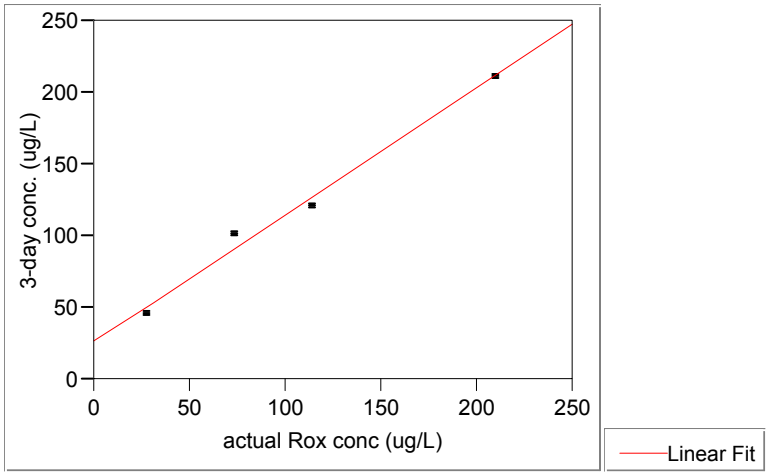
C.1a Ap-MC initial sorption equilibrium experiment

For this set of initial sorption experiments, there appears to be no significant difference between the sorption that occurs by the Ap-MC soil when the experiment was run for 3, 6, 9, and 12 days. For the 3-day experiment, R^2=0.989, P-value=0.005, and slope of regression line=0.886 ±0.065; for the 6-day experiment, R^2=0.981, P-value=0.009, and slope of regression line =0.670 ±0.064; for the 9-day experiment, R^2=0.971, P-value=0.014, and slope of regression line =0.537 ±0.065; and for the 12-day experiment, R^2=0.999, P-value=0.002, and slope of regression line =0.854 ±0.003.

Initial Equilibrium Results: 3-, 6-, 9-, and 12-day

Spiked Rox conc. μg/L	Actual Rox conc. (μg/L)	3-day conc. (μg/L)	6-day conc. (μg/L)	9-day conc. (μg/L)	Actual Rox conc for 12-day only	12-day conc (μg/L)
10	27.9	46.7	60.2	58.1	NA	NA
50	73.6	101.6	76.4	92.0	NA	NA
100	113.7	121.6	118.6	96.5	76.5	68.0
200	209.6	212.4	177.3	159.1	148.8	130.4
500	NA	NA	NA	NA	316.5	273.2

Bivariate Fit of 3-day conc. (μg/L) by Rox conc (μg/L)



Linear Fit

3-day conc. (μg/L) = 26.427009 + 0.8865159 actual Rox conc (μg/L)

Summary of Fit

RSquare	0.989469
RSquare Adj	0.984204
Root Mean Square Error	8.662158
Mean of Response	120.575
Observations (or Sum Wgts)	4

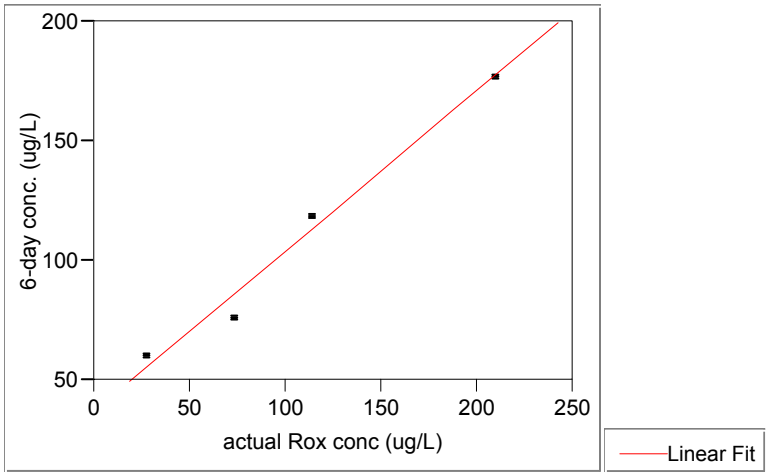
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	14100.382	14100.4	187.9224
Error	2	150.066	75.0	Prob > F
C. Total	3	14250.448		0.0053

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	26.427009	8.119471	3.25	0.0828
actual Rox conc (ug/L)	0.8865159	0.064669	13.71	0.0053

Bivariate Fit of 6-day conc. (μg/L) by Rox conc (μg/L)



Linear Fit

6-day conc. (μg/L) = 36.987326 + 0.6698463 actual Rox conc (μg/L)

Summary of Fit

RSquare	0.981952
RSquare Adj	0.972928
Root Mean Square Error	8.601211
Mean of Response	108.125
Observations (or Wgts)	Sum 4

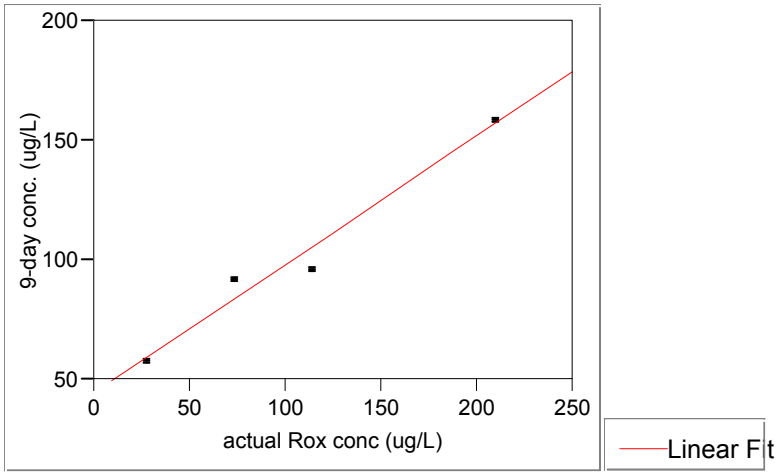
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	8050.2258	8050.23	108.8150
Error	2	147.9617	73.98	Prob > F
C. Total	3	8198.1875		0.0091

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	36.987326	8.062343	4.59	0.0444
actual Rox conc (ug/L)	0.6698463	0.064214	10.43	0.0091

Bivariate Fit of 9-day conc. (μg/L) by Rox conc (μg/L)



Linear Fit

9-day conc. (μg/L) = 44.444754 + 0.5365372 actual Rox conc (μg/L)

Summary of Fit

RSquare	0.971466
RSquare Adj	0.957199
Root Mean Square Error	8.709228
Mean of Response	101.425
Observations (or Sum Wgts)	4

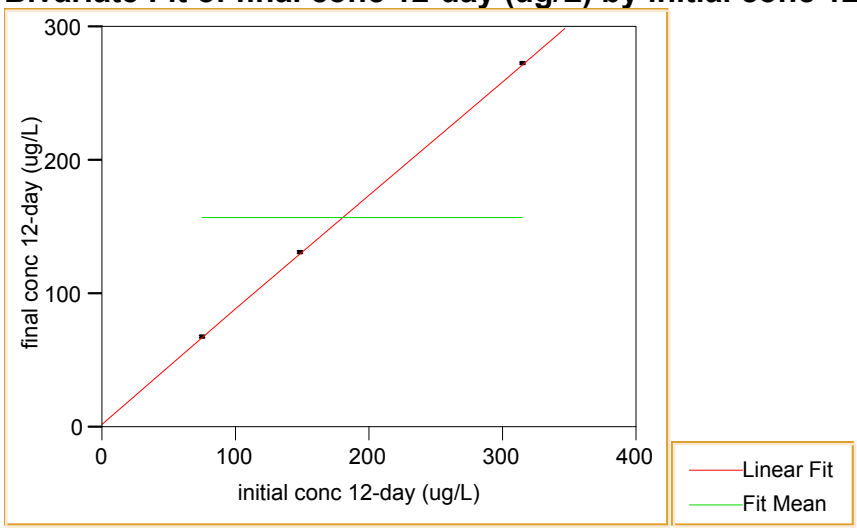
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	5164.8462	5164.85	68.0923
Error	2	151.7013	75.85	Prob > F
C. Total	3	5316.5475		0.0144

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	44.444754	8.163592	5.44	0.0321
actual Rox conc (ug/L)	0.5365372	0.065021	8.25	0.0144

Bivariate Fit of final conc 12-day (ug/L) by initial conc 12-day (ug/L)



Linear Fit

final conc 12-day (ug/L) = 2.8975354 + 0.854388 initial conc 12-day (ug/L)

Summary of Fit

RSquare	0.99999
RSquare Adj	0.999981
Root Mean Square Error	0.464354
Mean of Response	157.2
Observations (or Sum Wgts)	3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	22130.664	22130.7	102635.1
Error	1	0.216	0.2	Prob > F
C. Total	2	22130.880		0.0020

Parameter Estimates

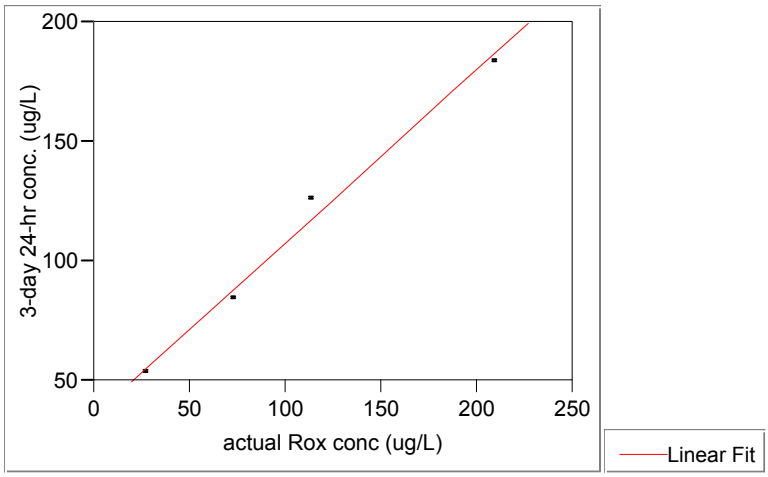
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.8975354	0.55123	5.26	0.1197
initial conc 12-day (ug/L)	0.854388	0.002667	320.37	0.0020

For the second set of initial experiments, the 3-day, 24 hour (shaking) experiment shows no significant difference from the 3, 6, and 9-day sorption trials (R^2=0.99, P-value=0.006). The no-shaking experiment shows a slight significant difference from the 3, 6, and 9-day trials (R^2=0.95, P-value=0.024).

Comparison of Actual Roxarsone Concentration to Concentrations after 3-day/24 hr Shaking and With No Shaking

Spiked Rox conc μg/L	Actual Rox conc. (μg/L)	3-day/24hr conc. (μg/L)	No shaking conc. (μg/L)
10	21.8	53.8	56.3
50	55.9	84.2	65.8
100	107.5	126.1	121.9
200	180.8	183.8	175.9

Bivariate Fit of 3-day 24-hr conc. (μg/L) by Rox conc (μg/L)



Linear Fit

3-day 24-hr conc. (μg/L) = 35.064932 + 0.7242003 actual Rox conc (μg/L)

Summary of Fit

RSquare	0.989022
RSquare Adj	0.983534
Root Mean Square Error	7.22639
Mean of Response	111.975
Observations (or Sum Wgts)	4

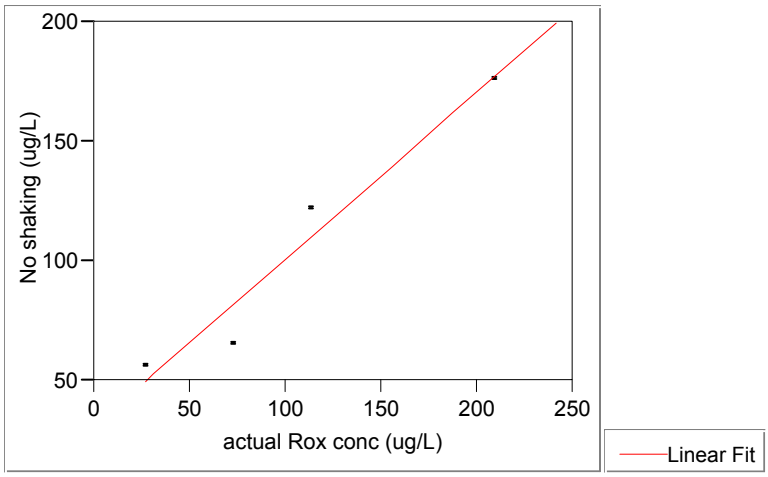
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	9409.6861	9409.69	180.1907
Error	2	104.4414	52.22	Prob > F
C. Total	3	9514.1275		0.0055

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	35.064932	6.773655	5.18	0.0353
actual Rox conc (ug/L)	0.7242003	0.05395	13.42	0.0055

Bivariate Fit of no shaking (μg/L) by Rox conc (μg/L)



Linear Fit

No shaking (μg/L) = 30.694678 + 0.6994381 actual Rox conc (μg/L)

Summary of Fit

RSquare	0.951897
RSquare Adj	0.927846
Root Mean Square Error	14.89196
Mean of Response	104.975
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	8777.2063	8777.21	39.5779
Error	2	443.5412	221.77	Prob > F
C. Total	3	9220.7475		0.0243

Parameter Estimates

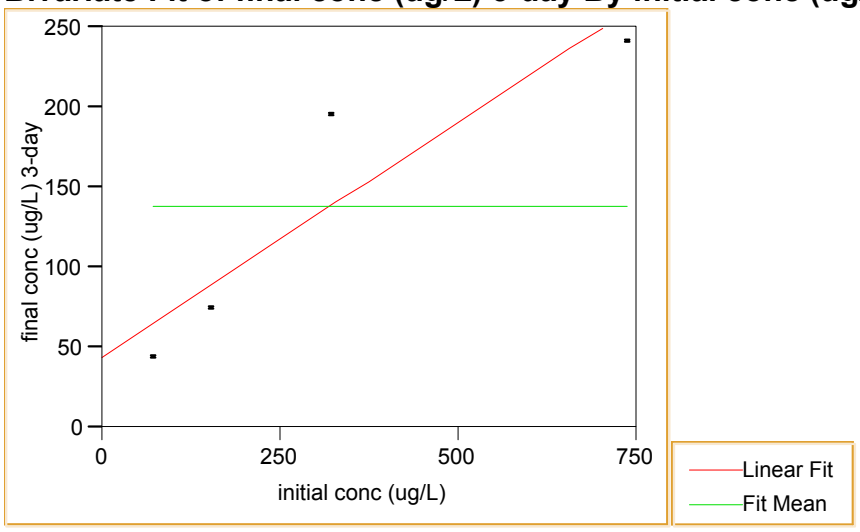
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	30.694678	13.95898	2.20	0.1589
actual Rox conc (ug/L)	0.6994381	0.111179	6.29	0.0243

C.1b Bt1-MC initial equilibrium experiment

For this set of initial sorption experiments, there appears to be no significant difference between the sorption that occurs by the Bt1-MC soil when the experiment was run for 3, 6 or 9 days. For the 3-day experiment, R^2=0.841, P-value=0.083, and slope of regression line=0.293 ±0.083; for the 6-day experiment, R^2=0.986, P-value=0.007, and slope of regression line=0.324 ±0.027; and for the 9-day experiment, R^2=0.919, P-value=0.041, and slope of regression line=0.323 ±0.068.

Spiked Rox conc. μg/L	Actual Rox conc. (μg/L)	3-day conc. (μg/L)	6-day conc. (μg/L)	9-day conc. (μg/L)
100	72.0	43.4	42.4	18.3
200	154.0	74.3	82.5	61.0
500	323.0	195.2	149.7	162.1
1000	739.0	241.1	264	240.0

Bivariate Fit of final conc (ug/L) 3-day By initial conc (ug/L)



Linear Fit

final conc (ug/L) 3-day = 44.312682 + 0.2925072 initial conc (ug/L)

Summary of Fit

Rsquare	0.841426
RSquare Adj	0.762139
Root Mean Square Error	46.18876
Mean of Response	138.5
Observations (or Sum Wgts)	4

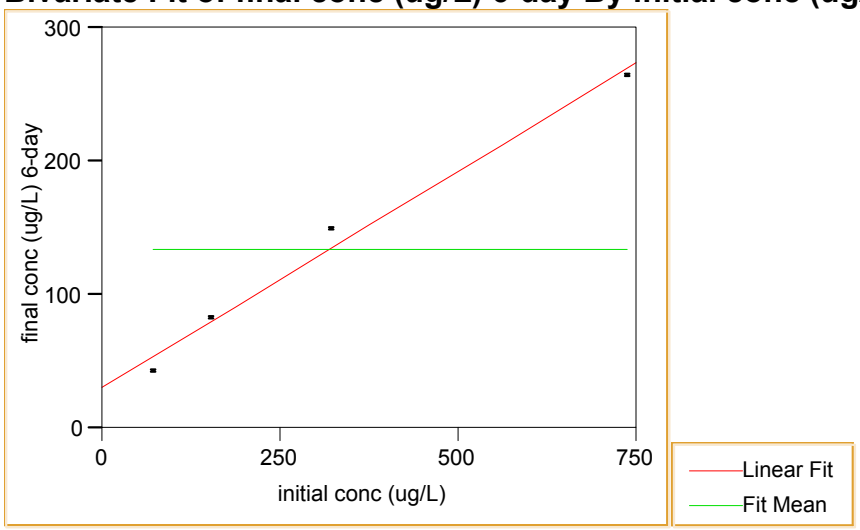
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	22640.496	22640.5	10.6124
Error	2	4266.804	2133.4	Prob > F
C. Total	3	26907.300		0.0827

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	44.312682	37.00383	1.20	0.3538
initial conc (ug/L)	0.2925072	0.08979	3.26	0.0827

Bivariate Fit of final conc (ug/L) 6-day By initial conc (ug/L)



Linear Fit

final conc (ug/L) 6-day = 30.269962 + 0.3241616 initial conc (ug/L)

Summary of Fit

RSquare	0.986456
RSquare Adj	0.979684
Root Mean Square Error	13.81616
Mean of Response	134.65
Observations (or Sum Wgts)	4

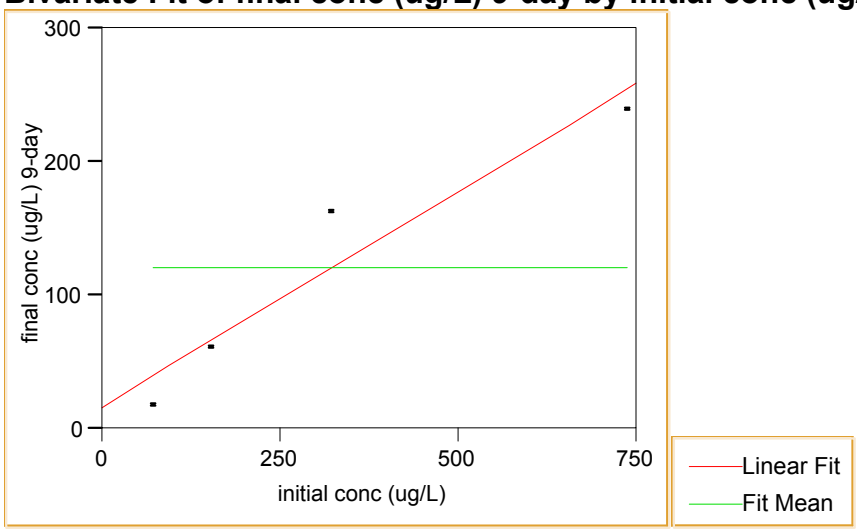
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	27805.837	27805.8	145.6670
Error	2	381.773	190.9	Prob > F
C. Total	3	28187.610		0.0068

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	30.269962	11.06873	2.73	0.1117
initial conc (ug/L)	0.3241616	0.026858	12.07	0.0068

Bivariate Fit of final conc (ug/L) 9-day by initial conc (ug/L)



Linear Fit

final conc (ug/L) 9-day = 16.40633 + 0.3228064 initial conc (ug/L)

Summary of Fit

RSquare	0.919256
RSquare Adj	0.878884
Root Mean Square Error	34.79925
Mean of Response	120.35
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	27573.835	27573.8	22.7697
Error	2	2421.975	1211.0	Prob > F
C. Total	3	29995.810		0.0412

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	16.40633	27.8792	0.59	0.6158
initial conc (ug/L)	0.3228064	0.067649	4.77	0.0412

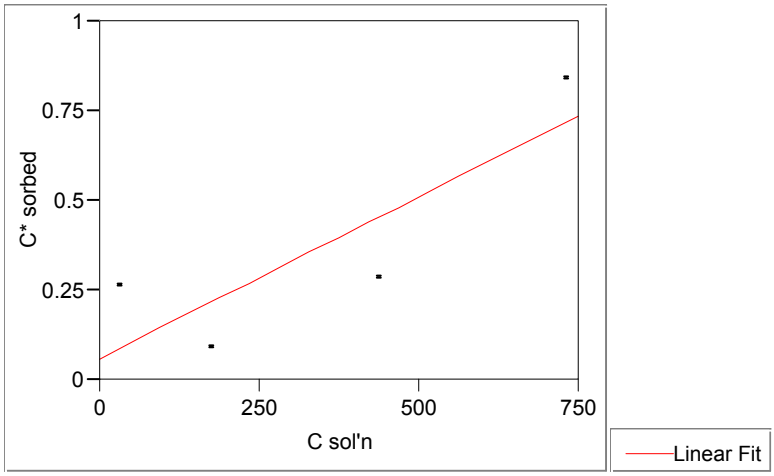
C.2 Linear isotherms

For the sorption experiments with varying concentrations, the concentration of roxarsone in the final solution (μg/L) and the C* sorbed (μg/L) are plotted to derive the linear isotherms for the soils. The R^2, P-value, and K_d values for the soils are: Ap-MC soils: R^2=0.720, P-value=0.152, and K_d=0.001; Bt1-MC soils: R^2=0.964, P-value=0.018, and K_d=0.231; Ap-Control soils: R^2=0.980, P-value=0.010, and K_d=0.004; Bt1-Control soils: R^2=0.929, P-value=0.036, and K_d=0.198; and Bt2-Control soils: R^2=0.178, P-value=0.578, and K_d= -0.410.

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Ap-MC Soil

Rox. Conc. (μg/L)	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
No Rox	0	NA	27.0	NA
100	100	66.07	33.93	0.264
200	200	23	177	0.092
510	510	71.9	438.1	0.288
943	943	210.7	732.3	0.843

Bivariate Fit of C* sorbed (μg/g) by C sol'n (μg/L) for Ap-MC soil



Linear Fit

C* sorbed = 0.0610208 + 0.0008998 C sol'n

Summary of Fit

RSquare	0.720022
RSquare Adj	0.580033
Root Mean Square Error	0.211308
Mean of Response	0.37175
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.22965867	0.229659	5.1434
Error	2	0.08930208	0.044651	Prob > F
C. Total	3	0.31896075		0.1515

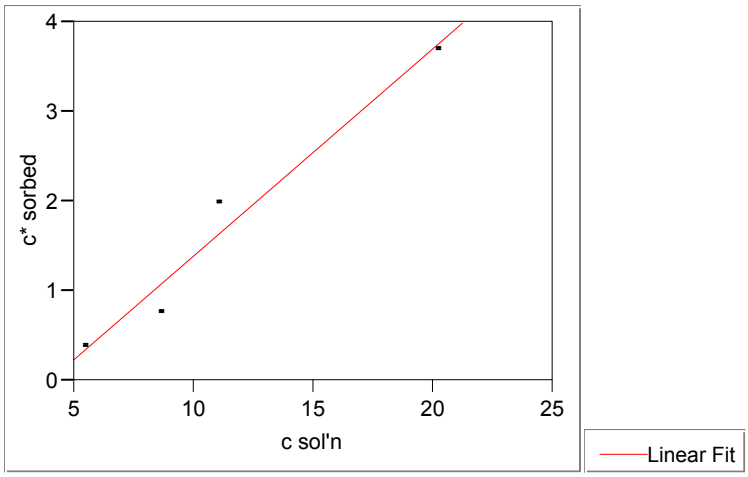
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0610208	0.173017	0.35	0.7580
C sol'n	0.0008998	0.000397	2.27	0.1515

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of Soil (C* sorbed) for Bt1-MC Soil

Rox. Conc. (μg/L)	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
No Rox	0	NA	0.8	NA
100	100	94.5	5.5	0.378
200	200	191.27	8.73	0.765
510	510	498.88	11.13	2.00
943	943	922.73	20.27	3.691

Bivariate Fit of C* sorbed (μg/g) by C sol'n (μg/L) for Bt1-MC soil



Linear Fit

c* sorbed = -0.926266 + 0.2309679 c sol'n

Summary of Fit

RSquare	0.964433
RSquare Adj	0.94665
Root Mean Square Error	0.344552
Mean of Response	1.7085
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	6.4382686	6.43827	54.2324
Error	2	0.2374324	0.11872	Prob > F
C. Total	3	6.6757010		0.0179

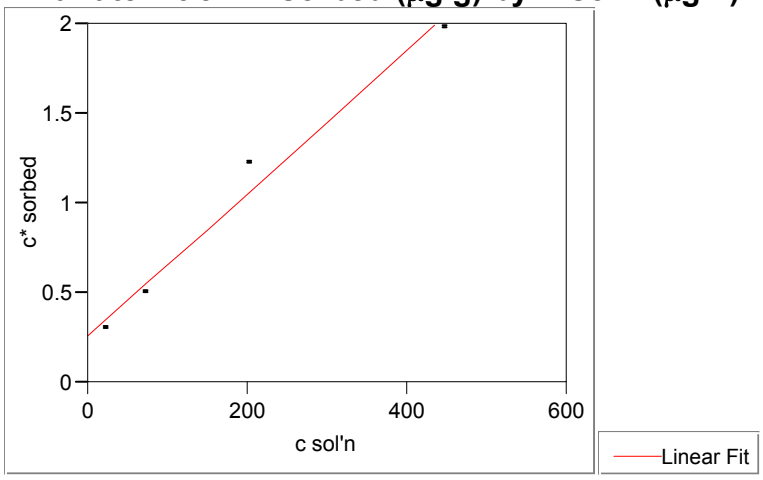
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.926266	0.397094	-2.33	0.1449
c sol'n	0.2309679	0.031363	7.36	0.0179

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Ap-Control

Rox. Conc. (μg/L)	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
No Rox	0	NA	13.9	NA
100	100	75.63	24.37	0.303
200	200	125.9	74.1	0.504
510	510	307.3	202.7	1.229
943	943	495.1	447.9	1.980

Bivariate Fit of C* sorbed (μg/g) by C sol'n (μg/L) for Ap-Control soil



Linear Fit

c* sorbed = 0.2573951 + 0.0039868 c sol'n

Summary of Fit

RSquare	0.97951
RSquare Adj	0.969266
Root Mean Square Error	0.13369
Mean of Response	1.004
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.7088558	1.70886	95.6105
Error	2	0.0357462	0.01787	Prob > F
C. Total	3	1.7446020		0.0103

Parameter Estimates

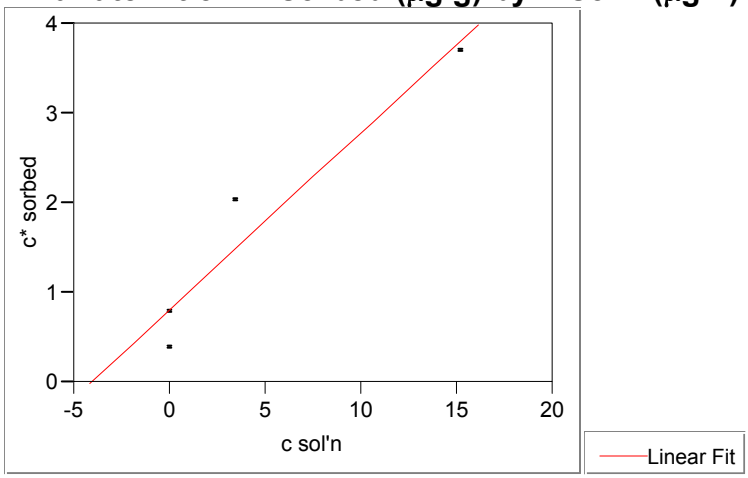
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.2573951	0.101481	2.54	0.1266
c sol'n	0.0039868	0.000408	9.78	0.0103

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of Soil (C* sorbed) for Bt1-Control

Rox. Conc. (μg/L)	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
No Rox	0	NA	bdl	NA
100	100	100	0	0.4
200	200	200	0	0.8
510	510	506.53	3.47	2.03
943	943	927.7	15.3	3.71

bdl= below detectable limit NA = not applicable

Bivariate Fit of C* sorbed (μg/g) by C sol'n (μg/L) for Bt1-Control



Linear Fit

c* sorbed = 0.8074668 + 0.1976629 c sol'n

Summary of Fit

RSquare	0.929429
RSquare Adj	0.894143
Root Mean Square Error	0.484192
Mean of Response	1.735
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	6.1752163	6.17522	26.3401
Error	2	0.4688837	0.23444	Prob > F
C. Total	3	6.6441000		0.0359

Parameter Estimates

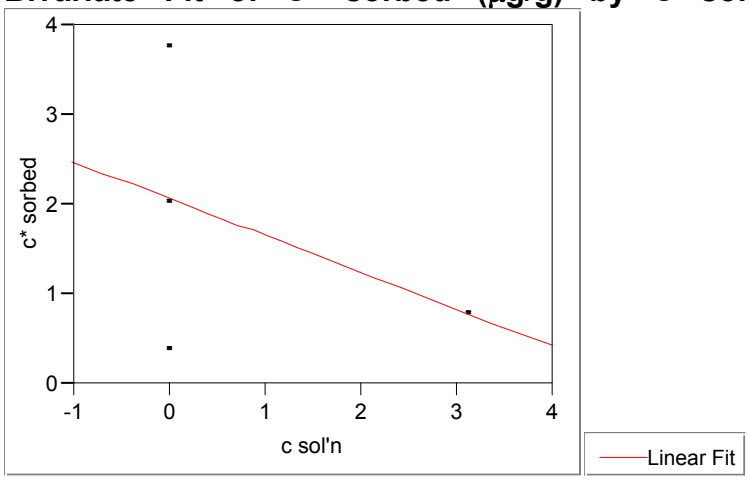
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.8074668	0.302113	2.67	0.1161
c sol'n	0.1976629	0.038514	5.13	0.0359

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of Soil (C* sorbed) for Bt2-Control

Rox.. (μg/L)	Cadded (μg/L)	Csorbed(μg/L)	C sol'n (μg/L)	C*sorbed(μg/g)
No Rox	0	NA	bdl	NA
100	100	100	0	0.4
200	200	196.87	3.13	0.788
510	510	510	0	2.04
943	943	943	0	3.772

bdl = below detectable limit NA = not applicable

Bivariate Fit of C* sorbed (μg/g) by C sol'n (μg/L) for Bt2-Control



Linear Fit

c* sorbed = 2.0706667 - 0.4097977 c sol'n

Summary of Fit

RSquare	0.178299
RSquare Adj	-0.23255
Root Mean Square Error	1.686209
Mean of Response	1.75
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.2339253	1.23393	0.4340
Error	2	5.6866027	2.84330	Prob > F
C. Total	3	6.9205280		0.5777

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.0706667	0.973533	2.13	0.1673
c sol'n	-0.409798	0.622066	-0.66	0.5777

C.2a SAS output for regression analysis

Analysis of the 5 original soils (1)

The REG Procedure
Model: MODEL1
Dependent Variable: C*sorbed C*sorbed

NOTE: No intercept in model. R-Square is redefined.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	41.01213	8.20243	5.64	0.0040
Error	15	21.81415	1.45428		
Uncorrected Total	20	62.82628			

Root MSE	1.20593	R-Square	0.6528
Dependent Mean	1.31339	Adj R-Sq	0.5370
Coeff Var	91.81818		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Ap_MC		1	0.00101	0.00138	0.73	0.4764
Ap_control		1	0.00476	0.00242	1.97	0.0680
Bt1_MC		1	0.16497	0.04762	3.46	0.0035
Bt1_control		1	0.25923	0.07687	3.37	0.0042
Bt2_control		1	0.25132	0.38487	0.65	0.5236

Analysis of the 5 original soils (2)

The REG Procedure
Model: MODEL1

Test Bt1_MC_vs_Bt1_control Results
for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	1.58029	1.09	0.3137
Denominator	15	1.45428		

Analysis of the 5 original soils (3)

The REG Procedure
Model: MODEL1

Test Bt1_MC_vs_Bt2_control Results
for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	0.07210	0.05	0.8268
Denominator	15	1.45428		

Analysis of the 5 original soils (4)

The REG Procedure
Model: MODEL1

Test Bt1_control_vs_Bt2_control Results
for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	0.00059124	0.00	0.9842
Denominator	15	1.45428		

Analysis of the 5 original soils combining the Bt soils (5)

The REG Procedure
Model: MODEL1
Dependent Variable: C*sorbed C*sorbed

NOTE: No intercept in model. R-Square is redefined.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	39.39665	13.13222	9.53	0.0006
Error	17	23.42963	1.37821		
Uncorrected Total	20	62.82628			

Root MSE	1.17397	R-Square	0.6271
Dependent Mean	1.31339	Adj R-Sq	0.5613
Coeff Var	89.38475		

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Ap_MC		1	0.00101	0.00135	0.75	0.4633
Ap_control		1	0.00476	0.00236	2.02	0.0594
Bt		1	0.19177	0.03919	4.89	0.0001

Analysis of the 5 original soils combining the Bt soils (6)

The REG Procedure
Model: MODEL1

Test Ap_MC_vs_Ap_control Results for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	2.63567	1.91	0.1846
Denominator	17	1.37821		

Analysis of the 5 original soils combining the Bt soils (7)

The REG Procedure
Model: MODEL1

Test Ap_MC_vs_Bt Results for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	32.60874	23.66	0.0001
Denominator	17	1.37821		

Analysis of the 5 original soils combining the Bt soils (8)

The REG Procedure
Model: MODEL1

Test Ap_control_vs_Bt Results
for Dependent Variable Csorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	31.26143	22.68	0.0002
Denominator	17	1.37821		

C.3 Adsorption envelope

For the sorption experiments with varying pH, the pH and C* sorbed [C* sorbed = concentration of final solution (μg/L) * concentration of the solution added to the soil sample (L) / weight of the soil sample (g)] are plotted to derive the adsorption envelope for the soils.

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Ap-MC at varying pHs

pH	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
3	157.6	0	161.1	0
5	193.6	47.8	145.8	0.191
7	165.7	37.2	128.5	0.149
9	158.4	0	163.2	0
11	154.8	0	169.2	0

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Bt1-MC at varying pHs

pH	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
3	157.6	152.5	5.1	0.61
5	193.6	185.9	7.7	0.744
7	165.7	160.6	5.1	0.642
9	158.4	152.7	5.7	0.611
11	154.8	145.2	9.6	0.581

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Ap-Control at varying pHs

pH	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
3	162	107	55	0.428
5	162	108.1	53.9	0.432
7	165.7	95	70.7	0.38
9	152.8	82.8	70	0.331
11	158.4	34.3	124.1	0.137

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Bt1-Control at varying pHs

pH	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
3	162	162	0	0.648
5	162	162	0	0.648
7	165.7	165.7	0	0.663
9	152.8	152.8	0	0.611
11	158.4	156.3	2.1	0.625

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Bt2-Control at varying pHs

pH	C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
3	162	162	0	0.648
5	162	162	0	0.648
7	165.7	165.7	0	0.663
9	152.8	152.8	0	0.611
11	158.4	158.4	0	0.634

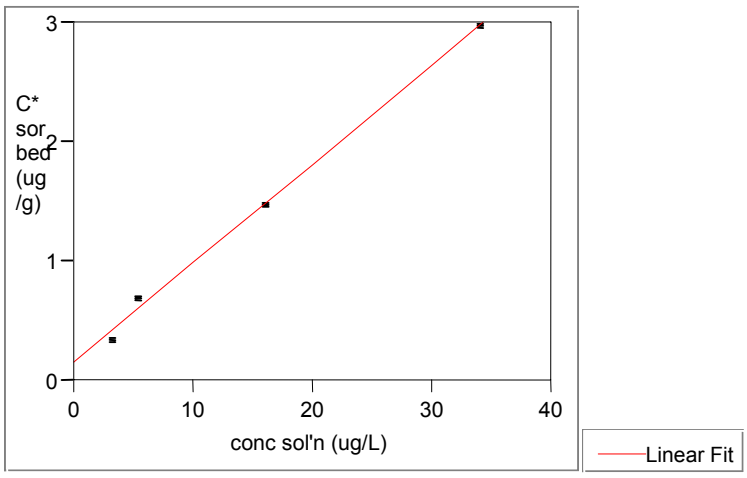
C.4 Kaolin sorption

For the kaolin sorption experiments, the kaolin sorption shows a linear fit, and K_d(=slope)=0.083, R^2=1.00, P-value=0.0018.

Concentrations of Roxarsone added to system(C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Kaolin

C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
91.6	88.37	3.23	0.35
180.4	174.97	5.43	0.7
384	367.9	16.1	1.47
780	745.87	34.13	2.98

Bivariate Fit of C* sorbed (μg/g) by conc sol'n (μg/L) for Kaolin



Linear Fit

C* sorbed (μg/g) = 0.158382 + 0.0826366 conc sol'n (μg/L)

Summary of Fit

RSquare	0.996418
RSquare Adj	0.994626
Root Mean Square Error	0.085605
Mean of Response	1.375
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	4.0766434	4.07664	556.2878
Error	2	0.0146566	0.00733	Prob > F
C. Total	3	4.0913000		0.0018

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.158382	0.067029	2.36	0.1419
conc sol'n (ug/L)	0.0826366	0.003504	23.59	0.0018

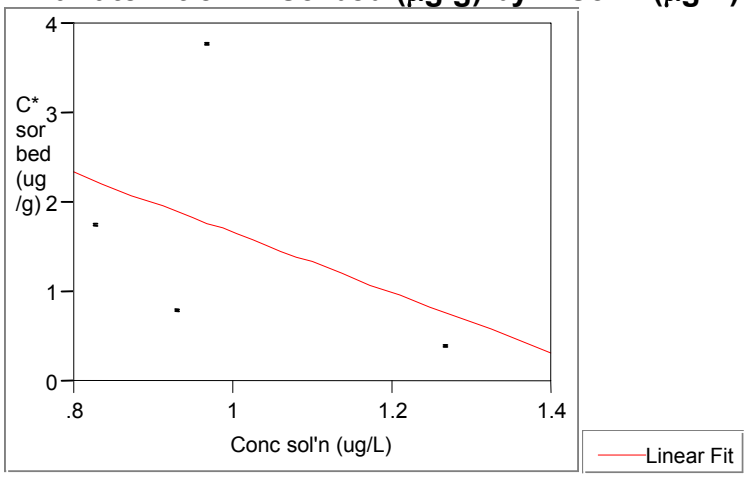
C.5 Goethite sorption

For the goethite sorption experiment, the sorption does not show a linear fit (R^2=0.18, P-value=0.58).

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of mineral (C* sorbed) for Goethite

C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
99.2	97.93	1.27	0.39
195.2	194.27	0.93	0.78
439.5	438.67	0.83	1.75
941	940.03	0.97	3.76

Bivariate Fit of C* sorbed (μg/g) by C sol'n (μg/L) for Goethite



Linear Fit

C* sorbed (μg/g) = 5.0120074 - 3.3420074 Conc sol'n (μg/L)

Summary of Fit

RSquare	0.176603
RSquare Adj	-0.23509
Root Mean Square Error	1.6738
Mean of Response	1.67
Observations (or Wgts)	Sum 4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.2017859	1.20179	0.4290
Error	2	5.6032141	2.80161	Prob > F
C. Total	3	6.8050000		0.5798

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.0120074	5.170845	0.97	0.4347
Conc sol'n (ug/L)	-3.342007	5.10267	-0.65	0.5798

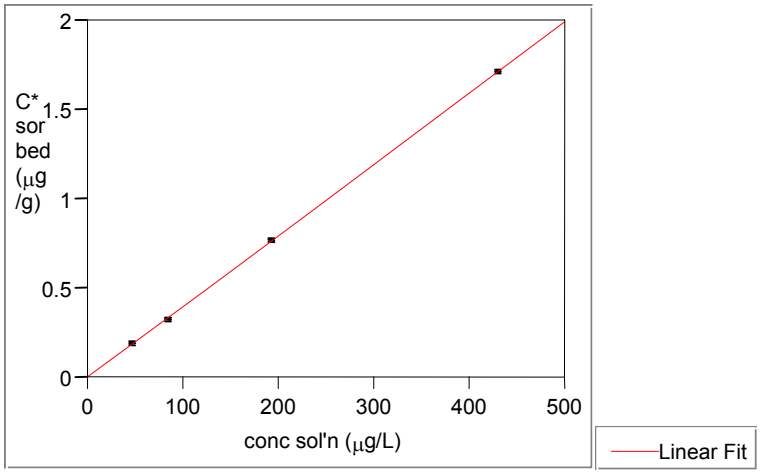
C.6 Ap soils with organic matter removed sorption

For the sorption experiments with the organic matter removed, the Ap soils show a linear fit with Ap-MC soils having K_d(=slope)=0.004, R^2=1.00, P-value=0.0001 and Ap-Control soils having K_d=0.004, R^2=1.00, P-value=0.0001.

Concentrations of Roxarsone added to system(C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Ap-MC With Organic Matter Removed

C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
96.5	50.1	46.4	0.19
175.6	92	83.6	0.33
421.2	228.2	193	0.77
838.2	407.7	430.5	1.72

Bivariate Fit of C* sorbed (μg/g) by conc sol'n (μg/L) for Ap-MC with organic matter removed



Linear Fit

C* sorbed (μg/g) = 0.0003789 + 0.0039927 conc sol'n (μg/L)

Summary of Fit

RSquare	0.999974
RSquare Adj	0.99996
Root Mean Square Error	0.004354
Mean of Response	0.7525
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.4312371	1.43124	75508.01
Error	2	0.0000379	0.00002	Prob > F
C. Total	3	1.4312750		<.0001

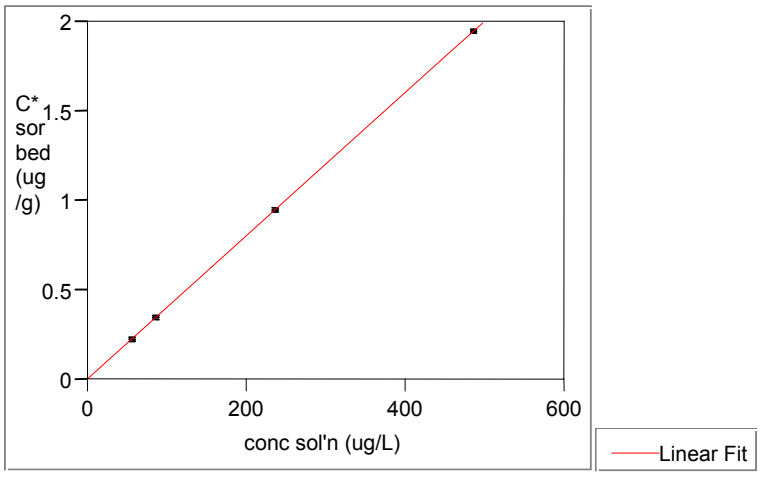
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0003789	0.003497	0.11	0.9236
conc sol'n (ug/L)	0.0039927	0.000015	274.79	<.0001

Concentrations of Roxarsone added to system (C added), Roxarsone sorbed onto soil (C sorbed), Roxarsone left in solution (C sol'n), and the concentration of Roxarsone by weight of soil (C* sorbed) for Ap-Control with Organic Matter Removed

C added (μg/L)	C sorbed (μg/L)	C sol'n (μg/L)	C* sorbed (μg/g)
114.6	58.1	56.5	0.23
186.8	100.3	86.5	0.35
446.4	210.2	236.2	0.95
852.5	366.6	486.2	1.95

Bivariate Fit of C* sorbed (μg/g) by conc sol'n (μg/L) for Ap-Control soil with organic matter removed



Linear Fit

C* sorbed (μg/g) = 0.0039495 + 0.004003 conc sol'n (μg/L)

Summary of Fit

RSquare	1
RSquare Adj	1
Root Mean Square Error	0.000444
Mean of Response	0.87
Observations (or Sum Wgts)	4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	1.8527996	1.85280	9386259
Error	2	0.0000004	0.00000	Prob > F
C. Total	3	1.8528000		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0039495	0.00036	10.99	0.0082
conc sol'n (ug/L)	0.004003	0.000001	3063.7	<.0001

C.6a SAS output for comparison of Ap soils before and after the treatment to remove organic matter

Analysis of the treated Ap-soils (9)

```
The REG Procedure
Model: MODEL1
Dependent Variable: C*sorbed C*sorbed

NOTE: No intercept in model. R-Square is redefined.

                          Analysis of Variance

                                    Sum of           Mean
Source                     DF       Squares         Square  F
Value    Pr > F

Model                       4       13.07803       3.26951
 127.50    <.0001
Error                      12        0.30773        0.02564
Uncorrected Total          16       13.38575


Root MSE              0.16014    R-Square     0.9770
Dependent Mean        0.72212    Adj R-Sq     0.9693
Coeff Var            22.17602
```

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Ap_MCafter		1	0.00396	0.00033269	11.91	<.0001
Ap_MCbefore		1	0.00101	0.00018360	5.50	0.0001
Ap_ctrlafter		1	0.00317	0.00029098	10.88	<.0001
Ap_ctrlbefore		1	0.00476	0.00032167	14.81	<.0001

Analysis of the treated Ap-soils (10)

The REG Procedure
Model: MODEL1

Test Ap_MC(after)_vs_Ap_MC(before) Results for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	1.54930	60.42	<.0001
Denominator	12	0.02564		

Analysis of the treated Ap-soils (11)

The REG Procedure
Model: MODEL1

Test Ap_control(after)_vs_Ap_control(before) Results for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	0.34852	13.59	0.0031
Denominator	12	0.02564		

The REG Procedure
Model: MODEL1

Test Ap_MC(after)_vs_Ap_control(after) Results for Dependent Variable C*sorbed

Source	DF	Mean Square	F Value	Pr > F
Numerator	1	0.08353	3.26	0.0962
Denominator	12	0.02564		

APPENDIX D: Removal of organic matter method

The method used is a modified procedure found in Jackson (1960). Homogenize the soil by air drying, then sieving (2 mm sieve). Place 100 g of soil in a 1-liter beaker. Saturate soil with nanopure water. Add 20 mL 30% H_2O_2 to soil. It will react with the organic matter in an effervescent manner. Heat in 85°C water bath. Do not allow it to boil. Stir occasionally with glass rod. When no more reaction is occurring, remove from water bath. Allow the sample to cool to room temperature. Add another 20 mL 30% H_2O_2 to soil. Observe reaction (does it fizz?). Put in water bath again. When the reaction stops, remove from bath and allow to cool. If vigorous reaction occurred when adding 30% H_2O_2 the second time, add an additional 20 mL. If reaction is mild, add only 10 mL 30% H_2O_2. Place in water bath until it no longer fizzes. Repeat this step until no reaction occurs when adding 30% H_2O_2. When no reaction has occurred, heat for 4 hours to get rid of the H_2O_2. Stir occasionally and do not allow the sample to dry out. Add nanopure water to prevent desiccation. Allow soil to air dry or dry in oven at low temperature (<70°C).

APPENDIX E: Sequential extraction experiments

E.1 Introduction to sequential extraction of arsenic

To study sorption of organic or inorganic arsenic, it is necessary to use sequential extraction procedures in order to determine where arsenic is being sequestered in the soil. Tessier et al. (1979) developed sequential chemical extractions for the partitioning of particulate trace metals (not including arsenic) in five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual. Keon et al. (2001) modified Tessier's procedure and developed a sequential extraction procedure to differentiate between six pools of solid phase arsenic: loosely and strongly adsorbed As; As coprecipitated with metal oxides or amorphous monosulfides; As coprecipitated with crystalline iron (oxyhydr) oxides; As oxides; As coprecipitated with pyrite; and As sulfides.

Turpeinen at al. (1999) used a modified Tessier procedure to determining the partitioning of arsenic to the following soil fractions: exchangeable, bound to easily reducible metal oxides, bound to organic matter, bound to crystalline Fe- and Al-oxides and residual fraction. Over seventy-five percent of the arsenic was bound to the residual and Al- and Fe-oxides fractions.

Chunguo and Zihui (1988) separated nine fractions of arsenic, consisting of soluble As(III) and total soluble arsenic, loosely bound arsenic, aluminum arsenate, iron arsenate, calcium arsenate, arsenic occluded on iron oxides, organic compounds of arsenic and residual arsenic using a sequential extraction procedure. In the water and sediment from the Xianjiang River in China, the majority of arsenic was in the form of aluminum arsenate, iron arsenate, and calcium arsenate.

Mucci et al. (2000) determined the partitioning of dissolved and particulate arsenic in the water column and sediments of the Saguenay Fjord in Canada. They applied a sequential extraction procedure to determine where the arsenic is bound to four fractions: exchangeable, oxyhydroxides (amorphous and crystalline), organic, and total arsenic. They concluded that the arsenic, in the

form of arsenate, is sorbed by the iron oxyhydroxides in the oxic environment near the sediment-water interface. Upon burial, the amorphous iron oxyhydroxides are reduced and dissolved, releasing arsenic into the porewaters. It is reabsorbed onto more resistant iron oxyhydroxides, which then undergo reductive dissolution in the presence of sulfides. Arsenite is then sorbed by the sulfides.

Breit et al. (2001) applied chemical extraction treatments to sediments from Bangladesh. Sequential extractions were done for the following extractions: exchangeable, manganese oxides, amorphous iron and aluminum oxides, crystalline iron and aluminum oxides, organic matter and sulfides, and residual arsenic. A single extraction procedure was also done to determine the total arsenic bound to reducible iron oxides. Extraction results indicate that ninety percent of the arsenic was tightly bound to the crystalline iron-aluminum oxides. Rutherford at al. (2002) used various extraction methods to determine binding mechanisms for arsenic. The mobile arsenic component and the arsenic bound to natural organic matter and metal oxyhydroxides were determined. Results suggest that the mobile arsenic is being released from the organic matter and that most of the arsenic in the soil is sequestered in the metal oxyhydroxides.

E.2 Extraction procedure for defining the partitioning of arsenic to soil fractions

For the sequential extraction experiment, we used the method by Turpeinen et al. (1999). This modified Tessier extraction procedure is used to delineate the arsenic sequentially as exchangeable, easily reducible metal-oxide bound, organic matter bound, Fe- and Al-oxide bound and residual (see table below). To extract the exchangeable arsenic fraction, dry soils at 100°C for 1½ hrs. Crush the soil in mortar and pestle. Measure 2.5-g soil into centrifuge tube. Add 25 mL 1 M $MgCl_2$ and shake well. Place on wrist shaker for 4 hours. Centrifuge for 30 minutes at 3500-rpm (25°C). Pipette supernatant into 35-mL bottle. After each extraction, the residual soil was washed with 8 mL of Nanopure water, centrifuged for 30 minutes, and the supernatant was discarded. The residual solid was then used for the next extraction.

To extract the easily reducible metal-oxides arsenic fraction, add 10 mL 0.1 M $(NH_2)OH{\cdot}HCl$ and 15 mL 0.01 M HNO_3 to the soil sample. Shake well then place on wrist shaker for 30 minutes. Centrifuge for 30 minutes at 3500 rpm. Pipette supernatant into 35-mL bottle.

To extract the organic matter-bound arsenic fraction, add 22.5 mL 0.02 M HNO_3 and 2.5 mL 30% H_2O_2 to the soil sample and shake well. Loosen the lids on the centrifuge tube and heat at 85°C for 2 hours. Add 3.5 mL NH_4Ac and 1.5 mL 25% HNO_3 and shake well. Heat at 85°C for 5 hours (with loosened lids) and then at 25°C for 30 minutes. Centrifuge for 30 minutes at 3500 rpm. Pipette supernatant into 35-mL bottle.

To extract the crystalline Fe- and Al-oxides bound arsenic fraction, add 12.5 mL 0.175 M $(NH_4)_2C_2O_4$ and 12.5 mL 0.1 M $C_2H_2O_4$ and shake well. Heat at 95°C for 30 minutes (with loosened lids). Centrifuge for 30 minutes at 3500 rpm. Pipette supernatant into 35-mL bottle.

To extract the residual arsenic fraction, add 10 mL 32.5% HNO3 and shake well. Transfer mixture to 100-mL autoclave bottles and crimp shut. Autoclave the sample at 120°C (1 atm) for 30 minutes. Transfer mixture back to centrifuge tubes. Centrifuge for 30 minutes at 3500 rpm. The supernatant was removed and diluted with Nanopure water to give a final HNO_3 concentration of 5%. All samples were analyzed for total arsenic by ICP-AES or GFAAS.

Two alternate methods of analyzing the amount of As partitioned onto organic matter and iron oxide soil fractions were employed (see table below). For the alternate organic matter extraction, a step from the sequential extraction procedure by Breit et al. (2001) was implemented. To 0.5 g of soil, 0.5 g $KClO_4$ and 10 mL of concentrated HCl were added into a glass tube. The mixture was shaken by hand, allowed to settle for 10 min, and then placed on the wrist shaker for 30 min. After 5 min (equivalent to a total time of 45 min of intermittent shaking), the sample was centrifuged (3500 x g) for 5 min. The supernatant was analyzed by GFAAS. For the alternate iron oxide extraction, an oxalic-dithionite extraction procedure (Rutherford et al. 2002) was used. In an acid-washed centrifuge tube, 0.5 g of soil, 10 mL of ammonium oxalate (0.11 M) and 10 mL of

oxalic acid (0.09 M) were added. The mixture was heated at 80ºC for 45 min. Next, 0.4 g of sodium dithionite was added, and the mixture was again heated at 80ºC for 45 min. The sample was allowed to cool, and was then placed on the wrist shaker overnight. The mixture was centrifuged, and the supernatant was analyzed by GFAAS.

The Modified Tessier Extraction Procedure; modified by Brown (2002)

Target Phase	**Extraction procedure**
Exchangeable	1 M $MgCl_2$ (25 mL, pH 7); 4 h (25°C); shaker
Easily reducible metal-oxides	0.1 M $NH_2OH{\cdot}HCl$ (10 mL) + 0.01 M HNO_3 (15 mL); 30 min (25°C); shaker
Bound to organic matter	0.02 M HNO_3 (22.5 mL) + 30% H_2O_2 (2.5 mL); 2 h (85°C), 3.0 M NH_4Ac (3.5 mL) + 25% HNO_3 (1.5 mL; pH 2.5); 5 h (85°C) + 30 min (25°C)
Method II - Bound to organic matter	To 0.5 g of soil, add 0.5 g $KClO_4$ and 10 mL of concentrated HCl; 45 min (25°C) intermittent shaking
Bound to Fe- and Al-oxides	0.175 M $(NH_4)_2C_2O_4$ (12.5 mL) + 0.1 M $C_2H_2O_4$ (12.5 mL); pH 3.25; 30 min (95°C)
Method II – Bound to Fe-oxides	To 0.5 g of soil, add 0.11 M $(NH_4)_2C_2O_4$ (10 mL) + 0.09 M $C_2H_2O_4$ (10 mL); 45 min (80°C), add 0.4 g $Na_2S_2O_4$; 45 min (80°C), cool, rotate overnight, centrifuge
Residual	32.5% HNO_3 (10 mL); autoclave 30 min (120°C, 1 atm)

E.3 Data for the extraction experiments

Data for sequential extraction experiments

As total conc. (mg/kg)	**Ap-control**	**Ap-MC**	**Bt1-control**	**Bt1-MC**	**Bt2-control**
Sequential Extraction					
Exchangeable	bdl	0.067	bdl	bdl	bdl
Easily reducible metal-oxides	bdl	0.086	bdl	bdl	bdl
Bound to organic matter	0.0156	0.511	bdl	bdl	bdl
Method II – OM bound	5.642	7.242	NA	NA	NA
Bound to Fe- and Al-oxides	0.0224	0.407	bdl	bdl	bdl

Method II – Fe-oxide bound	0.256	0.212	0.50	0.052	0.258
Residual	0.110	3.625	bdl	bdl	bdl
Sequential Extraction TOTAL	1.340	4.696	bdl	bdl	bdl
Seq. Ext. with Method II TOTAL	*6.008*	*12.303*	*0.50*	*0.052*	*0.258*
Total Digestion Extraction	**6.58**	**7.03**	**5.10**	**10.58**	**14.82**

Notes: NA = not available, bdl = below detection limit of 0.0056 mg/kg

Appendix F: BET particle size analysis

A surface area analysis of the Ap soils was performed using N_2-BET (Quantachrome NOVA 1200 BET Analyzer).

The surface area (in square meters per gram) for the Ap soils

	Specific Surface Area (m^2/g)
Ap-MC	5.1733
Ap-Control	6.3328

VITA

Brenda Lee Brown

Current Address
Department of Geological Sciences
VPI&SU
Blacksburg, VA 24061
(540) 231-2404 (office)
(540) 989-1423 (home)
brbrown@vt.edu

Permanent Address
3221 Brandywine Ave
Roanoke, VA 24018
dlh3436@juno.com

Education

Virginia Polytechnical Institute and State University
Blacksburg, VA 24061
M.S. in Geological Sciences (Emphasis Contaminant Hydrogeology)
- Graduated May 2003

Thesis Topic: The Sorption of Roxarsone, an Organoarsenic Animal Feed Additive
Thesis Advisor: Dr. Madeline Schreiber
Cumulative GPA 3.79

Virginia Polytechnical Institute and State University
Blacksburg, VA 24061
M.A.Ed. in Education (Secondary, Earth Science) – Graduated August 1998
Cumulative GPA 3.80

The College of William and Mary
Williamsburg, VA 23185
B.S. in Geological Sciences
Cumulative GPA 3.15

Research/Work Experience

Master's Research, Department of Geological Sciences, Virginia Polytechnical Institute and State University (January 2001 through May 2003)

- Conducted ASTM batch experiments to determine the amount of roxarsone sorbed onto Ap and Bt horizon soils
- Used a modified Tessier extraction procedure to define the partitioning of roxarsone to soil fractions
- Compared microwave, ultraviolet and dry ashing digestion techniques to determine which was most suited for determination of arsenic content
- Preparing manuscript for publication

Research Technician for Madeline Schreiber, Associate Professor of Hydrogeology, in the Department of Geological Sciences, Virginia Polytechnical Institute and State University (May 2002 through August 2002)

- Helped develop experiments for the new lab added to the Groundwater course
- Assisted in the design of equipment used in laboratory experiments

Instructional Experience

Teaching Assistant at Virginia Polytechnical Institute and State University (January 2001 through May 2003)

- Laboratory instructor for undergraduate introductory geology course: Resources Geology
- Teaching assistant for Groundwater Hydrology
- Planned weekly lectures introducing concepts, theories, and procedures pertinent to laboratory exercises
- Created and graded weekly quizzes, mid-term and final exams

High School Teacher (September 1998 through January 2001)

- Taught 9th grade Earth Science and 10th grade Biology at Nandua High School in Onley, VA (September 1998 through June 1999)
- Taught 9th grade Earth Science at Patrick Henry High School in Roanoke, VA (September 1999 through June 2000)
- Taught 9th grade Earth Science and 11th Grade Environmental Science at William Byrd High School in Vinton, VA (September 2000 to January 2001)

Community College Teacher (September 1999 through May 2000)

- Taught Historical Geology (evening classes), preparing lectures and laboratory exercises

Publications

Brown, B. L. and M. E. Schreiber. 2002. Examining the biotransformation and sorption of roxarsone, an organoarsenic animal feed additive. Proceedings of the 2002 Virginia Water Resources Research Symposium, Roanoke, Virginia, Nov 5-6, 2002.

Career Related Skills

- Skilled in the development and implementation of laboratory experiments
- Proficient teaching and presentation verbal communication skills
- Strong data collection and manipulation skills

- Proficient in the use of spreadsheet (Excel), word processing (Word, Word Perfect), statistics program (JMP)
- Skilled in the use of computer groundwater modeling programs (GMS 3.0 and 4.0 using MODFLOW, MODPATH, SEAM3D)
- Experience with graphite furnace atomic adsorption spectrometer (GFAAS), Milestone ETHOS PLUS Microwave (for MW assisted acid digestion of soils), and Quantachrome NOVA 1200 BET Analyzer