Reactive Metal Partitioning in INL Vadose Sediment

Reactive Metal Partitioning in Idaho National Laboratory (INL) Vadose Sediment

By

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Abstract

The legacy of disposal practices for radioactive and other heavy metals has resulted in highly contaminated soils at the INL facility in Idaho Falls. Microbial effects on the mobility of a suite of metals (U, As, Cr, Co and Zn) within INL vadose sediments were assessed over a range of solution metal concentrations and biological activity through batch experiments. Sediment associated metals in the bulk sample as well as individual soil compartments increased as a function of total solution metal concentration. System specific trends in partitioning coefficients emerged, reflecting complex interrelations among biological activity, solution metal concentration and the specific metals involved.

Results of this study clearly show that cyclic linkages between metal concentration and biological activity play a role in metal sediment biogeochemical behaviour. Differential impacts of biological activity on metal solid retention as a function of solution metal concentration were observed. This result may reflect feedback of metals on the microbial population such that the extent and/or nature of microbial activity is concentration dependent. Typically biological activity has a stronger effect with increased concentration, changing from a beneficial/neutral impact to an increasingly negative effect across the concentration range. The degree of this effect, and whether positive or negative on soil metal retention was however, element specific and dependent on the degree of biological activity.

This is one of the first studies to evaluate the relative affinities of a suite of metals for the solid vs. solution phases over varying metal cocktail concentrations and levels of biological activity. My results indicate that differing affinities occur across this suite of metals, and that their relative affinities are non-linearly dependent on both the levels of metal and biological activity present. These results indicate that successful prediction of metal behaviour in complex natural systems, based on mono-metal laboratory experiments is likely limited.

Acknowledgements

I would like to take this opportunity to thank those people that have made important contributions to my work as well as helping me grow professionally. Foremost I would like to thank my supervisor, Dr. Lesley Warren, who was patient and understanding and only ever voiced words of encouragement and praise. I would also like to thank Dr. Craig Cooper from INL, who has been a wealth of knowledge with expedient responses by email. Each member of my lab has offered advice and come to my aid during crises, for which I am very grateful.

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Table of Contents

List of Figures

List of Tables

1.0 Introduction

Acceptable radioactive and hazardous waste disposal once included disposal of waste through injection wells, as well as placing liquid waste in unlined disposal ponds and disposal of solid waste into unlined landfills (Cooper et al., 2003; Holdren et al., 2002; Makhijani and Boyd, 2001). While these practices were once routine and completely acceptable by law, current environmental law prohibits such practices. Furthermore, while these practices may have been very inexpensive at the time, they have come with great external costs, resulting in the degradation of human and ecosystem health as well as requiring large amounts of funding and prolonged efforts to clean disposal areas (CWI, 2005; Zachara et al., 2004; Marsh et al., 2000).

The Idaho National Laboratory (INL; formerly the Idaho National Engineering and Environmental Laboratory - INEEL), located in Idaho Falls, is a Department of Energy (DOE) facility and has been responsible for the disposal of radioactive and hazardous waste resulting from the production of nuclear weapons and research materials (CWI, 2005; Makhijani and Boyd, 2001). Historical disposal practices, including those stated above, have resulted in widespread inorganic and radionuclide contaminants in the soils surrounding this facility (Nimmo et al., 2004; Cooper et al., 2003; Holdren et al., 2002; Makhijani and Boyd, 2001), thus an INEEL affiliate (CH2M Washington Group Idaho (CWI)) has been designated the

Figure 1.1: Site map of INEEL Facilities within Idaho. Source: Mattson et al., 2004.

responsibility of cleaning the contaminated soils on which the facility is situated.

Of specific concern is the Subsurface Disposal Area (SDA), which is located within the Radioactive Waste Management Complex (RWMC; Nimmo et al., 2004; Cooper et al., 2003, refer to Figure 1.1). The SDA contains pits and trenches containing drums of radionuclide-waste from fission related work that are corroding and losing integrity over time (Cooper et al., 2003; Makhijani and Boyd, 2001). Flooding of the SDA has occurred in the past resulting in surface water drainage and inorganic contamination extending as far as 2500m outside the SDA (Nimmo et al., 2004; Markham et al., 1978).

Of utmost concern in Idaho Falls is the quality of the Snake River Plain Aquifer, one of the most productive aquifers in the United States, supplying approximately 2500 billion litres of water for drinking and nearly 2.5 billion cubic meters of water for industry and agriculture annually (Francis, 2005).

The importance of groundwater, in general, has led to many studies on the mobility of contaminants within the saturated zone of soil (Gu et al., 2003; Gego

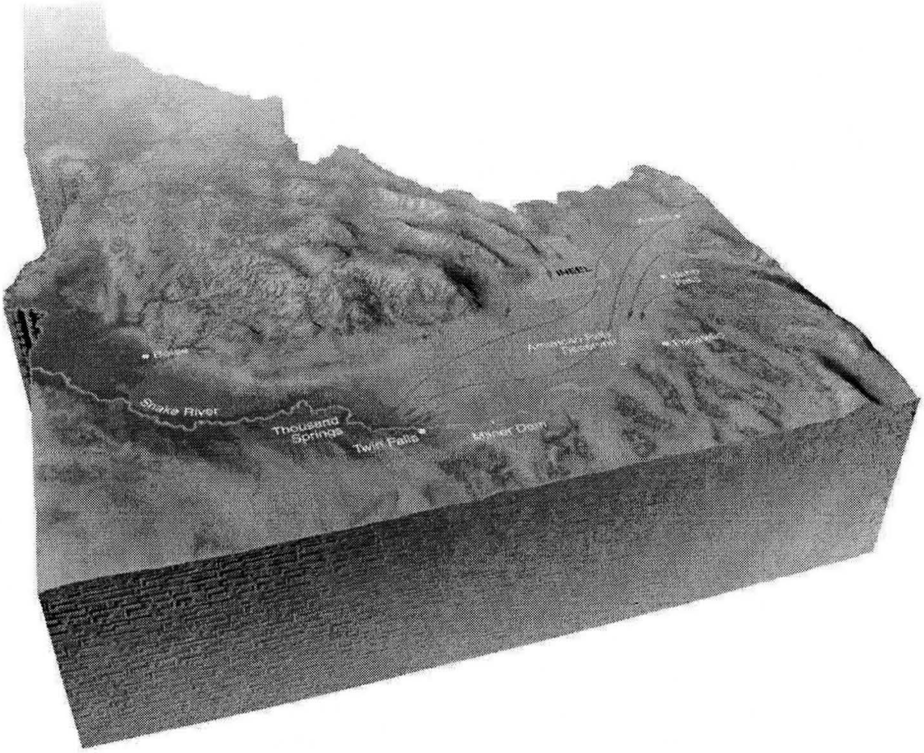

Figure 1.2: Map of Idaho delineating the Snake River aquifer in connection with the INL facility (Identified on this figure by its former name – INEEL). Source: Cooper, 2004.

et al., 2002; Ryu et al., 2002; Loyaux-Lawniczak et al., 2001; Roback et al., 2001; Su and Puls, 2001; Nickson et al., 2000; Morin et al., 1988). However, the vadose zone, which was once believed to be a fairly inactive layer within the soil and thus not of great concern, has been coming into light as a continual source of contamination, where inorganic contaminants do indeed have the potential to move through this layer to reach the water table after which mobility is greatly enhanced (Mattson et al., 2004; Nimmo et al., 2004; Smith, 2004; Zektser et al., 2004; Cooper et al., 2003; Selim and Kingery, 2003, Makhijani and Boyd, 2001). Given that the SDA directly overlies the Snake River Aquifer (Figure 1.2), separated only by a 200m vadose zone, only limited contaminated migration would have to occur through this vadose zone to reach this key aquifer (Nimmo et al., 2004).

Specific contaminants of interest at the INL site are numerous, but given the current contaminants of concern at the INL facility, U, As, Cr, Co and Zn were considered in this study. While these inorganic contaminants are specific to INL, they also have great relevance at other locations within the United States, which has led to regulation under the United States Safe Drinking Water Act (U, As and Cr), regulation under the Resource Conservation and Recovery Act (As and Cr) and under the Secondary Drinking Water Regulation (Zn). And while this specific circumstance may apply predominantly to the United States, the problem of contamination spans worldwide, again, largely a result of previous practices for the production or disposal of hazardous wastes.

The issues of contaminant concentrations and mobility are further complicated by the contributions of microorganisms. U, As and Cr have more than one valence state, and their overall properties, including mobility, are dependent on the oxidation state as well as the speciation of the metal in question, which can be influenced by microbial metabolism (Arias and Tebo, 2003; Elias et al., 2003; Haack and Warren, 2003; Fredrickson et al., 2002; Warren and Haack, 2001; Cooper et al., 2000; Lovley and Phillips, 1992). Mobility of Co and Zn may also be influenced by microbial activity as a result of microbes attacking the potential binding substrates for these metals or changing geochemical conditions through metabolic activity, such that metal sorption is affected (Warren and Haack, 2001; Cooper et al., 2000).

While the immensity of this problem at INL and at other US DOE sites has attracted many researchers and led to numerous studies evaluating metal behaviour in vadose systems (Mattson et al., 2004; Nimmo et al., 2004; Smith, 2004; Zektser et al., 2004; Cooper et al., 2003; Makhijani and Boyd, 2001; Cooper et al., 2000; Doughty, 2000), one of the deficiencies of current published research is the lack of complexity designed into experiments attempting to assess likely contaminant behaviour. It is important to focus on fundamental concepts, a basic understanding of potential processes is indeed necessary, and the general approach has been to evaluate behaviour in a simple manner to constrain mechanisms. However, natural systems are highly complex and in order to truly understand these systems in a manner that will help to produce effective remediation strategies it is important to include greater complexity into research studies. For example, natural systems are rarely only contaminated with a single metal and the interaction of multiple metals is likely not additive; metals directly compete for binding sites and may contribute to differing geochemical gradients which in turn impact the behaviour of alternate metals (Voegelin et al., 2002; Warren and Haack, 2001; Sauvé et al., 2000). For this reason it is essential that studies be done on larger 'suites' or cocktails of metals, to examine the interaction of these metals, making the results more relevant to natural systems.

Furthermore, behaviour of metals in natural systems is not only the result of abiotic controls, biological activity can also impact metals through microbial influence on both sorption processes as well as redox reactions; both of which can be directly or indirectly affected by metabolic activity. A number of studies on vadose zone metal biogeochemistry have clearly demonstrated this link (Nimmo et al., 2004; Mattson et al., 2004; Cooper et al., 2003, 2000; Makhijani and Boyd, 2001; Smith, 2004). Thus to accurately assess the behaviour of metals in natural systems, this component must also be scrutinized.

This study incorporates multiple levels of complexity by evaluating metal sequestration in INL vadose soils under varying levels of (1) metal concentration and (2) biological activity for a suite of metals (U, As, Cr, Co, Zn) added as metal cocktails in laboratory batch experiments. The objective is to determine the linkages among solution metal concentration, metal identity and levels of biological activity in controlling vadose soil metal sequestration.

2.0 Background Information – Metal Behaviour

The behaviour of metals in a porous medium is largely controlled by solid-solution partitioning which is governed by two major types of processes; redox reactions and solid surface sorption reactions, both of which can be impacted by biological activity (Figure 2.1; Turpeinen et al., 2004; Krumholz et al., 2003; Selim and Kingery, 2003; Su and Puls, 2001; Tokunaga et al., 2001; Warren and Haack, 2001; Sauvé et al., 2000; Turner, 2000; Tessier et al., 1996; Rae and Parker, 1993; Tsezos, 1984). The relative extent and importance of these two types of processes are dependent on the types and concentrations of the reactive solids, as well as the types and concentrations of the specific element(s) involved (Warren and Haack, 2001; Sauvé et al., 2000).

2.1 Redox Implications

Redox processes are essential to all life processes and while defined as the transfer of electrons, these processes ultimately describe the transfer of energy. The low flux of elements does little to describe the importance of redox processes, considering the geochemical cycles of elements are largely driven by redox reactions (Krivtsov and Sigee, 2005; Orphan et al., 2003; Arias and Tebo, 2003; Cooper et al., 2000; Fredrickson et al., 2000; Bhumbla and Keefer, 1994). The types of elements involved in such reactions are those that are most abundant in living matter, which include carbon, nitrogen and sulfur as well as some metals, however abundance within the geosphere does not dictate elemental importance in redox reactions (Morel and Hering, 1993) since this is largely controlled by biological processes (Islam et al., 2004; Arias and Tebo, 2003; Cooper et al., 2003; Elias et al., 2003; Fredrickson et al., 2003; Ahmann et al., 1994).

While thermodynamics would favour many reactions, without microbial mediation redox reactions are often extremely sluggish, typically occurring over slow (i.e. geologic) timescales abiotically (Morel and Hering, 1993). Therefore, reactions that are microbially catalyzed are often much more relevant for environmental geochemical behaviour (Warren and Haack, 2001; Morel and Hering, 1993). The thermodynamically unfavourable energy capturing process of photosynthesis becomes the fundamental process driving virtually all redox cycles (Equation 1; Morel and Hering, 1993):

$$CO_2(g) + H_2O \xleftrightarrow{\text{sunlight}} CH_2O + O_2(g) \qquad \Delta G = +478\ \text{kJ mol}^{-1} \quad (1)$$

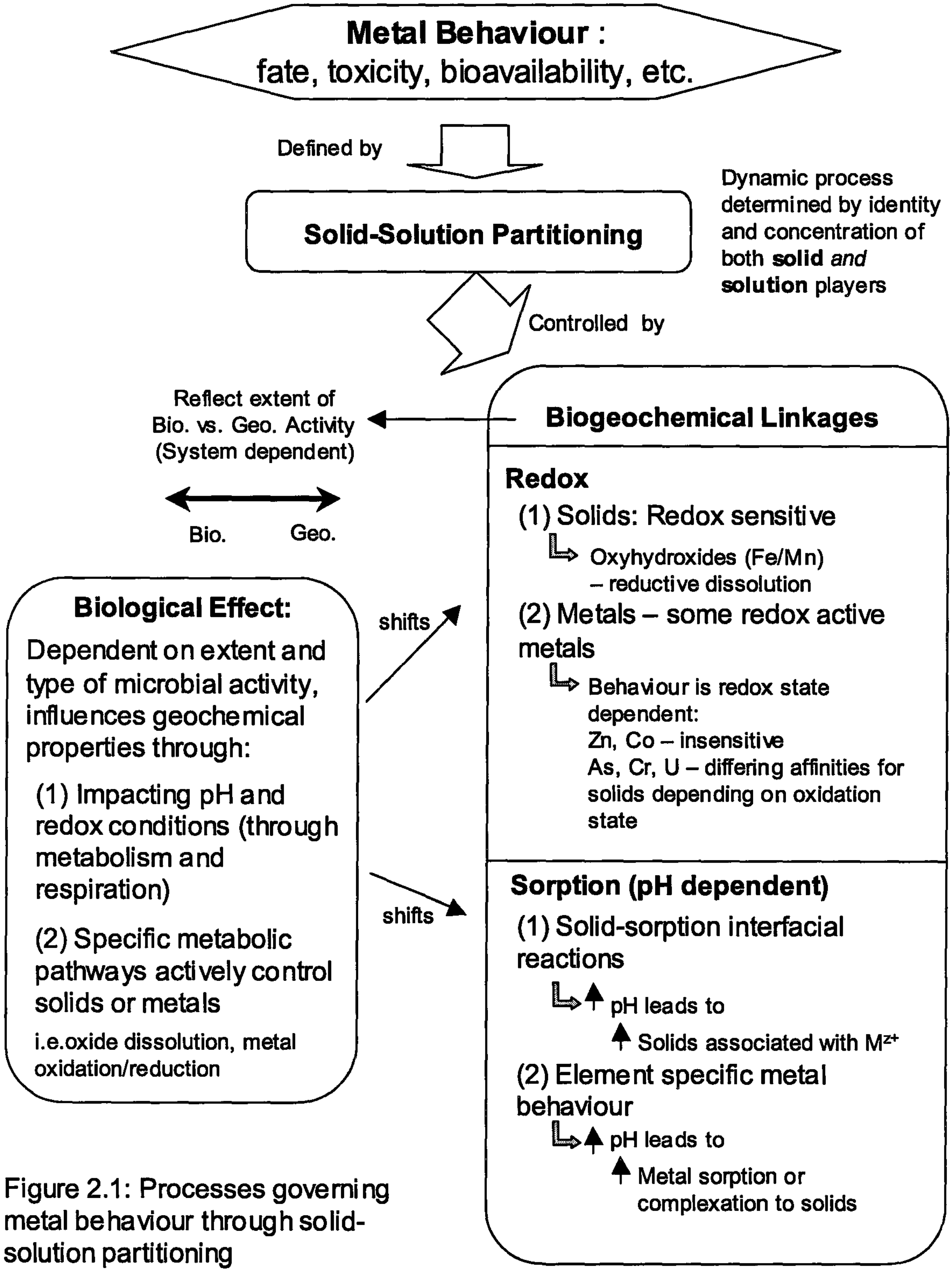

Figure 2.1: Processes governing metal behaviour through solid-solution partitioning

where oxygen is the ultimate oxidant and organic matter (represented here by "CH_2O" as a generic sugar molecule) is the ultimate reductant. The cycling of carbon, and energy between CO_2 and CH_2O is often coupled by microorganisms to many other reductants (for CO_2 reduction) and oxidants for decomposition (for "CH_2O" oxidation). The microbial control on redox reactions and their ability to couple carbon cycling to many elements important for life (e.g. N, Mn, Fe, S) as well as to those of geochemical relevance (e.g. H_2, redox-active metals, redox reactive minerals) means that the control on geochemical behaviour associated with redox driven processes will be determined by the location, nature and extent of microorganism activity, rather than classic geochemical constraints such as O_2 status or pH (Warren and Haack, 2001; Nealson and Stahl, 1997; Morel and Hering, 1993).

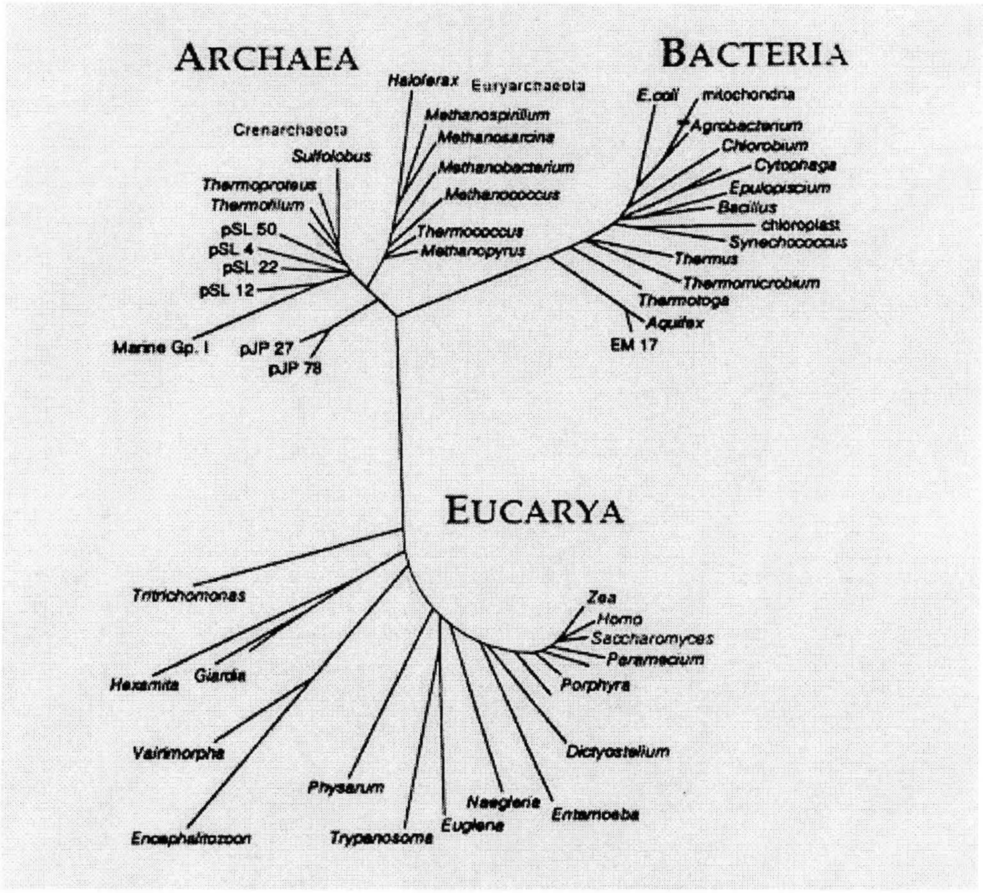

Figure 2.2: Phylogenetic tree based on evolutionary change according to gene sequence analysis. Source: Pace, 1997.

2.1.1 Microbial Mediation on Redox

Bacteria, single cellular organisms lacking nucleus and organelle membranes, have evolved in metabolically diverse ways to take advantage of the energy available by redox processes. While eukaryotes, which are characterized by the presence of nucleus and organelle membranes, have developed a wide variety of shapes and sizes, where physical abilities determine behaviour (such as predator-prey relationships), bacteria have evolved quite differently. Bacteria have remained morphologically small and simple, expressing their diversity in terms of physiology and metabolism (Nealson and Stahl, 1997). Bacteria are typically 0.5 to a few micrometers in diameter with surface area to volume ratios 100-1000 times higher than eukaryotic cells (Nealson and Stahl, 1997). Due to their small size, bacteria rely on diffusion for food uptake and elimination of waste products, thus the large surface area to volume ratio is essential (Holt and Leadbetter, 1992; Beveridge, 1989, 1984). Their simple structure and rigid cell wall, eliminate the need for intracellular organelles, tissues and organs, leaving them with very few complex parts that may fail, thus bacteria have been able to survive in many extreme environments where eukaryotes could not (Nealson and Stahl, 1997).

Relatively recent studies on microbial diversity and behaviour have revealed complex ecosystems and have demonstrated the limited wealth of knowledge we have on microbes (Kirk et al., 2004; Pace, 1999, 1997). By 1992, only approximately 5000 non-eukaryotic organisms had been formally described (Pace, 1999; Bull et al., 1992), and this value can be largely attributed to the development of gene sequencing (Schwartz and Dayhoff, 1978). Through this sequencing, a molecular sequence-based phylogenetic tree was created that could relate all organisms and the order of evolution (Figure 2.2), although no time scale can be correlated with the sequence changes since evolution does not occur over a given time (Woese, 1987). This tree changed the view of how organisms evolved as well as pointing at the misconception of diversity based on morphology. The further a branch is away from another, the more dissimilar they are genetically. Humans show 98% similarity with corn (Zea – next 'twig' over from Homo). The rest of the phylogenetic tree showing enormous genetic diversity is comprised of single celled organisms, mostly Bacteria (note that Bacteria denotes both the Archeal and Bacterial domains of the tree). This tree transformed our understanding of both the occurrence of unicellular organisms in the environment as well as the substantive diversity in their metabolism. Somewhat paradoxically, it does, however, make complete geochemical sense. Bacteria have evolved over billions of years in intimate contact with the geosphere. Their needs for carbon and energy are balanced by a seemingly endless facility to adapt via a variety of possible mechanisms by which to gain these necessities for life (Nealson and Stahl, 1997). The geochemical environments where water, energetic gradients (e.g. disequilibrium), amorphous minerals phases and redox active elements occur, are those that would provide bacteria with a niche in which to grow and survive via a myriad of potential pathways; these are common conditions in metal contaminated systems (Macur et al., 2004; Turpeinen et al., 2004; Haack and Warren, 2003; Krumholz et al., 2003; Loyaux-Lawniczak et al., 2001; Warren and Haack, 2001).

Improvements in environmental microbial research have determined that organisms do not simply fade out as resources become sparse as was once believed (Nealson and Stahl, 1997). While bacteria will compete for these limited resources they may also be functioning as a productive community supplying nutrients to the system by oxidizing a substrate, rendering it available to other organisms which in turn may provide byproducts that are desirable to the original bacteria, supporting a symbiotic community. This form of cyclical feedback as well as simultaneous redox pair processes may occur (Arias and Tebo, 2003; Elias et al., 2003; Haack and Warren, 2003; Fredrickson et al., 2002; Warren and Haack, 2001; Cooper et al., 2000; Lovley and Phillips, 1992).

2.1.2 Redox Implications for Metal Behaviour

Redox reactions, which are largely driven by microbes, are intimately involved in determining metal behaviour for a large number of elements either through direct redox reactions or through establishment of redox conditions which impact their behaviour. While some reactions proceed without the aid of bacterial metabolism, many reactions (while favourable) proceed very slowly thus many redox reactions observed within dynamic natural systems are chiefly biologically controlled (Islam et al., 2004; Arias and Tebo, 2003; Elias et al., 2003; Haack and Warren, 2003; Fredrickson et al., 2002, 2000; Cooper et al., 2000; Madsen, 1998).

Some metals are redox active, and can occur in natural environments in more than one oxidation state (i.e. U, As, Cr), altering their speciation and hence behaviour. Others, having only one stable oxidation state (e.g. Co and Zn), can still be impacted indirectly by redox processes due to affected properties of redox sensitive metal sorbing substrates or general geochemical conditions in the local environment.

2.2 Sorption Implications

Sorption reactions, where solution metal ions or molecules attach to solid surfaces through complexation reactions, are profoundly influenced by system pH (Selim and Kingery, 2003; Warren and Haack, 2001; Sauvé et al., 2000; Morel and Hering, 1993; Otabbong, 1990). The charge on the solution molecule as well as the charge of the solid surface binding site, which dictates binding site potential, are dependent on pH such that increases in pH result in increases in solids associated with metal uptake as well as increasing metal sorption reactions. Further, the concentration and species of metals in solution are also important since metal sorption can be competitive depending on sorbent types, concentrations and relative affinities of different elements for specific solids (Warren and Haack, 2001; Sauvé et al., 2000; Warren and Zimmerman, 1994a; Tessier et al., 1989).

2.2.1 Microbial Influence on Solid Metal Sequestration

Microbes can have significant impacts on solid metal sequestration processes through many different mechanisms: (1) acting directly as a sorbent themselves, (2) directly impacting oxidation states of metals and thus behaviour, and (3) indirectly impacting pH and redox gradients in their local microgeochemical environment.

The surfaces of microbes possess a heterogeneous variety of functional groups and under most environments, this surface carries a negative charge due to negatively charged functional groups (phosphoryl and carboxyl groups), thus bacterial cell walls attract cations such as metals (Warren and Haack, 2001; Schiewer and Volesky, 2000; Fortin et al., 1997; Ferris and Beveridge, 1985). Given the potential populations of microbes, sorption to microbial cells and extra cellular polysaccharides (EPS) can be a large sink for metals in localized pockets (Schiewer and Volesky, 2000; Pace, 1997; Urrutia, 1997). Microbes can also directly alter the redox state of a metal, by coupling the reduction of metals for use in their own metabolism (Islam et al., 2004; Fredrickson et al, 2002; Laverman et al., 1995; Ahmann et al., 1994; Bhumbla and Keefer, 1994; Lovley and Phillips, 1992; Lovley et al., 1991). This results in a change in the overall charge of the metal as well as the compounds in which this metal will be incorporated. Lastly, bacteria may indirectly impact pH and redox conditions through metabolism and respiration, which may not directly involve any use of a metal, but does alter geochemical conditions which can impact the ability of substrates to attract or release metals (Warren and Haack, 2001; Cooper et al., 2000). Research at the INL facility has identified microbial populations within the vadose sediment to be composed primarily of heterotrophs (oxidation of organic carbon is the dominant metabolic pathway), therefore it would be expected that microbial activity would result in greater organic matter decomposition which would be associated with reduction in pH and increased CO_2 production (Cooper, 2004).

2.3 Assessing Solid Solution Metal Behaviour: Kds and Sequential Extraction Procedures

Metal behaviour is largely determined by the relative distribution between solid and solution phases, typically higher concentrations of metals in solution result in greater metal mobility and toxicity (Khan and Puls, 2003; Maiti and Banerjee, 2002; Ryu et al., 2002; Su and Puls, 2001; Mok and Wai, 1994). This behaviour is captured empirically through partitioning coefficients (Kds), where Kd is the ratio of solid metal concentration to solution metal concentration (L/kg), therefore higher Kds indicate greater partitioning of metals to the substrate, and hence reduced metal mobility. Kds have proven to be a useful parameter and are widely used in the scientific community (Curtis et al., 2004; Cooper et al., 2003, Echevarria et al., 2001; Sauvé et al., 2000; Buchter et al., 1989).

Further, evaluation of metal association with specific sediment components within a mixed sedimentary pool gives greater insight into overall behaviour, as different solids are differentially affected by changes in system geochemical behaviour. Metals show differential affinities for differing solid phases and solids show differing reactivities for different elements. In general, sequential metal extraction procedures (SEPs) characterize the relative abundance of metals associated with key sediment fractions known to be important in metal sequestration (i.e. they capture sedimentary metal dynamics which is important for assessing the potential reversibility of metal sequestration). Since its inception in 1979 (Tessier et al.), SEPs have gained increasing credibility in the scientific field (Haack and Warren, 2003; Kneebone et al., 2002; Bostick et al., 2001; Hall et al., 1996; Howard and Shu, 1996; Real et al., 1994; Mahan et al., 1987) and have proven to be very useful in our understanding of important sorbent phases and controls on metal uptake (Haack and Warren, 2003; Kneebone et al., 2002; Bostick et al., 2001; Real et al., 1994). SEPs have been successfully applied to a variety of environments analyzing any number of metals (i.e. wetlands – Bostick et al., 2001, estuaries – Turner, 2000, mine tailings – Haack and Warren, 2003). The use of any SEP requires caution when comparing results since reagents used for various extraction steps vary among researchers (Haack and Warren, 2003; Hall et al., 1996; Howard and Shu, 1996; Real et al., 1994; Nirel and Morel, 1990; Mahan et al., 1987). However, the information that can be obtained from an SEP can dramatically improve our understanding of likely metal behaviour within any given system (Haack and Warren, 2003; Real et al., 1994).

While metal behaviour within the dynamic vadose system is still governed by redox and sorption processes, vadose zone complexity (note that the physical characteristics of vadose zone settings are discussed subsequently in Section 2.4.1, page 17) facilitates an overview of these processes in relation to these specific sediment compartments. Many of the substrates identified as key metal sorbents in most soil and sediment settings, are susceptible to changes in geochemical properties associated with changing redox conditions. Redox gradients are critical for determining potential metal retention or release to the various substrates (Figure 2.3) and as pH is the dominant factor controlling sorption to the various compartments, changes in pH will be reflected in changing metal sorption behaviour (Figure 2.4).

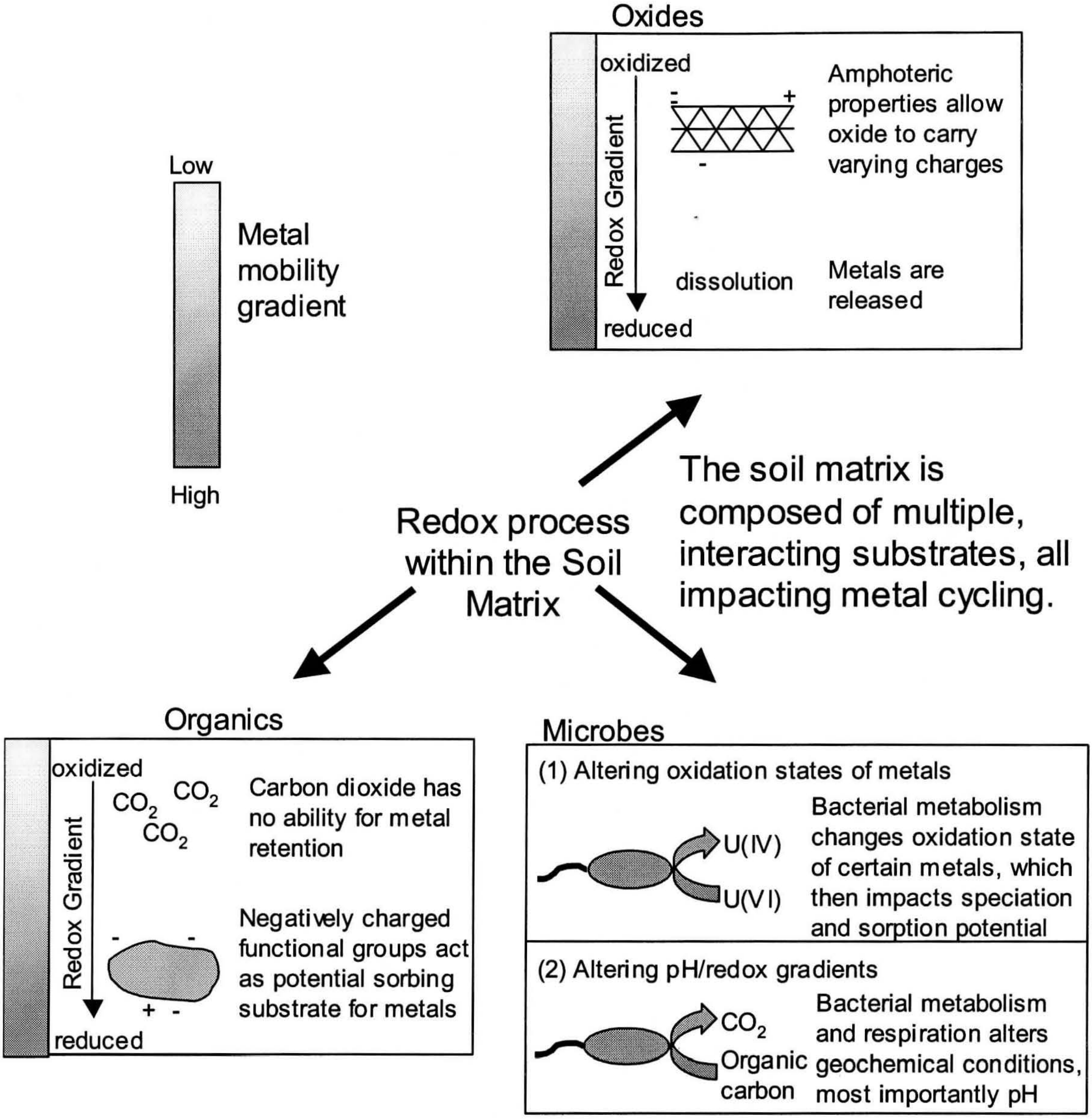

Figure 2.3: Impact of redox gradients on various soil compartments showing highly reducing conditions to be detrimental to oxide metal retention capacity, and highly oxidizing condition to be detrimental to solid organic metal retention.

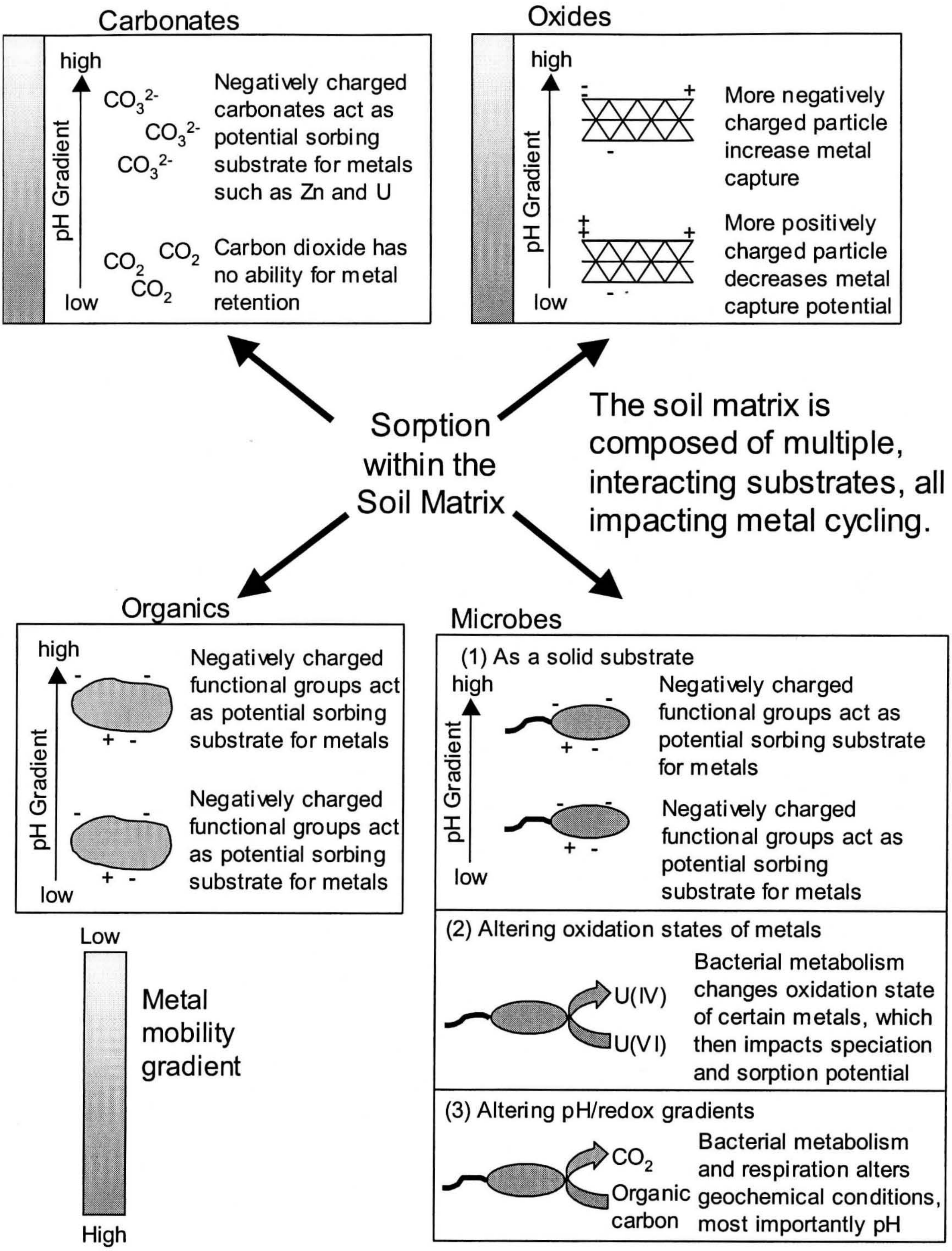

Figure 2.4: Sorption within the soil compartments shows pH as the dominant control for carbonates and oxyhydroxides in dictating metal mobility. Note: Surface charge of organic and microbial 'substrates' will remain negative over a circumneutral pH range.

2.3.1 Leachable/Exchangeable

Metals held by the leachable/exchangeable soil fraction are those held through electrostatic attraction on exchange sites both on the surface and interface of negatively charged complexes of soils (Hall et al., 1996). The force of attraction between the metal cations and the negatively charged soil particles will increase as the charges on the molecules become larger and the distance between the molecules becomes smaller (Kotz and Treichel, 1996). However, these metals are only weakly sorbed and any change in ionic strength is likely to change sorption processes resulting in uptake or release of metals (Mahan et al., 1987).

2.3.2 Carbonates

Solid carbonates are a key metal sequester in alkaline, carbonate rich systems (Krumholtz et al., 2003; Song et al., 1999; Tessier et al., 1979). $CaCO_3(s)$ dynamics are influenced by pH (Tessier et al., 1979) – increasing pH can drive $CaCO_3$ precipitation (Krumholtz et al., 2003; Reeder, 1996; Xu et al., 1996) and decreasing pH can induce carbonate dissolution as well as decreasing relative sorptive ability (Song et al., 1999). While changes in pH will affect all metals bound to the carbonates, certain metals are more likely to form carbonate complexes (i.e. uranium and zinc; Duff et al., 2002; Bostick et al., 2001; Gabriel et al., 1998) thus mobility of these metals becomes strongly pH dependent.

2.3.3 Oxides: Amorphous and Crystalline

Mineral oxides, such as Fe and Mn oxides, are ubiquitous and widespread in nature and often exist as colloids or surface coatings on mineral substrates such as clays and carbonates (Warren and Haack, 2001; Zachara et al., 2001; Robertson and Leckie, 1998; Cornell and Schwertmann, 1996). Therefore, despite their relatively small fraction of the total mass of soil, mineral oxide surfaces are extremely important sorbents for metals (Warren and Haack, 2001; Turner, 2000; Chao and Theobald, 1976). Oxides exist both as amorphous and crystalline solids, and while many SEPs separate these two fractions due to their differential metal sorptive capacities, the fundamental characteristics of these minerals are similar so will be discussed together.

Oxide surfaces possess a single functional group (hydroxyl group) however; these sites have a range of binding affinities reflecting the physical variation in their molecular coordinational environment (e.g. edge sites are typically more reactive), and are amphoteric, having the ability to carry an overall positive, negative or neutral charge depending on the pH and redox conditions of the system. The pH_{ZPC} (zero point of charge) is defined as the pH where an amphoteric molecule (in this case an oxide molecule) has net zero charge, therefore, at pH levels above the pH_{ZPC} the oxide becomes negatively charged, while at pH levels below this the oxide becomes positively charged. This characteristic has implications for metal sorption since metals occurring as cationic species (e.g. Cd^{2+}) will sorb when oxide surfaces are negatively charged (i.e. as pH increases above the surface pH_{ZPC}) while metals occurring as oxyanions, (e.g. CrO_4^{2-}) will generally sorb at low pH values below the oxide pH_{ZPC} when the surface is net positively charged. pH_{ZPC}'s vary considerably for oxide materials depending on the type of metal oxide (i.e. Mn vs. Fe) and a number of other characteristics including age, crystallinity as well as wetting and drying regimes of the sediment (ranging from 2.5-9, Kennedy et al., 2004; Robertson and Leckie; 1998; Catts and Langmuir, 1986; Murray, 1974; Parks, 1965).

Further, metals may become incorporated into the mineral by surface reactions such as ion exchange reactions, coprecipitation and precipitation in addition to sorption at the oxide surface (Warren and Haack, 2001). As mentioned above, sorption of cationic metals is much higher in the alkaline region than in the acidic region (although the speciation of the metal complexes is also important in determining sorption characteristics). In addition, these minerals are strongly influenced by redox processes, the reduction of either Fe^{3+} in Fe oxides or $Mn^{3+/4+}$ in Mn oxides results in the dissolution of the mineral releasing any bound metals back into the pore water (Kneebone et al., 2002; Cummings et al., 1999; Spliethoff et al., 1995; Kuhn and Sigg, 1993; Lovley, 1991).

While early literature on oxides focused on crystalline structures, environmental studies have shown that amorphous forms are often more relevant for metals. It has been determined that amorphous oxides are more reactive sorbents and are more relevant in aqueous environmental settings for metals, than crystalline oxides (Robertson and Leckie, 1998; Hall et al., 1996; Tessier et al, 1996). As a result of the differences in structure, the sorption of metals to amorphous oxides will differ from sorption to more crystalline structures indicating the need for separate evaluation of amorphous and crystalline oxides during SEPs.

2.3.4 Organic Matter

In addition to metal oxides, organic matter (including organic solids and natural organic matter components such as humic and fulvic acids) is a key sorbent for metals. Sorption to this substrate is dependent on both the species the metal has formed in solution, and the overall charge of that resulting molecule (Warren and Haack, 2001; Sauvé et al., 2000; Tessier et al., 1996; Warren and Zimmerman, 1994a; Buffle, 1988). Humic and fulvic organic substances are especially important for the sorption of metals due to their ability to complex inorganic cations, their redox sensitivity and their potential to form coatings on solid surfaces (Zuyi et al., 2000; Buffle, 1988; Kerndorff and Schnitzer, 1980). The importance of organic solids, whether live or dead, has been emphasized in low pH systems, where these types of sorbents would be more effective as metal sorbents than Fe oxides, due to the presence of carboxylic groups on organic surfaces ($pK_a \cong 4\text{-}6$) which render them negatively charged even in low pH systems (Warren and Haack, 2001; Tessier et al., 1996). Metals sorbed by solid organic matter are less susceptible to being released back into the soil porewater by changes in pH, compared to oxides, due to their lower pH_{ZPC} values resulting from carboxylic functional groups, degradation of the organic matter may lead to metals being released (Warren and Haack, 2001).

2.3.5 Residual

The residual fraction primarily consists of minerals that retain trace metals within their crystal structure. These metals are bound very strongly, and as such, are not expected to be released to the porewater over a reasonable time span under conditions normally encountered in nature (Sauvé et al., 2000; Tessier et al., 1979). Therefore, it can be assumed that in most vadose zone systems, the metals bound to this fraction are inert, not likely to pose a threat to groundwater regardless of natural changes in ionic strength, redox conditions or pH.

2.4 Metal Behaviour within the Vadose Zone

2.4.1 The Dynamic Vadose Zone

The vadose zone is defined specifically as the water-unsaturated zone extending from the land surface to the regional water table (Fetter, 1999) and is characterized by variable moisture levels that will impact local geochemical conditions. This zone presents a highly dynamic physico-chemical system where the behaviour of metals is dictated by biological and geochemical processes variably occurring at both micro and macro scales that will reflect the localized physical setting.

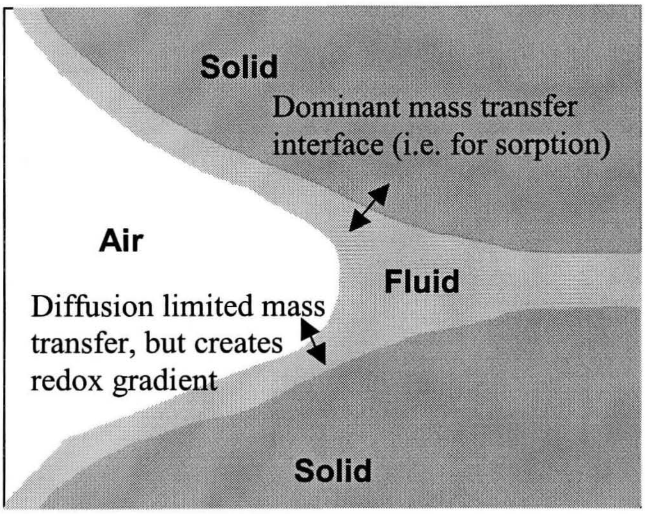

Figure 2.5: Pore scale schematic of the three phases present in the vadose zone. Modified from Ainsworth et al., 2000.

Three components interact in the vadose zone: the soil phase, the solution phase and the gas phase. When moisture levels are low and the gas phase is dominated by O_2, a highly oxidizing environment as shown in Figure 2.5 generally occurs (Ainsworth et al., 2000). When moisture levels are high and microbial activity levels increase, the gas phase will correspondingly show greater CO_2 concentrations, and more reducing conditions are engendered. A key characteristic of the vadose zone is the shifting spatial and temporal gradients in moisture and gas compositions, creating a highly dynamic environment with gradients occurring at both the macro and microscale (Zausig et al., 1993). Metal behaviour thus is dependent on more than just bulk vadose geochemical conditions since pore scale geochemistry can be radically different from that of the bulk system (Tokunaga et al., 2001; Zausig et al., 1993). Depending on the size of the pore, the gas phase may be limited by diffusion, thus solid-solution interactions will dominate. The mobility and migration of heavy metals in such a system will be dependent on solid-solution interactions as well as the overall migration of the solution. The vadose zone is further complicated by fluctuations in soil moisture content, which in turn results in dramatic changes in solution migration as well as changes in redox gradients and therefore biogeochemical behaviour. The behaviour of any given metal within this dynamic environment will be dependent on the specific metal's properties, which are discussed subsequently.

2.4.2 Uranium

Throughout North America, the contamination of soils with uranium has resulted from a history of uranium mining (McCready et al., 1986; Cahalan, 1963), irrigation of agricultural lands and disposal of nuclear waste (CWI, 2005; Makhijani and Boyd, 2001; Lovley et al., 1991). While uranium is a radioactive heavy metal, its toxicity poses a greater threat to human health and the environment than its radioactive decay, due to its long half-life (4.5 X 10^9 years for ^{238}U, Fetter, 1999).

Redox Properties and Speciation:

Uranium is a redox sensitive element with three oxidation states; U(IV), U(V) and U(VI), however U(V) is uncommon (Morin et al., 1988) and thus will not be considered further. Speciation information on uranium has been used as a means to evaluate potential *in-situ* remediation strategies for contaminated soils such as microbial reduction and natural bioattenuation (Fredrickson et al., 2000; Lovley et al., 1991). The dominant aqueous species of the oxidized form, U(VI), are $UO_2(SO_4)_2^{2-}$, $UO_2CO_{3(aq)}$ and UO_2^{2+}, while the dominant aqueous species of the reduced form, U(IV), are $U(OH)_3^+$, $U(OH)_4$ and $U(SO_4)_2$ (Morin et al., 1988). The sorption of the various species of uranium is largely dependent on pH. Significant sorption of U(VI) occurs in the more acidic range (pH 4-5.5) reflecting the negative charge on the dominating $UO_2(SO_4)_2^{2-}$ species, however, there is a significant decrease in sorption in the alkaline pH region (8-9.5) since U(VI) is believed to exist as the neutrally charged UO_2CO_3 species (Hsi and Langmuir, 1985).

While both U(IV) and U(VI) can exist as cations or neutrally charged species, U(VI) is generally more soluble and thus more mobile than its reduced counterpart (Duff et al., 2002; Morin et al., 1988). In oxidized environments uranium is often present as the highly soluble UO_2^{2+} species (Duff et al., 2002), which hydrolyzes to form a number of aqueous hydroxyl species (McKinley et al., 1995). Therefore, the reduction of uranium is an important mechanism for the immobilization of uranium (Lovley et al., 1991). Under reducing conditions, soluble U(VI) species can undergo biotic reduction to the sparingly soluble U(IV) species $UO_{2(s)}$ through abiotic reactions, bacterially facilitated passive bioreduction reactions (by using their cell walls to provide a reactive surface), as well as biologically active reduction reactions where microorganisms can gain energy by direct electron transport to U(IV) (Figure 2.6a: Duff et al., 2002; Fredrickson et al., 2000; Lovley and Phillips, 1992; Lovley et al., 1991; Langmuir, 1978).

Potential Sorbing Substrates:

The behaviour of uranium in the vadose zone will be dependent on both the redox state and speciation of the metal involved, mechanisms by which uranium mobility can be retarded are shown in Figure 2.6. While the charge of the molecule creates a potential for sorption to the leachable fraction, as previously stated, U uptake in this fraction is typically negligible. The solid carbonate fraction, however, will likely be a large reversible sink/source for the uranium (Figure 2.6 f: Duff et al., 2002; Gabriel et al., 1998). Since in the alkaline pH region, uranium often precipitates as UO_2CO_3 this will likely be a major sink for uranium (although it may become a potentially large source given a change in pH).

Oxides are also likely sinks for uranium (Figure 2.6 d-f). Field and laboratory studies have shown uranium to be associated with Fe oxides in natural systems (Gabriel et al., 1998; Thomas et al., 1993; Hsi and Langmuir, 1985), not surprising considering their high adsorption capacity and their existence as surface coatings in soils where uranyl adsorption reactions are significant (McKinley et al., 1995). Lack et al. (2002) has shown that anaerobic bio-oxidation of Fe(II) and precipitation of Fe oxides by *Dechlorosoma sp.* resulted in rapid adsorption and removal of uranium from solution. Results of this study indicated that the sediment sorbed uranium continued to be stable even upon exposure to oxygen, however, in the absence of the Fe(II) exposure to oxygen resulted in rapid oxidation to U(VI). Thus, while oxides may be important for uranium sorption, the redox status of the system may enhance or hinder

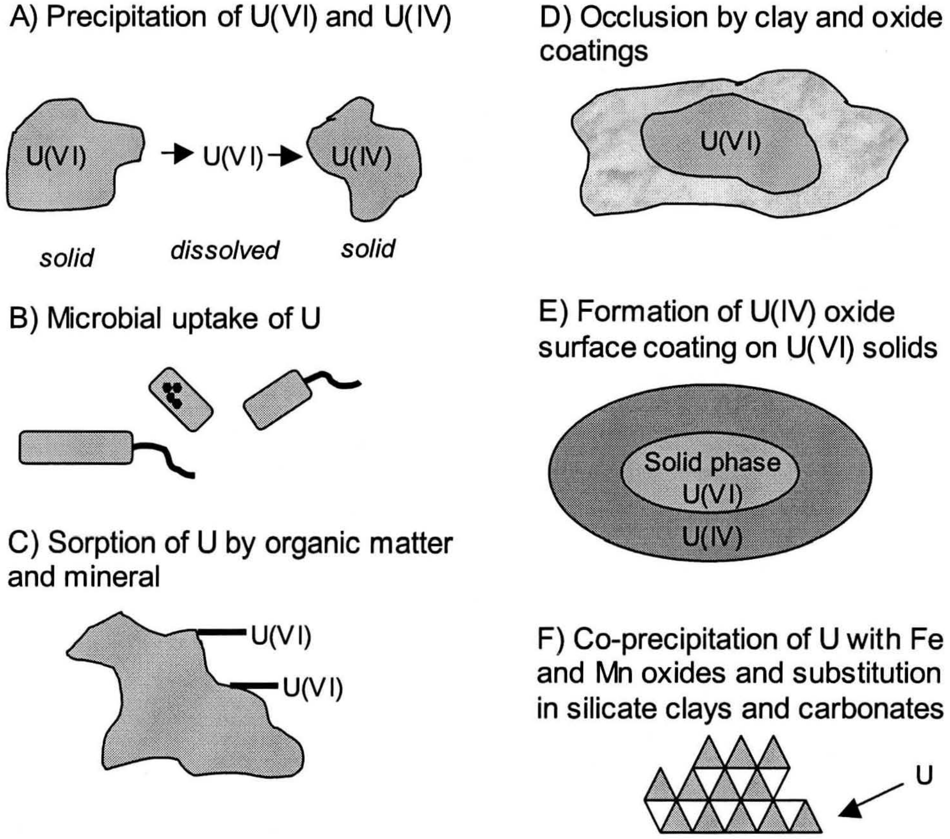

Figure 2.6: Mechanisms by which uranium mobility can be retarded in the surface and subsurface geologic environment. Source: Duff et al., 2002.

uranium sorption. To further complicate matters, the presence of dissimilatory metal-reducing bacteria can be a deciding factor on whether soluble U(VI) will be converted to the reduced form in the presence of oxides. In a laboratory study by Fredrickson et al. (2002), it was determined that in the absence of bacteria, various Mn oxides were responsible for the oxidation of $UO_{2(s)}$ to the soluble U(VI) species, indicating some Mn oxides may actually hinder uranium sorption.

In addition to the carbonates and oxides, organic matter may present a third significant sink for uranium. A study conducted by Zuyi et al. (2000) found that humic substances effected sorption of uranium to oxides, indicating that these two fractions do not behave independently. The conclusions of this study indicated that the effect of humic substances on sorption to oxides was dependent on pH, the nature of the oxide, the fractionation of the humic substance, the relative strength of the complexes of both the soluble and sorbed humic substances and the concentration of the humic substance. Additional studies have found organic matter to be an important medium for the removal of uranium from anoxic water columns, although it is uncertain whether this is due to the reduction to U(IV) or the complexation of U(VI) (Figure 2.6 c: Cochran et al., 1986; Baturin and Kochenov, 1973). Furthermore, uranium can actually be taken up by microbial biomass as is shown in Figure 2.6 b (Duff et al., 2002; Tsezos, 1984).

Based on the geochemical behaviour of this element, an organic- and metal oxide-rich, reducing environment would be best suited for site remediation.

2.4.3 Arsenic

Arsenic often contaminates the subsurface as a result of weathering of rocks, industrial waste discharges, pesticides, etc. (Oremland and Stolz, 2003; Bhumbla and Keefer, 1994), having severe effects on both human health as well as the health of terrestrial plants, freshwater and marine communities (Ryu et al., 2002; Su and Puls, 2001; Mok and Wai, 1994).

Redox Properties and Speciation

Inorganic arsenic occurs in two oxidation states, arsenate (As(V)) and arsenite (As(III)) that exhibit differing toxicity, mobility, and bioavailability (Gao et al., 1998; Cullen and Reimer, 1989). The relative abundance of each species is primarily regulated by redox potential (refer to Figure 2.7: Bose and Sharma, 2002; Ryu et al., 2002). Arsenate exists predominantly as H3AsO4, which has three pKa values: pK1 = 2.20, pK2 = 6.97 and pK3 = 11.53, therefore, in the

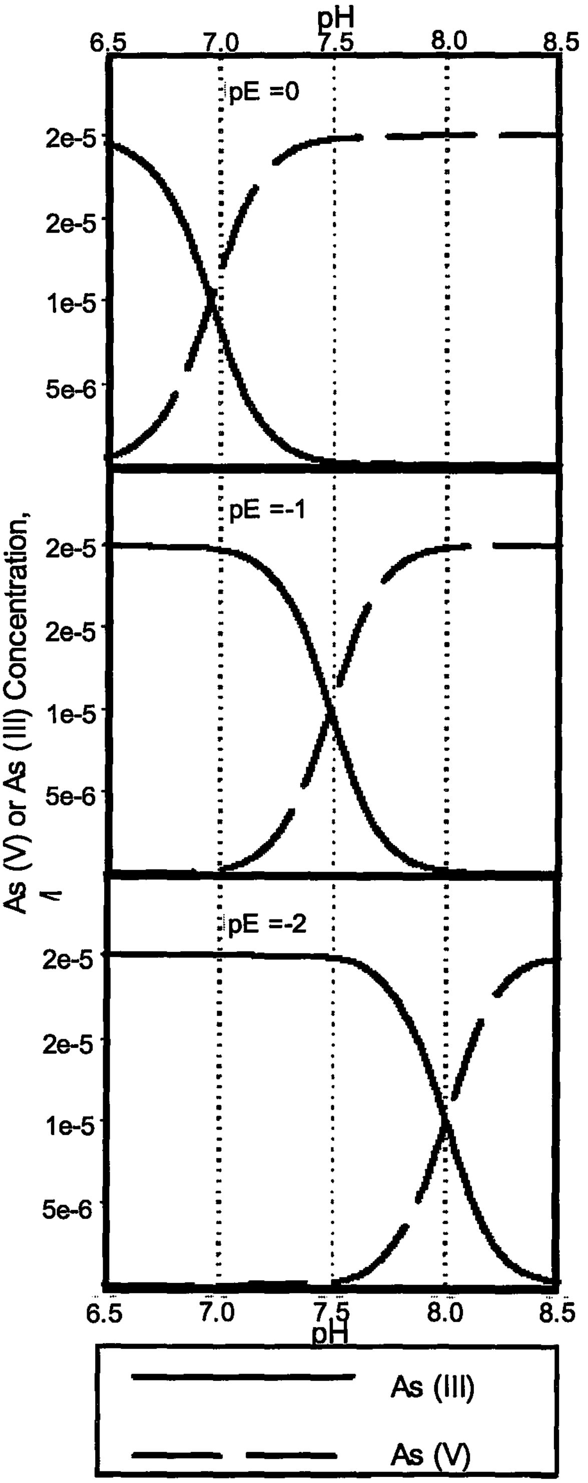

Figure 2.7: Effect of pH and pE on relative distribution of arsenic between As(III) and As(V). Source: Bose and Sharma, 2002.

circumneutral pH range, it would be expected that $HAsO_4^{2-}$ would dominate with potentially some $H_2AsO_4^-$ (Su and Puls, 2001). Arsenite also has three pKa values, however, they are much higher than those of arsenate so the dominant species below pH 9 is H_3AsO_3, which carries a neutral charge (Su and Puls, 2001). Various studies have identified that in sediment As(V) is strongly sorbed, thus the mobility of As(V) is reduced (Price and Pichler, 2005; Oremland and Stolz, 2003; Su and Puls, 2001; Mok and Wai, 1994; Holm et al, 1979). Conversely, the overall neutral charge on As(III) species, renders reduced forms of arsenic highly soluble inhibiting its ability to persist in soils (Sadiq et al., 1983). In terms of toxicity, As(III) is considered to be substantially more toxic than As(V) to terrestrial organisms (National Research Council, 1977); and the former is the predominant species in many groundwaters, thus remediation efforts are generally directed toward the oxidation of As(III) to As(V) and immobilization of both species by adsorption or coprecipitation (Su and Puls, 2001).

Potential Sorbing Substrates

Iron and manganese oxides provide a carrier phase for the deposition of arsenic in sediment (Smedley et al., 2005; Craw et al., 2004; Gault et al., 2003; Bose and Sharma, 2002; Kneebone et al., 2002; Belzile and Tessier, 1990), however, the potential for arsenic sorption is pH dependent, where higher pH causes desorption of As(V) and sometimes As(III) from the oxide (Bose and Sharma, 2002). Furthermore, the type and age of oxide is also important. While it is clear that Fe and Mn oxides exhibit different properties and affinities for metals (Warren and Haack, 2001; Tessier et al., 1996), aging of oxides can often result in increased crystallinity and associated changes in relative sorptive capacity. For instance, Fuller et al. (1993) demonstrated that as ferrihydrite (amorphous Fe oxide) crystallized to goethite (crystalline Fe oxide), there was a reduction in binding sites resulting in a release of sorbed arsenic.

Under reducing conditions, oxides dissolve and any bound arsenic is released into the pore water (Kneebone et al., 2002; Spliethoff et al., 1995; Kuhn and Sigg, 1993; Peterson and Carpenter, 1986). Studies in Bangladesh have attributed the reduction of Fe oxides to the mass release of arsenic causing severe contamination of the groundwater (Bose and Sharma, 2002; Nickson et al., 2000). It is believed that this reduction of Fe oxides is driven by microbial metabolism of organic matter (Nickson et al., 2000). Therefore, while little literature exists on the effect organic matter has on the direct sorption of arsenic, the presence of organic matter is likely an important factor driving As behaviour, due to its potential impact on oxides and thus As sorption to the soil.

2.4.4 Chromium

Chromium is also a common metal contaminant in soil due to its use in metal plating, tanneries and wood preservation (Khan and Puls, 2003; Adriano, 1986).

Redox Properties and Speciation:

Chromium has two stable oxidation states, namely Cr(III) and Cr(VI) where again, each state exhibits different properties. While Cr(III) is an essential element for living beings (Otabbong, 1990), Cr(VI) is a human carcinogen in cases of long exposure (Khan and Puls, 2003; Norseth, 1981). The toxic, oxidized form of chromium (Cr(VI)) is much more soluble and mobile than Cr(III) which often exists as a stable solid (Rai et al., 1989). In uncontaminated soils chromium is often present as Cr(III), thus Cr(VI) is largely restricted to contaminated sites (Tokunaga et al., 2001). Between pH 6 and pH 12, the dominant speciation of Cr(III) is the precipitated chromic $Cr(OH)_3$ or mixed Cr-Fe oxide phases (Khan and Puls, 2003; Rai et al., 1987), whereas Cr(VI) exists in solution in high redox potential environments as H_2CrO_2, $HCrO_4^-$, and CrO_4^{2-} or $Cr_2O_7^-$ if the total Cr(VI) concentration exceeds 10mmol/L (Loyaux-Lawniczak et al., 2001).

Reduction of Cr(VI) to Cr(III) is desirable for remediation of contaminated soils. Reduction of Cr(VI) would potentially increase both sorption and precipitation of the non-toxic Cr(III) form and this can be achieved through the metabolism of many microbial species (Arias and Tebo, 2003). Factors that are usually involved in Cr(VI) reduction include the presence of Fe(II) both in solution and as minerals, sulfides and organic matter (Loyaux-Lawniczak et al., 2001; Wittbrodt and Palmer, 1995). Microorganisms can also reduce Cr(VI) indirectly through the depletion of oxygen, reduction of Fe and S and production of organic ligands and reductants (Tokunaga et al., 2001). While it is advantageous to reduce Cr(VI), oxidants also exist in the sediment that may counter the reduction of Cr(VI) by promoting the oxidation of Cr(III) (Schroeder and Lee, 1975). Mn oxides as well as dissolved oxygen play a role in the oxidation of Cr(III) in the environment (Chung et al., 2001; Schroeder and Lee, 1975). This may be particularly important in unsaturated soils, which have a larger oxygen content and a large water-air interface within the pores for oxygen to diffuse across, thus Cr(III) oxidation would be expected to be common for the bulk sample. However, it has been determined that bulk samples characterization is inadequate for understanding the controlling biogeochemical process since these processes occur at the pore scale (Tokunaga et al., 2001). On the pore scale, the outer surfaces of soil aggregates would generally experience oxidizing conditions favouring the stability of Cr(VI), however, interior regions would likely experience reducing conditions, favouring transformation to Cr(III) (Tokunaga et al., 2001).

Potential Sorbing Substrates

In low pH systems, metal oxides play a role in sorption of Cr(VI), since oxides carry an overall positive charge (at pH levels below their pH_{ZPC}) attracting the negative charge of the various Cr(VI) species. In circumneutral to high pH systems on the other hand, metal oxides would no longer carry this overall positive charge, thus the ability to sorb Cr(VI) species is significantly reduced (Davis and Leckie, 1980). Researchers have also considered the importance of organic matter for the reduction of chromium, but with natural samples it is difficult to separate the effect of organic matter from the effect of oxides on chromium reduction (Anderson et al., 1994). However, studies performed specifically on organic matter have been able to determine that organic compounds do not directly reduce Cr(VI) very rapidly at pH's greater than 2 (Anderson et al., 1994; Brauer and Wetterhahn, 1991; Connett and Wetterhahn, 1986).

2.4.5 Cobalt

Cobalt contamination is often the result of urban stormwater runoff, mining operations, nuclear reactor operations and industrial effluents (Krumholz et al., 2003; Triantafyllou et al., 1999; Leonard et al., 1993). While cobalt is essential to life in small concentrations, larger concentrations are detrimental to both human health and the environment (Triantafyllou et al., 1999).

Redox Properties and Speciation:

Typically in aqueous systems, cobalt is considered to have only a single stable oxidation state, namely Co^{2+} (Fetter, 1999). However, Co actually has two oxidation states, Co(II) and Co(III), with the predominant form being Co(II) in natural waters (Qian et al., 1998). Cobalt as Co(III) is reported to only occur in strong organic complexes or in association with particulate matter, especially Mn-oxides (Kay et al., 2001; Qian et al., 1998; Moffett and Ho, 1996; Murray and Dillard, 1979). Therefore, the geochemical behaviour of this metal may be directly affected by redox reactions but will also be indirectly affected by redox processes due to sorption onto substrates that are strongly redox dependent.

Potential Sorbing Substrates

Many researchers have proposed that Fe and Mn oxides are the dominant controlling factors for the distribution of cobalt in the subsurface (Kay et al., 2001; Rae and Parker, 1993; McLaren et al., 1986; Kinniburgh et al., 1976). Studies have shown that Co^{2+} has a strong affinity for Mn, and Fe oxides (Kay et al., 2001; Rae and Parker, 1993; McLaren et al., 1986; Kinniburgh et al., 1976). However in reducing environments Mn and Fe oxides may dissolve, releasing Co^{2+} back into the water column. Other potential sorbents for Co^{2+}, due to the free ion's positive charge, are organic matter (McLaren et al., 1986; Kerndorff and Schnitzer, 1980) and smectite-rich clays, which generally carry a negative charge, however sorption to clays is limited by the cation retention capacity (Triantafyllou et al., 1999; McLaren et al., 1986).

2.4.6 Zinc

Accumulation of zinc in soil is largely a result of mining activities (Roberts et al., 2002; Dudka and Adriano, 1997), but has also resulted from the application of fertilizers and sewage sludge on agricultural lands (Madyiwa et al., 2002).

Although this metal is essential for humans, it is toxic at high concentrations (Maiti and Banerjee, 2002). Environmental studies have shown a decrease in the abundance of soil microorganisms, reduced soil fertility and damaged vegetation as a result of high zinc levels (Adriano, 1986).

Redox Properties and Speciation:

Although zinc only has a single stable oxidation state (Zn^{2+}), the potential binding substrates for this heavy metal are generally pH and redox dependent rendering the mobility of zinc in natural systems to be highly dependent on these properties as well.

Potential Sorbing Substrates

Zinc partitioning is largely due to two soil fractions, carbonates and oxides. Binding to carbonates often occurs in anoxic environments whereas zinc sorbs strongly to oxides in aerated systems (Bostick et al., 2001). Sorption of zinc to metal oxides tends to increase with pH through the acidic to circumneutral pH ranges (Cooper et al., 2000), however, in the alkaline region and at high initial zinc concentrations, precipitates of $Zn(OH)_2$, $Zn(CO)_3$ and $ZnFe_2O_4$ may control solubility (Sadiq, 1991). Since carbonates are highly pH dependent and oxides are redox sensitive, partitioning of Zn can undergo extreme fluctuations (Bostick et al., 2001).

2.5 Research Directions

Contamination of the vadose zone has resulted from many different processes and introduced a variety of heavy metals. Therefore, when considering potential remediation strategies it is important to understand the behaviour of each heavy metal as well as having an understanding of how the metals in the system may interact.

Many studies have proposed the theory of creating a reducing environment through the introduction or stimulation of microbial communities so that heavy metals will be reduced (Arias and Tebo, 2003; Elias et al., 2003; Khan and Puls, 2003; Krumholz et al., 2003; Loyaux-Lawniczak et al., 2001; Fredrickson et al., 2000; Anderson et al., 1994). While this is a potential strategy for metals such as uranium and chromium, which become generally less soluble in their reduced form, it may cause the reduction of arsenic to the more toxic, mobile form. In addition, in highly reducing environments, metal oxides may also be reduced, which has important implications for metal mobility since oxide surfaces are generally strong sorbents for metals (although this is pH dependent), which upon reduction would release the metals back into solution (Kneebone et al., 2002; Spliethoff et al., 1995; Kuhn and Sigg, 1993).

Therefore, in order to characterize the behaviour of metals in a natural environment it is necessary to assess both the geochemical behaviour in terms of how strongly each metal sorbs to different components of the vadose zone (i.e. oxides versus organic matter) as well as the bacterial community to gain an understanding of the potential redox reaction that would be occurring. The objective of this research is to evaluate the mobility and geochemical behaviour of a suite of metals (U, As, Cr, Co, Zn) in INL vadose soil microcosms under varying levels of (1) metal concentration and (2) biological activity to determine the linkages among solution metal concentration, metal identity and levels of biological activity in controlling vadose soil metal sequestration.

3.0 Materials and Methods

Appropriate microbially sterile and metal-clean techniques were used throughout this study. Water used for sequential metal extractions and solutions was ultrapure (UPW: 18.2 mΩ/cm, Milli-Q, Millipore, Billerica, MA, USA). New plastic ware was first soaked in a dilute detergent solution (Sparkleen 1) then rinsed in UPW. All plastic ware was then immersed in an acid bath (4% HCl) where it soaked for at least eight hours and was subsequently rinsed seven times with UPW. Where sterile conditions were required, all plastic/glass ware was first washed as outlined above and then autoclaved. All reagents used for analyses were analytical grade or better.

3.1 Experimental Design:

Five experimental treatment systems were evaluated to identify abiotic and biotic impacts on metal behaviour (also illustrated in Figure 3.1):

- **Sterile** INL sediment, no additional treatment (abiotic control)
- **Non-Sterile** INL sediment, no treatment (biotic control)
- Non-Sterile INL sediment with addition of 1mM carbon mix (biotic **Amendment-1**)
- Non-Sterile INL sediment with addition of 5mM carbon mix (biotic **Amendment-2**)
- Non-Sterile INL sediment with addition of 10mM carbon mix (biotic **Amendment-3**)

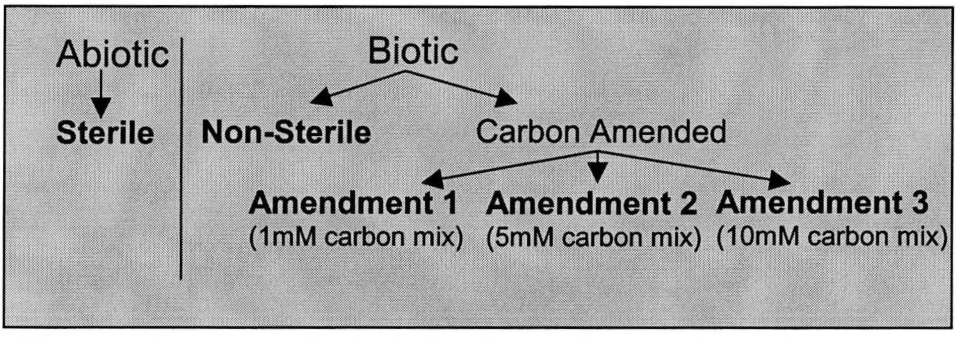

Figure 3.1: Schematic depicting the systems to be analyzed within the current study (biotic vs. abiotic).

Stock carbon amendments were prepared using equal proportions of ethanol, glucose and lactose in solution for total carbon concentrations of 1mM, 5mM and 10mM. These stock solutions were autoclaved prior to be added to the INL sediments.

For each of the above five experimental systems, three total metal concentrations (equimolar U, As, Cr, Co and Zn) of 50 µM, 200 µM, and 1000 µM, were also evaluated (refer to Table 3.1 for metal salt added). All experiments were run at a nominal starting pH of 7.1. In total, there were 5 treatments X 3 metal concentrations X 3 replicates, as well as 1 control for each of the treatments (no metals added) for a total of 50 experimental microcosm systems. An additional 10 microcosms were run for bacterial analysis, 5 treatments X 2 metal concentrations (0 µM and 1000 µM).

Table 3.1: Metal salts added to stock solution and their corresponding valence states.

Metal	**Salt**	**Valence State**	**Generally Least Harmful Valence State**	**Generally Most Harmful Valence State**
U	$UO_2(NO_3)_2{\bullet}6H_2O$	U^{6+}	U^{3+}	U^{6+}
As	$NaAsO_2$	As^{3+}	As^{5+}	As^{3+}
Cr	$NaCr_2O_7{\bullet}2H_2O$	Cr^{6+}	Cr^{3+}	Cr^{6+}
Co	$CoCl_2{\bullet}6H_2O$	Co^{2+}	N/A	N/A
Zn	$ZnCl_2$	Zn^{2+}	N/A	N/A

3.2 Experimental Set Up

Batch experiments were run in 50 mL polyethylene bottles using 4 grams of INL soil taken from a region know as Spreading Area B, an uncontaminated region directly North East of the Radioactive Waste Management Complex (RWMC) with basic soil mineralogy being very similar to that of the RWMC. For the three biotic amendments, the carbon amendments were added as a 5mL mixture of ethanol, glucose and lactose at the designated concentrations (1mM, 5mM and 10mM) and incubated at room temperature on an orbital shaker (120 rpm) for two weeks prior to metal additions. After removal of the carbon amendment by decanting the liquid (centrifugation was unnecessary), a 40 mL synthetic groundwater solution specific for INL vadose conditions (refer to Table 3.2) spiked with the desired metal concentration was added to each microcosm and incubated at room temperature for 3 weeks on an orbital shaker (120 rpm). The large volume to sediment ratio (i.e. 40mL groundwater:4g INL sediment) was designed to induce highly saturated conditions for analysis as an endmember in possible scenarios of moisture content.

The sterilized treatments were prepared by autoclaving to kill any indigenous bacteria present in the soil, and upon addition of the metal solutions, a one time addition of sodium azide was added at a concentration of 0.5mM, to prevent bacterial re-growth during the course of the experiment.

Upon completion of the 3 week period, the sediment was firmly pelleted into the bottom of the bottles, allowing for the remaining solution to be removed by decanting without centrifuging the samples.

Table 3.2 Groundwater composition. Modified from Roback et al., 2001.

Salt Added	**Concentration (mmol/L)**
KNO_3	0.04
$MgSO_4$	0.914
$CaCl_2$	1.748
$NaHCO_3$	0.016
$KHCO_3$	0.062

3.3 pH and Redox

pH values for the bulk liquid of each microcosm were measured (Micro pH electrode, Lazar Research Laboratories, Inc., Los Angeles, CA, USA) immediately after metal additions and are reported as an average for each designated system (3 replicate samples at 3 solution metal concentrations for each system, therefore values reported for each system are the average of 9 pH measurements). After completion of the metal exposure phase, porewater pH and redox (ORP electrode; Lazar Research Laboratories, Inc., Los Angeles, CA, USA) were measured for each microcosm and reported as average values for each system (reported values are the average of the 3 replicates at 3 solution metal concentrations for a total of 9 measured values for each system). These results are reported in Table 4.1.

3.4 Metal Partitioning Analyses

Using 1g of wet sediment from the top of each microcosm, metal partitioning was evaluated by the SEP outlined in Table 3.3 using a 20:1 ratio of reagents-to-dry weight sediment sample (with a INL sediment wet/dry conversion of 1.43). The sediment was placed in digester vessels (Microwave Labstation: Ethos Plus, Milestone Inc. with easyWAVE version 3.2.1.0 software MLS GmbH, Germany) and processed according to the conditions specified in Table 3.3. After each step of the extraction procedure, the resulting slurries were poured into

Table 3.3: Reagents and conditions for each of the fractions used for the sequential extraction procedure. Each step was preformed in sequence on three sub samples of each experimental system at each metal concentration, as developed by Haack and Warren, 2003.

(Sequence) Fraction	**Reagents**	**Conditions**
(1) Leachable	1M sodium acetate, pH 8.2 (with HOAc)	1hr shaking on orbital shaker (120 rpm), room temperature
(2) Carbonates	1M sodium acetate, pH 5.0 (with HOAc)	Microwave Program 1 (MP-1): Heat to 150°C over 8 minutes. Maintain temperature for 5 minutes. 30 minute cool-down.
(3) Reducible: Amorphous Fe/Mn Oxides	0.25M Hydroxylamine hydrochloride in 0.25M HCl.	MP-1
(4) Reducible: Crystalline Fe/Mn Oxides	1.0M Hydroxylamine hydrochloride in 25% (v/v) acetic acid.	MP-1
(5) Oxidizable: Organic and Sulfides	3:2 ratio 30% H_2O_2: 0.02M HNO_3 + 1:4 ratio 3.2M NH_3OAc:UPW	MP-1
(6) Residual	Concentrated HNO_3	Microwave Program 2 (MP-2): Heat to 180°C over 20 minutes. Maintain temperature for 10 minutes. 45 minute cool-down.

Nalgene centrifuge tubes, and centrifuged (16,000 rpm) for 20 minutes and the solutions decanted. The resulting solutions were diluted in a 2% HNO_3 solution and analyzed for U, As, Cr, Co and Zn by Inductively Coupled Mass Spectrometry (ICP-MS; Perkin Elmer SCIEX ELAN 6100, Woodbridge, ON, Canada). The remaining pellet was mixed into solution with the appropriate volume of the subsequent reagent and poured back into the microwave vessel for the next step of the procedure. Analytical accuracy during extractions was assessed by extraction of certified reference material (NIST Buffalo River Sediment, SRM 1577b) and comparison of known metal content to the metals found in all sediment fractions combined (i.e. sum of all fractions). Triplicate analyses for each fraction showed minimal variation (±10%) for all metals analyzed (U, Cr, Co and Zn) for the total sediment (i.e. sum of all fractions).

All metal data are reported as mean values plus or minus one standard error (S.E.) defined as the standard deviation divided by the square root of sample size or "n" (Zar, 1984).

Partitioning coefficients were calculated according to the following equation:

$$Kd = C^*/C \qquad \text{Equation 3.1}$$

Where Kd is the partitioning coefficient (L/kg), C* is the mass of metal sorbed per dry unit weight of solid (mg/kg) and C is the concentration of metal in solution (mg/L). Sediment compartmental Kds were calculated for the relevant metal sorbing fractions: oxides (combined amorphous and crystalline), carbonates and leachable, as the concentration of a given metal bound to the concentration of the specific sediment compartment. The amount of oxides and carbonates present within each sample were estimated according to previous characterization of the INL sediment (Table 3.4: Cooper et al., 2003), where the largest representative values reported by Cooper et al. (2003) were chosen to represent the sediment in the study as the most conservative values. The leachable Kd values were calculated using the entire soil mass as theoretically all sediment surfaces would be available for leachable interfacial reactions.

Table 3.4: Previous INL sediment characterization allowed the estimation of potential binding substrate for the current investigation. * Cooper et al., 2003

Substrate	**Reported Values ***	**Assumed values**
Leachable	---	100% wt.
Carbonates	0.1-10% wt.	10% w.t.
Oxides	5-20mg/g	20mg/g

Statistical analyses were performed to compare averages values across systems, treatments and elements using ANOVA and the Tukey test with a significance value of $p<0.1$.

3.5 Bacterial Analyses

Cell enumeration for the evaluation of bacterial densities across the five treatments were performed by visual inspection using the fluorescent stain 4',6-diamidino-2-phenylindole (DAPI). DAPI is a noninterchalating, DNA-specific stain which fluoresces blue or bluish-white when bound to DNA, and allows for the identification of bacteria both on the basis of colour as well as size and shape (Kepner and Pratt, 1994). Microcosms from each of the 5 treatments, both without the addition of metals (0 μM) and with high metal loading (1000 μM) were analyzed using the procedure outlined by Bernier and Warren (2005).

For each system, 0.5mL of wet sediment was immersed in 1.5mL of 4% paraformaldehyde, vortexed for 30 seconds and allowed to fix for one hour at 4°C. Upon removal of paraformaldehyde by centrifugation (10,000 rpm for 10 minutes), samples were washed twice with 1x Phosphate-buffered saline (1xPBS) and then resuspended in 0.7mL of 1xPBS and 0.7mL of 96% ethanol. Samples were stored at -20°C.

Sonication of samples in cold water (19°C) was required in order to separate bacterial cells from lithic fragments and soil aggregates (Craig et al., 2002; Schallenberg et al., 1989), however it was necessary to remove ethanol from solution prior to sonication, thus samples were centrifuged (10 minutes at 10,000 rpm). The supernatant was discarded and 1mL 1xPBS was added. Samples were sonicated for a total of 10 minutes (Craig et al., 2002), although the water within the sonicator was replaced with fresh cold water after the first five minutes (temperature had risen to 21.5°C) and then sonicated for the remaining 5 minutes. Samples were centrifuged (4,500 rpm for 3 minutes) and the supernatant carefully siphoned for cell analysis. An equal volume of ethanol (1mL) was added to the supernatant and the samples were stored at -20°C for up to 1 week.

Enumeration of bacterial populations was conducted using cell staining and subsequent visual analysis on an epifluorescent microscope. On each well of a gelatin-coated slide, 3 µL of sample was smeared with the end of the pipette tip to fill the entire well, and air-dried in the biological fume hood (Class II A/B3 Biological Safety Cabinet, Model 1284, Forma Scientific Inc., Ohio, USA) for at least one hour at room temperature. The slides were then dehydrated by submersion in an increasing ethanol series (50%, 80%, 95% - 3 minutes at each stage) and then air-dried for an additional hour. The slides were then stained by adding 20 µl of 1 µg/mL DAPI (Molecular Probes Inc., Eugene, OR, USA) to each well on the slide and incubated in a dark at room temperature for 30 minutes after which the slide was handled only under dark conditions to prevent photodegradation. The slide was washed thoroughly with ultrapure water after which 1 drop per well of mountant (Component A SlowFade Light – Molecular Probes Inc.) was added to minimize photobleaching.

Visual analysis of the DAPI-stained cell was then performed using the bright field, DIC and epifluorescence under a 100X objective with a Leica DMRA (Leica Microsystems, Richmond Hill, ON) with digital imaging carried out with OpenLab 2.2.5 (Improvision, Coventry, UK). Slides were also examined without the DAPI stain to determine the influence of background autofluorescence. At least 200 cells were counted per slide, and this value was converted to bacterial cells per gram of sediment according to the following equation:

$$\text{Bacteria (cells per gram)} = (N \times A_t)/(d \times V_f \times G \times A_g) \qquad \text{Equation 3.2}$$

Where N is the number of cells counted, A_t is the area of the slide well, d is the dilution factor (g/ml), V_f is the sample volume (ml), G is the number of grids counted, and A_g is the area of the counting grid (Kepner and Pratt, 1994).

4.0 Results and Discussion

4.1 Metal Geochemistry

4.1.1 pH and Redox

Table 4.1: pH and Redox values recorded in the supernatant solution at the beginning and end for pH and at the end of the experiment for redox.

	System	Initial	Final
pH	S	7.1±0.2	7.1±0.3
	NS	7.1±0.1	7.0±0.1
	A1	7.1±0.1	7.1±0.2
	A2	7.1±0.2	7.2±0.1
	A3	7.1±0.1	7.0±0.2
Redox (mV)	S		180±30
	NS		200±30
	A1		190±20
	A2		220±20
	A3		200±20

No statistically significant difference in either solution pH or redox values (mV) were found either across treatments at the end of the experiment, or for pH, for a given treatment from initiation to the end of the experiment. The pH values in the overlying aqueous phase in the batch microcosms remained circumneutral, ranging between 7 and 7.2 across treatments and time, indicating that no major shifts in solution [H^+] occurred. Redox values were only measured at the end of the experiment, but showed similar, mildly reducing values (~200mV) across all treatments, suggesting that some reduction in the sediments had occurred to generate oxygen consumption from the overlying water, as typically well oxygenated waters would have an ORP value in the range of 400-600 mV (Wetzel, 1983). The values recorded are consistent with NO_3^- – Fe(III) reduction zones (Drever, 1997) and likely indicate more reducing conditions would have been present in the sediments. In both instances, no treatment specific trends were evident in the recorded solution values of these parameters. These results may be interpreted to suggest that either sediment associated values were also identical across treatments (i.e. identical impacts on pH and redox occurred within sedimentary compartments across treatments) or that treatment dependent sediment dynamics were not sufficiently marked to be observed in the overlying water values (i.e. microgeochemical pockets of intense activity occurred within the sediments that do not translate into bulk H_2O characteristics).

4.1.2 Solid-Solution Partitioning

While overlying aqueous pH and redox conditions showed no discernible differences across microcosms or time, increasing solution concentration of metals (50 µM, 200 µM and 1000 µM total concentrations) corresponded to increasing total solid concentration for all individual elements (Figure 4.1). This result would suggest that the sorbent capacity of the INL sediment was not exceeded over the concentration range examined. This trend is consistent with the classic conceptual geochemical understanding of solid-solution metal dynamics, i.e. when sorbent concentrations are not limiting, solid phase metal concentrations increase concomitant with increasing solution concentrations (Figure 4.2; Fetter, 1999).

However, while the total solid concentration of metal increased as a function of solution concentration, the solid-solution partitioning of each metal shows a more complex non-linear pattern, manifesting as an interactive function of (1) solution concentration, (2) level of biological activity and (3) the specific element involved. To date, our limited conceptual understanding of the influence of biological activity on metal solid-solution behaviour based on literature evidence would suggest, in the most simple representations, two scenarios as the 'end members' to possible behaviour (Figure 4.3). One, an increase in solid metal uptake with increasing biological activity, and two, a decrease in solid metal uptake with increasing biological activity (Islam et al., 2004; Macur et al., 2004; Arias and Tebo, 2003; Elias et al., 2003; Gu et al., 2003; Khan and Puls, 2003; Krumholz et al., 2003; Nicholas et al., 2003; Tokunaga et al., 2001; Fredrickson et al., 2000; Lovely et al., 1991). These conceptual 'end members' to potential behaviour, develop from the understanding of microbial activity impacts on geochemical conditions (principally redox and pH conditions) creating microgeochemical environments that enhance or diminish solid metal capture (Islam et al., 2004; Turpeinen et al., 2004; Fredrickson et al., 2003; Krumholz et al., 2003; Roden, 2003; Zachara et al., 2001) and/or in conjunction with direct impacts on metal capturing solid phases with the solid pool (i.e. mineral formation or dissolution (Islam et al., 2004; Fredrickson et al., 2003; Roden 2003; Wazne et al., 2003; Warren and Haack, 2001; Zachara et al., 2001)). However, the extent of these impacts will also likely be dependent on the specific metal involved, given elemental differences in behaviour under differing geochemical scenarios as well as relative affinities for different solid phases . The results of this study support a more complex third scenario, and not yet considered

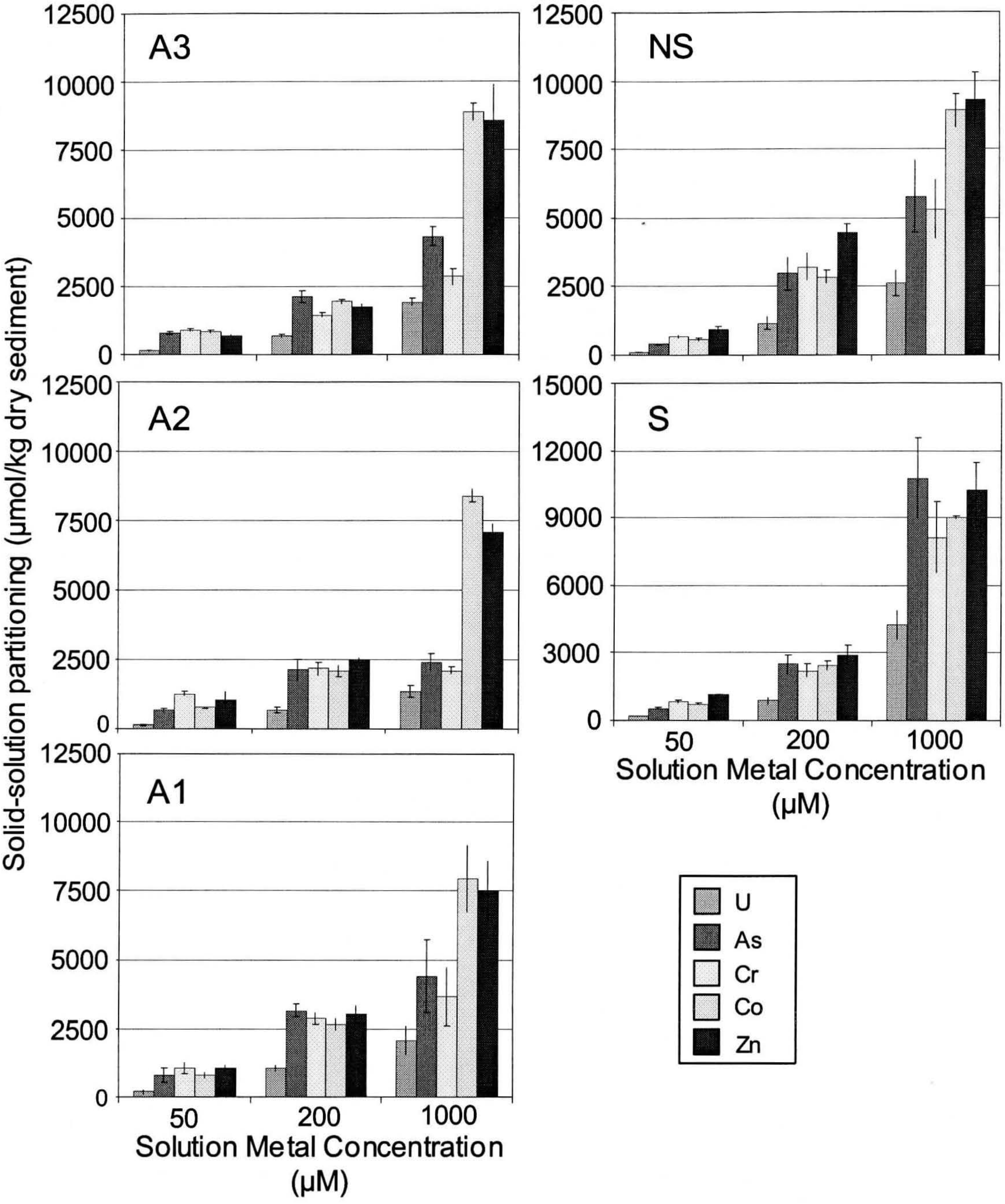

Figure 4.1: All sediment associated metal concentrations increase in a non-linear manner as a function of solution metal concentration for each system. Mean values ± standard error are shown (n=3).

in any published research: interactive effects of metal concentration and biological activity i.e. non-linear trends in solid metal concentration as a function of solution metal concentration are observed (Table 4.2, Figure 4.1 and 4.4).

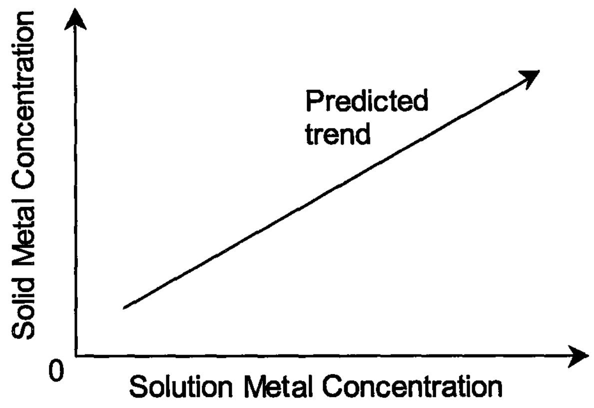

Figure 4.2: Conceptual representation of classic geochemical model for increased solid metal capture with increased metal in solution (linear sorption isotherm).

No consistent linear trends emerge for solution concentration, level of biological activity or for a given element in the results (Table 4.2) indicating that complex, non-linear interactions amongst all three parameters are likely occurring. Further, relatively few statistically significant differences were noted (which is likely induced by the relatively low power of the analyses) nonetheless; results should only be conservatively interpreted to show differences amongst biotic and abiotic treatments. Given the low power and the occurrence of some interesting trends in behaviour contrasting either different metals at a given solution concentration or across different solution concentrations for a given metal, a more detailed discussion of the trends follows.

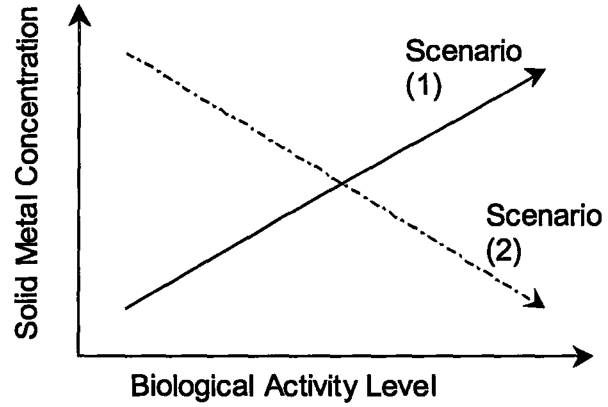

Figure 4.3: Conceptual representation of biological impact on metal capture presenting two potential and opposite impacts.

Table 4.2: Metal partitioning trends across systems for each metal according to solution metal concentration. Abbreviations are as follows: S=Sterile, NS=Non-Sterile, A1=Lowest Amendment, A2=Intermediate Amendment, A3 = Highest Amendment.

Metal	**Solution Metal Concentration (μM)**		
	50	**200**	**1000**
Uranium	NS≤A3≤A2≤S≤A1	A2≤A3≤S≤A1≤NS	A2≤A3≤A1≤NS≤S **A2,A3,A1≠S**
Arsenic	NS≤S≤A2≤A3≤A1	A2≤A3≤S≤NS≤A1	A2≤A3≤A1≤NS≤S **A2,A3,A1≠S**
Chromium	NS≤S≤A3≤A1≤A2 **NS≠A2**	A3≤A2≤S≤A1≤NS **A3≠A1,NS**	A3≤A2≤A1≤NS≤S **A3≠S**
Cobalt	NS≤S≤A2≤A1≤A3 **NS≠A3**	A3≤A2≤S≤A1≤NS **NS≠A3**	**A1<A2<NS≤S<A3**
Zinc	A3≤NS≤A2≤A1≤S	**A3≤A2≤S≤A1<NS** **A3≠A1**	A2≤A1≤NS≤A3≤S **A2,A1≠S**

Element specific trends occurs at all three solution metal concentrations (Figure 4.5). At the lowest solution metal concentration (50 μM total metal), As, Cr and Co show similar results where biological activity (A treatments; three levels of activity through carbon amendments) appear to stimulate solid capture compared to the S (Sterile) and NS (Non-Sterile) treatments (Table 4.2, Figure 4.4, 4.5). In contrast, both U and Zn solid concentrations are high in the S treatments compared to the biologically stimulated treatments at this low solution concentration. At the highest solution metal concentration (1000 μM total metal), all metals examined (with the exception of Co) show the same result, where any biological activity (A treatments) reduces solid capture compared to the abiotic S treatment (Figure 4.4, 4.5). The intermediate metal solution concentration of 200 μM, unlike the other metal solution concentrations examined, shows differing biological impacts on solid associated concentrations, according to the level of biological activity. For all metals studied, low levels of biological activity (NS and A1) appear to stimulate metal capture compared to the S treatment, whereas high levels of biological activity (A2 and A3) inhibit metal capture compared to the abiotic system (Figure 4.4, 4.5). Thus, biological stimulation has a threshold level for positive impact on metal capture, after which stimulating further has a

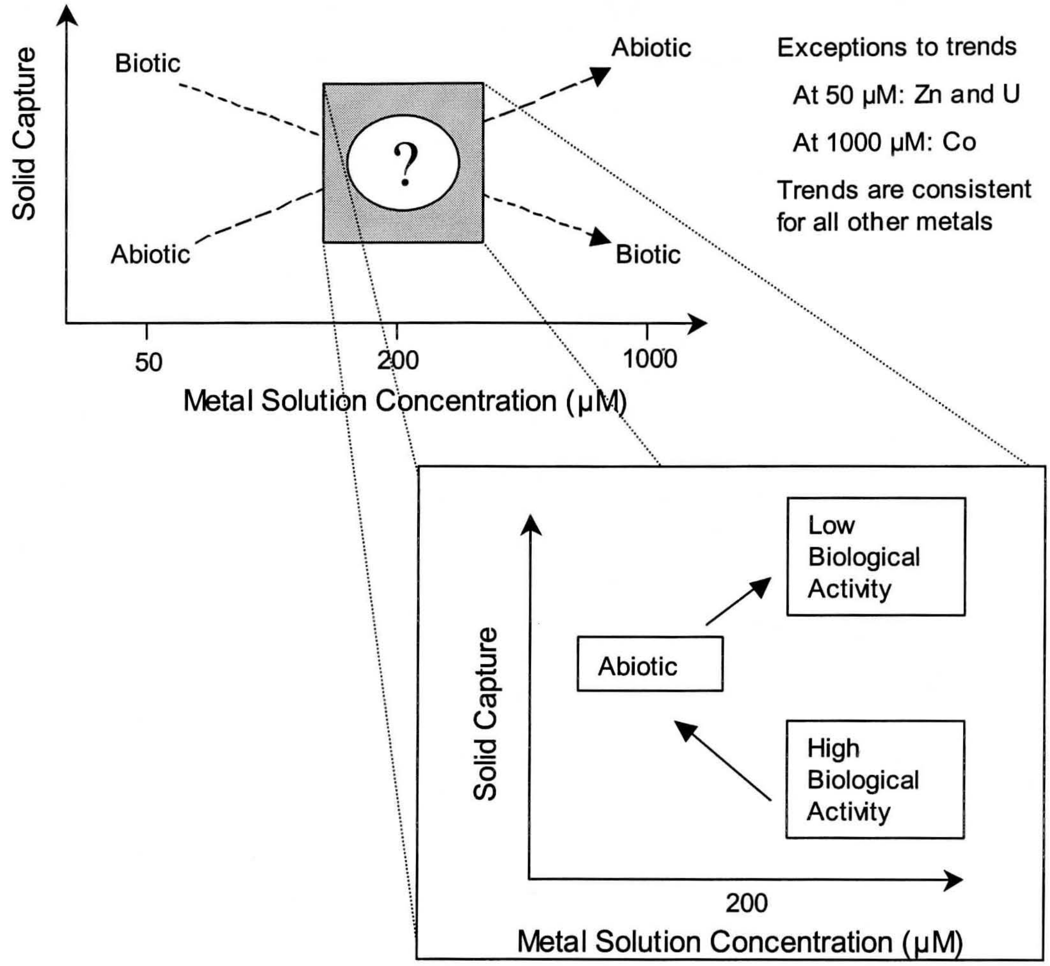

Figure 4.4: Conceptual representation of general trends observed at each solution metal concentration.

negative impact (as represented by level of carbon amendment and associated presumed levels of biological activity). Therefore, the results at this metal concentration clearly show that behaviour will depend on the interaction amongst specific elements, solution metal concentration and level of biological activity, in a non-linear, non-additive manner.

The shift from increasing to decreasing metal uptake associated with increasing levels of microbial activity with increased solution metal concentration may represent shifts in the active players within the associated microbial communities. Heavy metals have been documented as having both toxic effects (Macur et al., 2004; Oremland and Stolz, 2003; Kelly et al., 1999) as well as energy generating effects (Macur et al., 2004; Elias et al., 2003; Oremland and Stolz, 2003, Ahmann et al., 1994). While it is not possible to ascertain the exact change in the microbial community of the current study, given the concentration of metals added and the toxicity of these metals, one could infer that some microbial species would have experienced a negative or toxic effect through exposure to higher levels of metals. However, had the entire community been either shut down or killed through exposure to high metal loads, it would be expected that in the highest metal loaded microcosms (1000 μM) the biotic treatments would then behave identically to the abiotic treatment, but this was not the case. Therefore, the concentrations of metals added were not sufficient enough to kill or shut down the entire microbial community, thus it is more likely that a shift has occurred in the metabolically active bacteria species that are either resistant to the metals added, or are shifting their oxidant to the metals added for organic carbon decomposition (e.g. metal reduction), a phenomenon that is frequently observed in the literature (Gremion et al, 2004; Macur et al., 2004; Turpeinen et al., 2004; Oremland and Stolz, 2003; Cooper et al., 2000; Kelly et al., 1999).

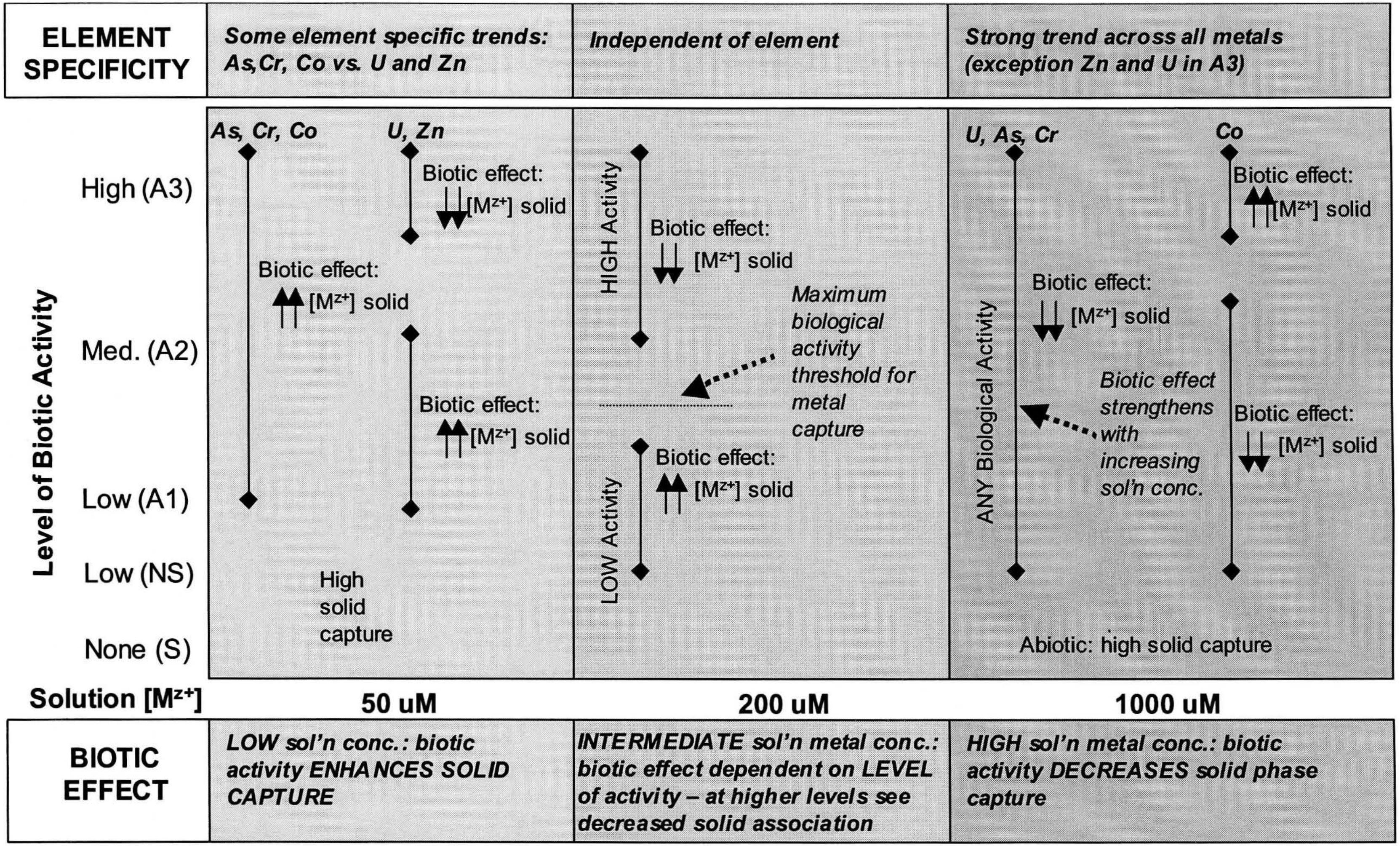

Figure 4.5: Complex interaction of biotic activity level, solution metal concentration and element involved in determining observed metal partitioning trends. In general, increasing solution concentration leads to increased solid associated metal; however biotic activity will impact the overall pattern dependent on both the amount of activity (inferred from level of carbon amendment) and the solution concentration. In general, increasing levels of biotic activity negatively impact metal retention by sediments; this effect strengthens as solution metal concentration increases.

4.1.3 Total Partitioning Coefficients

Distribution coefficients (Kd - solid metal concentration/solution metal concentration (L/kg)) permit evaluation of any shift in the relative affinities of elements for the solid vs. solution compartments independent of absolute concentration and are used widely to estimate system impacts (Curtis et al., 2004; Prokop et al., 2003; Sauvé et al., 2000; Janssen et al., 1997; Buchter et al., 1989). Typically the higher the Kd, the higher the proportion associated with the solid and therefore greater retardation of the metal (decreased transport). Total Kd values calculated for each metal in each treatment across each solution concentration synthesize the trends reported above more clearly.

In general, Kd calculations at the two extreme solution concentrations of 50 and 1000 µM indicate that values decrease as solution concentration increases; i.e. while the absolute mass of metal in the solid phase is higher at the highest solution concentration, the relative distribution or percentage in the solid phase is lower – solid phase capture efficiency decreases – for all metals (Table 4.3). However, element specific trends reflecting the complex interplay of level of biological activity and solution metal concentration are also observed in Kd values that result in a non-linear pattern from lowest to highest metal concentration treatments. For instance, the Kd values calculated for the intermediate solution metal concentration frequently do not show an intermediate value to those calculated for the low and high solution metal concentration values. That is to say that relative distribution of these elements between solution and sediment compartments is not simply a linear function of the solution concentration.

Further, element specific patterns in Kd trends also emerge, indicating that the behaviour of the specific metals evaluated differs. Most notably, Kd values for U (2.31-2.94: ave. 2.67) are the lowest observed of the 5 metals (Table 4.3, Figure 4.6) indicating a lower relative solid affinity. Zn typically showed the highest affinity (3.05-3.54: ave. 3.32), while As (2.79-3.39: ave. 3.15), Co (3.09-3.42: ave. 3.26) and Cr (2.52-3.50: ave. 3.16) showed considerable variation in their relative affinities exhibiting a non-linear dependency on both treatment as well as solution concentration (Table 4.3; Figure 4.6). Kd values are system specific, dependent on element concentration, substrate type and concentration, moisture levels and flow conditions, etc. (Curtis et al., 2004; Cooper et al., 2003; Sauvé et al., 2000; Buchter et al., 1989). Kd values within the literature often span as much as 5 orders of magnitude for any given metal, whether it be for a mono-metal study or a suite of metals (Curtis et al., 2004; Gomes et al., 2001; Sauvé et al., 2000). This makes a comparison between literature values and those observed in the current study less relevant, and comparisons of Kd values at a given experimental solution concentration impossible, however no values

Table 4.3: Total Log Kds according to metal, treatment and solution metal concentration in units of L/Kg. Values reported are ±5% standard error. * indicates significant difference for a particular metal across the concentration range.

System	**Sol'n Metal Conc. (µM)**	**Co**	**Zn**	**As**	**Cr**	**U**	**Trends**
A3	50	3.42*	3.35	3.39*	3.45*	2.63	**U<Zn≤As≤Co≤Cr**
	200	3.19	3.14	3.22*	3.05*	2.72* (wrt1000)	**U<Cr≤As≤Co≤Zn Cr≠Co,Zn**
	1000	3.19	3.22	2.83*	2.65*	2.48* (wrt200)	**U≤Cr≤As<Co≤Zn U≠As**
A2	50	3.38*	3.48	3.33	3.60*	2.63	**U<As≤Co≤Zn≤Cr As≠Cr**
	200	3.21	3.29	3.20	3.23*	2.70	**U<As≤Co≤Cr≤Zn**
	1000	3.12	3.05*	2.57*	2.52*	2.31*	**U≤Cr≤As<Zn≤Co U≠As**
A1	50	3.39	3.52	3.36	3.50	2.73	**U<As≤Co≤Cr≤Zn**
	200	3.32	3.37	3.39	3.35	2.91* (wrt1000)	**U<As≤Co≤Cr≤Zn**
	1000	3.09*	3.06*	2.79*	2.72*	2.48* (wrt200)	**U≤Cr≤As<Zn≤Co U≠Zn,Co**
NS	50	3.25	3.45	3.10	3.33	2.54	**U<As≤Co≤Cr≤Zn As,Co≠Zn As≠Cr**
	200	3.35* (wrt1000)	3.54	3.35* (wrt1000)	3.39	2.94*	**U<Co≤As≤Cr≤Zn**
	1000	3.14* (wrt200)	3.16*	2.94* (wrt200)	2.91*	2.60	**U≤Cr≤As<Co≤Zn**
S	50	3.35	3.55*	3.23	3.43* (wrt1000)	2.71	**U<As≤Co≤Cr≤Zn**
	200	3.28	3.35	3.28	3.23	2.83	**U<Cr≤As≤Co≤Zn**
	1000	3.15*	3.20	3.21	3.09* (wrt50)	2.81	**U≤Cr≤Co≤Zn≤As U≠Co,Zn,As**
Trends	50	NS≤S≤A2≤ A1≤A3 **NS≠A3**	A3≤NS≤A2 ≤A1≤S	NS≤S≤A2≤ A1≤A3 **NS≠A1,A3**	NS≤S≤A3≤ A1≤A2 **NS≠A2**	NS≤A3≤A2 ≤S≤A1	
	200	A3≤A2≤S≤ A1≤NS **A3≠NS**	A3≤A2≤S≤ A1<NS **A3≠S,A1**	A2≤A3≤S≤ NS≤A1	A3≤A2≤S≤ A1≤NS **A3≠A1,NS**	A2≤A3≤S≤ A1≤NS	
	1000	A1≤A2≤NS ≤S≤A3	A2≤A1≤NS ≤S≤A3	A2≤A1≤A3 ≤NS≤S **A2,A1≠S**	A3≤A2≤A1 ≤NS≤S	A2≤A3≤A1 ≤NS≤S **A2,A3,A1≠ S**	

observed for this study fall outside the range of Kd values expressed in the literature (Curtis et al., 2004; Cooper et al., 2003; Gomes et al., 2001; Sauvé et al., 2000).

Comparisons between the results of this study and predicted selectivities of metals poses another challenge due to the number of elements and potential element combinations in conjunction with the limited number of studies evaluating metal cocktails in sorption experiments. In addition, studies that have been conducted on a suite of metals often examined different sets of metals under different system conditions (i.e. type of sediment, pH and redox conditions etc.). Several researchers have predicted Co to form stronger bonds with soil than Zn based on differing mechanisms (i.e. ionic radius and ionization potential (Sposito, 1989) and either electronegativity or electrostatics (McBride, 1994)). However, Schwertmann and Taylor (1989) and Hsu (1989) predict the opposite in the presence of oxides, a substrate that is clearly present in INL soil (Cooper et al., 2003). Therefore the slightly higher averaged Kd values observed for Zn may potentially be attributed to interactions with oxide materials. Relative selectivities for the other metals within comparable environmental settings were not found within the literature. Thus, this is one of the first studies to evaluate the relative affinities of a suite of metals for the solid phases over varying metal concentrations and levels of biological activity. My results indicate that differing affinities occur across this suite of metals, and that further, their relative affinities are dependent on the concentrations and biological activity present. These results would indicate that predicting metal behaviour based on mono-metal behaviour experiments is likely to be with limited success.

A notable trend also emerged for the relative affinities of Cr across the concentration range examined in this thesis. Unlike the other metals, the relative affinity of Cr decreases with increasing solution concentration for all treatments. For instance, at the lowest nominal solution metal concentration of 50 µM, Cr has the highest or second highest Kd across all treatment systems (compared to the other metals in solution), however at the highest solution concentration examined, 1000 µM, Cr has the second lowest Kd compared to the other metals for all systems (Table 4.3). This result may reflect competition amongst various metals; as the solute to potential binding site ratio increases Cr is being out competed by the other metals for increasingly less frequent available binding sites.

Trends in Kd values across systems indicate that the influence of biological activity is metal specific (i.e. some metal behaviour is influenced by biological activity while others appear independent of biological activity; Figure 4.6). Log Kd values for Co and Zn show little to no significant differences across systems and across the concentration range, with no clear non-significant trends either (Table 4.3). Trends do, however, emerge for U, Cr and As. For these metals at the lowest nominal solution metal concentration (50 µM), the amended treatments typically have the greatest Kd values indicating that biological activity is stimulating soil metal retention (with the exception of Kd values for U in the Sterile system which are similar to the amended values, Table 4.3). However the Kds for all three of these elements decrease with increasing solution concentration, showing that observable biological activity impacts on sediment retention are concentration dependent (Table 4.3, Figure 4.6). Thus, at the highest solution concentration (1000 μM), the amended treatments typically have the lowest Kds, while the Sterile system has the highest Kd. However, at the intermediate solution metal concentration of 200 μM, the most biologically active systems (A2 and A3) have the lowest Kd values for these metals, while the low biologically active systems (Non-Sterile and A1) have the highest total partitioning coefficients (Table 4.2, Figure 4.6), which is consistent with the shift in partitioning illustrated in Figure 4.4.

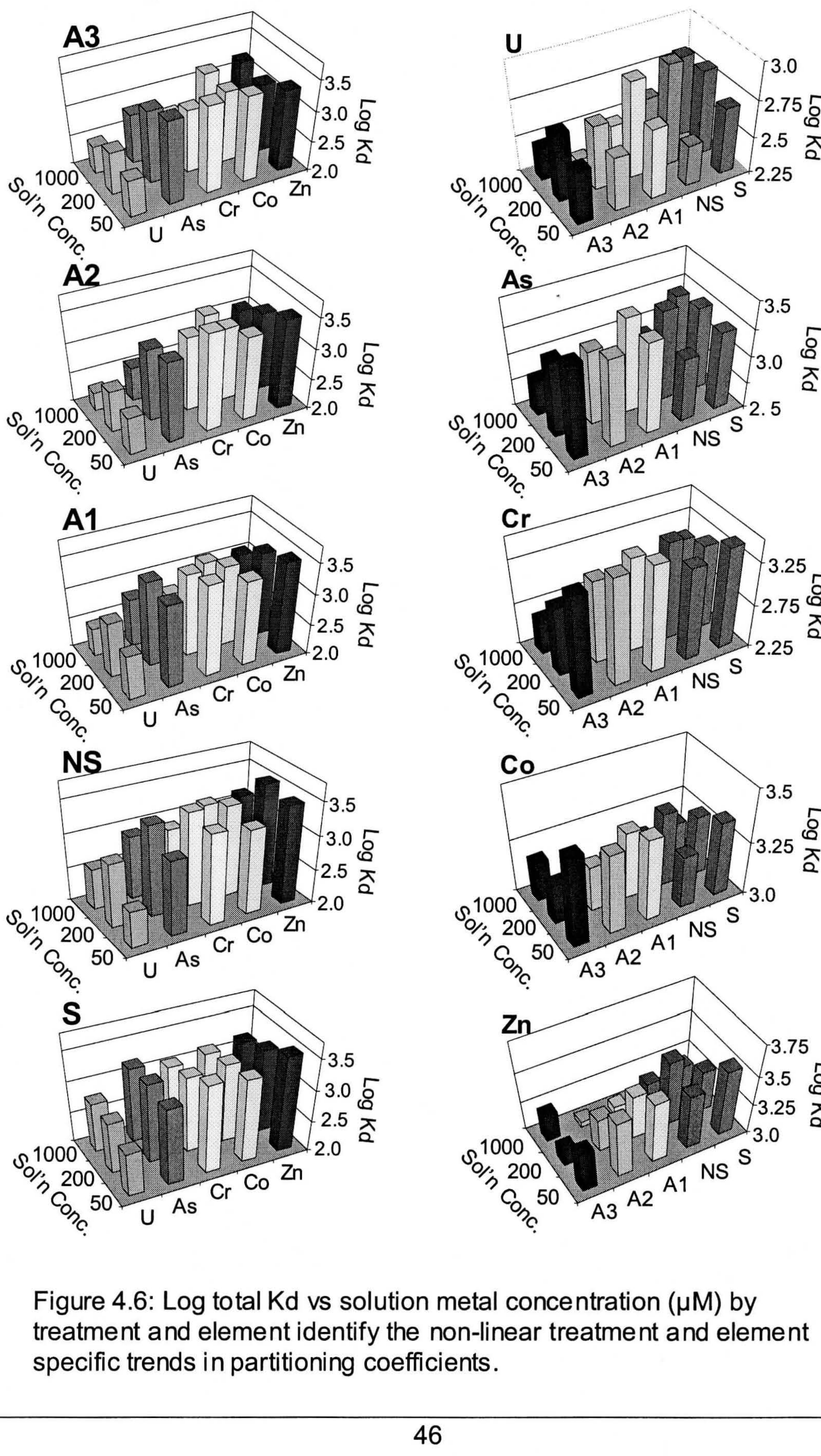

Figure 4.6: Log total Kd vs solution metal concentration (µM) by treatment and element identify the non-linear treatment and element specific trends in partitioning coefficients.

4.2 Fractional Compartment Sediment Associations

Evaluation of metal partitioning for the suite of 5 metals examined amongst potential sediment fractions in the INL soil also shows a dependence on the total metal solution concentration (Figure 4.7). While detectable levels of metals were observed in all sediment fractions, the two dominant metal sequestration phases were the amorphous oxide and carbonate fractions (Figure 4.7).

Bulk Kd values indicate the oxidizable fraction was an efficient metal sequester and current literature has emphasized the reactivity of this substrate (Warren and Haack, 2001; Sauvé et al., 2000; Zuyi et al., 2000; Tessier et al., 1996; Warren and Zimmerman, 1994a; Buffle, 1988; Kerndorff and Schnitzer, 1980). However, Kd values are normalized to substrate mass and indicate relative affinity. Very little organic sediments actually occur in the INL sediment (0.25% wt. Cooper et al., 2003), thus this fraction, while highly metal reactive, is only a very small sink for metals (typically less than 5%) due to its low absolute mass in the INL soil.

Total metal (i.e. sum of all elements) sorbent-specific Kd values show oxides (combined amorphous and crystalline oxides) to also be the most efficient metal sequesters (average log Kd of 4.62 ± 0.04; Table 4.4) while carbonate Kd values were approximately a log unit lower (average log Kd of 3.67 ± 0.05; Table 4.4) indicating a lower relative affinity for metals to carbonates compared to the oxides. Given the larger mass of these substrates compared to the oxidizable fraction (approximately 10% wt. carbonates and 2% wt. oxides vs. 0.25% wt. organic, Cooper et al., 2003), both of these compartments were large metal sinks for the INL sediments; which one was most important depended on the solute concentration. The relative importance of the different substrates shifts from the amorphous oxides at the lowest solution concentration (amorphous oxides – accounts for approximately 50% of solid associated metals, carbonates – approximately 20%) to the carbonates at the highest solution concentration (amorphous oxides – approximately 30% of solid associated metals, carbonates – approximately 50%; Figure 4.8) and this trend is consistent across all treatments. This result indicates that the amorphous oxides have a high affinity for the metals at low concentrations, but that as total solution concentration increases, their

Table 4.4: Total metal bulk Kds for each of the assessed sediment fractions (sorbents). Values reported are the average for total metals across all solution metal concentrations and treatments ± standard error.

Sorbent	Bulk Kd
Leachable	1.56 ± 0.06
Carbonate	3.67 ± 0.05
Oxide	4.62 ± 0.04
Organic	4.4 ± 0.1

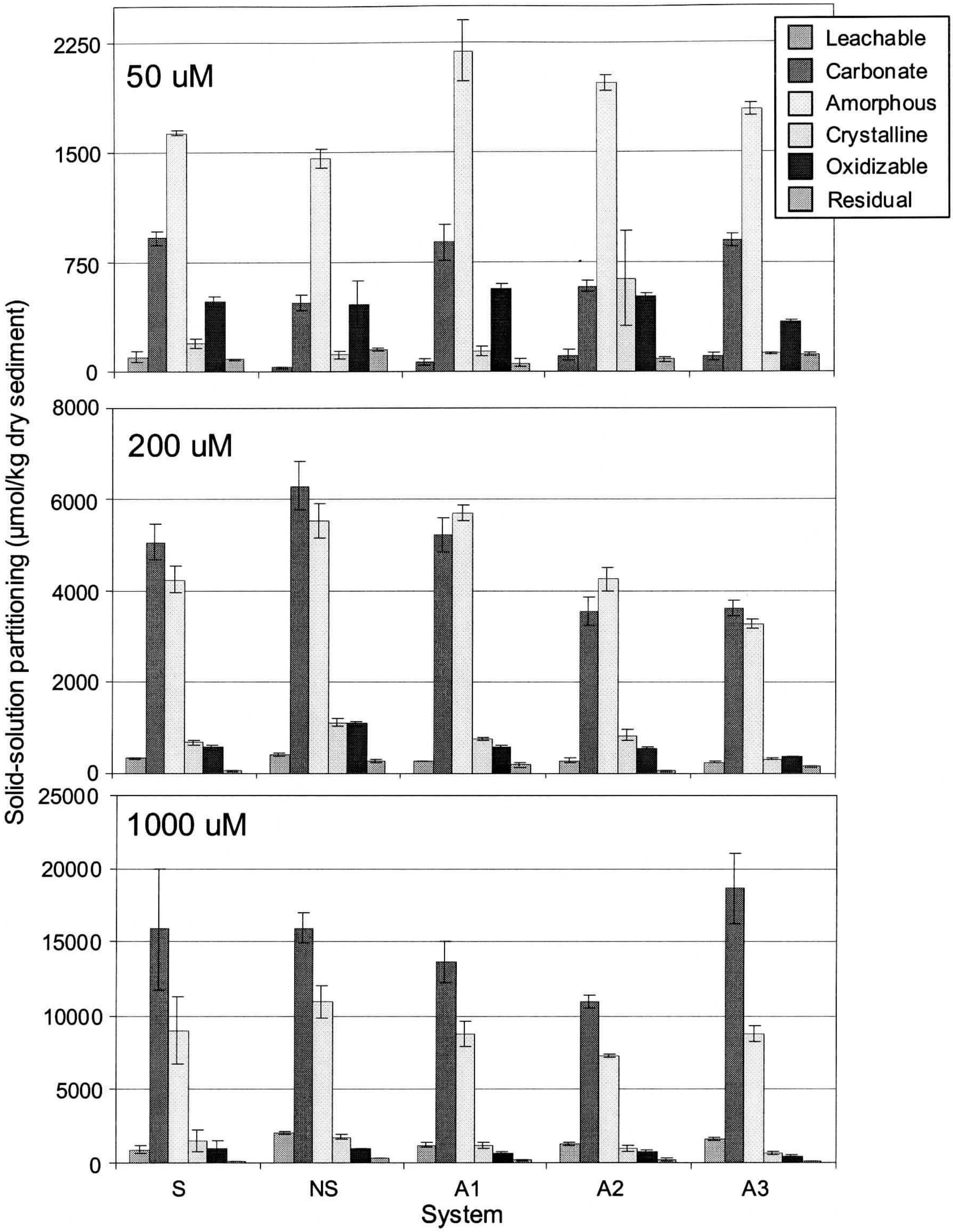

Figure 4.7: Total metal partitioning by sediment phase for each system at each total metal solution concentration.

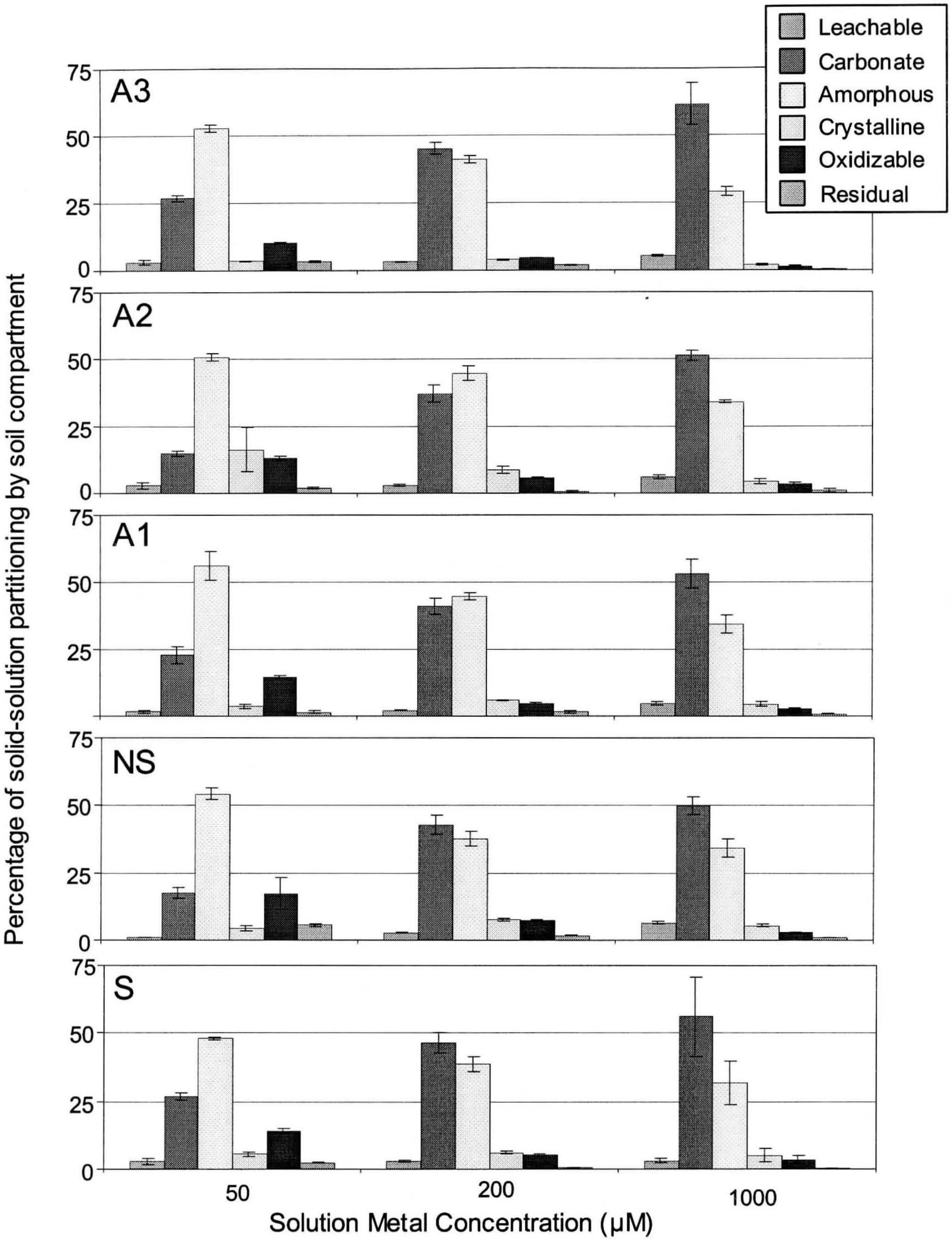

Figure 4.8: Relative partitioning of total metal (sum of individual elements) to each of the compartmental fractions.

relative affinity in relation to the carbonate fraction decreases. This result may indicate substrate saturation (or approaching saturation) for the oxide fraction, with resulting metal "spillover" then being sorbed by the carbonates. This result also indicates that sorbent metal uptake dynamically shifts as a greater concentration of metals in solution occurs, independent of biological activity (i.e. the same trend was seen in all treatment systems). The implication of this result is that subsequent behaviour of solid associated metals will shift also as the mechanisms that affect metal carbonates are different from those that affect metal oxides (i.e. changes in pH vs. changes in redox selectively affect carbonate and oxide reactivity (Kneebone et al., 2002; Spliethoft et al., 1995; Kuhn and Sigg, 1993; Wetzel 1983; Tessier et al., 1979).

Element specific solid associations within the sediment fractional compartments typically show the classic trend of increased partitioning with increased solution metal concentration (Figure 4.9). This result indicates saturation did not occur over the solute concentration range examined. The only exception to this trend occurred for Cr associations with the oxidizable and residual fractions, which remained constant irrespective of solution metal concentration (including soil samples processed prior to any metal addition in order to assess background metal concentrations) suggesting Cr was associated with these fractions prior to the present study in a very robust manner, remaining bound to these components despite other metal additions and system manipulations (Figure 4.9).

Individual element sediment associations show three major trends (Figure 4.9). As, Zn and Co partitioned predominantly to the carbonate (typically 20-50%) and amorphous fractions (typically 30-70%), whilst the dominant fractions for U were the leachable (10-20%) and carbonate fractions (80-90%), and Cr shows a unique pattern; whilst the majority of Cr partitioned to the amorphous oxide fraction (30-50%), there was substantial spread across all fractions examined (at least 5% in all fractions) across treatment systems.

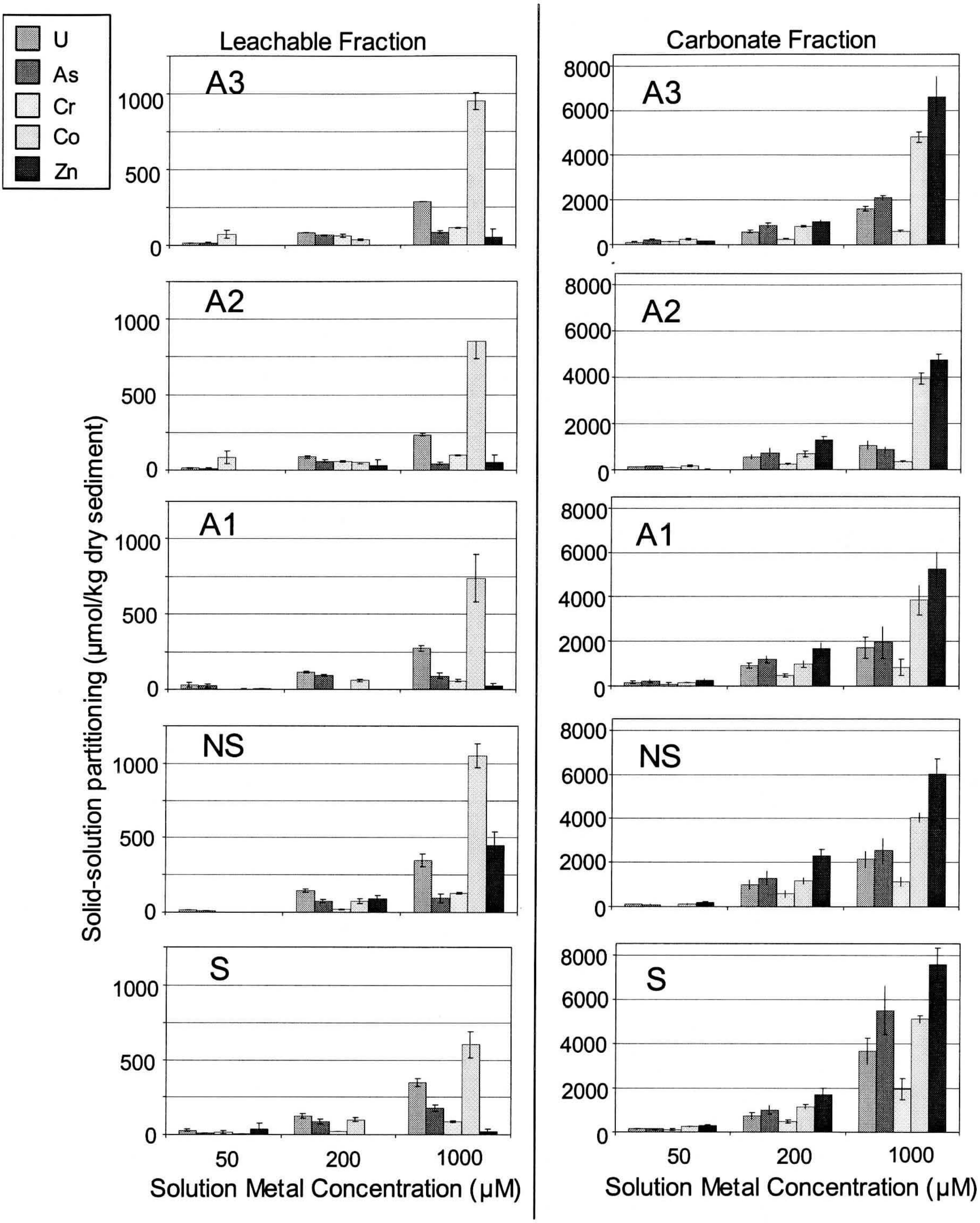

Figure 4.9a: Solid associated concentration of individual metals in the **leachable** and **carbonate** fractions for each treatment as a function of solution concentration. Mean values ± standard error are shown (n=3).

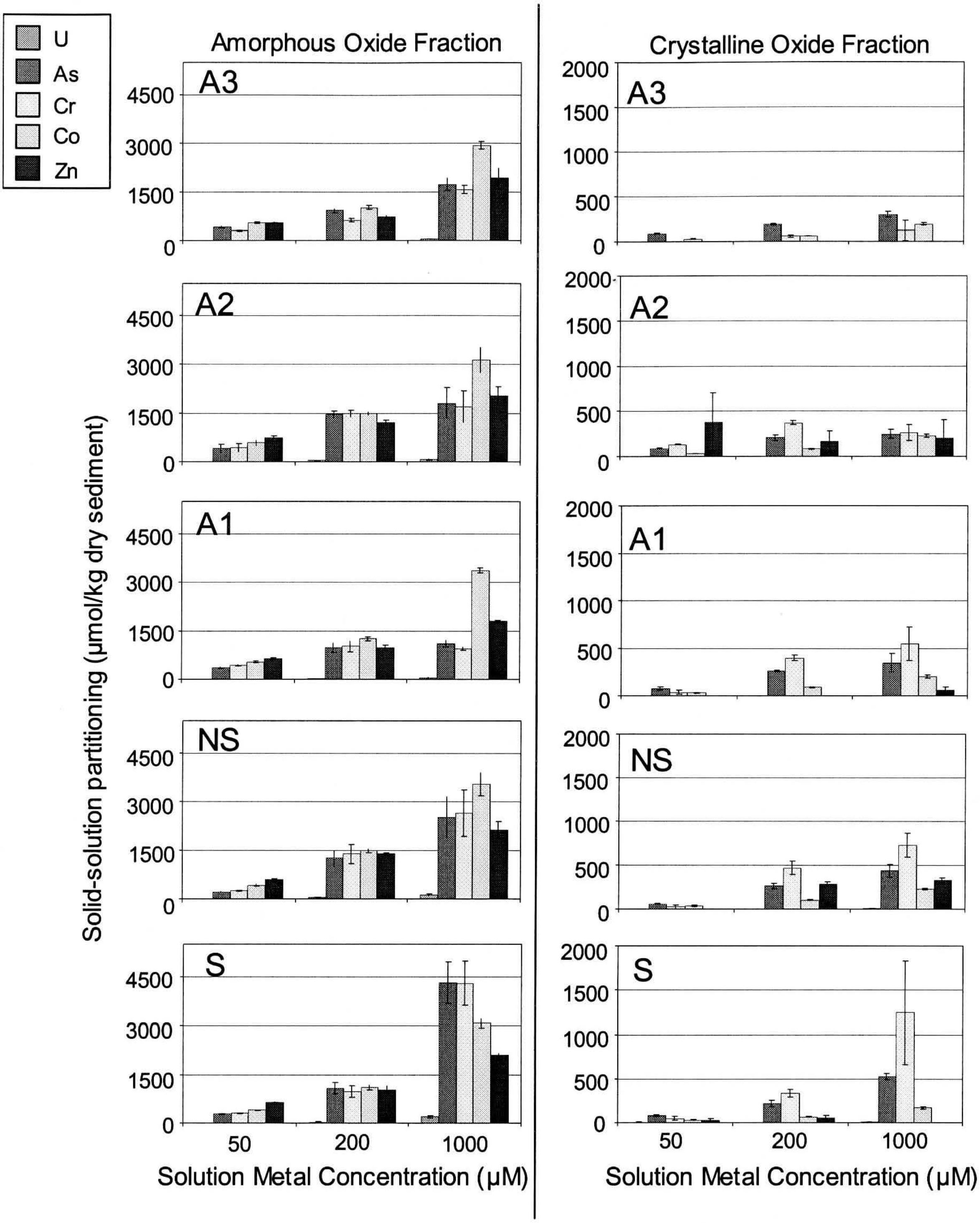

Figure 4.9b: Solid associated concentration of individual metals in the **amorphous** and **crystalline oxide** fractions for each treatment as a function of solution concentration. Mean values ± standard error are shown (n=3).

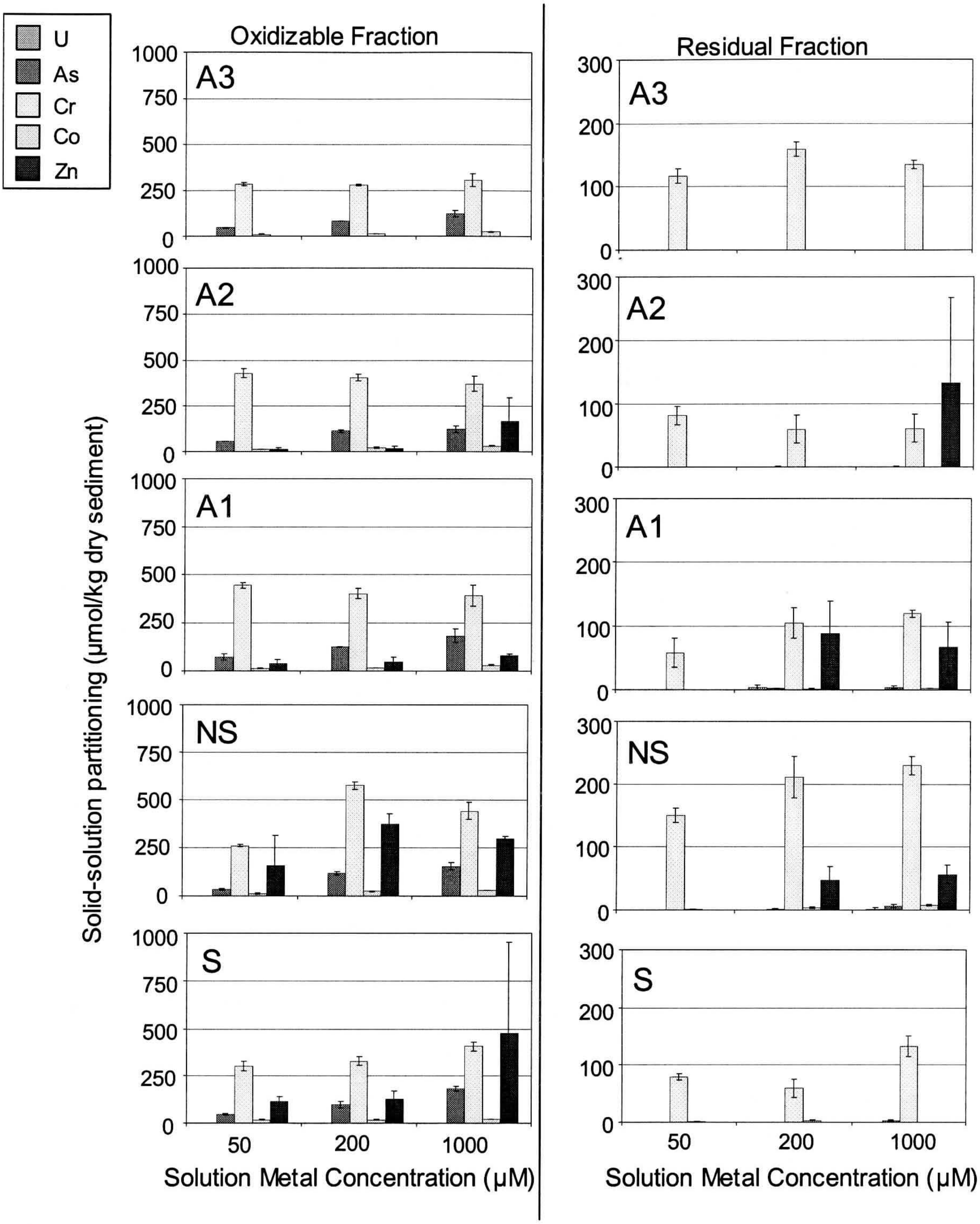

Figure 4.9c: Solid associated concentration of individual metals in the **oxidizable** and **residual** fractions for each treatment as a function of solution concentration. Mean values ± standard error are shown (n=3).

The dynamic shift observed in relative importance of fractional compartments for total metals associated with the sediment, in conjunction with the general trend discussed previously of complex interplay between solute concentration, the element involved and the level of biological activity facilitates the need for discussion of these dominant compartments in order to assess the effects of (1) solid-metal associations, (2) metal competition, as well as (3) the impact of biological activity on element specific sorbent associations.

4.2.1 Oxide Fraction

Oxide material within INL sediments only accounts for approximately 2% of the total sediment mass (Cooper et al., 2003); however this fraction proved highly reactive reporting the highest average log Kds for all the sediment compartments analyzed for all metals (ranged from approximately 4-5 – with the exception of U which did not partition to this fraction – Table 4.5). This result is consistent with the well documented high reactivity of this sediment compartment within the literature (Warren and Haack, 2001; Turner, 2000; Robertson and Leckie, 1998; Hall et al., 1996; Tessier et al, 1996; Chao and Theobald, 1976). The tendency of oxides to form surface coatings on sediments would also account for the importance of this fraction for metal sequestration despite the relatively small mass of the oxides within the sediment (Warren and Haack, 2001; Robertson and Leckie, 1998).

Amorphous and crystalline oxide values were combined for a more comprehensive assessment of this oxide sink. No statistically significant difference in average Kd values (averages calculated include all systems and nominal solution metal concentration values) was observed for individual metals associated with the oxide compartment. Log values ranged from 4.6 ± 0.3 to 4.7 ± 0.2, indicating that all metals examined (As, Cr, Zn and Co) had a similar affinity for this substrate. Comparison of these values to those reported in the literature pose some difficulty given the highly system specific nature of Kds and their sensitivities to the composition of reactive particulate matter, as well as redox and pH properties (Prokop et al., 2003; Ferris et al., 2000; Sauvé et al., 2000; Radovanovic and Koelmans, 1998; Warren and Zimmerman, 1994b). However, these values fall within the range of values typically reported for oxide metal reactivities (log Kd values range from approximately 2 to 5; Ferris, 2005 (U); Wazne et al., 2003 (U); Ferris et al., 2000 (U); Lofts and Tipping, 2000 (Co, Zn)).

However, substantial decreases (and often statistically significant decreases) in partitioning coefficients were observed for all metals and systems for the oxide compartment as a function of solution metal concentration (Table

Table 4.5: Log Kd values for Oxide compartment according to metal, treatment and solution metal concentration in units of L/Kg. Values reported are ±5% standard error. * indicates significant difference for a particular metal across the concentration range.

System	**Sol'n Metal Conc. (µM)**	**Co**	**Zn**	**As**	**Cr**	**Trends**
A3	50	4.96*	4.93*	4.89*	4.67*	**Cr<As≤Zn≤Co**
	200	4.62*	4.45*	4.64*	4.42*	**Cr≤Zn<Co≤As**
	1000	4.41*	4.24*	4.20*	4.12*	Cr≤As≤Zn≤Co
A2	50	4.95*	5.17*	4.85	4.95*	As≤Cr≤Co≤Zn
	200	4.72*	4.65*	4.66	4.73*	**Co<Zn≤As≤Cr**
	1000	4.45*	4.19*	4.02*	3.98*	**As≤Cr≤Zn<Co**
A1	50	4.97*	5.07*	4.85	4.82	**Cr<As≤Co≤Zn**
	200	4.79*	4.68*	4.83	4.87	**Zn<Co≤As≤Cr**
	1000	4.41*	4.21*	4.20*	4.20*	As≤Zn≤Co≤Cr
NS	50	4.84	4.96*	4.63	4.63	Cr≤As<Co≤Zn
	200	4.79	4.81*	4.76	4.84* (wrt1000)	As≤Co≤Zn≤Cr
	1000	4.47*	4.28*	4.34*	4.40* (wrt200)	Zn≤As≤Cr≤Co
S	50	4.84*	5.03*	4.74	4.73	**Cr≤A<Co<Zn**
	200	4.66*	4.63*	4.69	4.70	Zn≤Co≤As≤Cr
	1000	4.41*	4.22*	4.57	4.62	**Zn<Co<As<Cr**
Trends	50	S≤NS≤A2≤A3≤A1	A3≤NS≤S≤A1≤A2	NS≤S≤A2≤A1≤A3	NS≤A3≤S≤A1≤A2	
	200	A3≤S≤A2≤NS≤A1 **A3≠A2,NS,A1** **S≠NS,A1**	**A3<S≤A2≤A1<NS**	A3≤A2≤S≤NS≤A1	**A3<S≤A2≤NS≤A1**	
	1000	S≤A1≤A3≤A2≤NS	A2≤A1≤S≤A3≤NS	A2≤A1≤A3≤NS≤S **A2,A1,A3≠S**	A2≤A3≤A1≤NS≤S **A2,A3,A1≠S**	

4.5). Thus, as the concentration of metals in solution increases, the relative importance of this fraction (compared to the other fractions examined) decreases, irrespective of biological activity. This may be a result of (1) physical alteration of the oxide fraction, where the mass of the substrate may be decreasing, or (2) metal sorption saturation for this solid at higher solution concentrations. In reducing environments oxide materials can be reduced resulting in dissolution of the oxide (i.e. decrease in substrate mass) which would result in the decreased ability to partition metals which is consistent with the results observed. However, the literature indicates reduction of oxides is often the result of biological activity (Kneebone et al., 2002; Zachara et al., 2001; Spliethoft et al., 1995; Khun and Sigg, 1993) and given that the trend was consistent across all treatments (including the abiotic system), any decrease in mass would have to be attributed to abiotic processes. The more likely alternative scenario is that with increased metals in solution, oxide binding sites are being filled and the fraction may be nearing or exceeding the saturation point, thus the ratio of sorbate to sorbent would be decreasing and the Kd value would also decrease to reflect this ratio.

The impact of biological activity on partitioning to the oxide compartment proved to be complex, depending on solution metal concentration and element identity, again necessitating the in depth discussion of the biological impact according to each metal which can be found in section 4.3.

4.2.2 Carbonate Fraction

Metals showed a much lower affinity for the carbonate fraction compared to the oxide fraction, exhibiting log Kd values an order of magnitude lower than for that of the oxide fraction (average log Kd values for specific elements ranging from 3.3 ± 0.3 to 3.9 ± 0.2 for the carbonates; Table 4.6). Furthermore, the suite of metals partitioned to the carbonate fraction show differing affinities to this substrate, a result that was not observed for the oxide fraction. In general the log Kd values for the carbonate fraction increase in the following order: Cr<U<As<Co<Zn (Table 4.6).

Another distinctive difference from trends observed for the oxides is that the carbonate Kd values do not simply decrease with increased solution metal concentration. Trends for carbonate Kd values across the concentration range are complex, exhibiting a dependency on biological activity. Kd values for systems with little to no biological activity (S, NS and A1) peak at the intermediate 200 µM solution metal concentration for all metals; however this trend breaks down for the systems with higher biological activity (this trend fails for 2 of the 5 metals for A2, and 4 of the 5 metals for A3 – Table 4.6). The influence of biological activity on carbonate metal sequestration shows a strong solution concentration dependence. While no consistent trend can be identified across the different elements for biological activity at the 50 µM concentration, at the 200 µM concentration high levels of biological activity (A2 and A3) are detrimental to metal sequestration to this fraction (for all metals) and at the 1000 µM concentration any level of biological activity impedes metal capture for all elements considered (Table 4.6). These results indicate that the sequestration of metals specifically by the carbonate pool is dynamically impacted by biological activity.

Current literature suggests that biological activity can decrease the pH of localized microbially active areas through microbial respiration (production of CO_2), which in turn could lead to the dissolution of solid carbonates and the release of metal cations (Song et al., 1999; Mahan et al., 1987; Wetzel, 1983). The results of this study are consistent with the hypothesis of either increasing dissolution of carbonates or decreased sorptive abilities (Prokop et al., 2003) occurring in the vicinity of microbial activity, as the level of biological activity increases and associated pH decreases. A potential decrease in either the carbonate substrates available for metal sequestration or their relative sorptive capacity would account for the observed lower Kd values. While this study cannot directly assess which mechanism is occurring, the results clearly support the links between microbial activity and metal retention for the carbonates in this INL soil.

Aerobic microbial respiration

$$CH_2O + O_2 \longrightarrow CO_2 + H^+ \quad \text{Equation 4.1}$$

These results, also clearly show that cyclic linkages between metal concentration and biological activity also need to be considered in any interpretation of metal sediment biogeochemical behaviour. Differential impacts of biological activity on metal solid retention as a function of solution metal concentration was observed for the carbonate fraction (Figure 4.9a). This result would suggest that the metals within the microcosms are either impacting levels of metabolism/respiration directly, or that microbial activity is being differentially altered by the varying levels of metal in solution (Gremion, et al., 2004; Kelly et al., 1999). Higher concentrations of metals in solution may in fact be having toxic effects on some microbial strains, which could shift the selective advantage to other more metal resistant strains with different metabolisms (Macur et al., 2004; Turpeinen et al., 2004; Kelly et al., 1999).

Table 4.6: Log Kd values for Carbonate compartment according to metal, treatment and solution metal concentration. Values reported are ±5% standard error. * indicates significant difference for a particular metal across the concentration range.

System	**Sol'n Metal Conc. (µM)**	**Co**	**Zn**	**As**	**Cr**	**U**	**Trends**
A3	50	3.91	3.71* (wrt1000)	3.85	3.61*	3.58	**U≤Cr≤Zn≤As≤Co** **U,Cr,Zn≠Co** **U,Cr≠As**
	200	3.81	3.91	3.82	3.32*	3.66	**Cr<U≤Co≤As≤Zn** **U≠Zn**
	1000	3.88	4.01* (wrt50)	3.52*	2.98*	3.39*	**Cr<U≤As<Co≤Zn**
A2	50	3.71	3.18*	3.72	3.52*	3.58	**Zn<Cr≤U≤Co≤As**
	200	3.71	4.00	3.74	3.26*	3.62	**Cr<U≤As≤Co≤Zn** **U≠Zn**
	1000	3.79	3.87	3.13*	2.75*	3.20*	**Cr<As≤U<Co≤Zn**
A1	50	3.70	3.92	3.78	3.42	3.66	**Cr<U≤Co≤As≤Zn**
	200	3.88	4.10	3.97* (wrt1000)	3.55	3.84* (wrt1000)	**Cr<U≤Co≤As≤Zn** **U≠Zn**
	1000	3.77	3.91	3.42* (wrt200)	3.02	3.39* (wrt200)	**Cr≤U≤As≤Co≤Zn**
NS	50	3.50*	3.74* (wrt200)	3.41	--	3.46	**Cr<As≤U≤Co≤Zn**
	200	3.95*	4.25* (wrt50)	3.97*	3.61*	3.86*	**Cr≤U≤Co≤As≤Zn** **Cr≠Zn**
	1000	3.80	3.97	3.58	3.23*	3.51	**Cr≤U≤As≤Co≤Zn** **Cr≠As,Co,Zn** **U≠Co,Zn** **As≠Zn**
S	50	3.89	3.97	3.61* (wrt1000)	3.49	3.61	**Cr≤U≤As<Co≤Zn**
	200	3.95	4.11	3.88	3.56	3.75	**Cr≤U≤As≤Co≤Zn** **Cr≠As,Co,Zn** **U≠Zn**
	1000	3.91	4.07	3.92* (wrt50)	3.46	3.75	**Cr<U≤Co≤As≤Zn** **Cr≠Co,As,Zn** **U≠Zn**
Trends	50	NS≤A1≤A2≤S≤A3 **NS≠S,A3**	A2≤A3≤NS≤A1≤S **A2≠A1,S**	NS≤S≤A2≤A1≤A3 **S≠A1,A3**	NS≤A1≤S≤A2≤A3	NS≤A3≤A2≤S≤A1	
	200	A2≤A3≤A1≤NS≤S	A3≤A2≤A1≤S≤NS	A2≤A3≤S≤NS≤A1	A2≤A3≤A1≤S≤NS	A2≤A3≤S≤A1≤NS	
	1000	A1≤A2≤NS≤A3≤S	A2≤A1≤NS≤A3≤S	A2≤A1≤A3≤NS<S **A2≠A3,NS**	A2≤A3≤A1≤NS≤S **A2≠NS,S** **A3≠S**	A2≤A1≤A3≤NS≤S **A2≠NS,S** **A1,A3≠S**	

4.2.3 Leachable Fraction

The leachable fraction typically represents the most labile metal fraction of the sediment metal pool. In general, this fraction is interpreted to represent weakly surface associated metal species that are easily released given changes in pH or ionic strength (Hall et al., 1996; Mahan et al., 1987). Not surprisingly, results from this study indicate that partitioning to the leachable fraction is highly variable, showing distinct differences in affinity across the metals for this fraction (Table 4.7). Average Kd values for individual metals (averaged from all solution metal concentration values across all systems) ranged from 1.4 ± 0.4 (for Cr) to 1.8 ± 0.1 (for U) - excluding Zn, as partitioning of Zn to this fraction was negligible. Relative affinities across the solution concentration range are element specific, unlike either the oxide fraction (which showed decreasing Kd values across the concentration range) or the carbonate fraction, which showed a fairly consistent trend for all metals examined, of generally the highest Kd value at the 200 µM concentration (Table 4.7). Both As and U leachable Kd values typically peaked at the 200 µM concentration and for As the lowest Kd values are reported at the 1000 µM concentration for all systems (Table 4.7). Co Kd values increased with increased metal in solution for all systems, and Cr was completely treatment dependent preventing a simple trend description.

Biological activity was also a factor for the association of metals with this fraction (Table 4.7). However, the influence of biological activity was complex being both element and solution concentration dependent, precluding a simple description, therefore biological impact will be discussed in the following section according to element.

Table 4.7: Log Kd values for Leachable compartment according to metal, treatment and solution metal concentration. Values reported are ±5% standard error. * indicates significant difference for a particular metal across the concentration range.

System	**Sol'n Metal Conc. (µM)**	**Co**	**As**	**Cr**	**U**	**Trends**
A3	50	--	1.75	2.31*	1.63	**Co<U≤As<Cr**
	200	1.47*	1.73	1.68*	1.83	**Co<Cr≤As≤U** **Cr≠U**
	1000	2.20*	0.75*	1.26*	1.65	**As≤Cr≤U≤Co** **As≠Co**
A2	50	0.28*	1.59	2.33	1.63	**Co<As≤U<Cr**
	200	1.59	1.66	1.65	1.83*	**Cr≤As≤U≤Co**
	1000	2.12	0.82*	1.19	1.56	**As≤U≤Co≤Cr**
A1	50	0.39*	1.80	--	1.88	**Co≤Cr≤As≤U** **Co≠As,U**
	200	1.68	1.87	--	1.96	**Cr<Co<As≤U**
	1000	2.04	1.14*	0.97*	1.62	**Cr<As≤U≤Co** **As≠Co**
NS	50	0.16*	1.50	--	1.71	**Cr<Co<As≤U**
	200	1.76*	1.76	1.17	2.05*	**Cr<As≤Co≤U**
	1000	2.22*	1.14*	1.30	1.72	**As≤Cr<U<Co**
S	50	1.25*	1.60	1.18	1.94	**Cr≤Co≤As≤U**
	200	1.87	1.82*	1.25	1.98* (wrA1000)	**Cr<U≤As≤Co**
	1000	1.97	1.44	1.13	1.73* (wrA200)	**Cr≤As<Co≤U**
Trends	50	A3≤NS≤A2≤ A1≤S **A3,NS,A2≠S**	NS≤A2≤S≤ A3≤A1	NS=A1<S≤ A3≤A2	A3≤A2≤NS≤ A1≤S	
	200	A3≤A2≤A1≤ NS≤S **A3≠NS,S** **A2≠S**	A2≤A3≤NS≤ S≤A1 **A2≠A1**	A1<NS≤S< A2≤A3	A3≤A2<A1≤S ≤NS	
	1000	S≤A1≤A2≤A3 ≤NS	A3≤A2≤A1≤ NS≤S **A3≠S**	A1≤S≤A2≤A3 ≤NS	A2≤A1≤A3≤ NS≤S **A2≠NS**	

4.3 Element Specific Behaviour

While discussion of the dominant fractional compartments allows for examination of general trends for a given substrate and has demonstrated a dynamic shift in substrate importance for total metals as well as some individual metals, the different properties of each element as well as clear element specificity within these fractions warrants the discussion of each individual metals in order to clearly assess (1) changes in the sorbent importance and (2) impact of biological activity over the concentration range for each element in the suite of metals.

4.3.1 Uranium

Despite varying U concentrations and levels of microbial activity associated with the sediment phase across experimental treatments, the relative partitioning of U a was constant with approximately 80% of total U sequestered by the carbonate soil fraction and the remaining 20% partitioned to the leachable fraction (Figure 4.10). These results indicate an equilibrium distribution of U, independent of metal concentration and/or microbial activity, under the given experimental conditions.

An unexpected result of this study was the lack of U sequestration by the oxide fraction, given that many researchers have emphasized the strong affinity of U for oxide minerals (Lack et al., 2002; Gabriel et al., 1998; Thomas et al., 1993; Hsi and Langmuir, 1985). However, most literature studies examining U sorption behaviour have not done so using metal cocktails. These results may reflect metal competition for a limited oxide pool within the sediment, U may have a lower affinity to the oxides compared to the other metals examined. Another explanation for this result may be that microbial respiration involving a coupled reduction of Mn oxides and U oxidation is responsible for the oxidation of $UO_{2(s)}$ to the soluble U(VI) species (Fredrickson et al., 2002). However the quantity of Mn oxides is quite small in the INL soil (Cooper et al., 2003) compared to that of the Fredrickson et al. study. Furthermore, had this been a dominant mechanism, one would expect either of two outcomes: (1) with increased metal solution concentration the ability of Mn oxides to oxidize all of the U present would decrease, thus we would see increased relative immobilization at higher solution metal concentrations, or (2) a higher level of oxide associated U for the abiotic treatment for all concentrations, and neither of these outcomes were observed. The most likely explanation comes from recent work by Wazne et al. (2003), which showed the same result (i.e. little to no U associated with the oxide

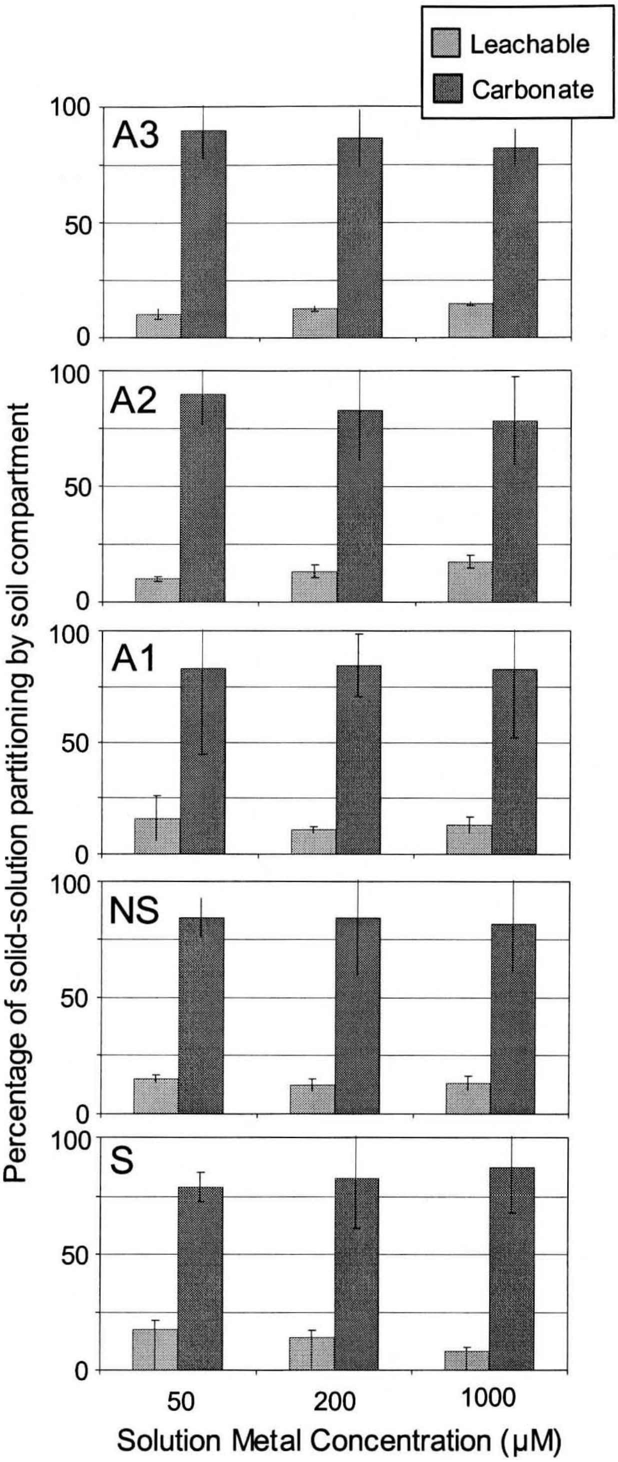

Figure 4.10: Percentage of U partitioning to the relevant fractions showing robust partitioning across all solution metal concentrations and treatments.

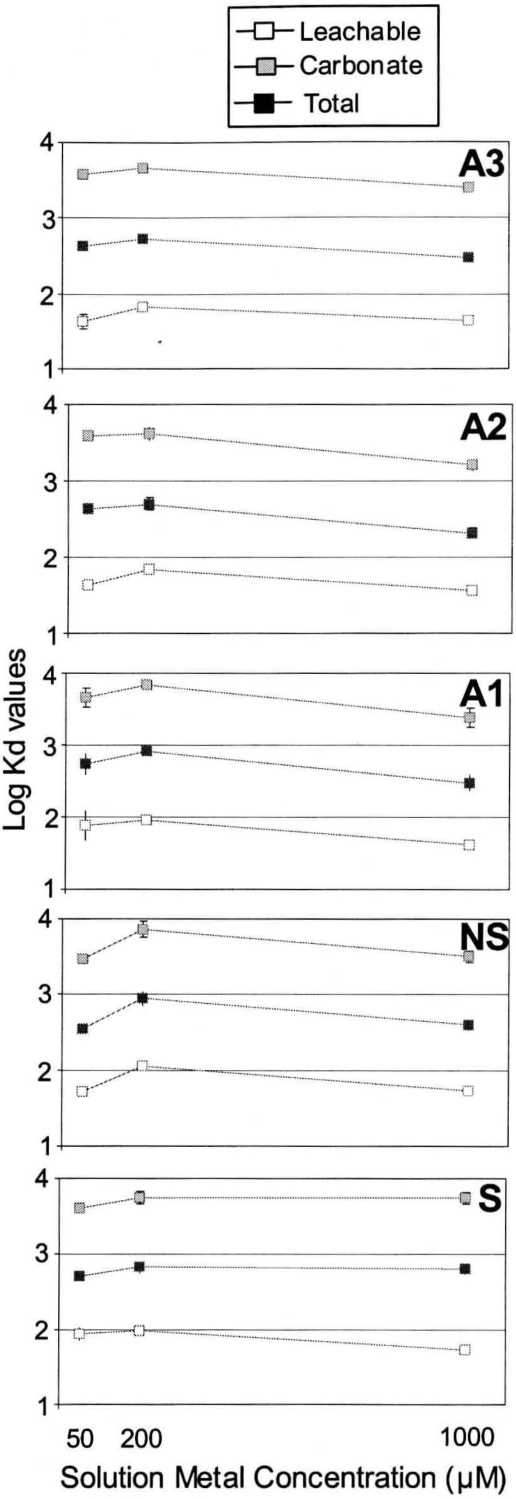

Figure 4.11: Log Kd values for U partitioning to each of the relevant fractions showing a clear preference for the carbonate fraction.

fraction) without the presence of other metals. Wazne et al. (2003) as well as Echevarria et al. (2001) found that the presence of carbonate dramatically reduced sorption of U onto iron hydroxides due to the much stronger affinity of solution U to form carbonate complexes, and that in the circumneutral pH range speciation of U was dominated by UO_2CO_3, $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ which may then hinder subsequent solid sorption due to steric hindrances associated with the significant size of these U-carbonate molecules. Given the carbonate content of the INL soil (up to 10% wt.) and the circumneutral pH reported (7.0-7.2) in the current study, these same species would likely be present and would be expected to be either soluble, or associated with the carbonate or leachable fractions, which is consistent with the results observed.

Literature also suggests the presence of U would be expected in the organic fraction, either bound to the organic matter or associated with microbial uptake, but again this was not observed in my results (Duff et al., 2002; Zuyi et al., 2000). Again, my interpretation of this result is the relatively high carbonate content of the soil and its relatively greater affinity for U under the experimental conditions compared to the organic fraction with potentially other metals outcompeting U for the relatively few organic binding sites available.

All systems showed the same trend in partitioning coefficients as a function of concentrations for the carbonate and leachable fractions, with an increase in Kd from the 50 µM to 200 µM solution metal concentration, and then typically showing a significant decrease to the 1000 µM concentration (Table 4.6, 4.7, Figure 4.11, 4.12). These results indicate that the relative partitioning of U to both the carbonate and leachable fractions is not sorbent limited at the lower U concentrations, i.e. Kd values increase indicating relatively greater solid-U concentrations with higher dissolved concentrations. However, Kd values decrease at the highest dissolved metal concentration (1000 µM) indicating that some saturation of the solid fractions may have occurred (i.e. less sites available for uptake). The decrease in Kd values at the higher solution metal concentration may reflect the decrease in sorbent to sorbate ratio as previously discussed or may be the result of other metals having a higher affinity for the carbonates given that it has been demonstrated that these more labile fractions (i.e. leachable and carbonates) become substantially more important for metal sequestration at the higher solution metal concentrations. Comparison of these results to the literature revealed the limited wealth of knowledge on uranium partitioning to soil compartments. The strong influence of pH on solid U partitioning is discussed extensively (Wazne et al., 2003; Echevarria et al., 2001; Kelly et al., 2001; Sauvé et al., 2000; Song et al., 1999). Studies reporting compartmental specific Kds for U partitioning to the oxide fraction are limited to a single solution metal concentration, and often at lower concentrations than examined in the current study. Wazne et al. (2003) reported U oxide log Kd values in the range of 4 – 5 for laboratory batch experiments using synthesized ferrihydrite, however their solution metal concentrations were 2.94 µM, an order of magnitude lower than

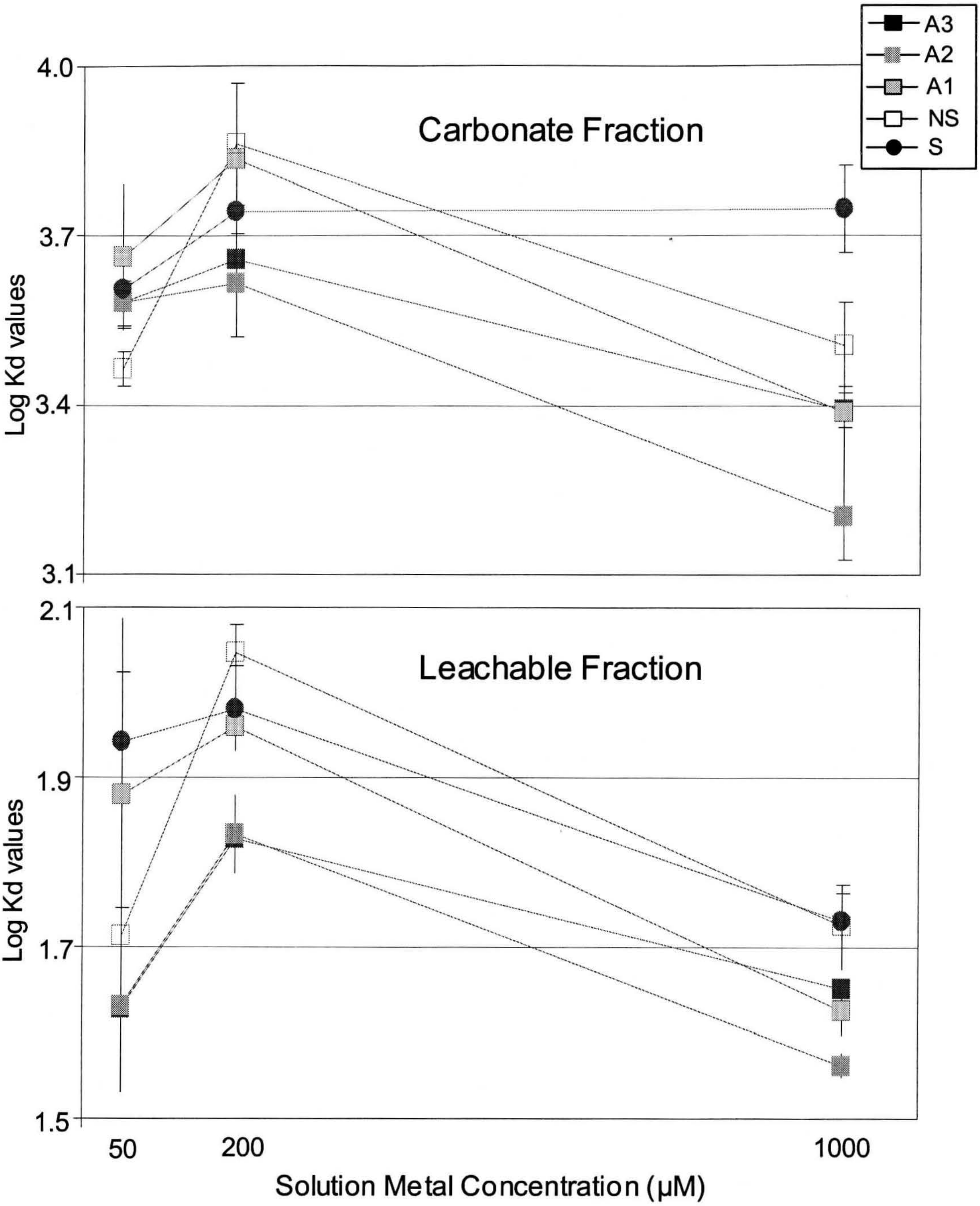

Figure 4.12: Log Kd values for U partitioning to the Carbonate and Leachable fractions according to system.

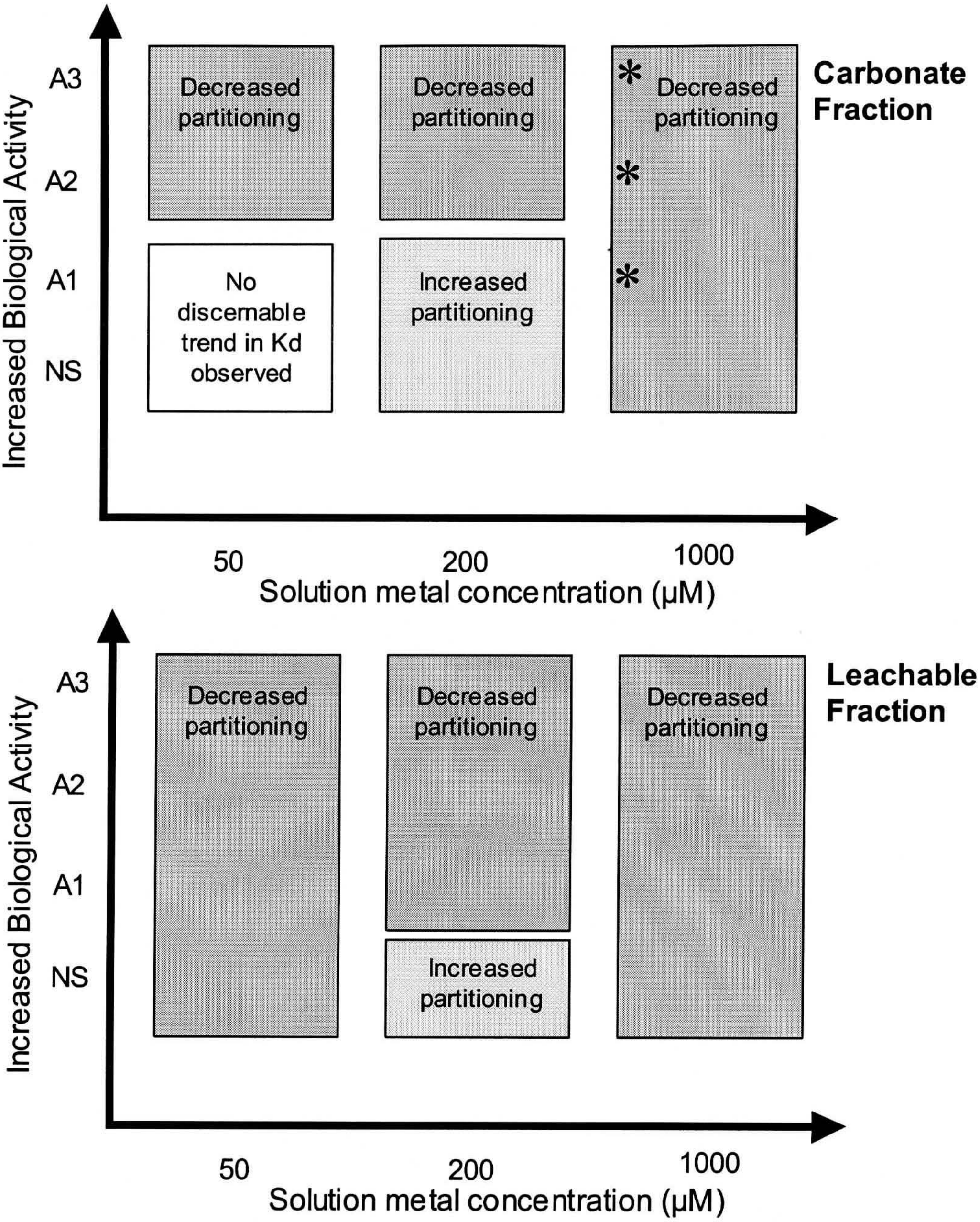

Figure 4.13: Uranium partitioning coefficients relative to the abiotic system for the **Carbonate** and **Leachable** fractions, * designates a statistically significant difference from the abiotic system.

the lowest concentration examined in this study. Ferris et al., (2000) found similar U oxide log Kd values for a subterranean mine environment in Sweden groundwater (approximately 3.8 - 5) but at even lower concentrations (0.045 - 0.06 µM). While these studies at least allow for the evaluation of typical Kds associated with the oxide fraction, this current study points to the effect of solution concentration, a subject that has significant impacts on U partitioning and one that has not been addressed in the literature.

While no biological impact can be discerned from relative partitioning values (80% carbonate, 20% leachable across all treatments and concentrations; Figure 4.10) there is an apparent biological imprint on the sorbent specific Kd values for both the carbonate and leachable fractions as a function of solution metal concentration (Figure 4.12, 4.13).

Carbonate Fraction

U partitioning coefficients for the carbonate fraction of the abiotic system show a classic langmuir partitioning pattern of increasing as a function of solution concentration to a maximum value whereby the Kd value reaches a plateau, becoming independent of solution metal concentration (Figure 4.12, Table 4.6). For U, abiotic partitioning to this fraction becomes independent of concentration at the higher concentrations studied (i.e. 200 µM and above). Results for the biotic systems, however, do not show this trend, instead these systems show significant decreases in U Kd values from the 200 to 1000 µM concentration for the amended treatments, suggesting that microbial activity decreases the amount of U associated with the carbonate fraction (Figure 4.12, Table 4.6).

Examination of Kd values also indicate biological activity is influencing U partitioning to the carbonates in a metal concentration dependent manner. While it is important not to over interpret these results given the limited number of statistically significant relationships, it does appear that the systems with higher biological activity (A2 and A3) consistently partition less U than the abiotic system (Table 4.6; Figure 4.12, 4.13). This relationship is significant at the 1000 µM solution metal concentration for all of the amended treatments. At the 200 µM concentration, low microbial activity (NS, A1) has a positive impact on partitioning, resulting in higher Kd values than the abiotic system (Table 4.6, Figure 4.12, 4.13). Collectively, these results imply the microbial community is impacting U partitioning to soil carbonates and that it can increase or decrease U partitioning relative to abiotic processes in a metal concentration dependent manner.

While many researchers have identified microbes themselves to be potential substrates for metal capture (Duff et al., 2002; Warren and Haack, 2001; Fowle et al., 2000; Schiewer and Volesky, 2000; Fortin et al., 1997; Ferris and Beveridge, 1985), had this been the case here, a greater amount of U would have been detected within the organic fraction for the amended treatments, however negligible U was found within this fraction for all treatments. Therefore, the microbial community must be impacting U partitioning through either changing the geochemical properties (i.e. pH or redox) of the environment or through direct U associated metabolism, and while this current study cannot ascertain which scenario is occurring, it is apparent that the impact becomes greater at high solution metal concentrations (Figure 4.12, 4.13). As measured in this study, bulk geochemical properties (pH and redox) remained constant across all treatments, however, microbial communities may have altered localized areas, through metabolism and respiration and these types of localized, microgeochemical effects would not have been detected at the resolution of the bulk measurements (Tokunaga et al., 2001). Respiration alone would be expected to minutely decrease pH values. A study by Song et al. (1999) showed the release of U from carbonates is pH dependent, reporting a non-linear increase in U release with decreasing pH over the range of 8.5-6, below pH 6 the rate of release for U began to decrease. Given the observed bulk pH results of the current study (7.0-7.2), it unlikely that pore-scale pH values dropped below pH 6. Therefore, based on the findings of Song et al. (1999) it can be inferred that any minute decrease in pH would result in the release of some U from the carbonate fraction and this would be consistent with the results observed in the current study. Microbial metabolism, whereby the microbes reduce U(VI) to U(IV) as a coupled reaction, often with the oxidation of organic matter, could also have occurred (Yun-Juan et al., 2005; Duff et al., 2002; Lovley and Phillips, 1992; Lovley et al., 1991), however this is less likely given the high nitrate concentrations that occur in the INL soil (i.e. nitrate reduction is more energetically favourable than the reduction of U(VI): Elias et al., 2003).

Leachable Fraction

The fraction specific partitioning coefficients for the leachable fraction are approximately 2 log units lower than for the carbonate fraction (approximately 1.6-2.05 for the leachable fraction; Figure 4.11, 4.12). However, approximately 20% of the sequestered U has been captured by this fraction, thus it is important in the consideration of U sediment associations. Given the high affinity U has for carbonates and the likelihood of forming carbonate complexes in the presence of carbonate rich sediments (Wazne et al., 2003; Echevarria et al., 2001; Bargar et al., 2000; Song et al., 1999), it is likely that the U associated with this fraction is also in the form of a carbonate complex. These carbonate complexes would be governed by solubility constants – this would account for the consistent partitioning between the leachable and carbonate fractions. And while Figure 4.10 shows robust partitioning between the carbonate and leachable fraction with no apparent shift in relative partitioning to either of these fractions as a function of solution concentration, Kd values indicate partitioning to the leachable fraction is not independent of solution concentration. Leachable Kd partitioning typically mimic that of the carbonate fraction, showing an increase in partitioning coefficients from the solution metal concentration of 50 to 200 µM, and then a distinct decrease in Kd for the 1000 µM solution metal concentration (statistically significant for 3 of the 5 systems). However, there is a notable difference in observed results for the leachable fraction compared to those observed for the carbonate fraction; U partitioning to the leachable fraction in the abiotic treatment does not become independent of solution metal concentration at the highest metal concentration. Rather, results show the same distinct decrease in Kd from the 200 to 1000 µM solution metal concentration as observed in the biotic treatments. This decrease at the highest solution metal concentration may reflect metal competition. There will be greater competition among the metals for available uptake sites as solution concentration increases, therefore the most metal reactive fraction (the oxides) will take up its preferred ions, leaving the remainder of the metal ions to compete for the remaining available sediment fraction uptake sites. Under high solution metal concentrations, U, especially as a large complex $UO_2(CO_3)_x$ molecule, may be "outcompeted" by the other metals for sorption sites, as the sorbent to sorbate ratio decreases, which is consistent with the overall decrease in oxide Kds observed at the higher solution concentrations (Figure 4.11, 4.12, Table 4.5). While no strong U associations with the oxide fractions were observed in the current study, other metals (specifically As, Cr, Co and Zn, which did show strong oxide associations; Table 4.5) showed a shift to the more labile fractions at the higher solution concentrations consistent with a scenario of saturated available oxide sites and greater competition amongst solution metals for remaining sorption sites associated with the leachable and carbonate sediment fractions. As the oxide fraction reaches or exceeds sorptive capacity, relatively more As, Cr, Co and Zn will be left in solution which then more successfully compete for remaining sites within the carbonate and leachable fraction compared to U.

While leachable Kd values for the amended treatments at each of the solution metal concentrations are not significantly different from the abiotic system, there is a consistent trend of lower partitioning values indicating that stimulating biological activity may be negatively impacting partitioning to this fraction, independent of solution metal concentration.

4.3.2 Arsenic

Unlike U, relative partitioning of As was more dynamic indicating that solution concentration impacted sediment As behaviour. Across all systems, a shift in relative partitioning from the oxide to the carbonate fractions was observed as a function of solution metal concentration; oxide associated As concentrations decreased (amorphous: 50-55% of total solid associated As to 40%, crystalline: 10-15% to 5-10%) while carbonate associated As increased

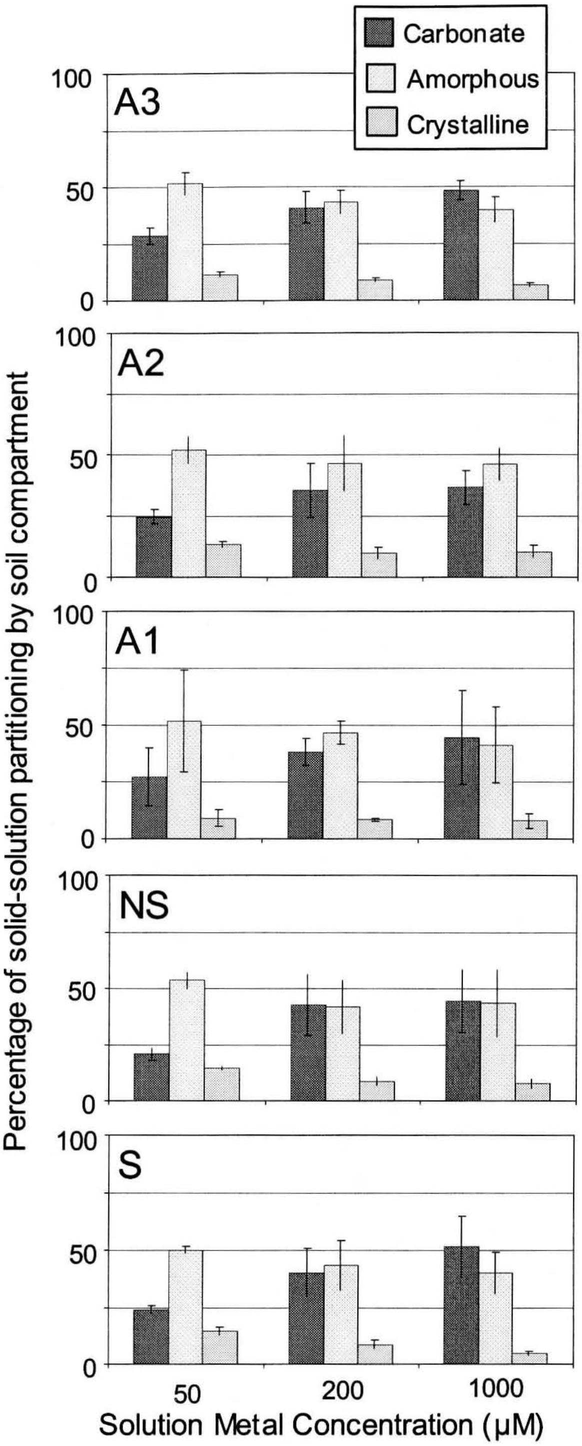

Figure 4.14 Percentage of As partitioning to the relevant fractions showing a dynamic shift from the oxide to the carbonate fraction with increasing solution metal concentration.

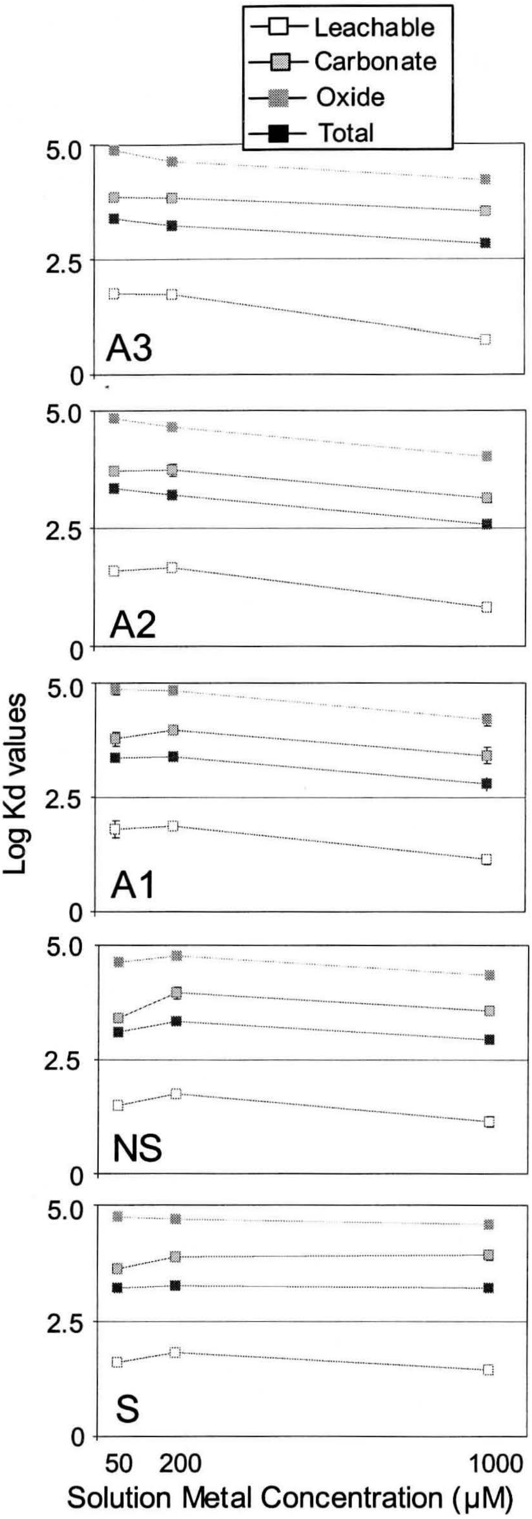

Figure 4.15: Log Kd values for As partitioning to each of the relevant fractions showing a clear preference for the oxide fraction.

with increasing solution metal concentration (approximately 25% at the 50 µM concentration to 35-50% at the 1000 µM concentration: Figure 4.14). Trace amounts of As were also found within the leachable fraction (>5%), however, given the limited partitioning to this fraction, trends are difficult to ascertain. Thus, while U results for relative partitioning indicated an equilibrium distribution, As results indicate a potential saturation of the most reactive substrate, the oxide fraction, resulting in a heightened importance of the carbonate fraction as the solution metal concentration increases. This shift in compartmental distribution is important since the oxide and carbonate fractions have different abilities to retain these metals given any changes in geochemical properties (i.e. redox and pH).

Across all systems and concentrations examined, As partitioning coefficients for INL sediment was greatest for the oxides fraction (average Kd values of 4.6±0.3; Table 4.5) followed by the carbonates (average Kd value of 3.7±0.2; Table 4.6, Figure 4.15). It has been well documented that oxides play a prominent role in sorption reactions (Smedley et al., 2005; Craw et al., 2004; Gault et al., 2003; Bose and Sharma, 2002; Kneebone et al., 2002; Belzile and Tessier, 1990). A study conducted by Gault et al. (2003) on As contaminated sediment at an English brook found that 50-84% of all As partitioned to the sediment was associated with the oxide fraction, unfortunately the mass of the oxide fraction was not reported precluding the ability to calculate Kd values to compare to this current study. However, Gault et al. (2003) found only 3-4% of the sediment captured As to be associated with the carbonate fraction. Therefore, it is consistent with the relative importance of fractional compartments observed in the current study (i.e. oxides most important followed by carbonates).

As behaviour shows relatively static fractionation in the Sterile system, indicating that under abiotic conditions carbonates and oxides are the most important sediment fractions, independent of solution concentration. While the same fractions were important for the biotic systems, partitioning typically showed a non-linear trend as a function of solution metal concentration where the highest solution metal concentration (1000 µM) yielded the lowest partitioning coefficients. Therefore, while compartmental partitioning of U has differed from that of As, both of these elements show an overlay of biologic activity on partitioning. Substantial differences in Kd values were observed for the oxide and carbonate fractions across the concentration range for the abiotic and biotic systems (Figure 4.15, Table 4.5, 4.6).

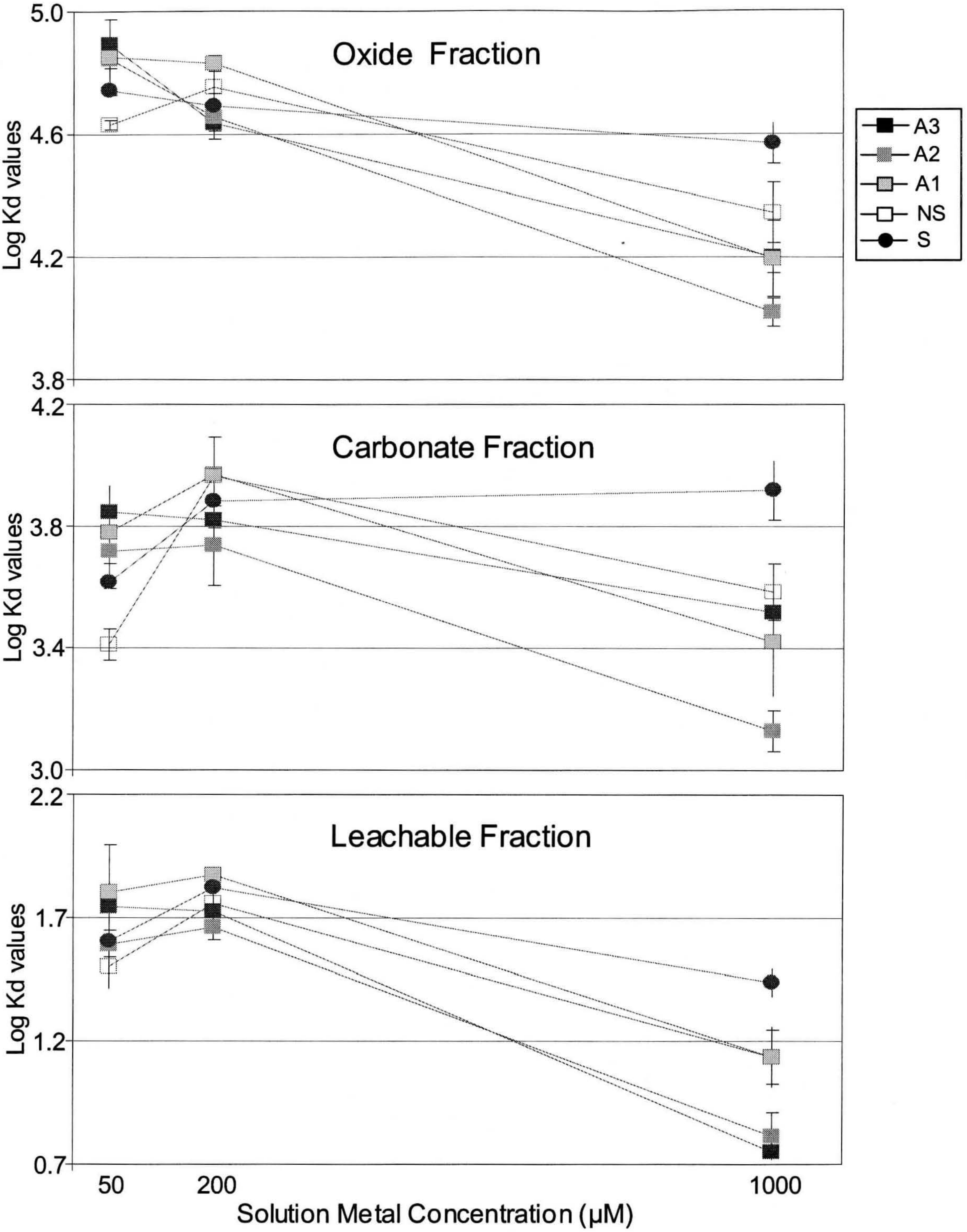

Figure 4.16: Log Kd values for As partitioning to the Oxide, Carbonate and Leachable fractions according to system.

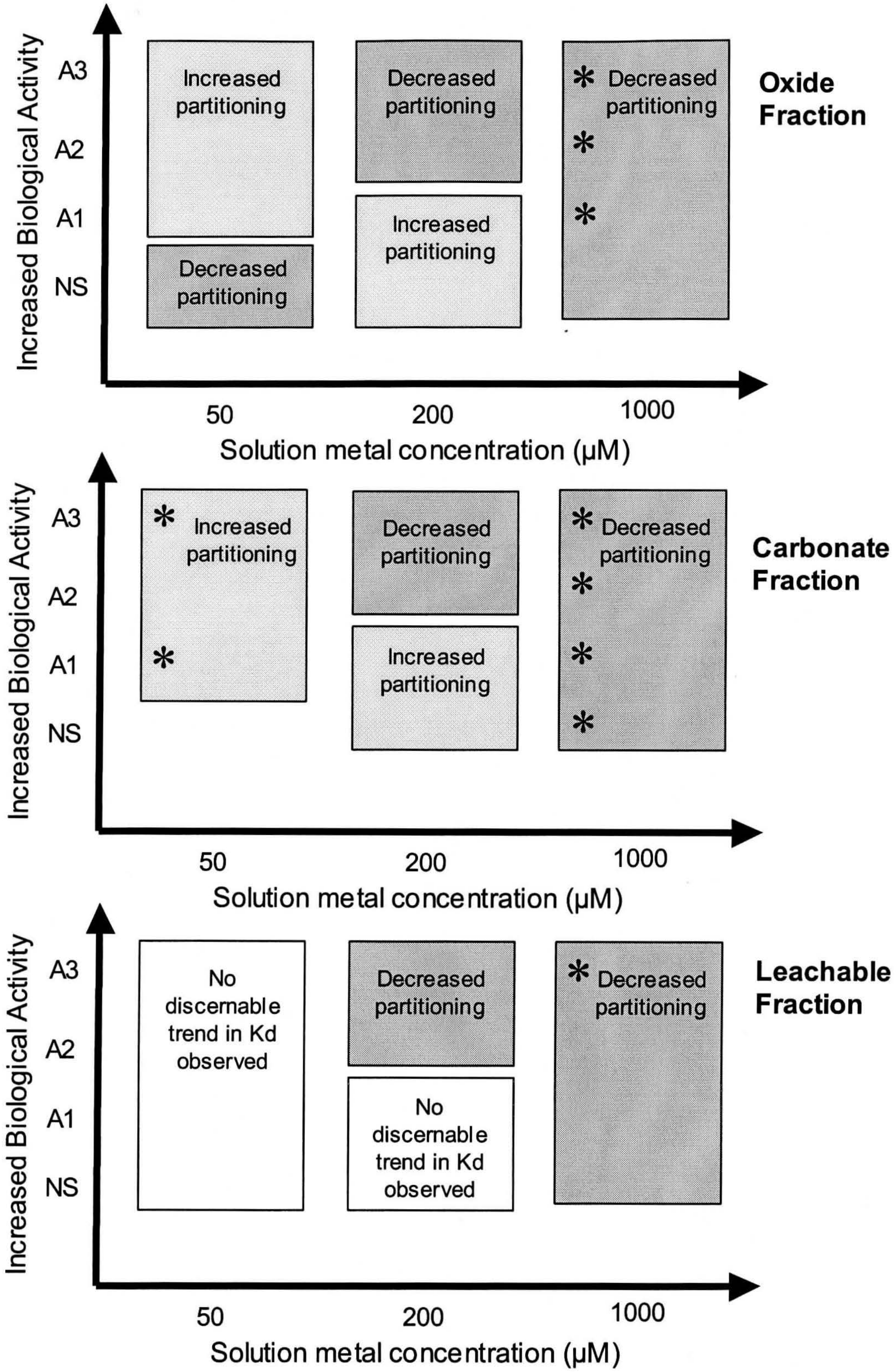

Figure 4.17: Arsenic partitioning coefficients relative to the abiotic system for the **Oxide**, **Carbonate** and **Leachable** fractions, * designates a statistically significant difference from the abiotic system.

Biological activity can have two distinct and opposite effects on As, some microbes can oxidize As(III), while others can use As(V) as a nutrient and couple its reduction with the oxidation of organic matter (Islam et al., 2004; Oremland and Stolz, 2003; Laverman et al., 1995; Ahmann et al., 1994). Given that As(III) is typically more mobile than As(V) (Su and Puls, 2001; Sadiq et al., 1983), and the results show a decrease in partitioning to all fractions as a function of solution metal concentration for all fractions (leachable, carbonate and oxides) it is possible that the INL microbes are, in fact, using As as a terminal electron acceptor, and converting As that would be in the As(V) form to As(III) in association with organic matter decomposition. Since the dominant species of arsenite below pH 9 is H_3AsO_3, a neutrally charged species, As in this form is highly soluble and mobile (Sadiq et al., 1983). In addition, since this effect is much stronger at higher solution metal concentrations it would suggest that the metabolism of As reducing bacteria is increasing in the presence of this so called 'nutrient', or that dynamic changes are occurring within the microbial community where some species (those able to reduce As) are becoming more metabolically active which was observed in contaminated aquifer sediments in Bangladesh (Islam et al., 2004).

Oxide Fraction

Partitioning of As within the oxide fraction is a function of both solution metal concentration and biological activity. The abiotic system exhibits very little variation in partitioning over the concentration range compared to the other systems (Table 4.5, Figure 4.15, 4.16), reporting log Kd values that decrease slightly as a function of concentration. Biological systems exhibited much greater variation in oxide Kd values over the concentration range, indicating the biologic impact on oxide associated As behaviour is heavily dependent on solution metal concentration (Table 4.5, Figure 4.15, 4.16).

Partitioning of As to the oxide fraction demonstrates a dependence on biological activity following the same overall trend exhibited in Figure 4.4. At the 50 µM concentration, although not statistically significant, all amended treatments reported higher partitioning values than the abiotic system, while at the highest solution metal concentration any biological activity resulted in significantly lower partitioning coefficients (Table 4.5, Figure 4.16, 4.17). And at the 200 µM concentration, relative partitioning among the systems is dependent on level of biological activity (Table 4.5, Figure 4.16, 4.17); low biological activity resulted in higher partitioning coefficients, whereas high activity (A2 and A3) resulted in lower partitioning coefficients than that observed for the abiotic system. While these trends are not all significant, the consistency of this overall trend with that illustrated in Figure 4.4 shows it is an important result, one that may have been significant with a larger sample size.

This non-linear result complicates any remediation approach. First of all, given the large amount of As associated with the oxide fraction, it is important to prevent highly reducing conditions as this often results in large scale As release from the sediments (Bose and Sharma, 2002; Kneebone et al., 2002; Nickson et al., 2000; Spliethoff et al., 1995; Kuhn and Sigg, 1993). However, the non-linear result for the effect of biological activity (beneficial vs. detrimental) indicates increased solution metal concentration is changing the microbial community, which is causing two possible scenarios: (1) the oxide fraction is being decreased, and (2) microbial species are changing their metabolism to include arsenic as a terminal electron acceptor.

In the case of the first scenario, decreasing the mass of the oxide fraction would increase the sorbate to sorbent ratio and may give the false impression of decreased reactivity for the oxide fraction (Kd values would decrease). Reduction of oxides are often stimulated by microbial activity (Kneebone et al., 2002; Zachara et al., 2001; Spliethoft et al., 1995; Khun and Sigg, 1993), however, given the behaviour of the entire suite of metals within this study, as discussed in Section 4.2.1, whereby the importance of the oxide fraction decreases with increasing solution metal concentration independent of biological activity, this possibility has already been ruled unlikely.

As for the second possible scenario, most microbial species will be unable to change their metabolism such that arsenic is utilized as a terminal electron acceptor (i.e. changing the redox couple from which the microbes gain energy), given the limited number of microbial species that can achieve this feat (Turpeinen et al., 2004; Ormeland and Stolz, 2003). However, the microbial community may be shifting to species that can not only survive in a highly toxic As rich environment but thrive by using As as unique energy source (Macur et al., 2004; Turpeinen et al., 2004; Nicholas et al., 2003; Oremland and Stolz, 2003). Bacteria with this ability have been referred to as dissimilatory arsenate-reducing prokaryotes (DARPs) and have been found in a number of environments including freshwater sediments, estuaries, hot springs and gold mines, they have also been found in subsurface aquifer materials, and they have been found in these environments with solution metal concentrations consistent with the range used in this study (Islam et al., 2004; Nicholas et al., 2003; Oremland and Stolz, 2003; Herbel et al., 2002; Ryu et al., 2002). Further, DARPs have been found to grow well in As concentrations up to 10 mM, an order of magnitude higher than the highest metal concentration examined in this study (Oremland and Stolz, 2003). Therefore, if DARPs are present in the current study, through their metabolism As(V) would be reduced to the more soluble As(III) resulting in a decrease in sediment associated arsenic, which is consistent with the results observed for the oxide compartment.

Carbonate Fraction

While a general decrease in As partitioning coefficients was observed across the concentration range for the oxide compartment, As associated with the carbonate fraction showed more complex behaviour with changes in partitioning coefficients across the concentration range being dependent on biological activity (Figure 4.16). Similar to U partitioning to this fraction, abiotic As partitioning follows the classical nonlinear langmuir isotherm; a large increase in partitioning coefficients from the 50 to 200 µM concentrations, nearing a plateau at the 1000 µM concentration (Figure 4.16). This indicates that As partitioning to carbonates under abiotic conditions is a fairly robust process and is not affected by the competition of other metals as it appears that at high concentrations abiotic partitioning to the carbonate fraction is independent of solution metal concentration.

Microbial activity however, has a profound effect on As behaviour, and the strength of this effect is concentration dependent. Like the oxide compartment, at the low solution metal concentration, amended treatments result in higher partitioning values indicating that at this concentration the stimulation of biological activity is beneficial (Figure 4.16, 4.17). At the highest solution metal concentration, the biotic systems report lower partitioning coefficients, thus, with high metal loads, biological activity results in decreased partitioning to this fraction. And at the 200 µM concentration partitioning is dependent on the level of biological activity; low levels of biological activity (NS and A1) result in greater partitioning coefficients, and high levels of activity (A2 and A3) result in lower partitioning coefficients compared to the abiotic system although the values are not statistically significant. Similar to the oxide fraction, this result can be attributed to two scenarios: (1) a change in the carbonate fraction (mass or reactivity), and (2) a change in the microbial community (DARPs) metabolism. As previously discussed, the carbonate fraction is strongly influenced by pH, to the extent that even slight changes in pH resulting from respiration of the microbial population may be sufficient to locally dissolve carbonates or alter their reactivities. A decrease in the mass of carbonates would be consistent with the observed decrease in Kd values. The second scenario has already been discussed in the context of the oxide fraction, whereby the microbial community is shifting to one in which DARPs thrive obtaining energy through reducing As(V) to As(III) – the more soluble arsenic species. Therefore, this is also a possible explanation for the results observed. Given that both scenarios are plausible, it is not possible to determine which mechanism is occurring, or whether both are occurring simultaneously.

Leachable Fraction

The relative amount of As bound to the leachable fraction is very small compared to the carbonate and oxide compartments (i.e. less than 5% of the total As partitioned to the solid), thus has not been included in Figure 4.14. However, compartmental specific Kds were calculated to ensure system specific trends were not overlooked. Log Kds for the leachable fraction range from 0.8 – 1.8 and are characterized by the dramatic decrease in values from the 200 to 1000 µM concentrations across all treatments (Figure 4.16). This result may have occurred due to greater competition among metals for this labile fraction.

No clear trend emerged for differences in Kd values for the biotic and abiotic systems at the 50 and 200 µM concentrations, however at the 1000 µM concentration biological activity results in significantly lower Kd values compared to the abiotic system. This may also reflect increased metal competition, which is heightened for the biologically active systems since these systems have exhibited lower As association with the oxide and carbonate fractions, which would leave a greater concentration of As in solution to react to any leachable sites. More likely, the decrease in leachable Kd values is the result of DARPs altering the redox state of the As to more soluble species (As(III)) since this is consistent with the results observed for both the oxide and carbonate fractions.

4.3.3 Chromium

Relative Cr partitioning was highly variable showing shifts in compartmental partitioning with increased solution metal concentration as well as differences in relative partitioning with different levels of biological activity indicating Cr is dependent on a complex set of interactions (Figure 4.18). Across the solution metal concentration range, an increase in the relative importance of the carbonate fraction (for all systems) and amorphous fraction (3 of the 5 systems) was observed (Figure 4.18).

It is important to note that Cr is the only metal of the suite of metals evaluated to demonstrate a trend of increasing importance for the oxide fraction (as is illustrated by the increase in percentage of Cr found in this fraction) across the concentration range. One possible explanation for this result would be that Cr has a higher affinity for the oxide fraction than the other metals used in this study; however, other studies have found that chromate sorption to iron oxides is significantly reduced by the presence of arsenate due to competition for adsorption sites and electrostatic effects (Balasoiu et al., 2001; Khaodhiar et al., 2000). Another possibility for the increased relative importance of the oxide fraction could be attributed to the unique properties of Cr. Unlike many other metals that compete for binding sites as cations, C(VI) often exists as an oxyanion (i.e. bichromate – $HCrO_4^-$, chromate – CrO_4^{2-}, and dichromate – $Cr_2O_7^{2-}$; Hellerich and Nikolaidis, 2005; Zachara et al., 2004; Loyaux-Lawniczak et al., 2001), and at sufficiently low pH levels, oxides may become positively charged (Kennedy et al., 2004; Robertson and Leckie; 1998; Catts and

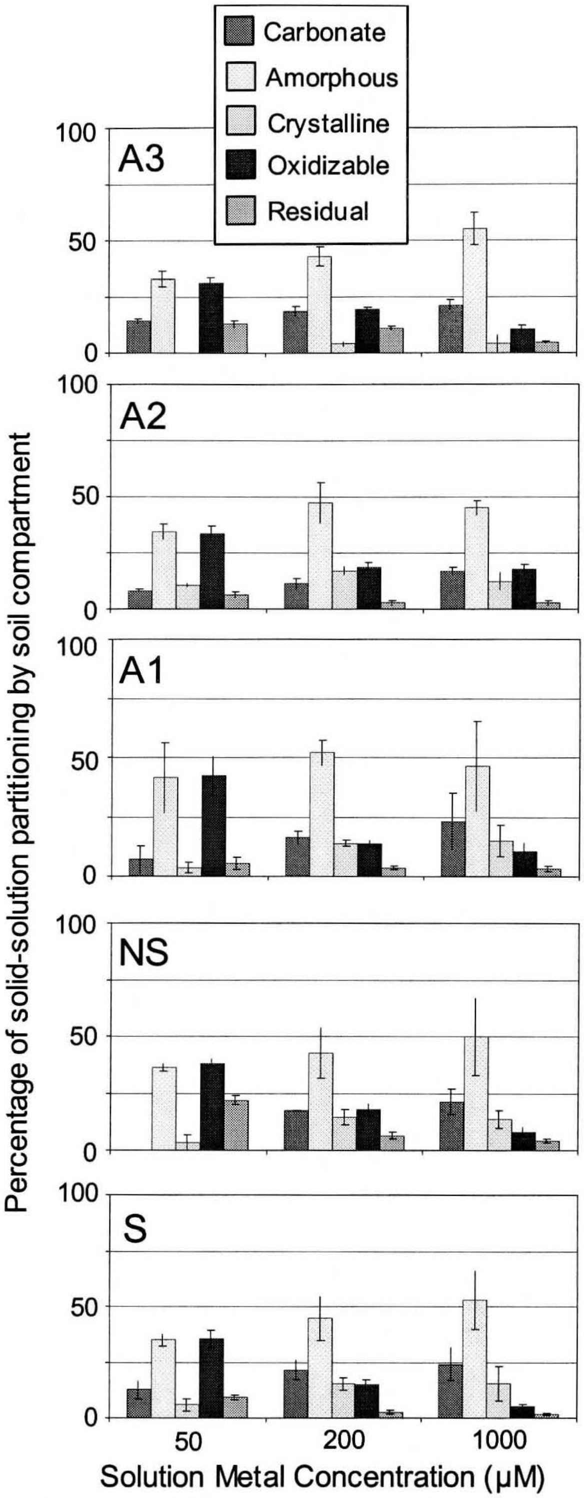

Figure 4.18: Percentage of Cr partitioning to the relevant fractions showing a complex dynamics across many fractions.

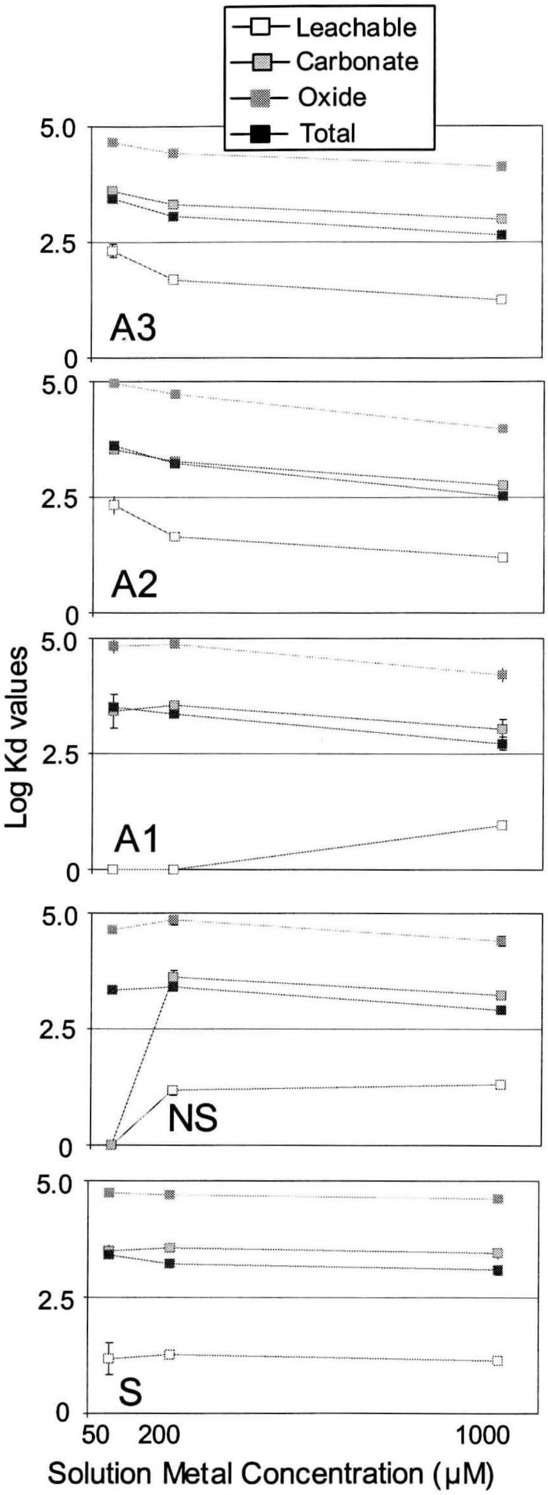

Figure 4.19: Log Kd values for Cr partitioning to each of the relevant fractions showing a clear preference for the oxide fraction.

Langmuir, 1986; Murray, 1974; Parks, 1965) which would enhance Cr partitioning to the oxides, while hindering other positively charged metals. However, the pH of the soil would have to drop below the pH_{ZPC} of the oxides present in order to enhance capture of the negatively charged chromate. While the biologically active systems have a mechanism to decrease pH, it is unlikely the decrease would be sufficient to create positively charged oxide surfaces; therefore this is also an unlikely explanation for increased Cr associations with the oxide fraction. The most plausible explanation for the increased Cr associated with this fraction would be a change in the oxidation status of this metal. Reduction of Cr(VI) to Cr(III), which typically precipitates at pH levels greater than 5 (as is the case in this study; Hellerich and Nikolaidis, 2005), could account for increased association with the solids (both to the carbonates and oxides fractions).

The oxidizable and residual fractions decrease in terms of relative partitioning (oxidizable: 30-45% of solid associated As to 5-15%, residual: 10-15% to 5%; data not shown). However, absolute values indicate that the moles of Cr partitioned (per gram of sediment) were constant across the concentration range, and therefore the Cr present in these fractions is independent of concentration, and was likely bound to these fractions prior to the experiment. This Cr is likely part of the mineral matrix, and given that this portion of the Cr withstood the various steps of the sequential extraction procedure, which is much more evasive than any potential environmental changes in pH and redox conditions, this Cr can be considered enduringly immobile.

Of all metals analyzed, Cr shows the greatest spread of occurrence across the sediment compartments, associating with each of the fractions analyzed within my SEP (at least 5% in each fraction; Figure 4.9, 4.18). While the application of a SEP in the investigation of Cr behaviour is rare, a similar extraction to that used in the current study was performed by Balasoiu et al. (2001) with similar results. Balasoiu et al. extracted Cr from an artificially contaminated, laboratory derived soil (controlling soil composition), and found 0.1-18.8% in the leachable fraction, 7.9-14.2% in the carbonate fraction, 50.8-66.0 in the oxide fraction, 5.8-36.9 in the oxidizable fraction and 2.4-9.7 in the residual fraction –all of these reported values are comparable to those obtained in this current investigation.

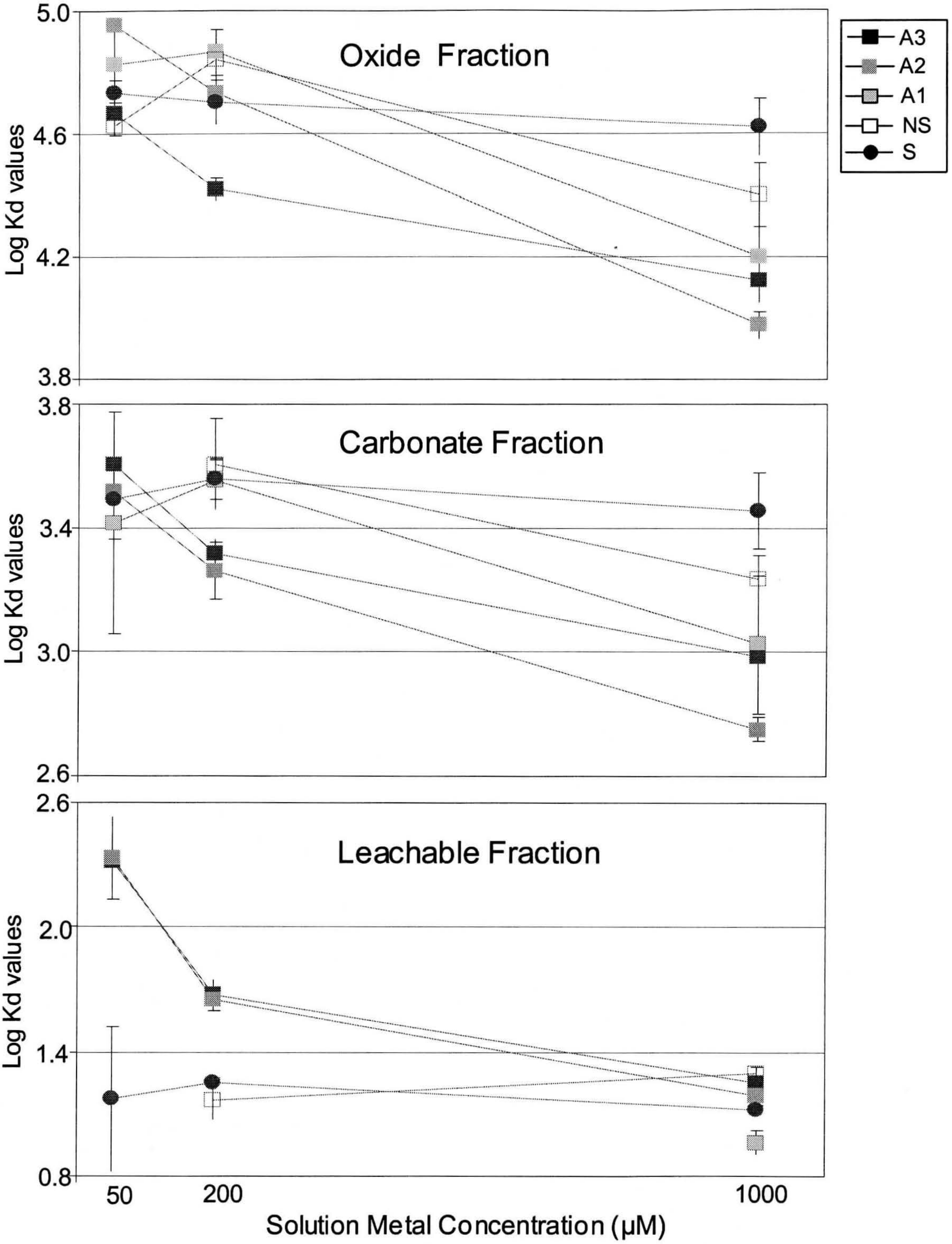

Figure 4.20: Log Kd values for Cr partitioning to the Oxide, Carbonate and Leachable fractions according to system.

The dominant compartmental sinks for Cr were the oxide fraction (crystalline and amorphous (log Kd values of 3.98-4.95)), the carbonate fraction (log Kd values of 2.75-3.61) and the leachable fraction (log Kd values of 0.97-2.33) (Table 4.5, 4.6, 4.7, Figure 4.19). Partitioning to the oxides was expected based on the literature reporting a high association between oxides and Cr (Zachara et al., 2004; Khan and Puls, 2003; Loyaux-Lawniczak et al., 2001; Tokunaga et al., 2001; Buerge and Hug, 1997), as well as an association with leachable fractions of sediments (Zachara et al., 2004; Balasoiu et al., 2001). However, partitioning to the carbonate fraction was not expected. With the exception of the work by Balasoiu et al. (2001), no other studies reporting an association between Cr and carbonates were found in the literature. As previously discussed, Balasoiu et al. reported relative Cr partitioning to the carbonate fraction of 7.9-14.2%, which is in strong agreement with my findings of 0-24% (average 15.6%). Unfortunately, Balasoiu et al. made no comment on this unique result and offered no explanation for this association.

Few studies can be found with published Kd values for Cr partitioning in environments comparable to my study limiting my ability to make comparisons (Zachara et al., 2004; McBride et al., 1999). However, one highly comparable study was performed on the Hanford vadose zone, a site that is associated with high-level nuclear waste contamination with porewater Cr concentrations extending beyond the confines of the current study (both higher and lower; Zachara et al., 2004). Typically the total log Kds reported by Zachara et al. are lower than those observed in this study (i.e. less than 1), and 2 of the 16 sites they analyzed had significantly higher values than their other sites (2.5 and 3.2), however, these values are still much lower than the average found in the current study (4.6±0.3). This result is not surprising given the site-specific nature of Cr partitioning, and the dependence on sediment characteristics (i.e. quantity of Fe in the sediment, types of sediments) as well as the geochemical and microbiological processes (Zachara et al., 2004). Unfortunately, no published reports of compartmental specific Kd values for Cr are available.

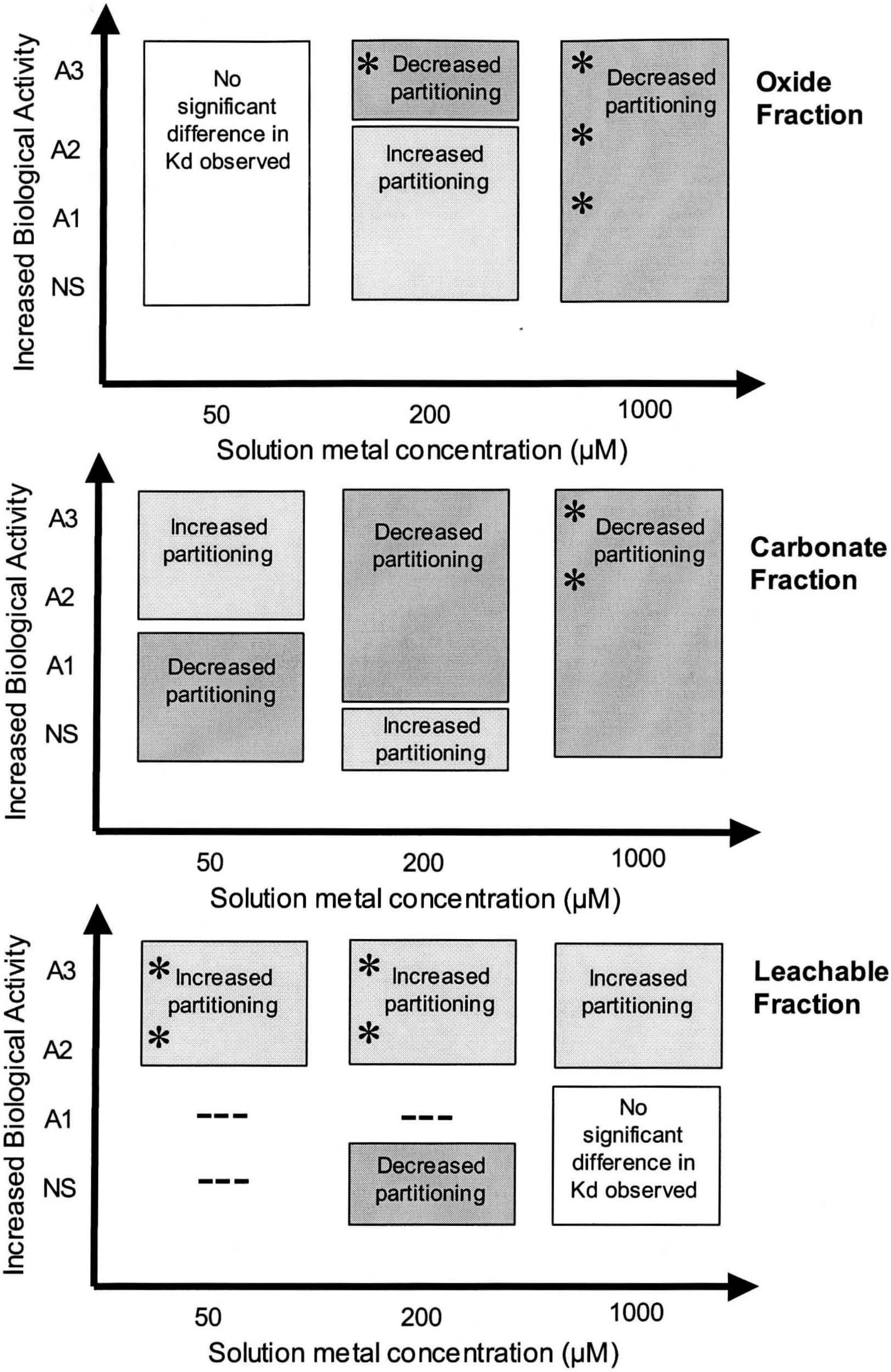

Figure 4.21: Chromium partitioning coefficients relative to the abiotic system for the **Oxide**, **Carbonate** and **Leachable** fractions, * designates a statistically significant difference from the abiotic system.

Oxide Fraction

It has been well documented that Cr often partitions to oxides within sediments (Zachara et al., 2004; Khan and Puls, 2003; Loyaux-Lawniczak et al., 2001; Tokunaga et al., 2001; Buerge and Hug, 1997). However, many researchers have cautioned that oxides' relative ability to partition chromate at circumneutral to high pH levels is greatly diminished (Zachara et al., 2004; Zachara et al., 1989; Davis and Leckie, 1980). This observation arises since oxides often carry negative charges at these higher pH levels preventing an attraction between the oxide and the dominant Cr(VI) oxyanionic species, ($HCrO_4^-$, and CrO_4^{2-} or $Cr_2O_7^-$ (Loyaux-Lawniczak et al., 2001)). Given that the pH of the current study is within the range of 7.0-7.2, such studies would suggest little Cr would be associated with this fraction, which is not consistent with the my results. Observed log Kd values in the range 4-5 for this current study clearly indicate the oxide fraction is not being rendered ineffective, instead proving to be an important sink for Cr. These results, therefore, can potentially be explained by (1) a change in the oxide substrate or (2) a change in the Cr redox (Cr(VI) to Cr(III)).

For a negative Cr species to be attracted to an oxide molecule, the oxide must be carrying a net positive surface charge, so the pH must be below the oxide's pH_{ZPC} for substantial Cr retention. While the bulk pH values range from 7.0 to 7.2, which may be high for the attraction of a negatively charged Cr molecule (depending on its actual pH_{ZPC}), it is possible that localized decreases in pH (on the porescale) could create environments more suitable for Cr attraction to the oxide fraction (Tokunaga et al., 2001). However, it would be expected that microbial metabolism would decrease pH values at the porescale, which, by this hypothesis, would increase Cr associations with the oxide fraction. This result was not observed, instead the microcosms with the greatest levels of microbial activity (which would have the greatest metabolism rate, and therefore the greatest decrease in pH – systems A2 and A3) showed a decrease in the reactivity (Kd) of the oxide fraction (Figure 4.20, 4.21).

Alternatively, a reduction of Cr from the original Cr(VI) to Cr(III) may also have occurred. The main factors influencing Cr reduction in natural systems are Fe(II) in solution or Fe(II)-bearing minerals, sulfides, and organic matter where the aqueous Cr(VI) species serves as the electron acceptor for oxidation of the reduced Fe, S and C in these substrates, and is itself reduced in the process and often immobilized in the solid phase (Loyaux-Lawniczak et al., 2001; Tokunaga et al., 2001). It has been reported that Cr(VI) reduction occurs predominantly as the result of such abiotic processes (Marsh et al., 2000). Alternatively, if microbial activity levels are sufficiently high to produce localized highly reducing microenvironments, Cr(III) species often occur as a number of cationic aqueous forms (e.g. Cr^{3+}, $Cr(OH)^{2+}$, $Cr(OH)_2^+$; Loyaux-Lawniczak et al., 2001), which may be potentially sorbed by negatively charged solid surfaces. Loyaux-Lawniczak et al. (2001) studied the chromium movement in contaminated groundwater and attributed reduction of Cr(VI) to Cr(III) by Fe(II) to the impediment of Cr migration. This was supported by the large amount of Cr(III) bound to the clay materials of the soils since the clays studied naturally contain large amounts of iron, but would also provide negatively charged surfaces for the Cr cationic species.

No significant difference in Cr oxide partitioning coefficients was observed for the abiotic system across the solution metal concentration range, reporting an average log Kd value of 4.69±0.06 (Table 4.5, Figure 4.19, 4.20) indicating that abiotic partitioning of Cr to the oxide fraction is very stable. Amended treatments, however, all show a decrease in Cr oxide partitioning coefficients as a function of increasing solution metal concentration, and this decrease is significant for the systems with the greatest biological activity (A2 and A3; Table 4.5). Therefore in biologically active systems, partitioning of Cr in systems is heavily dependent on solution metal concentration, facilitating the discussion of Cr partitioning behaviour at each solution metal concentration.

Consistent with the results observed for As, analysis of biotic vs. abiotic Cr partitioning at each concentration reveals a concentration dependent relationship similar to that shown in Figure 4.4, with the exception of the 50 µM concentration. At this lowest solution metal concentration, no significant differences or non-significant trends in Cr partitioning to the oxide fraction was observed. However, at the highest solution metal concentration examined (1000 µM), all biotic systems show lower partitioning coefficients than the abiotic system, and the amended systems are significantly lower than the abiotic system (Table 4.5). At the 200 µM concentration, the non-significant trend indicates that low microbial activity (NS and A1) increases partitioning above the abiotic system, whereas high microbial activity (A2 and A3) decreases partitioning compared to the abiotic system, results being significantly lower for A3 (Table 4.5). Clearly the microbial population is altering Cr partitioning and with a greater negative effect at higher solution metal concentrations. Biological reduction of Cr(VI) to Cr(III) is well documented within the literature (Turpeinen et al., 2004; Guha et al., 2003; Tokunaga et al., 2001; Marsh et al., 2000; Tebo and Obraztsova, 1998; Lovley and Phillips, 1994). However, this typically would result in a greater association of Cr with the oxide fraction, which was not observed. Instead, it appears that some factor is preventing the reduction of Cr, or oxidizing Cr once it is reduced releasing it back into solution. Studies indicate Mn-oxides are strong oxidizers of Cr and often the presence of these oxides prevents Cr reduction (Guha et al., 2003; Loyaux-Lawniczak et al., 2001; Powell et al., 1995). However, no Mn data was collected in this study, precluding any possible confidence in supporting this hypothesis as a viable potential retardation mechanism of oxide Cr uptake in this system.

Carbonate Fraction

The overall patterns for Cr partitioning to carbonates for each system across the various concentrations is very similar to that of the oxides, however, the values are approximately 1-1.5 log units lower, but carbonates still remain an important sink for Cr sequestration (Figure 4.19). As with the oxides, the abiotic system shows no significant difference in carbonate partitioning across the studied solution metal concentrations, whereas the amended treatments show a significant decrease from the lowest to highest solution metal concentrations (Figure 4.20).

At the 50 µM concentration, no significant difference in Kd values was observed across the systems. At the 200 µM concentration, while not statistically significant, all amended treatments reported lower partitioning coefficients than the abiotic system, and as the solution concentration increased to 1000 µM, any microbial activity resulted in significantly lower Kd values than the abiotic system (Table 4.6, Figure 4.20). Similar to oxide associated Cr for the abiotic system, Cr partitioning to the carbonate fraction for the abiotic system is independent of solution metal concentration exhibiting stable Kd values across the concentration range. However, biological processes are impacting partitioning, and the nature (enhancing or hindering Cr sequestration) as well as the degree of this impact are dependent on both solution metal concentration and the level of biological activity. The same biologic impact on partitioning to the carbonate fraction was observed for both uranium and arsenic. Given the strong relationship between carbonates and pH, as previously suggested, this decrease in partitioning with increased microbial activity may indicate that microbial metabolism is altering the carbonate substrate. Microbial metabolism, again, could be argued as a plausible means to decrease the pH in localized areas, which could result in localized dissolution of the carbonate substrate. However, it is questionable as to the type of complexes Cr is forming within this fraction. Cr is not reported to form carbonate complexes, although Balasoiu et al. (2001) did report Cr in their 'carbonate' fraction of their sequential extraction procedure, and in similar proportions to those found in this study. Given that the fractions within the SEP are operationally defined, this carbonate associate chromium may not actually be a carbonate, but rather a result of an acid/base reaction, unfortunately not knowing what type of complexes that are being formed limits my ability to hypothesize about possible mechanisms to account for the biotic impact.

Leachable Fraction

Partitioning to the leachable fraction was considerably lower than the oxide and carbonate fractions previously discussed, with log Kd values ranging from 0.97-2.33, which still accounts for an average of 3.3% and up to 8.6% of the sediment associated Cr. These findings are consistent with the research by Balasoiu et al. (2001), which found an association between Cr partitioning to the leachable fraction and the organic content of the soil. Balasoiu et al. reported percentage values of Cr partitioning to the leachable fraction within artificially contaminated soils ranging from 0.2%, for highly organic soils (peat), to 18.8%, for soils devoid of organic matter. The organic matter content of INL sediment has been reported as 0.25±0.14% wt. (Cooper et al., 2003) which falls within the broad range of Balasoiu et al.'s study, as do my the leachable Kd values.

Consistent with the two previously discussed fractions, Cr associated with the leachable fraction of the abiotic system is stable across all solution metal concentrations reporting a log Kd value of approximately 1.2 (Table 4.7; Figure 4.20), whereas the highly amended treatments (A2 and A3) show a significant decrease in partitioning across the concentration range. Therefore, the type (enhancing or hindering Cr attractions) and degree of impact is dependent on the level of biological impact as well as the solution metal concentration (Figure 4.20, 4.21). Greater Kd values were consistently reported for the systems with high biological activity (A2 and A3). However this result was most dramatic at the lower solution metal concentrations (Figure 4.20, 4.21). Kd values for the A2 and A3 systems at the 50 μM solution metal concentration were more than an entire log unit higher than Kd values for the abiotic system (2.31-2.33 for A2 and A3 versus 1.18 for the Sterile system). This gap narrows considerably at the 200 μM solution metal concentration, with a difference of only approximately 0.4 log units, although this difference is statistically significant (Table 4.7). Therefore, these results show that the importance of the leachable fraction to the systems with high microbial activity decreases with increased solution metal concentration.

It is important to consider the highly reversible nature of metals being sequestered by the leachable fraction. While these metals are considered to be associated with the sediments, only very small changes in geochemical properties (i.e. pH or redox) can result in the release of these metals back into the water column. Therefore, for the highly amended systems (A2 and A3) at the lower solution metal concentrations (50 and 200 μM), there is the potential to have a larger amounts of Cr released back into the water column compared to the abiotic system.

4.3.4 Cobalt

Similar to As, relative partitioning of Co to the sediment compartments was dynamic, indicating the behaviour of Co is also dependent on solution metal concentration. With increased solution metal concentration, partitioning shifted from the oxide compartment (55-75% of the solid associated Co at the 50 μM solution metal concentration to 30-45% at the 1000 μM concentration) to the more labile fractions for all treatments (Figure 4.22). Partitioning to both the carbonate and leachable fractions showed significant increases with increased

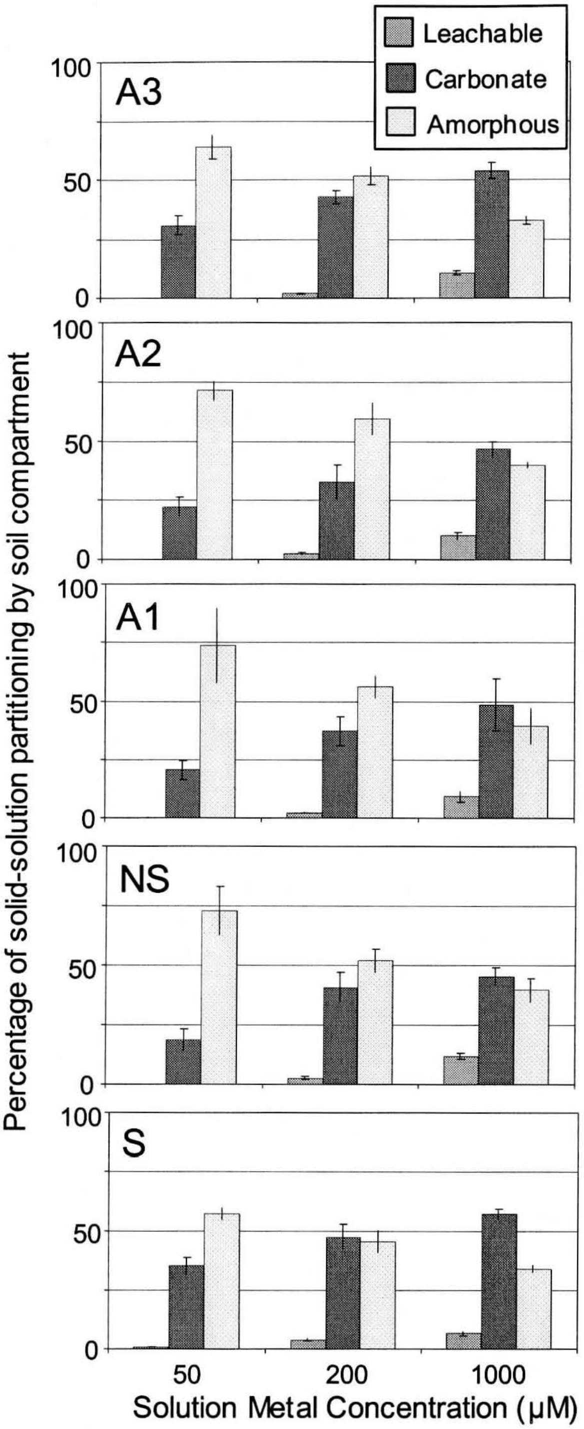

Figure 4.22: Percentage of Co partitioning to the relevant fractions showing a dynamic shift from the oxide to the carbonate fraction with increasing solution metal concentration.

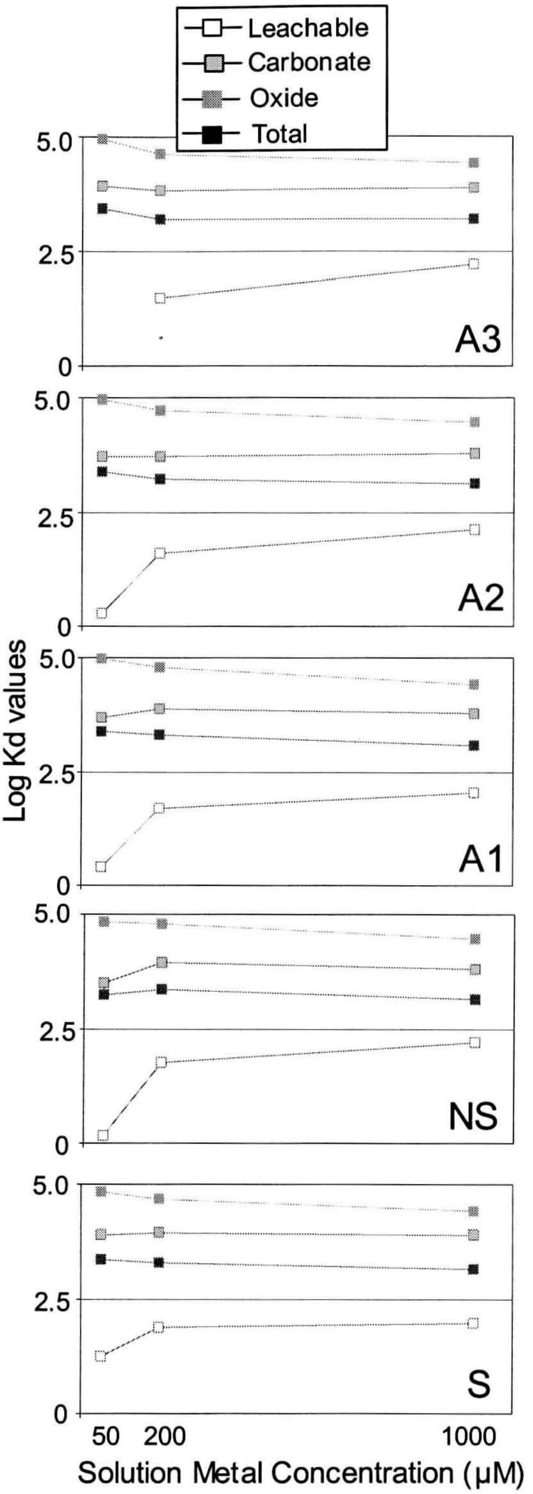

Figure 4.23: Log Kd values for Co partitioning to each of the relevant fractions showing a clear preference for the oxide fraction.

solution metal concentration (carbonate 20-35% to 45-55%, leachable 0% to 5-10%). Therefore, Co partitioning results indicate a potential saturation of the most highly reactive substrate, the oxide fraction, causing a shift to the labile fractions as the solution metal concentration increases. This result has important implications for metal mobility assessment, given that the labile fractions (carbonate and leachable fractions) are highly sensitive to changes in geochemical properties (pH and redox), and any changes in these properties may result in the release of metal back into the water column (Mahan et al., 1987).

Although a shift in relative partitioning was observed, the dominant Co sinks remained constant for all systems across the solution metal concentrations. Co partitioning was greatest for the oxide compartment (reporting log Kd values of 4.41-4.96; Table 4.5, Figure 4.23) followed by the carbonate fraction (log Kd values of 3.50-3.91; Table 4.6, Figure 4.23). Co was also associated with the leachable fraction however Kd values were considerably lower than the other two fractions and much more variable (log Kds values of 0.16-2.22). Unfortunately, very few published studies have examined partitioning of Co according to the different sediment fractions, and for those few studies currently published, compartmental specific Kd values were not reported. However, total Kds reported in the literature are consistent with those found in this study. Routson et al. (1987) examined trace Co sorption onto a variety of soil types from the Hanford Site and reported total Kd values ranging from 3.2-3.9, which is comparable to my findings of 3.1-3.4. And although compartmental specific Kd values could not be found in the literature, studies have been found emphasizing the oxides as the dominant player for Co partitioning (Kay et al., 2001; Rae and Parker, 1993; McLaren et al., 1986; Kinniburgh et al., 1976). The literature also reports carbonates, while not as substantial, to be important substrates for sediment associated Co as well (Krumholz et al., 2003; Bei et al., 1997; Reeder, 1996; Xu et al., 1996; Evans and Andrews, 1988).

An unexpected result of this research, was the observation of negligible partitioning of Co to the oxidizable fraction (Figure 4.9). This step of the sequential extraction procedure would be expected to extract Co from both organic and sulfide phases. And while the literature suggests strong Co associations to these phases (Krumholz et al., 2003; Qian et al., 1998; Bei et al., 1997), this was not observed in my results for these INL systems. Potential explanations for my observations are (1) insufficient metal exposure duration, (2) insufficient organic/sulfide contents within the sediment and (3) a greater affinity to the oxide and carbonate fractions than the oxidizable fraction. While it is possible that the 3-week metal exposure period was insufficient for Co to be incorporated into the oxidizable phases of the sediment, a similar study performed by Krumholz et al. (2003) on aquifer sediments found Co associated with the oxidizable fraction while using a similar timeframe to that in the current

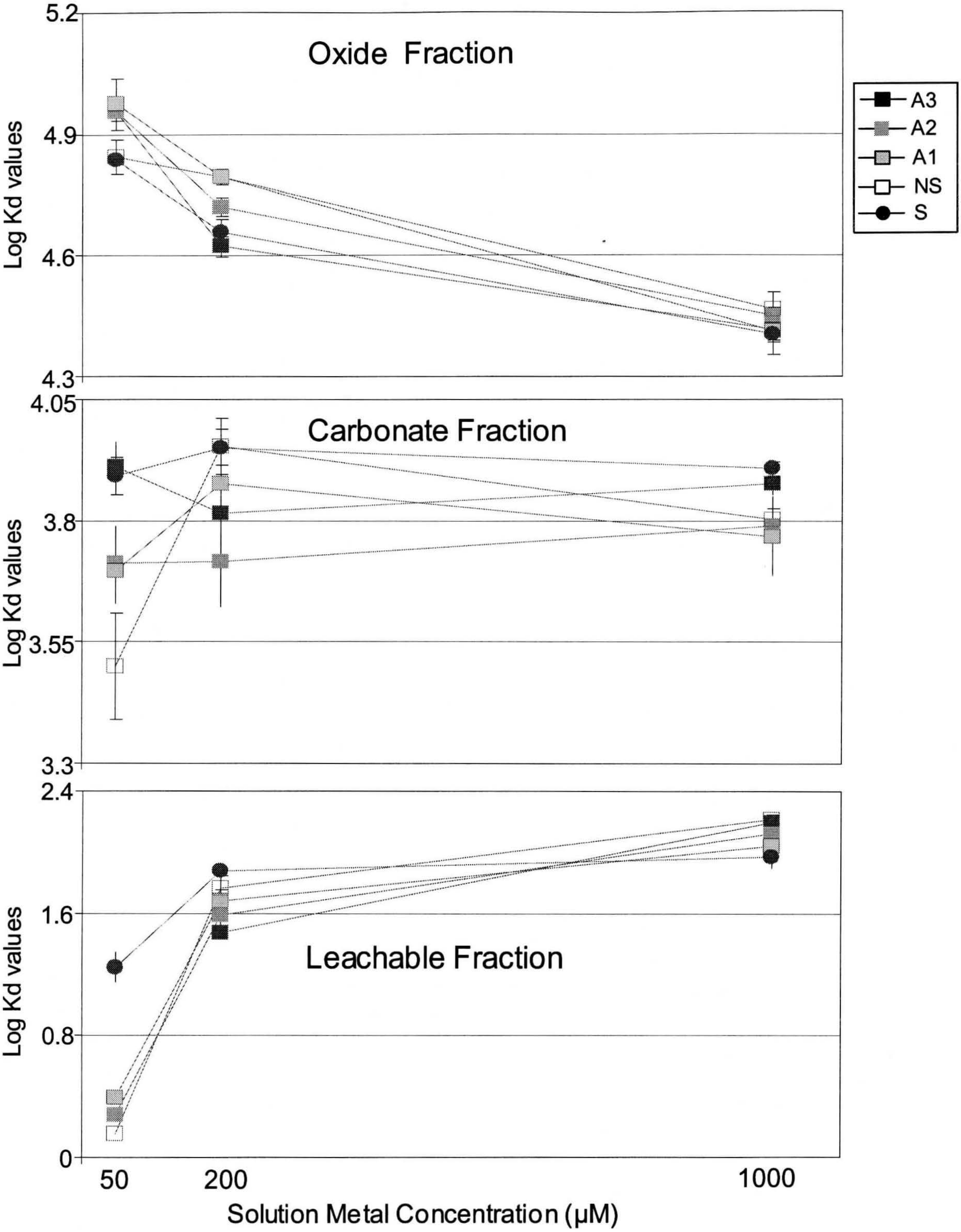

Figure 4.24: Log Kd values for Co partitioning to the Oxide, Carbonate and Leachable fractions according to system.

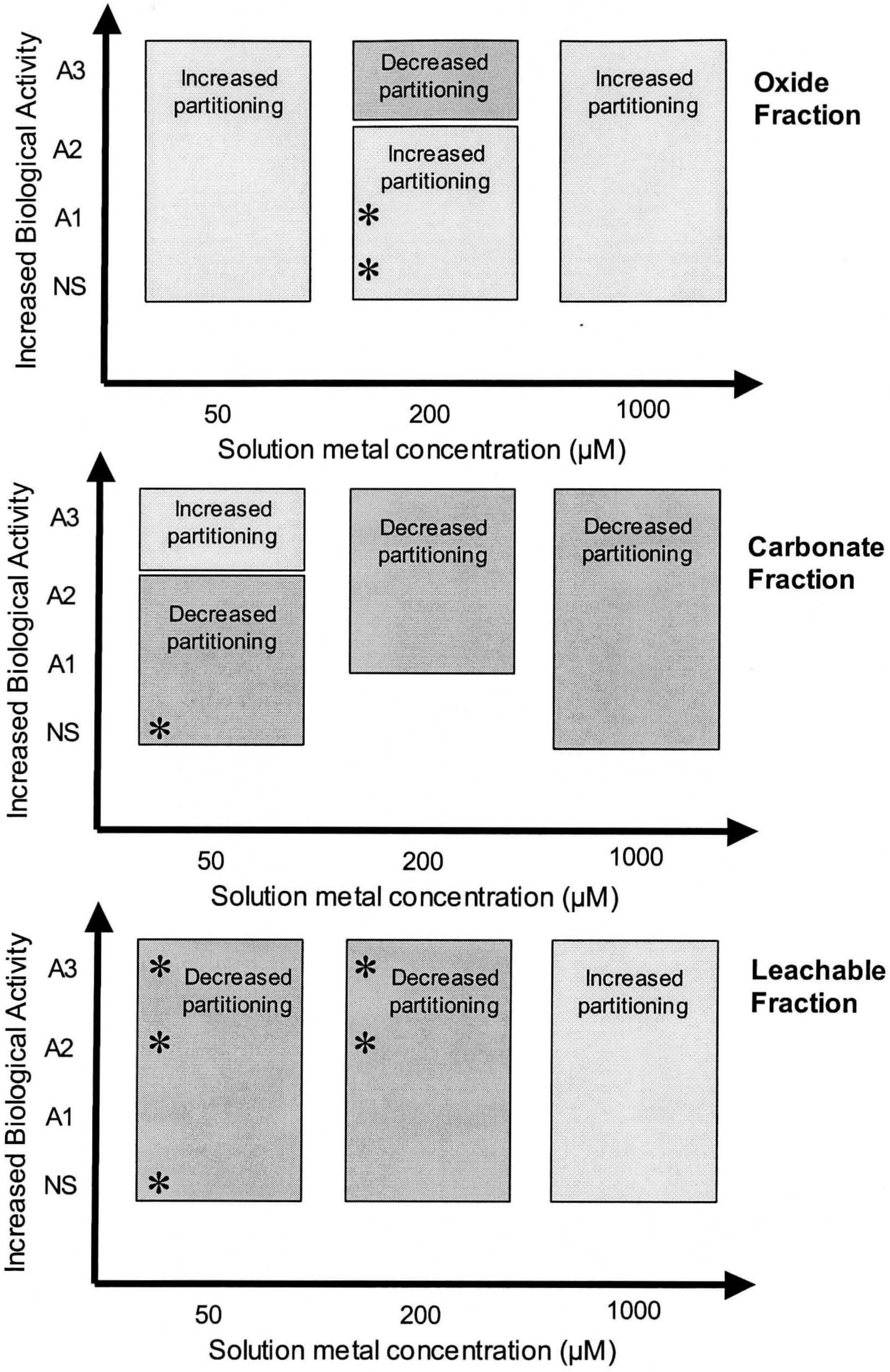

Figure 4.25: Co partitioning coefficients relative to the abiotic system for the **Oxide**, **Carbonate** and **Leachable** fractions, * designates a statistically significant difference from the abiotic system.

study. Therefore, this is not the most plausible explanation. However, it is possible that the low level of organic (0.25±0.14 %wt.) and sulfate (0.01±0.02 %wt; to generate sulfidic phases) material in INL sediment is insufficient to produce an effective Co sink (Cooper et al., 2003). Given the well documented dominance of oxides for Co partitioning (Rae and Parker, 1993; McLaren et al., 1986; Kinniburgh et al., 1976), it is also possible that the affinity of Co for the oxides (both Mn and Fe) surpassed that of the oxidizable phases. While this study cannot determine which of these possibilities is responsible for the observed result of negligible partitioning to the oxidizable fraction, it is clear that Co uptake in the INL soils is dominated by the oxide fraction.

Oxides

A non-linear decrease in oxide Co partitioning coefficients was observed for all systems across the concentration range, indicating potential Co saturation to oxide binding sites (Table 4.5, Figure 4.24).

Co oxide partitioning for the biotic systems follow the same general trend as the abiotic system across the concentration range, however partitioning values for all of the biotic treatments reported Kd values slightly above the Sterile system at all solution metal concentrations (with the exception of A3 at the 200 µM concentration; Figure 4.24, Table 4.5). These results indicate that biological activity stimulates Co partitioning to the oxide phase, which is quite different from the results observed for Cr and As showing biological activity greatly affected partitioning, both hindering and enhancing metal partitioning depending on the level of biological activity and the solution metal concentration.

This difference in observed behaviour across the various metals may be a result of partitioning to different types of metal oxides (i.e. Fe vs. Mn). While I have repeatedly commented on the importance of Fe oxides for partitioning of metals (U, As, Cr, and even Co), the contribution of Mn oxides for scavenging of these metals has not been directly addressed. While Mn oxides have been reported to be more reactive than Fe oxides (Tebo et al., 1997; Nelson, 1996), given the circumneutral pH of my current investigation and the amount of Fe oxides compared to Mn oxides (5-20 mg Fe_2O_3/g sediment vs. 0.13-0.9 mg MnOOH/g sediment; Cooper et al., 2003), partitioning of metals to the oxide compartment has generally been attributed to Fe oxides. However, Co has a very high affinity for Mn-oxides (Kay et al., 2001; Qian et al., 1998; Moffett and Ho, 1996; McLaren et al., 1986; Murray and Dillard, 1979), so may be responsible for the increased Co oxide associations. In support of this hypothesis, other studies have reported large amounts of Co uptake onto Mn oxides (Kay et al., 2001; Davies and Morgan, 1989) and suggested that oxidation of Co(II) to Co(III) on Mn oxides and the incorporation into Mn oxide minerals as a feasible explanation.

Carbonates

Partitioning of Co to the carbonate fraction shows a very different pattern than that of Co partitioning to the oxides. While partitioning coefficients for the oxide compartment decreased with increasing concentration, the carbonate Kd values, for all systems, showed stable partitioning coefficients with no significant differences across the concentration range (with the exception of NS at the 50 µM concentration, Table 4.6, Figure 4.24). This result indicates partitioning is independent of solution concentration and that carbonate sites were not saturated over the range of solution metal concentrations examined.

Co partitioning to this fraction demonstrates a clear, although non-significant, relationship between biotic and abiotic systems. At all concentrations the amended treatments exhibit lower carbonate Kd values than the biotic system (with the exception of A3 for the 50 µM concentration, Table 4.6, Figure 4.24, 4.25). Therefore, biological activity is decreasing partitioning of Co to the carbonate fraction. Xu et al. (1996) suggests a number of mechanisms for sorption of Co^{2+} on calcite ($CaCO_3$). Namely, Co^{2+} could (1) adsorb in exchange for Ca in the surface structure, (2) form surface complexes with CaOH and CO_3H groups, (3) precipitate as a carbonate or a hydroxide carbonate, (4) coprecipitate with $CaCO_3$ forming a solid solution, or (5) diffuse into the solid (Xu et al., 1996). Xu et al. concluded that that Co uptake by calcite is a highly complex and dynamic mechanism, exhibiting very different results for different experimental conditions where the mechanism was drastically influenced by pH. While the mechanism for Co sorption cannot be determined within the current study, given the decreased Kd values for Co partitioning to the carbonate fraction for the biologically active systems, it is possible that a change in mechanism for sorption is occurring. Given the strong influence of pH on Co sorption, and the likelihood of microbial respiration causing localized decreases in pH, this is a distinct possibility. This change in pH may be sufficient to dissolve some of the carbonate substrate, or change the dominant sorption mechanism. Further, a decrease in the amount of substrate available for partitioning in the biologically active system would result in decreased partitioning to this fraction, as well, alteration of the sorption mechanism could result in decreased partitioning. Therefore both possibilities are feasible although it is not possible to determine which is occurring within the current study.

Leachable

Considerably less Co partitioned to the leachable fraction (log Kd values of 0.39-2.22; Table 4.7, Figure 4.23) compared to the oxides and the carbonate fraction (log Kd values of 4.41-4.96 for oxides; Table 4.5, and 3.50-3.95 for carbonates; Table 4.6, Figure 4.23), and Kd values were also much more variable than those reported for the two dominant fractions. However, the leachable fraction exhibited a consistent increase in Co partitioning coefficients across the concentration range for all systems (Table 4.7, Figure 4.24). This result supports the notion that a shift in relative partitioning from the oxides (for which there was a decrease in partitioning coefficients across the concentration range) to this more labile fraction actually occurs with increasing solution metal concentrations.

There is also a biological fingerprint on Co partitioning behaviour within the leachable fraction that is dependent on the solution metal concentration. At both the 50 and 200 μM concentrations, biotic systems show lower partitioning coefficients than the abiotic system and for many of the systems this result is significant (Table 4.7). Therefore, at these lower solution metal concentrations biotic process are either (1) decreasing the ability of this fraction to loosely capture Co, or (2) biotic processes are releasing Co from this fraction. At the 1000 μM concentration however, all biotic treatments report greater partitioning values compared to the abiotic system. Therefore, for the leachable fraction biotic activity for the high solution metal concentration results in an increase of lightly bound metals to the soil substrate (Figure 4.24, 4.25, Table 4.7). A more important consideration for this fraction is the potential for these metals to be released back into the water column since, as mentioned previously, this fraction is highly sensitive to changes in redox, pH and even concentrations of cations in solution. Therefore, at the higher solution metal concentrations, there is a greater quantity of metals associated with this leachable fraction for the biotic systems, compared to the abiotic system, and thus the biotic systems can potentially release a greater quantity of Co back into the water column if changes in geochemical status occur.

4.3.5 Zinc

A strong shift in the relative partitioning of Zn with increased solution metal concentration from the oxide compartment to the more labile carbonate fraction was observed (Figure 4.26). Relative amorphous Zn partitioning decreased with increased solution metal concentration (60-75% to 20-25%), while relative carbonate partitioning increased across this concentration range (3-25% to 65-75%) and this result was observed for all systems (Figure 4.26). These results are consistent with the results observed for As and Co, both of which indicated a decrease in relative partitioning to the oxide compartment (Figure 4.16, 4.24, Table 4.5). This observable decrease in the relative partitioning for these metals lends further support to the contention that the oxide fraction is saturated at the high solution metal concentration.

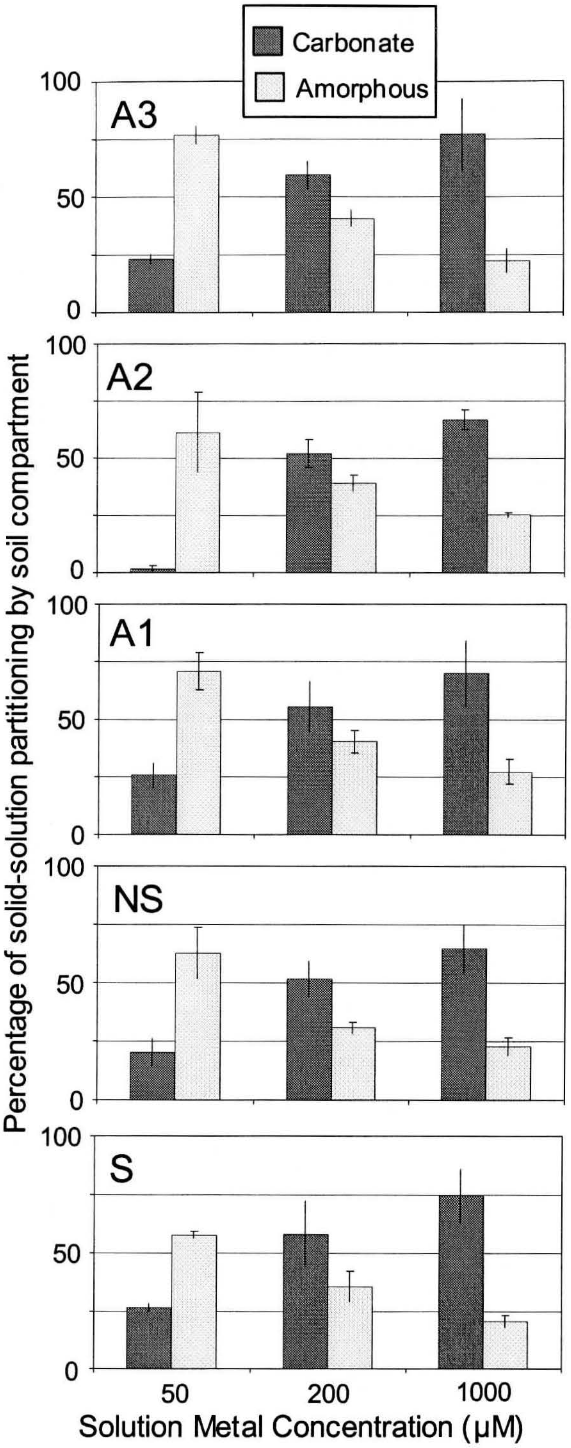

Figure 4.26: Percentage of Zn partitioning to the relevant fractions showing a dynamic shift from the oxide to the carbonate fraction with increasing solution metal concentration.

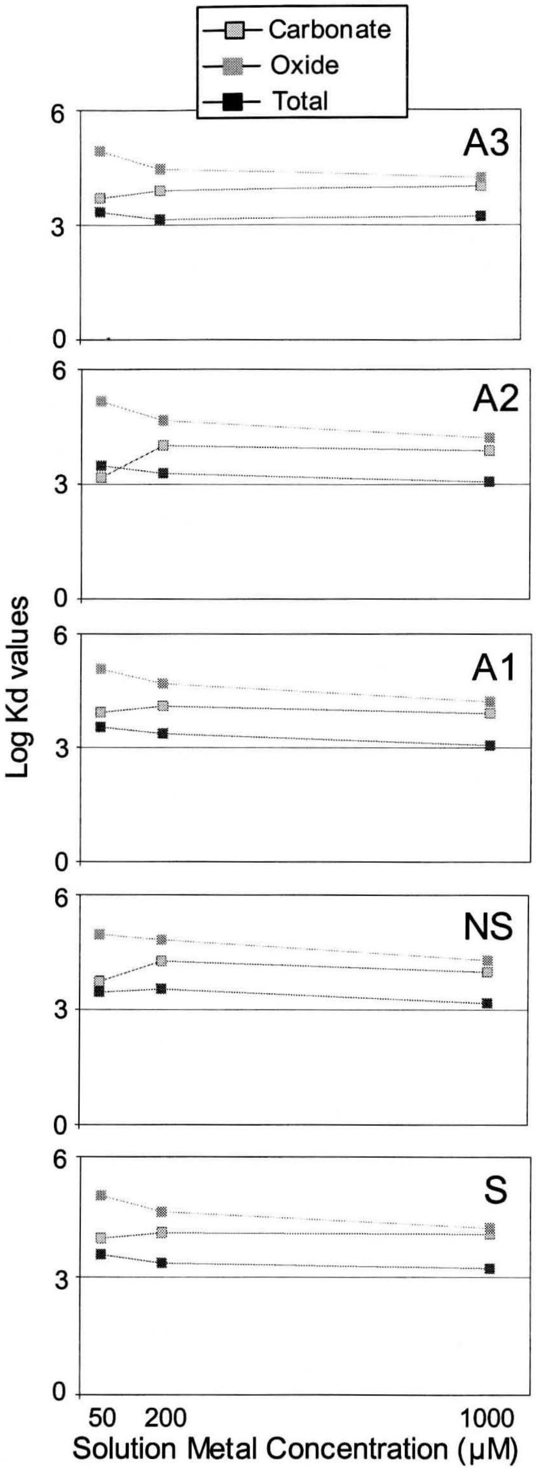

Figure 4.27: Log Kd values for Zn partitioning to each of the relevant fractions showing a clear preference for the oxide fraction.

Zn shows the highest affinity for the oxide fraction compared to the other sediment fractions (log Kd values ranged from 4.19-5.17, Table 4.5). Partitioning of Zn to the carbonate fraction was also substantial, although Kd values were approximately one log unit lower for the carbonates than the oxides (carbonate log Kd values ranged from 3.18-4.25, Table 4.6). These results are consistent with other published studies that have emphasized the strong carbonate association (Lin et al., 2004; Maes, et al., 2003; Warren and Zimmerman, 1994c; Tessier et al., 1989) and an even stronger affinity of Zn for iron oxides in sediments from a number of environments including aquifers (Lin et al., 2004; Magaritz et al., 1992), lakes (Tessier et al., 1996; Williamson and Parnell, 1994; Tessier et al., 1989), rivers (Warren and Zimmerman, 1994c; Johnson, 1986) and even peatlands (Bendell-Young, 1999).

Partitioning of Zn to the leachable fraction in this study was negligible (<5%) for all systems at all solution metal concentrations. This result is again, consistent with some literature results (Jonathan et al., 2004; Lin et al., 2004) but inconsistent with others that report associations of Zn to the leachable fraction to be greater than 25% of the total Zn partitioned to the sediment (Nyamangara, 1998; Peralta et al., 1996). The differences encountered in the literature is likely the result of differing study conditions, as solution metal concentrations, sediment composition and system pH play a crucial role in Zn behaviour in terms of solid associations (Prokop et al., 2003; Roberts et al., 2002, Bostick et al., 2001; Sauvé et al., 2000; Warren and Zimmerman, 1994c). Studies by Nyamangara (1998) and Peralta et al. (1996) analyzed lower pH systems (soil pH<6) that were characterized by high organic content, therefore it is not surprising that results from these studies are not in agreement with my findings. However, Lin et al. (2004) performed batch experiments on vadose sediments, inoculating with Zn for a solution metal concentration of 15 µM (compared to the current study with Zn concentration of 10 µM, 40 µM and 200 µM) over a circumneutral pH range, and reported negligible amounts of Zn associated with the leachable fraction. Therefore, the results of the study by Lin et al. are in strong agreement with my findings. Given that only negligible quantities of Zn are associated with the leachable fraction, there is less risk of inducing Zn mobility (compared to the other metals – U, As, Cr and Co which showed higher association with the leachable fraction) given slight changes in system geochemical properties.

A comparison of compartmental specific Kd values with other published values was precluded by a lack of literature reporting such sediment fraction specific Kd values. However, my total log Kd values (3.05-3.55) are in agreement with the range of values reported in the literature (3.1 – Prokop et al., 2003; 1.8-4.0 – Lin et al., 2004; approximately 2-4.5 – Sauvé et al., 2000 (review of over 300 reported Zn partitioning values)).

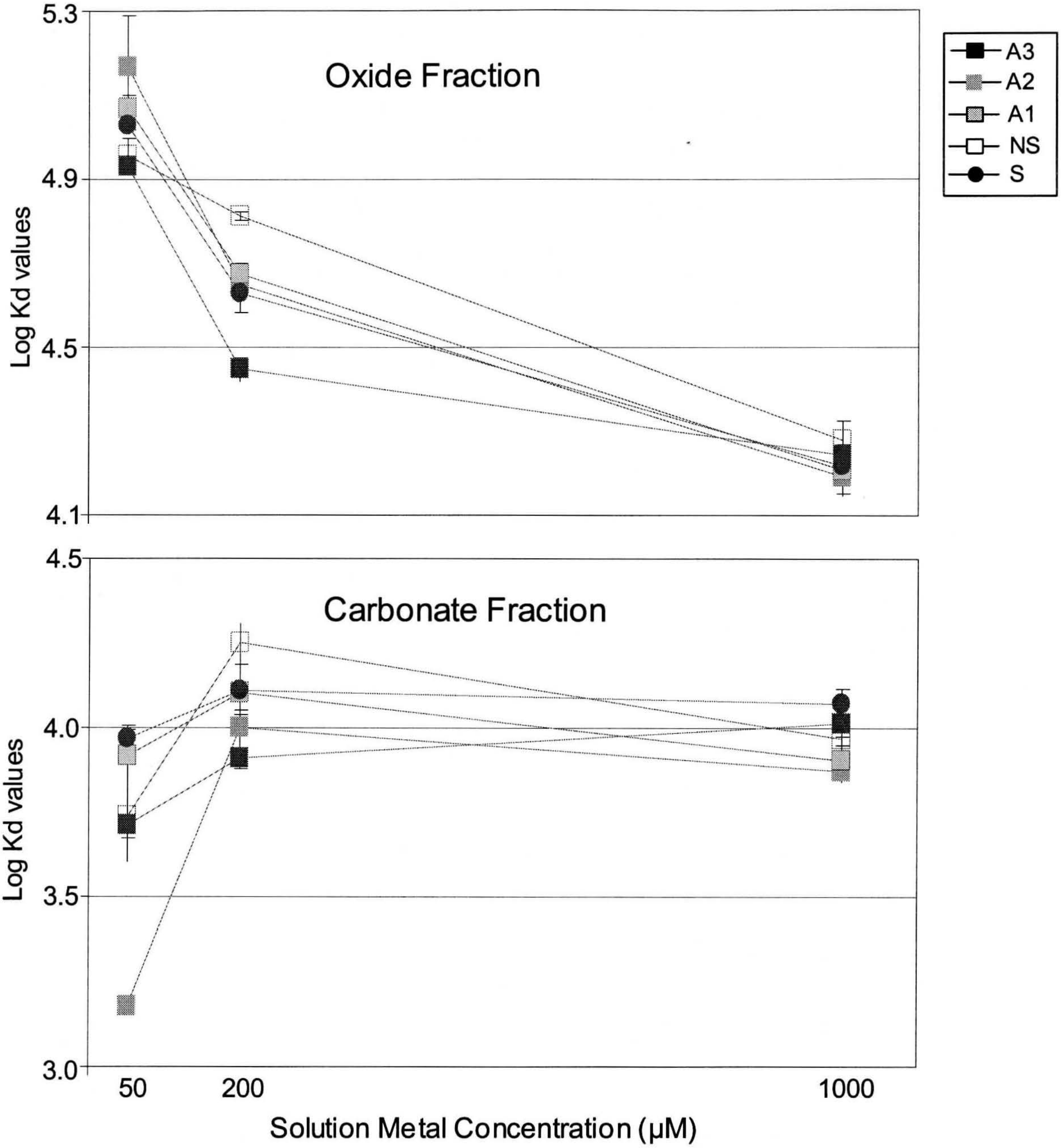

Figure 4.28: Log Kd values for Zn partitioning to the Oxide and Carbonate fractions according to system.

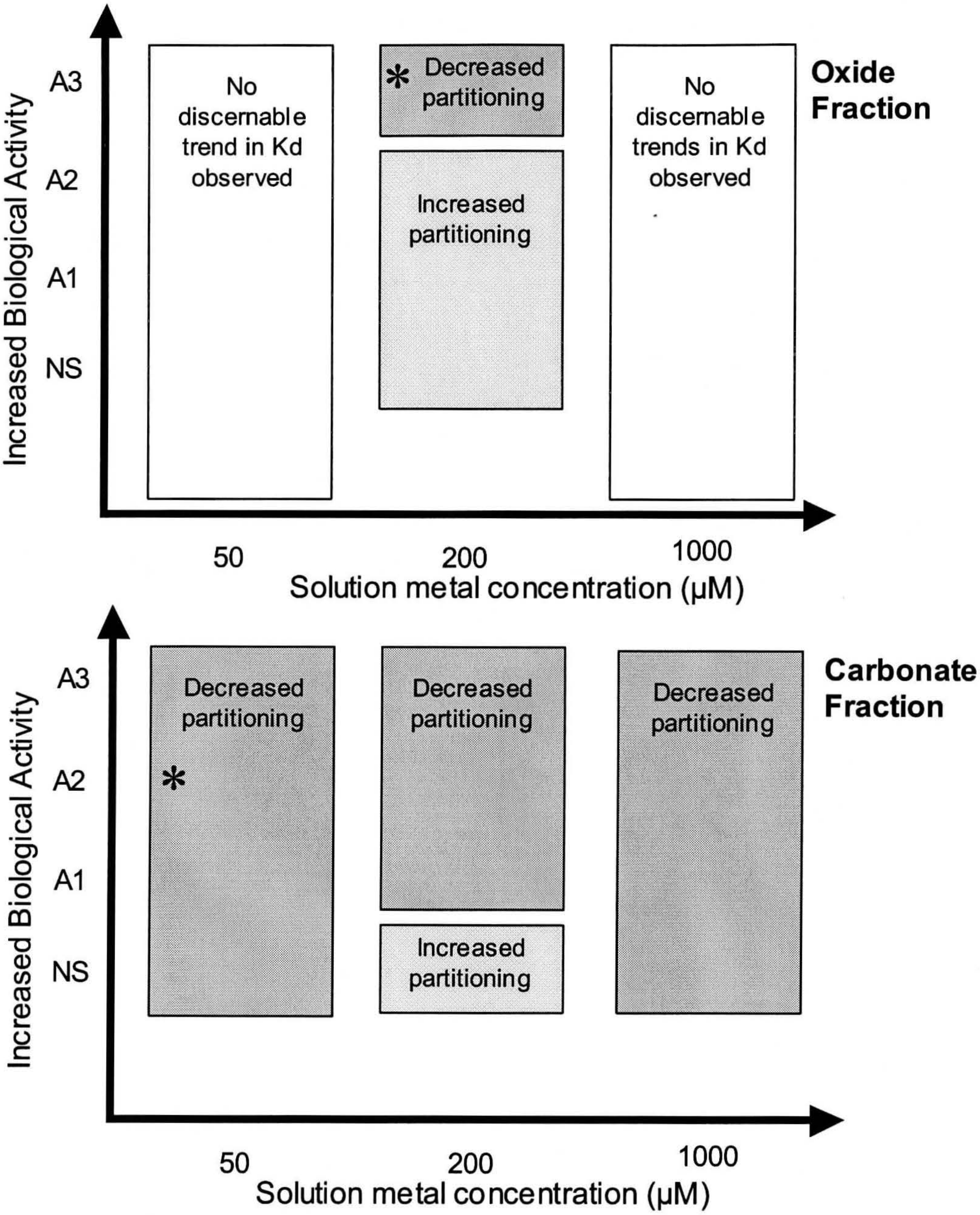

Figure 4.29: Zn partitioning coefficients relative to the abiotic system for the **Oxide** and **Carbonate** fractions, * designates a statistically significant difference from the abiotic system.

Oxides

As previously discussed, partitioning of Zn to the oxide compartment was consistent with Zn oxide partitioning for As and Co, with partitioning coefficients decreasing as a function of solution concentration for all systems (Figure 4.28, Table 4.5). This result indicates potential saturation of this highly reactive substrate and therefore, under the conditions of this current study (i.e. types and concentrations of metals), partitioning of Zn changes over the solution metal concentrations examined, likely as a result of this saturation.

No significant differences in Kd values were detected across the systems (biotic or abiotic) at either the 50 or 1000 µM solution concentration (Table 4.5). In the literature, Zn has been documented to have a toxic effect on some microbial communities. Kelly et al. (1999) found that after 15 days of incubation, a one time amendment of Zn (at a concentration similar to my highest solution metal concentration) to a soil microcosm resulted in 87% decrease in viable cell counts and a 47% decrease in microbial biomass. This could account for the similarity between the biotic and abiotic systems; however, results for the other metals investigated in this current study have shown clear biological impacts indicating the microbial community is still metabolically active, even if portions of the community were affected by metal loads. A study by Cooper et al. (2000) has shown that microbial activity can increase Zn immobilization which coincides with the production of Fe(II). This result has been attributed to the microbial reduction of Fe(III) to Fe(II), where Zn is believed to be incorporated into a mineral phase resulting from the presence of Fe(II). However, Cooper et al. (2000) found these results were not observed if concentrations of NO_3^- were sufficient enough to prevent the reduction of Fe(III), a finding that has been previously documented (Achtnich et al., 1995; DiChristina, 1992). Given the high concentration of NO_3^- in my simulated groundwater (which is consistent with INL groundwater, Roback 2001), it is possible that microbial reduction of Fe(III) has been inhibited. This would explain the similarity in oxide partitioning across the systems for the highest and lowest solution metal concentrations.

Carbonates

Zn carbonate partitioning coefficients were generally static across the concentration range (i.e. showed no significant differences in Kd values) for all systems (Table 4.6, Figure 4.28). Thus, partitioning of Zn to this fraction proved to be a robust process, not affected by potential metal competition.

Typically, biotic systems showed lower values than the abiotic system across all concentrations (except for NS at the 200 µM concentration, Table 4.6, Figure 4.28, 4.29). While the differences in reported values are quite small and generally non-significant, the decrease in partitioning to the carbonate fraction (relative to the Sterile system) was consistent for all amended systems. This finding supports the hypothesis that microbial respiration may be locally impacting geochemical properties. Microbial respiration may be sufficient to decrease pH causing some dissolution of the carbonate fraction. And while this hypothesis is consistent with the observed results, it is only speculative. However, the similarity in carbonate partitioning values for the systems (both abiotic and biotic) indicate abiotic processes dominate partitioning processes for Zn.

4.4 Soil Microbial Characteristics

Cell counts obtained by epifluorescence microscopy indicate that the Sterile system has the greatest number of cells per gram of sediment (7 X 10^6). However, cell counts across the systems showed very little variation - all treatments had cell counts on the order of 10^6 cell/g of sediment (Table 4.8). This observed higher cell count in the Sterile system compared to the biotic systems was not an anticipated result. Many studies have attributed decreases in soil biomass (Konopka et al., 1999; Roane and Kellogg, 1996; Fliessbach et al., 1994; Frostegard et al., 1993) and plate counts (Turpeinen et al., 2004; Kelly and Tate, 1998; Roane and Kellogg, 1996) to increased metal concentrations. However, it is possible that these cell numbers represent an artifact of the soil sterilization process that led to a higher cell count in the solution phase, as autoclaving may have loosened individual cells or decreased their adhesion to the soil matrix. Alternatively, biotic activity in the other treatments may have resulted in increased EPS and biofilm materials, which would decrease my ability to remove these cells even with sonication – resulting in lower cell counts for biotic systems. However, it is important to recognize that the difference in cell counts across the systems is not statistically different, so while not disregarding the results, it is important not to over interpret them. Further, cell counts do not necessarily reflect cell activity levels (Bernier and Warren, 2005), and thus attempting to infer direct correlations between the cell count number and level of metabolic activity would be highly inaccurate (Warren, 2005).

Table 4.8: Microbial cell counts per gram of dry sediment as determined by epifluorescent microscopy.

System	Cells
A3	4 X 10^6
A2	4 X 10^6
A1	2 X 10^6
NS	5 X 10^6
S	7 X 10^6

The range of cell count values observed is, however, consistent with values reported in literature for heavy metal contaminated aquifer/vadose sediments (10^6-10^7 cells/g, Turpeinen et al., 2004; Kelly et al., 1999), and INL sediments (10^6-10^7 cells/g, Cooper, 2004; Lehman et al., 2004; Geller et al., 2000). And while the consistency between reported values and those obtained in this study enhance the credibility of my methodology, the inability to detect significant differences between my systems identifies the limitations of this approach.

Current methods for evaluating microbial communities *in-situ* hinge upon either quantitative assessment of cell numbers (i.e. a proxy for activity as typically increased cell numbers reflect increased growth and division; Janssen et al., 2002; Garabetian et al., 1999; Bitton et al., 1993) or qualitative assessment of community phylogenetic structure through molecular biological approaches that evaluate the genetic diversity of the community (Bernier and Warren, 2005; Peccia et al., 2000; Derry et al., 1998; Konopka et al.,1998; Pace 1997; Wintzingerode et al., 1997). Both approaches are limited in their ability to directly constrain microbial metabolic influences on geochemical processes (Koizume et al., 2004; Haglund et al., 2003; Zopfi et al., 2001; Siering, 1998) and perhaps more seriously, both are hampered by methodological challenges and artifacts. While this newly emerging field continues to seek and develop more accurate tools to quantify microbial metabolism, current investigations can only use currently available techniques. My results, indicating that the Sterile system has the largest cell count (although not significant), identifies a fallacy in the supposition that metabolic activity can be inferred by total cell numbers.

The geochemical results clearly indicated that biological activity influenced metal behaviour in a complex manner, reflecting interaction of the level of biological activity and the solution concentration in an element specific manner (i.e. biological influence is not the same for all metals at all concentrations). However, microbial cell counts did not show significant differences across treatments (Table 4.8). While this result indicates that the number of microbial cells was statistically the same across treatments, it does not indicate whether all cells were metabolically active, or even of the same type of organism, and thus should not be taken to infer that no differences in microbial dynamics occurred. While it would be plausible to expect greater cell counts in systems where more microbial activity is occurring (e.g. in carbon amended systems where growth is presumably stimulated), the notorious inaccuracies in counting cells associated with soil particles may play a greater role in determining the observed results rather than actual trends in cell numbers associated with the various treatments (Kirk et al., 2004; Trevors, 1998; Wintzingerode et al., 1997). The geochemical results clearly show a treatment dependent overlay that argues against the microbial communities being (a) the same across treatments and/or (b) exhibiting similar levels of activity through similar metabolic pathways.

The artifacts with cell counts as applied to natural samples are beginning to clearly emerge in the literature (Kirk et al., 2004; Koizume et al., 2004; Maamri, 2000; Bitton et al., 1993; Torsvik et al., 1990a, 1990b). Originally derived for counts in laboratory pure cultures, the application to natural samples has been biased towards planktonic samples (Bernier and Warren, 2005; Zampino et al., 2004; Garabetian et al., 1999; Bitton et al., 1993). Increasingly, it is evident that microbes concentrate at solid surfaces (Bernier and Warren, 2005; Tabacchioni et al., 2000; Haack and Warren, 2003; Trevors, 1998) and that estimation of their densities in a solid-biofilm network architecture by cell count is hampered by extraction challenges (Kirk et al., 2004; Janssen et al., 2002; Wintzingerode et al., 1997).

DAPI staining and subsequent cell counts prior to sonication identified one of the major problems with this method when applied to environmental samples: sediment associate cell colonies (Figure 4.30). While this was not surprising

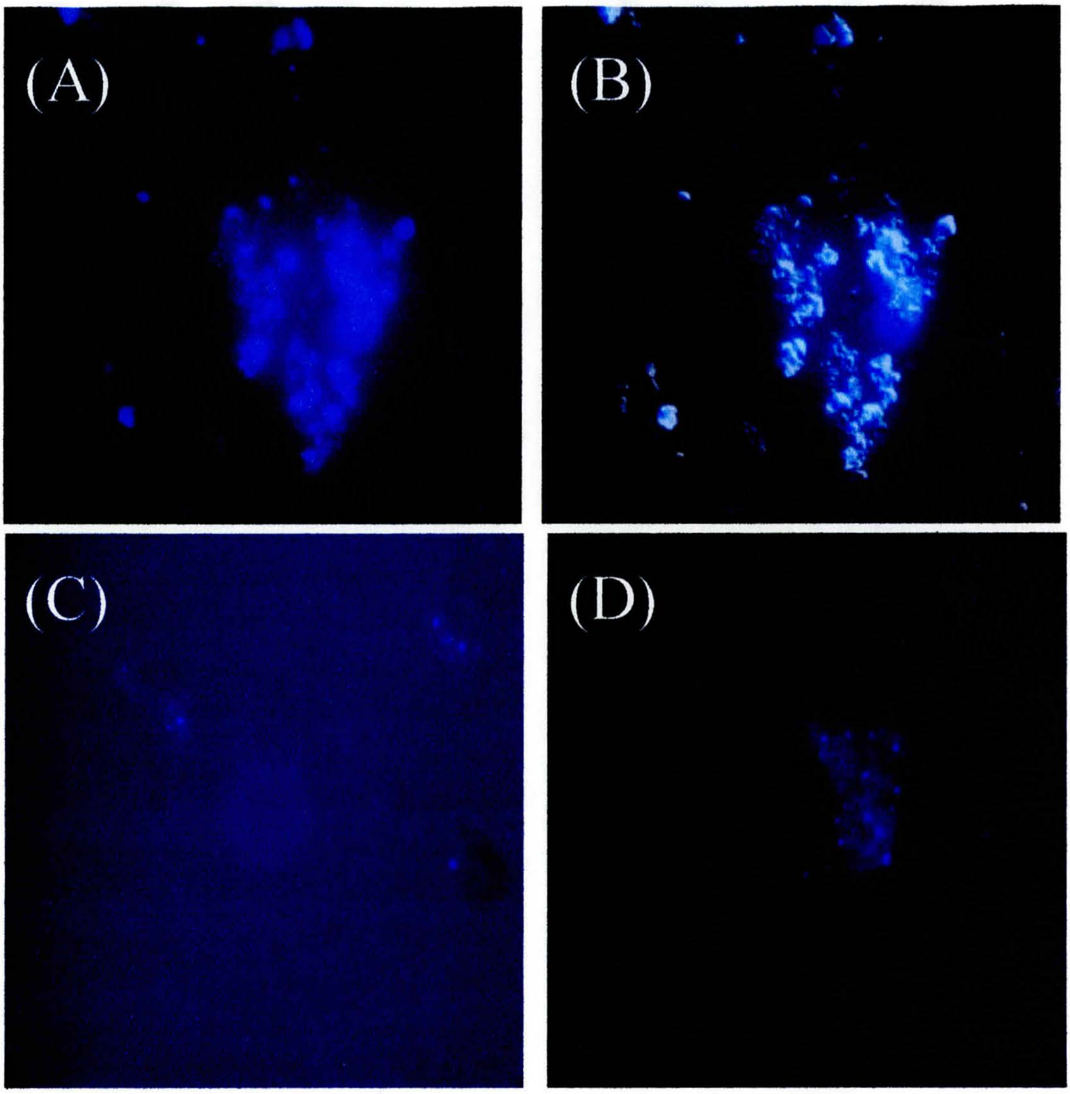

Figure 4.30: Epifluorescent microscopy images for direct cell counts using DAPI. Image (A) was obtained from the NS system prior to sonication and exhibits 'blurred' blue DNA stain preventing the ability to identify clear cells. Image (B) is a composite image combining the DAPI layer of Image A with a DIC layer showing sediment materials. This clearly illustrates the challenges of lithic fragments, sediment particles and biofilm associations as they can conceal cells and often result in large 'clumps' of fluorescing areas which are not suitable for cell counts. Images (C) and (D) were obtained on samples after sonication (from the NS and A3 systems respectively), a process which can separate cells from the sediment facilitating our ability to perform direct cell counts.

given that cells often adhere to sediment as biofilms within their desirable environment, it decreases the accuracy and applicability of this method. Counts performed after sonication reported cell counts of 10^6 cells/g, however, since this result was consistent across all systems, no further interpretation can be made using these values. This investigation provides further support for the limitations of this technique for application to environmental studies, for three reasons. First, there is question as to the effectiveness of the cell removal given that it is very difficult to remove bacteria from an established biofilm where cells are embedded in a network of biopolymers (EPS) that glues them to solid surfaces (Hanlon et al., 2006; Bernier and Warren, 2005; Daniels and Cherukuri, 2005; Schramm et al., 1996; Ramsing et al., 1993); e.g. cell numbers of the active microbial community involved in geochemical processes are not likely accurate. Secondly, there is no indication of the viability of the cells in each system (i.e. the Sterile and amendment treatments showed no significant difference in cell counts, however all cells in the Sterile system have been killed). And finally, if one were able to accurately count viable cells, the question remains as to whether they are dormant or active, and more specifically what is their metabolic function (Koizumi et al., 2004; Siering, 1998) and how to distinguish these different metabolic activities is the key to linking quantitatively to geochemically associated reactions.

5.0 Conclusions

Analysis of the effect of microbial activity on the mobility of a suite of metals (U, As, Cr, Co and Zn) in INL vadose sediments has shown that observed metal behaviour reflects a complex and dynamic set of interactions in laboratory batch experiments. While bulk water characteristics indicated that treatment dependent sediment pH and redox conditions were not sufficiently marked to be observed in the overlying water values, the relative solid-solution partitioning of each metal within the soil compartments of the batch systems, shows a more complex non-linear pattern, manifesting as an interactive function of (1) solution metal concentration, (2) level of biological activity and (3) the specific element involved.

This is one of the first studies to evaluate the relative affinities of a suite of metals for the solid phases over varying metal concentrations with a metal cocktail and levels of biological activity. My results indicate that differing affinities occur across this suite of metals, and that further, their relative affinities are non-linearly dependent on both metal concentration and level of biological activity. These results indicate that successful prediction of metal behaviour in complex natural systems, based on mono-metal laboratory experiments is likely to be limited.

My results clearly demonstrate a cyclic linkage between metal concentration and biological activity, presenting itself as a nonlinear interaction of increasing levels of microbial activity with increasing metal concentration such that at low metal concentration, increased microbial activity often leads to a decrease in metal retention compared to the abiotic system, while as total metal concentration increases from 50 to 1000 µm any biological activity hinders metal capture. This result may reflect feedback of metals on the microbial population such that the extent and/or nature of microbial activity is influenced by the level of metals present, thus it is important to consider this cyclic linkage in any interpretation of metal sediment biogeochemical behaviour.

Sediment compartments

The sediment associated oxide and carbonate fractions proved to be the dominant metal sequestering phases in INL sediment in terms of total metal partitioning; however their relative importance shifts from the amorphous oxides at the lowest (50 µM) solution concentration (amorphous oxides accounting for approximately 50% of total metals associated with the sediment vs. carbonates with approximately 20%) to the carbonates at the highest (1000 µM) solution concentration (carbonates with approximately 50% vs. amorphous oxides with approximately 30%) irrespective of biologic activity.

Oxides

The oxide compartment exhibited the highest Kd values for all metals (with the exception of U which showed no detectable partitioning to this fraction) indicating a high affinity of this sediment phase for these metals. Partitioning values substantially decreased (and often significantly) for all metals and systems for the oxide compartment as a function of solution metal concentration. Therefore, as the concentration of metals in solution increases, the relative importance of this fraction (compared to the other fractions examined) decreases, irrespective of level of biological activity. This observation most likely reflects saturation of available oxide sorption sites at higher solution metal concentrations. Increased metals in solution, corresponds to a decreasing ratio of sorbate to sorbent, and lower Kd values. Microbial activity affected partitioning to the oxide phase; however the effect was again dependent on the level of activity, the solution metal concentration as well as the metal identity. Such that no one trend emerged for all metals?? Again, try to be more specific with the general statement linking to a specific result(s) you are thinking of...

Carbonates

Partitioning coefficients for the carbonate fraction were an order of magnitude lower than for the oxides, and metal affinities for the carbonate fraction typically increased in the following order:

Cr<U<As<Co<Zn.

Metal retention associated with this fraction was again complex, showing differing trends according to metal solution concentration, biological activity and element identity.

Element Specific Behaviour

Uranium

In contrast to the other metals examined, U showed an equilibrium distribution, independent of metal concentration and/or microbial activity, under the given experimental conditions with approximately 80% of total U sequestered by the carbonate soil fraction and the remaining 20% partitioned to the leachable fraction irrespective of batch system conditions across all treatments. Partitioning values do, however, indicate non-linear changes in U affinity to both the carbonate and leachable fraction as a function of solution metal concentration, typically decreasing at the highest solution metal concentration possibly resulting from (1) the decrease in sorbent to sorbate ratio or (2) metal competition since it has been demonstrated that these more labile fractions (i.e. leachable and carbonates) become substantially more important for metal sequestration at the higher solution metal concentrations.

While microbial activity had no effect on shifts in relative U partitioning to sediment fractions, microbial impacts were observed both enhancing and hindering U partitioning where the type and magnitude of the impact was depended on the level of microbial activity as well as the solution metal concentration.

Arsenic

Across all systems, a shift in relative partitioning from the oxide to the carbonate fractions was observed as a function of solution metal concentration indicating a potential saturation of the most reactive substrate, the oxide fraction. This then resulted in the heightened importance of the carbonate fraction as the solution metal concentration increased.

As behaviour shows relatively static fractionation in the sterile system, indicating that under abiotic conditions carbonates and oxides are the most important sediment fractions, independent of solution concentration. While the same fractions were important for the biotic systems, partitioning typically showed a non-linear trend as a function of solution metal concentration, as well as varying results for microbial activity (beneficial vs. detrimental for metal retention) at each solution metal concentration. It is possible that the INL microbes are using As as a terminal electron acceptor, and converting As from As(V) to As(III) in association with organic matter decomposition. Since this effect is much stronger at higher solution metal concentrations it would suggest that the metabolism of As reducing bacteria is increasing in the presence of this so called 'nutrient', or that dynamic changes are occurring within the microbial community where some species (those able to reduce As) are becoming more metabolically active.

Chromium

Relative Cr partitioning was highly variable showing shifts in compartmental partitioning with increased solution metal concentration: an increase in the relative importance of the carbonate fraction (for all systems) and amorphous fraction (3 of the 5 systems) was observed. However, differences in relative partitioning was observed with different levels of biological activity indicating Cr is non-linearly dependent on a complex system of interactions.

Microbial activity has differential impacts (beneficial vs. detrimental) for Cr partitioning to the oxide compartment, at each solution metal concentration. However the strength of the biological effect increases with increased solution metal concentration, and results in an overall decrease in oxide associated Cr.

Biological activity negatively impacted Cr partitioning to this carbonate fraction and this effect was strengthened with both increasing biological activity and increasing solution metal concentration.

Cobalt

Similar to As, a non-linear decrease in Co oxide Kd values was observed irrespective of biological activity possibly suggesting potential reactive oxide sites are nearing saturation. The labile fractions showed increased importance at higher solution metal concentrations is likely the result of 'spillover' from the oxide fraction, a result which was observed for all systems.

Biological activity positively impacted Co oxide Kd values at all solution metal concentrations. However, biological activity decreased partitioning of Co to the carbonate at all concentrations. Microbial respiration would be expected to cause localized decreases in pH that may be sufficient to dissolve some of the carbonate substrate. This would, therefore, decrease the amount of substrate available for partitioning in the biologically active system, resulting in decrease partitioning to this fraction.

Zinc

Zn oxide partitioning coefficients decreasing as a function of solution concentration for all systems showing a strong shift in relative partitioning with increased solution Zn concentration from the oxide compartment to the more labile carbonate fraction.

Biological activity had no significant impact on Zn partitioning to the oxide phase at either the 50 or 1000 µM solution metal concentration, indicating that abiotic processes governed partitioning. Biotic systems typically reported lower carbonate Kd values than the abiotic system across all concentrations, however the differences in reported values are quite small and generally non-significant, so while biological activity may be decreasing Zn partitioning to the carbonate fraction, this impact is minimal.

Microscopy

The geochemical result clearly indicated that biological activity influenced metal behaviour in a complex manner, reflecting interaction of the level of biological activity and the solution concentration in an element specific manner (i.e. biological influence is not the same for all metals at all concentrations and/or at all levels of microbial activity (as assumed by level of carbon amendment)). However, microbial cell counts did not show significant differences across treatments. This lack of significant difference among experimental systems underscores the limitations of using cell counts as a sensitive indicator of metabolic activity. Further, notorious inaccuracies in cell counts associated specifically with soil particles, due to biofilm formation and difficulty in separating cells from solid particles, also likely precluded an accurate assessment of true microbial cell counts in these batch microcosms.

This research evaluated the effects of a complex suite of variables, commonly observed in natural systems (i.e. variable biological activity, variable metal concentrations, multi-element solutions), for metal partitioning behaviour. The results, which show that non-linear interrelationships amongst these factors influence the observed metal partitioning, support the necessity for greater complexity in laboratory-based experiments. From these results it is apparent that biological activity can affect metal partitioning in ways not captured solely by geochemical analysis, especially at high solution metal concentrations (i.e. 1000 µM). Metal partitioning behaviour is often considered to be site specific (which has been the general finding for metal Kd values reported within the literature). However, studies such as this thesis, which include multiple factors that may influence observed behaviour, increase our knowledge of these dynamic systems. By increasing our understanding of the interacting factors influencing metal partitioning we may potentially bridge the gap between laboratory-based metal partitioning results and metal behaviour observed in natural systems.

6.0 Future work

The results of this study have raised many new questions as well as pointing to gaps in the current knowledge of biogeochemical processes of multi-metal behaviour. In order to thoroughly cleanup the INL site, in a cost effective manner, more research is needed to understand the processes occurring within the subsurface so that we can manipulate environmental conditions to reduce metals from being released back into the porewater by enhancing in-situ metal retention. In order to achieve this we need to have a better understanding of the impact microbes are having on the oxide and carbonate fractions to determine if changes in substrate mass is occurring (i.e. decreases in Fe oxides or carbonates). We also need to improve our understanding of the processes affecting metals within the system. Secondary ion mass spectrometry (SIMS) as well as X-ray absorption fine structure (XAFS) and X-ray absorption near-edge structure (XANES) would be useful tools for the determination of the oxidation state and coordinational environments of these elements associated with the sediment, which would be useful for the understanding of processes governing U, As and Cr solid uptake as well as providing more information on their likely stability.

While this thesis has provided one of the first set of results evaluating multiple factors simultaneously, natural systems are still more complex, so it is important to increase the complexity of laboratory studies in an attempt to mimic natural conditions. Therefore, the next step would be to assess the impact of both flow and variable moisture levels on reactive metal partitioning, as these factors may affect pH and redox gradients as well as biological activity and metal migration.

7.0 References

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