Estimation of Human Availability of Arsenic in Contaminated Soils

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Estimation of Human Availability of Arsenic in Contaminated Soils

Albert Juhasz, Euan Smith and Ravi Naidu
CSIRO Land and Water, Adelaide
Note: please note that all authors are now based at Division of Information Technology, Engineering and the Environment, University of South Australia

ABSTRACT

It is generally understood that the bioavailability of a contaminant in soil may be significantly less than 100%. However, depending on the indicator organism being considered, quantification of contaminant bioavailability may be difficult. While acknowledging that there may be bioavailability constraints for contaminants, many health guidelines are currently based on total contaminant concentrations in soils. The greatest problem with this practice is that the risk to the environment may be overestimated which may lead to unnecessary and costly site remediation. Studies have been conducted in developed countries, notably the USA, into quantifying the extent of human bioavailability of soil-sorbed contaminants. Arsenic (As) is a soil contaminant where human bioavailability has been assessed using both *in vivo* swine and *in vitro* chemical extraction studies. This paper outlines biological and chemical methods available for bioavailability assessment, and evaluates measures of As bioavailability and its implications for human bioavailability. While this paper focuses on As bioavailability it could equally well relate to other inorganic contaminants.

1 INTRODUCTION

Arsenic (As) is widely distributed in the environment and is a common constituent of many foods and soils. Varying amounts of As are naturally present in many soils, with As concentrations ranging from 0.2 to 40 μg g^{-1} (Walsh *et al*., 1977). Weathering of regolith material and volcanic activity is the primary natural sources of As in soils. Anthropogenic activities also contribute to the deposition of As into the environment. The main anthropogenic activities that contribute to enhanced concentrations of As in soils occur mainly through the use of agricultural chemicals, such as pesticides, herbicides, cotton desiccants and wood preservatives, additives to animal feeds, in historical pharmaceutical products, as well as in the mining and smelting industries. In Australia, As contamination of soils occurs in many of the States and Territories and may range up to 15 000 μg As g^{-1} (Dowling *et al*., 2001).

In 1999, the National Environment Protection Council (NEPC) introduced the National Environmental Protection Measure (NEPM) for the Assessment of Site Contamination; a uniform framework and basis for assessing the risk associated with contamination across Australia. Within this framework the assessment of As-contaminated sites is based on the total concentration of As in soil. However, it is generally agreed that total concentration may not be indicative of the absolute bioavailability of the contaminant to a target organism. Regulatory policies generally permit but do not promote the inclusion of bioavailability adjustments in risk assessments and how it is determined varies considerably depending on which section of the scientific community is questioned.

2 BIOAVAILABILITY – DEFINITION

Although the term bioavailability has been used to a great extent in the scientific literature over the past few years, different definitions of bioavailability may arise depending on which sub-section of the scientific community is asked. For example, bioavailability may be described as a physiologically driven uptake process (or environmental bioavailability) (Peijnenburg *et al.*, 1997), the fraction of the total available dose absorbed by an organism which is distributed by the systematic circulation and ultimately presented to the receptor or sites of toxic action (toxicological bioavailability (Landrum *et al.*, 1992) or the extent to which a contaminant is available for biological conversion (bioremediation bioavailability) (Juhasz *et al.*, 2001). Other terms including pharmacological bioavailability, phytoavailability and bioaccessibility have also been coined (Landrum *et al.*, 1992).

However, no matter what definition of bioavailability is used, bioavailability is dependent on three factors. Firstly, the *opportunity* must arise where the receptor (or organism) is exposed to the matrix in which the contaminant resides. Secondly, within the matrix, there must be a fraction of the contaminant that is not irreversibly sequestered or bound to the matrix, *i.e.* the contaminant must be *potentially available*. Finally, the receptor (or organism) that is exposed to the contaminated matrix must have some *assimilative capacity* towards the potentially available fraction. Without fulfilling these three requirements, a contaminant is not bioavailable.

3 DETERMINATION OF BIOAVAILABILITY

The assessment of bioavailability has resulted in the development of numerous assays, including chemical extraction techniques, and tests employing microorganisms, invertebrates, amphibians, plants and higher organisms. Bioavailability may be estimated using a number of endpoints including a toxic or mutagenic response, by the inhibition of a metabolic function, by changes in microbial population structure, by mortality or malformations or by the accumulation of chemical species in organs or the bloodstream. In addition, bioavailability may be assessed by the dissolution of contaminants after extraction of particular mineral phases.

3.1 MICROBIAL TESTS

Bioavailability assays utilising microorganisms are popular due to the sensitivity of bacteria to numerous contaminant types. In addition, these assays are simple, rapid and low cost. Microbial assays can be divided into two groups, those that utilise indigenous or naturally occurring organisms and those that utilise genetically engineered microorganisms (GEMS). Assays employing indigenous microorganisms include determining changes in respiration, microbial diversity, soil ATP and enzyme activity (e.g. nitrification, phosphatase, urease) as a response to contaminant exposure. Popular GEM assays include the Ames Test (*Salmonella enterica* serovar Typhi strains) and SOS chromotest (*Escherichia coli*) which determine the mutagenic potential of contaminants, and microorganisms where the *lux* gene (bioluminescence) has been incorporated as a reporter system for contaminant toxicity, bioavailability etc.

The inclusion of the *lux* gene into microorganisms with specific responses to contaminants provides an opportunity for the determination of toxicity or bioavailability of a test substance by measuring changes in light output response when challenged by the contaminant.

Algal tests have also been developed for the determination of contaminant bioavailability. These tests are similar to bacterial tests where changes in respiration, algal diversity or enzyme activity are measured as a response to the bioavailability of a contaminant.

3.2 SOIL INVERTEBRATE TESTS

Because of their intimate relationship with the soil environment, soil invertebrates have been used in assays for determining contaminant toxicity and bioavailability. Earthworms are commonly used in these assays using methodologies as outlined in OECD guidelines (1984b). However, nematodes, collembola, daphnia and protozoa may also be used for bioavailability assessments. Contaminant bioavailability may be assessed by organism mortality, growth inhibition or accumulation of the contaminant in the organism.

3.3 AMPHIBIAN TESTS

It has been recognised for some time that amphibians, such as frogs, are sensitive indicators of pollution (Cook, 1981). Adverse effects such as malformations, disruption of metamorphosis and the decline of amphibian populations have been documented as a result of the presence of toxicants in the environment (Baringa, 1990; Cook, 1981; Herkovits *et al.*, 1989). Due to the high sensitivity of amphibians to 'environmental stress', their application for assessing environmental pollution has been realised through the development of a variety of toxicity tests. Tests vary in their application from the assessment of acute toxicity, short-term chronic and chronic toxicity and early life stage exposure assessment to hazardous substances and environmental samples. In turn, these tests may also be applied for assessing contaminant bioavailability. Frogs, toads, newts and salamanders have been utilised in toxicity/bioavailability tests, in fact, the use of over 35 species of frogs and toads have been reported in the literature. This may cause some confusion when selecting the most applicable species for site-specific toxicity/bioavailability assays.

3.4 PLANT TESTS

Plant assays are an important indicator of bioavailability or phytoavailability, in fact the determination of plant uptake is critical when determining exposure pathways for soil borne contaminants. Bio/phytoavailability may also be determined by assessing seedling emergence, root elongation or mutations using the Tradescantia micronucleus test. Currently there is no universal standard plant test for assessing bioavailability/toxicity, however, the OECD guideline No. 208 (OECD, 1984a) is often quoted for soil contaminants and the USEPA OPPS guidelines are utilised specifically for plant protection products.

The above information (sections 3.1-3.4) illustrates the variety of tests currently used for the assessment of contaminant bioavailability. While these tests may determine the potential impact of contaminants on various trophic levels in the environment, their application for the assessment of human health impacts as a result of contaminant bioavailability is questionable. Conceivably, some of these tests may be used as a rapid assay or form the basis of a battery of tests to assess the impact of contaminant bioavailability on ecosystem health and functioning. In the following sections (3.5 and 3.6), methods for the assessment of bioavailability, in the context of human health risk assessment, are outlined with specific reference to As-contaminated soil.

3.5 *IN VIVO* STUDIES OF HUMAN AVAILABILITY OF ARSENIC

Many methods are available for determining the extent of absorption of metals. Two of the most critical factors that need to be considered when designing absorption studies is that the material being tested closely resembles the nature of the material at the contaminated site and the nature of the exposure to be mimicked as closely as possible to what occurs during human ingestion. The geochemical forms combined with the absorption process results in a complex system that requires controlled studies to determine the bioavailability of As. Currently both *in vivo* and *in vitro* methods have been used to determine the bioavailability of As from ingested soil.

The ultimate test for human-based risk assessment is human experimentation, however, these tests are unethical. As such, a number of *in vivo* studies using animal models (monkey, pig, dog, rabbit, rat) have been developed for predicting human exposure to As in soil. *In vivo* bioavailability assessment involves animal dosing experiments where the test animal is dosed with soluble As (administered via gavage or intravenous injection) or As-contaminated material (administered orally). As absorption (or bioavailability) is determined by monitoring blood and urine As concentrations. Regardless of the animal model used, most studies indicate that As bioavailability in contaminated soils is much lower than the bioavailability of soluble As (as used for assessing the risk from As in drinking water). For example, Freeman *et al*. (1993) reported that urinary data collected from New Zealand White rabbits dosed with As-contaminated smelter material from the Anaconda smelter site in Montana, USA had an average relative absorption of 27 % (range 22-31 %). Freeman *et al*. (1995) also used a second animal model (monkeys) to study the relative bioavailability of As-contaminated smelter material also collected from residential areas of the Anaconda smelter site in Montana, USA. They reported that based on the urinary As data an average of 14 % of the As in the soil fed to the monkeys was absorbed.

The choice of an appropriate animal model necessitates the selection of an animal with the appropriate anatomical and physiological characteristics. Due to their close relatedness to man, monkeys are the first choice for *in vivo* bioavailability studies, however, the cost of such tests usually limits their use. Immature swine are considered remarkably similar to humans due to the similarity of the digestive tracts, nutritional requirements, bone development and mineral metabolism. Furthermore, young swine are considered to be a good physiological model for gastrointestinal absorption of a contaminant in children (Weis *et al*., 1991). Other advantages of *in vivo* swine trials include:

- Similar body size, weight and bone to body weight ratio to young children;
- Ease of contaminant delivery;
- Metabolism and excretion of As is similar to humans (pigs are monogastric omnivores);
- Adaptable to periodic feeding;
- Bioavailability may be assessed in a partially fasted state (unlike smaller animal studies);
- Possess a gall bladder which excretes bile into the small intestines when food is present;
- Coprophagia is not required to maintain normal nutritional status;
- Repeated blood sampling is possible (unlike in smaller animals);
- Rate of growth and maturation is slower than rats or rabbits; and
- Exceptionally high doses of As are not required to generate elevated levels unlike other animal studies (e.g. rats);

The bioavailability of As in soil has been assessed using the swine model and a number of soil types (Casteel *et al*., 1997). Arsenic bioavailability was found to be dependent on the form of As present in the sample; however, the results demonstrated that As in most soils and mine wastes studied are not as well absorbed as soluble As. Urinary As excretion data for the relative absorption factor for 14 soil and mining waste materials varied from near 0 to 0.52. Mining waste contained the least bioavailable As, presumably due to the presence of As in larger particle sizes and less soluble forms (e.g. sulfides) while smelter waste or tailing stream sediments contained the greatest amount of bioavailable As. The animal model evidence of reduced As bioavailability in contaminated materials is also supported by comprehensive *in vitro* studies.

3.6 *IN VITRO* STUDIES OF HUMAN AVAILABILITY OF ARSENIC

Although *in vivo* studies utilising animal models are an appropriate method for assessing bioavailability of As in soil, the time required for *in vivo* studies and the expense of animal trials precludes its use for routine bioavailability assessment. As a result, *in vitro* assays simulating gastrointestinal conditions in the human stomach have been developed. The predecessor of *in vitro* methods was developed for nutritional studies to assess the bioavailability of iron in food. These assessments are based solely on dissolution processes and as such are only applicable where the dissolution of the contaminant matrix controls the bioavailability of the contaminant of interest (Basta *et al*., 2001). However, where applied appropriately, these tests (PBET and IVG tests) act as a surrogate measurement of metal bioavailability that is quick and inexpensive compared to animal models.

The physiologically-based extraction test (PBET) and the *in vitro* gastrointestinal (IVG) method are *in vitro* screening-level tests used for predicting the bioavailability of metals from a soil matrix. Both methods simulate the physiological conditions of the human gut in glass reaction vessels; the amount of As solubilised during the dissolution reaction provides an estimate of the soil As potentially available for absorption. Studies to date have generally found good agreement between As availability obtained from the *in vitro* methods and from *in vivo* studies when similar As-contaminated materials have been compared. Rodriguez *et al.* (1999) evaluated the ability of the PBET and IVG methods to estimate As availability in contaminated soils compared to the swine animal model. They reported that there was close agreement between As bioavailability using the *in vitro* PBET and IVG methods and the *in vivo* swine animal model for non-calcinated slags and soils. However, both the PBET and the IVG methods underestimated the bioavailability of As in calcinated materials. In all contaminated materials studied using either the *in vitro* or *in vivo* methodology, As bioavailability in contaminated materials was markedly less than 100%.

4 BIOAVAILABILITY AND RISK ASSESSMENT

Risk assessment has become a central component of Australia's regulatory system, and the requirement for remediation has been determined on the basis of whether contamination was adversely affecting or potentially affecting human health or the environment. Although the NEPM guidelines moved towards a risk-based approach for the assessment of contaminated sites, soil criteria are still based on total metal concentrations. In the context of risk assessment and clean up decisions, bioavailability is fundamental in determining environmentally acceptable endpoints and potential clean up

options. As such there is a need to provide information on the role of bioavailability in evaluating human exposures to chemicals in soils and how this scientific information can be used to enhance risk assessment and remediation decisions at contaminated sites.

When quantifying exposure (i.e. the chemical daily intake) using the current guidelines, bioavailability of the contaminant is assumed to be 100%, however, this may result in the overestimation of exposure. The need to evaluate bioavailability arises from the fact that metal species vary in their solubility and capacity to sorb to soil constituents, which will influence their uptake by receptor organisms. As a result, the assumption that the relative availability of a metal is 1.0 may grossly overestimate exposure thereby influencing risk assessment and remediation decisions.

In light of this, the developers of the NEPM guidelines recognised the need for additional research to underpin future refinements especially related to risk assessment. In particular, there is a need to better understand the factors that affect the bioavailability (including human health implications, plant uptake and ecosystem health) of metals and whether significantly higher concentrations of metals (over NEPM guidelines) can be safely accepted after long-term natural processes or through active remediation.

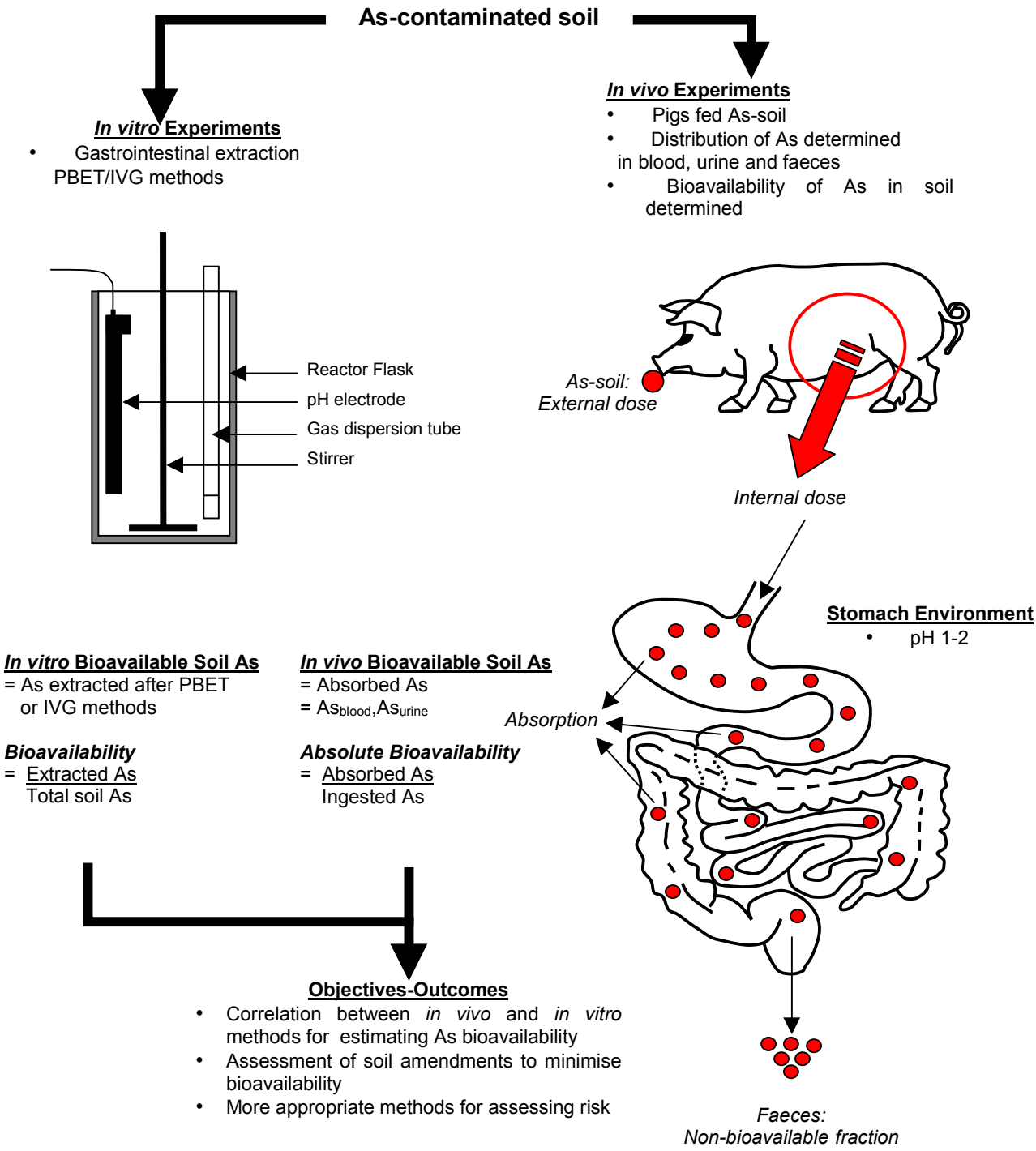

Figure 1. Schematic diagram of *in vivo* and *in vitro* trials.

5 CASE STUDY

Arsenic based herbicides were applied extensively to former railway tracks and sidings in South Australia to limit plant growth and reduce the subsequent risk of fire between the early 1940s and late 1960s. The former railway network was extensive, with approximately 1000 kilometres of disused railway corridor having been identified within the wheat and barley growing area of the mid-north of South Australia. Studies have revealed that total As concentrations may range up to 550 µg As g^{-1} and the potentially bioavailable As pool up to 20 µg As g^{-1} along the former railway corridors (Naidu *et al.*, 1997; Smith *et al.*, 2000, 2001). Development of regional areas close to major towns and cities has resulted in the intrusion of housing developments into areas adjacent to or in some cases onto the former railway corridors. *In vivo* studies using swine and *in vitro* chemical extraction studies are being undertaken to estimate the potential bioavailability of As from former railway corridors from the mid-north of South Australia. *In vivo* swine feeding trials are to be evaluated after the completion of the *in vitro* studies (Figure 1).

Both the PBET and IVG *in vitro* chemical extraction methods were studied using the soil particle size fraction less than 250 µm and elements in the extraction solutions analysed using either ICP-AES or FAAS coupled with hydride generation.

In vitro studies indicated that a significant amount of As in soils was not bioavailable (Figure 2) indicating that only a small portion of the total As may be absorbed in the gastric phase.

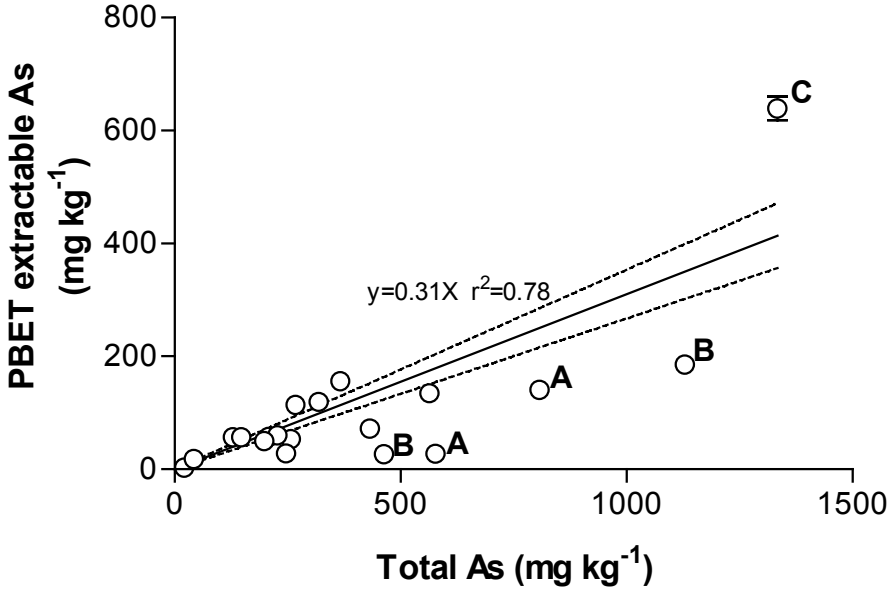

Figure 2. Comparison of potential bioavailable As against soil total As from former railway corridors, South Australia and mine sites, Victoria. For clarity, only the PBET extractable data is shown.

These results are similar to those published by authors outlined in Table 1 who utilised either *in vivo* or *in vitro* methods for assessing As bioavailability in soil. All studies noted that the relative bioavailability of As is considerably less than the total As content of the soil. In the case study presented above, the amount of As extracted from the soils using

the PBET method or the IVG method were similar. However, the percentage of extractable As varied considerably between the soils studied (ranging from 6 to 43% and 5 to 38% for the PBET and IVG methods, respectively), but did not exceed 45% of the total As in the soil irrespective of which bioavailable As extraction was studied.

Arsenic bioavailability was dependent on the mineral form of As, encapsulating matrix and site specific environmental conditions. Low As bioavailability was observed in mine site soils (labelled A, Figure 2) which is probably due to a high proportion of As being present as poorly soluble Fe-As-sulfides. A poorly soluble encapsulating matrix (calcinated material) may also be responsible for low As dissolution and therefore low bioavailability in soil collected from former railway corridors (labelled B, Figure 2). In soil C (Figure 2), where the highest proportion of bioavailable As was observed (43%), As bioavailability was influenced by As speciation and site-specific environmental conditions (pH). Arsenite comprised approximately 72% (182 μg g^{-1}) of the total As concentration in soil C. Under the alkaline conditions experienced at this site (pH = 8.9), arsenite is highly mobile, which greatly influences its bioavailability.

Table 1. Assessment of As bioavailability in contaminated soil.

Bioavailability Assay	Source of Material	As Bioavailability (%)	Reference
In vitro (PBET/IVG)	Railway corridors	5-43	This study
In vivo (rabbits)	Smelter soil	22-31	Freeman *et al*. (1993)
In vivo (monkeys)	Smelter soil	~14	Freeman *et al*. (1995)
In vivo (dogs)	Mine site	~8	Groen *et al*. (1994)
In vitro and *in vivo* (PBET, pigs)	Mine site	0-52	Casteel *et al*. (1997)
In vitro and *in vivo* (PBET/IVG, pigs)	Mine site	3-43	Rodriguez *et al*. (1999)

As bioavailability was site specific – bioavailability was dependent on As form, encapsulating matrix and soil properties.

If a "bioavailability factor" is included in the As risk assessment model used in determining Australian health-based investigation levels, the allowable concentration of As in soil changes considerably. Including a "worse case scenario" of 50% As bioavailability, the site-specific soil criterion increases by 100% (to 200 μg g^{-1}). However, in some instances As bioavailability was less than 10% which, if included in the risk assessment model would potentially equate to an allowable soil As concentration of 1000 μg g^{-1}. These results indicate the need for site-specific assessment of As bioavailability for inclusion in risk assessment models.

In the USA, results from bioavailability assessment have been used to adjust clean up targets for As and other elemental contaminants (Table 2). In the examples outlined in Table 2, As bioavailability was assessed on a site-specific basis and was found to be only a fraction of the total As concentration in the soil. These results led to a lowering of clean up targets and in one instance, clean up was not deemed necessary due to the unavailability of As in the calcinated material.

6 CONCLUSIONS

Numerous methods are currently available for the assessment of bioavailability, however, the applicability of some of these tests for human health risk assessment is questionable. Arsenic bioavailability is dependent on mineral form, encapsulating matrix, grain size, soil properties, environmental conditions as well as residence time in the soil and these site-specific factors must be taken into consideration when assessing As bioavailability and potential human health implications. Only a fraction of the total As concentration may in fact be bioavailable which may impact significantly on risk assessment and clean up decisions. However, if bioavailability factors are to be included in risk assessment guidelines, further scientific evidence is required to warrant its inclusion.

Table 2. Examples where As bioavailability adjustments have been included in remediation targets for contaminated sites in the USA (NFESC, 2000).

Site	Contaminant	Test	Bioavailability (%)	Clean up Target (µg g^{-1})	Regulator Agency
National Zn Co. Bartlesville, OK	Pb	*In vivo*, rat	40	925[1] (500)[2]	Oklahoma DEQ
	Cd	*In vivo*, rat	33	100[1] (30)[2]	
	As	*In vitro*, PBET	25	60[1] (20)[2]	
Anaconda, MT	As (soil)	*In vivo*, monkey	18	250[2]	USEPA Region VIII
	As (dust)	*In vivo*, monkey	26		
Crego Park, MI	As	*In vitro*, PBET	10	68[1] (6.8)[2]	Michigan DEQ
Union Pacific Railroad Yard, Sacramento, CA	As	*In vivo*, pig	< 0.1	No clean up (up to 1,800 µg g^{-1} As in slag)[1]	Cal-EPA

[1]Clean up target after site-specific bioavailability assessment.
[2]Clean up target prior to bioavailability adjustment.

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