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Bioavailability of metals and arsenic at contaminated sites from cattle dips, mined land and naturally occurring mineralisation origins

Jack C Ng[1], Barry Noller[1], Scott Bruce[1] and Michael R Moore[2]
[1] National Research Centre for Environmental Toxicology,
[2] National Research Centre for Environmental Toxicology, the University of Queensland, & Queensland Health Scientific Services.

ABSTRACT

Bioavailability of a contaminant, in this case a metal or metalloid, is influenced directly by the chemical species of the element and the form of wastes from an anthropogenic source or a naturally occurring origin. Currently in Australia there is no differentiation in the NEPM (National Environmental Protection Measures) for contaminated sites with regard to origin of waste material and presence of naturally occurring mineralisation. There is a clear need to develop soil criteria which are relevant to the range of wastes occurring at Contaminated sites. Of the range of contaminated sites which exist, important categories not adequately described are: cattle dip sites; mined land; and naturally-occurring mineralisation. This study evaluates case examples of each type incorporating a mammalian model for the determination of the bioavailability of the elements of interest. Our results indicate that the bioavailability of arsenic is higher from cattle dip sites representing an exclusive anthropogenic origin, whilst the bioavailabilities of arsenic and lead in soils are relatively low from mined land and a residential suburb built on a naturally occurring mineralisation zone. Importantly this is the first time that such an evaluation has been undertaken on mined land in Australia including the different kinds of mine wastes that occur such as tailings, heap leach waste and waste rocks. Also, naturally occurring mineralisation is included as such "contamination" may occur in urban areas arising from geochemical anomalies. Where this situation occurs, there is a need to have more closely developed criteria reflecting the true bioavailability of such material as being generally low. Bioavailability data are demonstrated to provide a useful adjunct tool for a realistic health risk assessment.

1 INTRODUCTION

Australia is a mineral-rich country and mining is a major industry which has done and continues to play an important role in generating economic activities. Contamination of mined land by specific type of mining waste is unavoidable but its adverse effects that impact upon the environment can be minimised through proper remedial actions and management (ANZMEC/MCA, 2000). Harmful metals, whether present in the naturally occurring mineralisation zone or generated from mining activities, of particular human health concern are arsenic, cadmium, chromium, nickel and lead and, to a lesser degree, other metals such as copper and zinc.

Urban development has sometimes inadvertently caused disturbance of naturally occurring mineralisation leading to contamination of residential land. In other instances, residential houses were built on or near tailings facilities. Another source of contamination is through the use of industrial chemicals / products containing metals or

metalloids such as lead and arsenic. For examples, lead-contaminated sites can be derived from the use/misuse of leaded-petroleum products, leaded-paint, lead acid batteries and from lead smelting activities; and arsenic-contaminated sites can be derived from copper-chrome-arsenate timber treatment process, pesticide use (e.g. tick control) and as a by-product of precious and base metal smelting activities.

Labile metal species are considered to be more biologically active than non-labile fractions. There are a number of tests for availability of toxic materials from single components and from mixtures (Tessier *et al*., 1979; Forstner, 1983) and arsenic species in mining wastes (Noller *et al*., 1997), which are generally based on the assumptions that greater solubility enhances bioavailability. The metal distribution value can usually be obtained using sequential extraction procedures, and several leaching schemes have been proposed and widely adopted. The chemical speciation obtained by sequential extractions is often believed to relate to bioavailability. However, such measures seldom give anything other than qualitative guidance on the likely uptake by organisms as we had demonstrated that *in vitro* leaching tests did not correlate with bioavailability data measured using an *in vivo* mammalian model (Ng and Moore, 1996; Ng *et al*., 1998). Quantitative data should be obtained based on actual measures of uptake of metals, for example in animals or humans in order to derive accurately bioavailability data.

Although chemical analyses of soil, air and water provide information on contaminant concentrations, they may be inadequate to assess the bioavailability of metal contaminants, or to predict their subsequent toxicity to wildlife and human health. Bioavailability of harmful elements from contaminated land has been carried out using small mammals including deer mice, meadow voles (Pascoe *et al*., 1994), dog (Groen *et al*., 1994), guinea pigs and rabbits (Freeman *et al*., 1993b) and laboratory rodents (Ng and Moore, 1996; Ng *et al*., 1998).

Bioavailability is defined as the fraction of the element from an ingested matrix such as soil, water or food that can be absorbed by an organism (e.g. humans). Bioavailability data can be broadly categorised as "comparative bioavailability" (Freeman *et al*., 1993b; Ng and Moore, 1996) and "absolute bioavailability" (Freeman *et al*., 1993b; Ng *et al*., 1998). "Comparative bioavailability" involves orally dosing animals with the metal-contaminated material (e.g. lead-contaminated soil) then the metal is measured in the blood several days after dosing and the elemental concentrations are compared to those of animals given an equivalent dose of the pure metal compound dissolved in water, presumably readily bioavailable (e.g. lead acetate). Whereas 'absolute bioavailability' involves orally dosing animals with the soil, keep them in metabolic cages for multiple samples of 24h urine collection over a long period of time (days to weeks). The area under the metal concentration curve over time is then compared to that obtained from animals given an intravenous injection of an equivalent amount of this element.

Bioavailability of metals from contaminated sites is a very important aspect of health risk assessment programs. In this report, we will present 3 case studies to illustrate how bioavailability data may be utilised in providing realistic risk assessment as a basis to begin derivation of soil criteria. More data of this type may help to establish relevant guideline values for metals in mined land and contaminated sites of natural mineralisation origin in urbanised areas.

2 MATERIAL AND METHOD

2.1 LABORATORY ANIMAL DOSING

For the comparative bioavailability measurement (Case study 1), male Wistar rats 6 weeks old of 170-190 g were obtained from the Central Animal Breeding House, The University of Queensland. Cattle dip soil samples suspended in 3 mL of water were dosed orally to groups of 4 rats for each soil sample at a dose rate of 5.0 and 0.5 mg As/kg body weight. For positive controls, 3 groups of 4 rats were given the equivalent amount of arsenic in the form of a solution of sodium arsenate or sodium arsenite, or a slurry of calcium arsenite spiked wheat flour respectively. Blood samples were collected from all rats 96 hours after dosing for the arsenic determination. The Comparative bioavailability (CBA) can be calculated using the following equation [1].

Equation [1]

$$CBA = \frac{SR - NCR}{PCR - NCR} * 100\%$$

Where: SR = average blood arsenic concentration of rats dosed with soil; NCR = average arsenic concentration of normal control rats; PCR = average of positive control rat blood arsenic concentration after an oral dose of sodium arsenate, sodium arsenite or calcium arsenite.

For the absolute bioavailability measurement (Case study 2), nine composite surface and sub-surface soils (C1-C9) and one rock composite sample (C10) from a residential area were orally dosed to groups of rats in the same manners. For positive controls, rats were given an intravenous injection of 0.5 mg As(III)/kg as sodium arsenite, 0.5 mg As(V)/kg arsenate, or 5.0 mg Pb/kg as lead acetate. All animals were kept in metabolic cages. Pooled 24h urine samples over 96 hours were collected free from contamination of faeces and measured for arsenic and lead. For the calculation of absolute bioavailability (AB), the urinary metal concentrations measured at 0, 24, 48,72 and 96h intervals were plotted. The area under the curve (AUC) was calculated using a graphics software package (GraphPad Prism; GraphPad Software, San Diego, CA, USA). The area under the curve for animals dosed with intravenous injection ($AUC_{i.v.}$) represented an arbitrary value of 100%. The AUC_{oral} derived for animals dosed by oral gavage, was then used to calculated the AB using the following equation [2]:

Equation [2] $\mathbf{\%AB = 100 * AUC_{oral} * Dose_{i.v} / AUC_{i.v} * Dose_{oral}}$

2.2 CATTLE GRAZING TRIAL

Two mine sites (referred to Site 1 and Site 2) were chosen for the metal uptake studies in which grazing cattle were allowed to graze on rehabilitated mined land consisting of various types of mining wastes including tailings, heap leach material and waste rock dump material (see Case Study 3 below).

2.3 ANALYTICAL TECHNIQUE

Atomic absorption spectrophotometer coupled with a hydride generation apparatus was used for the measurement of arsenic based on a method described by Ng *et al*., (1987) with

slight modifications. The analytical technique has been validated by the use of several reference materials and in-house rat blood and urine (Ng *et al.*, 1993). The determination of the oxidation state of arsenic (arsenic speciation) in the soil was described previously (Ng *et* al., 1998). ICP-MS was used for the multi-elemental analysis. As part of quality control/quality assurance (QC/QA) programme, QC samples were analysed after every 6-10 specimens in a run. The relative standard deviation (RSD) (n=20) of the ICP-MS results using a certified reference standard solution (ICPMO 111-1; EM Science, Gibbstown, NJ, USA) was 5.8%. An in-house rock digest gave an RSD of 3.2% (n=6). When the certified standard was analysed using ICP-AES, the corresponding RSD was 2.5% (n=4).

3 CASE STUDIES

3.1 CASE STUDY 1

This case study represents contamination from an anthropogenic source. Arsenic solutions for cattle tick control were widely used in Queensland and New South Wales from 1895 to 1955. Chemical investigations (Beard *et al.,* 1992) of arsenic contaminated soils obtained from some 1600 government owned cattle dip sites near north eastern NSW have revealed levels of As ranging up to 3000 mg/kg in the soil. Comparative bioavailability data were determined in 16 randomly selected soil samples with arsenic concentrations ranged from 700 to 2100 mg/kg. The arsenic concentrations of dip soils used for the animal dosing and blood arsenic of rats 96 hours post dosing are shown in Table 1.

Table 1. Arsenic speciation and blood arsenic (mg/L) in rats 96 hours after a single oral dose of arsenic at 5 mg As/kg b.w. from 16 various cattle dip soil samples, a solution of sodium arsenate, sodium arsenite or calcium arsenite-spiked wheat flour.

Soil I.D.	Total As (mg/kg)	As(III) %	Blood As (mg/L)
1	730	68	7.71±1.98
2	730	70	13.37±4.57
3	860	38	9.16±0.70
4	1300	75	15.51±4.29
5	700	70	7.15±1.61
6	2000	71	8.11±0.21
7	1400	57	7.65±0.62
8	980	72	10.80±2.58
9	750	30	2.28±0.76
10	2100	59	5.55±2.07
11	800	43	3.68±1.33
12	1000	74	5.74±0.69
13	1100	88	6.18±1.48(3)
14	2000	67	6.93±0.73
15	830	80	6.20±0.81(3)
16	900	57	6.24±0.52
Control groups			
Normal rats			0.85±0.32(6)
Sodium arsenate rats			11.14±1.83
Sodium arsenite rats			84.13±4.38(3)
Calcium arsenite rats			48.10±3.03

Note: 4 rats in each group unless specified by a number in ( ).

The CBA results for cattle dip soil samples are presented in Figure 1. The CBAs ranged from 1.7% to 15.0% with a mean of 8.1±4.0%, 3.0% to 31.0% (14.4±7.1%) and 13.9% to 142.5% (60.0±32.0%) relative to sodium arsenite (Figure 1 A), calcium arsenite (Figure 1 B) and sodium arsenate (Figure 1 C) respectively.

Assuming the arsenite in the test soil samples is in the calcium arsenite form, and the remaining fraction is in the sodium arsenate form, then the overall comparative bioavailability (OCBA) can be more accurately estimated by using equation [3]. In this case, the mean OCBA was found to be 31.6±14.8% ranging from 10.6% to 58.9%. Similarly, assuming the arsenite is sodium arsenite, then equation [4] is used for the calculation, giving a mean OCBA of 27.5±13.2% with a range of 0.2% to 48.8%. The OCBAs are shown in Figure 2 A and B.

Equation [3]

For example: OCBA for S1 is calculated as shown below:

$$OCBA = \frac{(SR-NCR)*AsIII\%}{48.1-NCR}*100\% + \frac{(SR-NCR)*(100\%-AsIII\%)}{11.14-NCR}*100\%$$

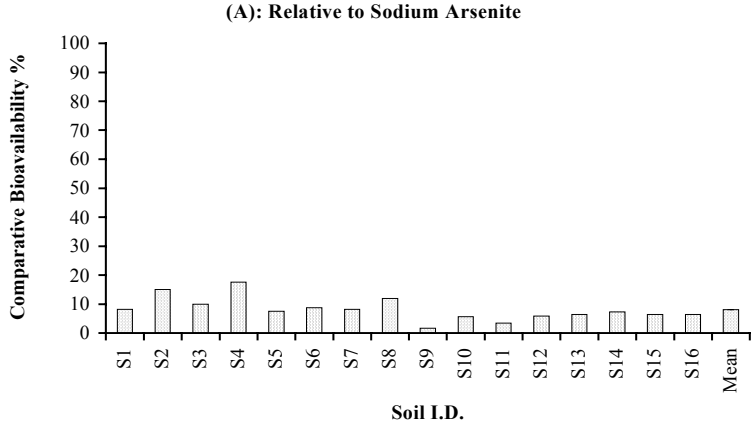
$$OCBA = \frac{(7.71-0.85)*68\%}{48.1-0.85}*100\% + \frac{(7.71-0.85)*(100\%-68I\%)}{11.14-0.85}*100\% = 31.2\%$$

Where: SR = average blood arsenic concentration of rats dosed with soil; NCR = average arsenic concentration of normal control rats; 48.1 is the average arsenic concentration in the blood of positive control rats after given an oral dose of calcium arsenite, 11.14 is the average arsenic concentration in the blood of the positive sodium arsenate-dosed rats

Equation [4]

$$OCBA = \frac{(SR-NCR)*AsIII\%}{84.13-NCR}*100\% + \frac{(SR-NCR)*(100\%-AsIII\%)}{11.14-NCR}*100\%$$

Where 84.13 is the average arsenic concentration in the blood of positive control rats after given an oral dose of sodium arsenite, 11.14 is the average arsenic concentration in the blood of the positive sodium arsenate-dosed rats



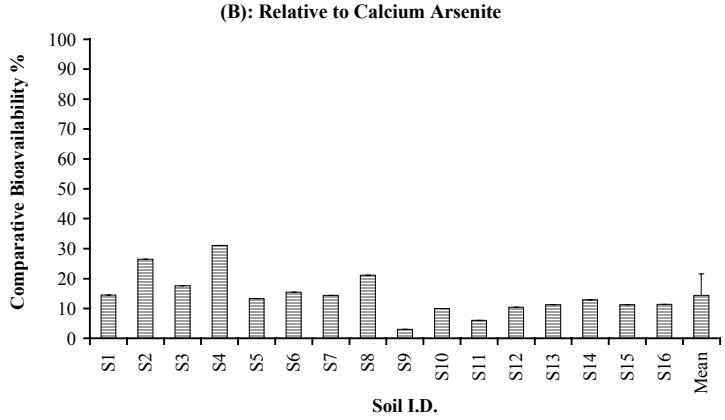


Figure 1 Comparative bioavailability (CBA) calculated from blood arsenic of rats dosed with 16 various cattle dip soils in comparison with blood arsenic of rats dosed with sodium arsenite (A), calcium arsenite (B) or sodium arsenate (C). Where CBA is greater than 100%, it indicates the contribution of the As(III) component in the sample. The last bar of each graph represents the mean±S.D. calculated from the 16 samples. The mean CBAs were 8.1±4.0%, 14.4±7.1% and 60.0±32.4% relative to sodium arsenite, calcium arsenite and sodium arsenate respectively.

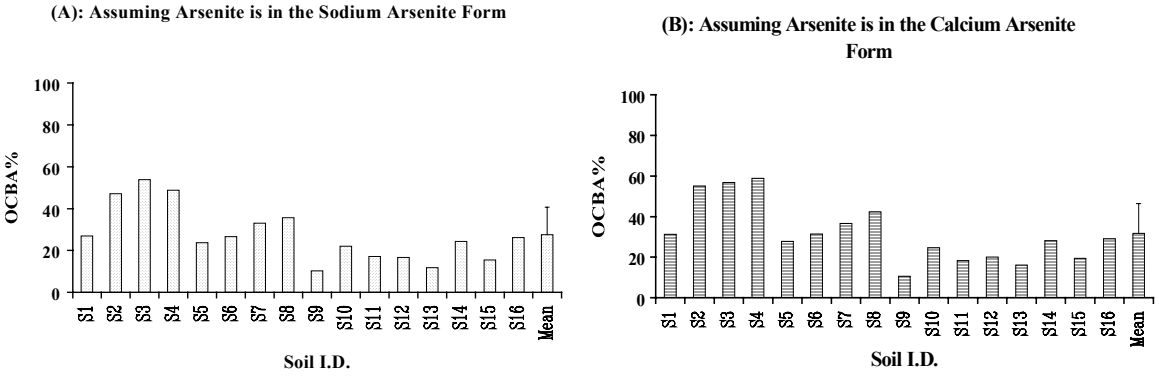


Figure 2 Overall comparative bioavailability (OCBA) based on speciation data where (A) assumes the arsenite in the soil is sodium arsenite and the remaining arsenic is sodium arsenate; (B) assumes the arsenite in the soil is calcium arsenite and the remaining arsenic is sodium arsenate. The mean OCBAs are 27.5±13.2% and 31.6±14.8% as shown in (A) and (B) respectively.

3.2 CASE STUDY 2

This case study represents arsenic and lead "contamination" which occurred in an urban area with houses built on a Gossan mineralisation zone in a capital city in Australia. Gossans are naturally occurring geological formations formed by the weathering zones of sulfide mineralisation and are known sources of arsenic, lead and other base metals. Arsenic and lead concentrations of 9 composite soil samples and one composite rock are shown in Table 2 and their respective absolute bioavailability data are shown in Table 3.

Table 2. Lead and arsenic speciation results obtained for nine composite soil (C1-C9) and one rock sample (C10).

Sample	Total arsenic (mg As/kg)	Arsenite [As(III)%]	Lead (mg Pb/kg)
C1	55	0.33	18
C2	32	1.38	10
C3	165	1.01	52
C4	295	0.73	1209
C5	67	56	20
C6	121	0.32	140
C7	1597	0.53	2585
C8	867	0.52	1582
C9	1325	0.54	347
C10	435	44.8	447

Table 3. Absolute bioavailability (AB) of arsenic relative to 0.5 mg As/kg i.v. of As(V) and As(III), and of lead relative to 0.5 mg Pb/kg i.v. of lead acetate.

Sample	%AB cf As(V)	%AB cf As(III)	%AB cf Pb
C1	2.31	8.50	8.89
C2	1.27	4.31	7.37
C3	2.68	9.87	9.41
C4	1.44	5.56	0.36
C5	2.98	9.58	14.74
C6	2.46	7.25	0.70
C7	0.55	1.86	0.05
C8	0.59	1.18	0.11
C9	0.67	1.96	0.33
C10	0.26	1.02	0.06

Since most soils contained about 99% of arsenate (As(V)) with the exception of C5 and C10, for practical purpose, one could calculate the %AB by comparing the urinary excretion curve of arsenate. Therefore %AB for these samples were found to range from 0.55 to 2.98% as shown in Table 3. The %AB for C10 was about 0.64% (average of 0.26 and 1.02) and 6.28% (average of 2.98 and 9.58) for C5 since the arsenite component in the soil was about 50% for both samples.

The %AB for lead in soils were highly variable and similar to %AB for the arsenic soils, and ranged from 0.06% (the rock, C10) to 14.74% (composite surface soil, C5). There appeared to be very little lead in the blood, liver and kidney in any of the soil-dosed animals (data not shown) supporting the relatively low bioavailability of the tested soils from a natural mineralisation origin.

3.3 CASE STUDY 3

This case study examined the transfer of metal and metalloid contamination in mined land. A study of metal and metalloid uptake was undertaken at the rehabilitated mine waste facilities at two mine sites (Site 1 and Site 2) in order to assess the suitability of the land for future pastoral application. The key objective was to assess the likelihood of transfer of trace elements through the food chain to cattle and subsequent contamination of the saleable meat.

A grazing trial was conducted over a period of 8 months at 3 experimental plots at each mine site.

At Site 1, 14 Brahman cross cattle in the feeding trial were divided into 3 groups: (i) Tailing Paddock 1 (5 head); (ii) Tailing Paddock 2 (5); and (iii) Background Paddock (4).

At Site 2, 3 groups of 4 Brahman cross cattle were allowed to graze for similar length of time at: (i) Heap Leach facility; (ii) Waste Rock Dump; and (iii) Undisturbed Adjacent Land, including naturally-occurring mineralisation.

All trials were approved by the University of Queensland Ethics Committee (NRC-10019). Cattle from a local supplier were initially acclimatised at the background (control) site before the commencement of metal uptake studies.

3.3.1 Mine Site 1

Two treatment tailing paddocks were chosen for the intensive grazing trial with Tailing Paddock 1 having a higher acid potential and hence regarded as a "hot spot". The grazed area was initially restricted to 0.8 ha for the first two months of the experimental period. Due to low consumption of the standing dry herbage over this period, it was decided to slash the existing 0.8 ha area to encourage fresh regrowth.

To ensure that sufficient pasture was available for stock, the restricted grazing area was enlarged. The restricted area tripled in 'Tailing Paddock 1', to become 2.4 ha and doubled in 'Tailing Paddock 2' to become 1.6 ha, based on the dry matter yield of each of the paddocks. This design was not intended to determine the potential for contamination under a lighter grazing intensity, as it was premised that the regulatory body usually only accepts proposed land use management based on the worst case scenario. If unacceptable levels of contamination occurred under the proposed trial design, recommendations for appropriate land management could be made based on a risk assessment.

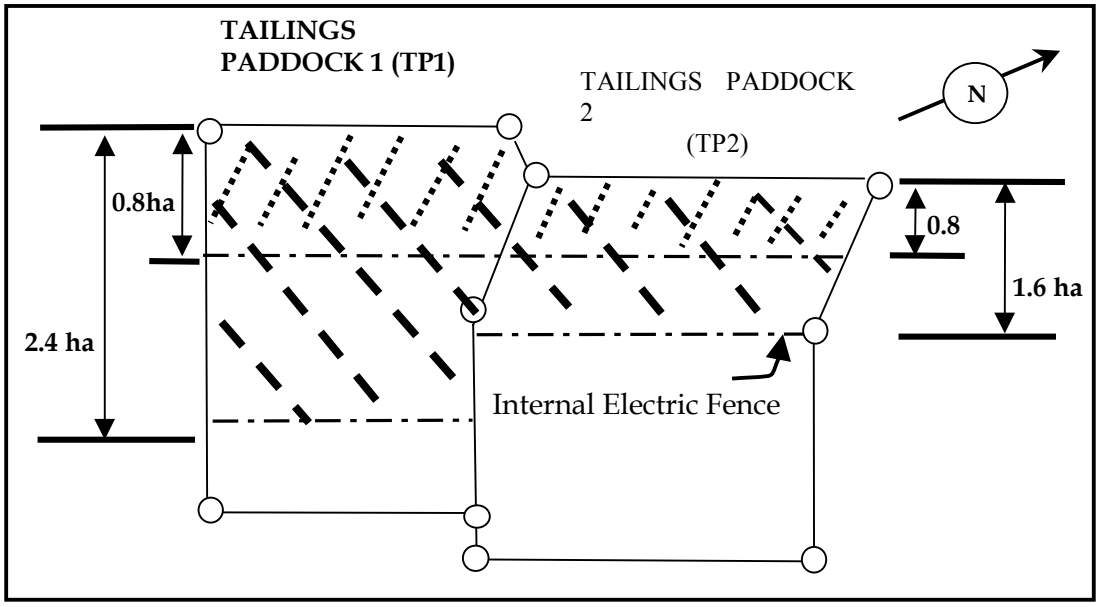


Figure 3 Schematic diagram showing the internal movable electric fence used to enlarge grazing area in the two tailing paddocks. The dotted-line area (▪▪▪▪▪)represents the initial enclosed area, the dash-lined area (_ _ .) represents the grazing area after the internal electric fence was moved and the grazing area enlarged.

Pasture condition, botanical composition, pasture nutrient concentrations and soil fertility parameters of the control and treatment sites were measured (data not shown).

The concentrations of metals in the background and two treatment paddocks are shown in Table 4.

Table 4. Average concentrations of metals in the composite samples of soils obtained from the background and two treatment paddocks at mine Site 1.

Element	Background Paddock (mg/kg)	Tailing Paddock 1 (mg/kg)	Tailing Paddock 2 (mg/kg)
As	<15	280	370
Ba	72	150	89
Be	<1	<1	<1
Cd	<1	6.3	16
Co	5	14	15
Cr	11	35	40
Cu	8.5	150	140
Hg	<0.2	<0.2	<0.2
Mn	290	720	920
Mo	<10	<10	<10
Ni	4	22	25
P	96	460	460
Pb	<10	170	150
S	63	11600	13200
Sb	<10	<10	<10
Se	<15	<15	<15
Sn	<15	<15	<15
V	24	38	39
Zn	36	750	1400

Although grass was found to contain adherent soil, uptake from grass consumption was much less than from soil as cattle ingesting soil through grazing activity received 90% of the total dose.

A key experimental technique was *in situ* measurement of uptake by periodic muscle and liver biopsy, and blood sampling. Several organ systems were sampled at necropsy when the trial was terminated after 8 months. Examples of liver biopsy results are shown in Figure 4.

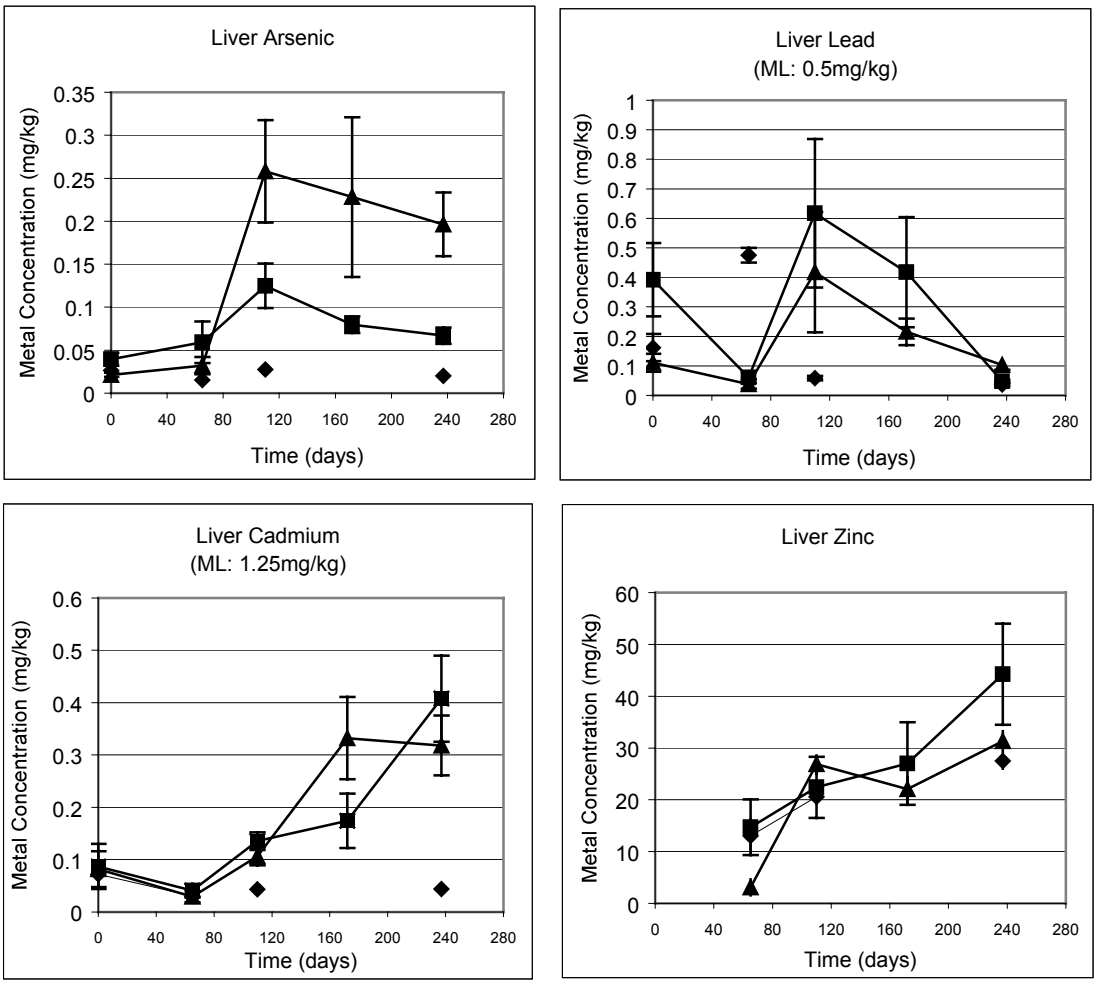


Figure 4 Arsenic, lead, cadmium and zinc concentrations in the liver of cattle grazing on Background Paddock (◆), Tailing Paddock 1 (▲) and Tailing Paddock 2 (■) over 8 months at mine Site 1. The ANZFA ML (maximum levels) for lead in liver is 0.5mg/kg, and 1.25mg/kg for cadmium in liver.

3.3.2 Mine Site 2

In this trial, the accumulation of metals and the subsequent potential for contamination were tested under a high intensity of grazing. This generated results based on a worst-case scenario, whereby cattle would have the maximum exposure to the soil similar to the trial at mine Site 1.

Based on biomass assessments conducted on site, and a daily stock consumption estimate of 2.5% of body weight, 5 ha was proposed as the paddock size for the trial including Heap Leach Pad (HLP), Waste Rock Dump (WRD) and Background paddock (undisturbed natural land).

All three paddocks were strip-grazed, using movable (electrified) internal fences to provide fresh feed on a controlled basis. This approach has the advantage of forcing exposure to the soil surface in a shorter period, thus maximising the potential for metal consumption to occur within the trial period. An area of 2 ha was provided for the initial

2 months within each of the three trial paddocks. Another 1ha was then provided at the end of the two months, and the final 2 ha was provided after 4 months.

Periodic blood, muscle and liver biopsies were taken for multi-elemental analyses as for mine Site 1. The metal concentrations of the 3 paddocks at mine Site 2 are shown in Table 5. Examples of liver biopsy results are shown in Figure 5.

Table 5. Concentrations of metals in the composite soils of background, heap leach paddock and waste rock dump paddock at mine Site 2.

Element	Background Paddock (mg/kg)	Heap Leach Paddock (mg/kg)	Waste Rock Paddock (mg/kg)
As	110	620	250
Ba	170	230	390
Be	1.3	3	2.5
Cd	1.9	4.5	3
Co	11	27	24
Cr	26	16	28
Cu	73	2600	1440
Hg	<0.2	0.5	<0.2
Mn	620	2300	3100
Mo	<10	36	<10
Ni	15	35	34
P	300	890	830
Pb	180	790	470
S	170	2700	170
Sb	<10	23	<10
Se	<15	19	<15
Sn	<15	71	<15
V	37	93	110
Zn	250	6800	3400

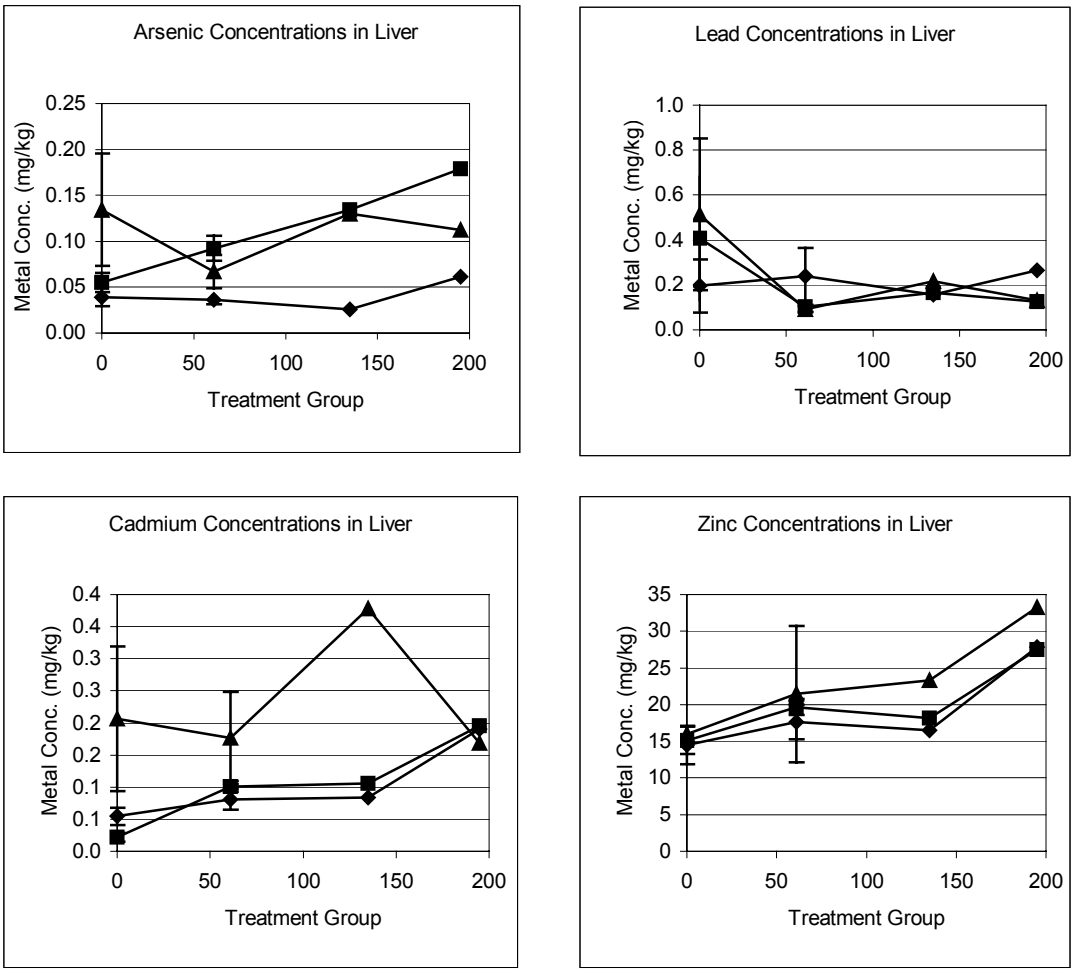


Figure 5 Arsenic, lead, cadmium and zinc concentrations in the liver of cattle grazing on Background Paddock (◆), Heap Leach (▲) and waste rock dump (■) over 8 months at mine Site2.

4 DISCUSSION

4.1 CATTLE DIP SITES - ANTHROPOGENIC SOURCE OF CONTAMINATION

It is well established that rats are different from other mammals in that they accumulate arsenic in the blood (Odanaka *et al.*, 1980; Chong *et al.*, 1989). The blood arsenic in the rat reaches a maximum 3 days after the animal is given an oral dose of arsenic and decreases with a biological half-life of 60 days (Ariyoshi and Ikeda, 1974). Hence the rat blood model was chosen for our study so that only a single blood sample was required for arsenic determination 4 days (96 h) after dosing for the calculation of CBA. The intra-group variations of blood arsenic levels were relatively small compared to inter-group variations (Table 2). The 16 different soil samples used were from sites where reported use of arsenicals occurred 35-40 years ago. Therefore, the age of soils used within this study was quite uniform and is unlikely to be a significant contributor to the inter-group variations. In addition, there is no correlation between the CBA and the amount of soil given to each animal (r=0.1). The variability is possibly due to soil types, to complex chemical and physical matrices, and/or to the speciation of arsenic found in soils.

For risk assessment, the 'worst-case' scenario is usually employed in that bioavailability of arsenic is compared only to soluble sodium arsenate (the least well absorbed of the 3 positive arsenic controls). In this study, CBA values were found to be 60.0±32.4% relative to sodium arsenate for cattle dip soils. Freeman *et al*., (1993a; b) reported the 'absolute bioavailability' of arsenic in the soil from a smelter site following oral administration in male rabbits and monkeys; this was 25.9 and 24.2%, respectively, when compared with animals given intravenous sodium arsenate. However, when their data were compared to animals given a gavage of sodium arsenate, their CBA values were calculated to be 70% and 43.6% for the rabbits and monkeys, respectively. In another study (US EPA, 1996), contaminated soil and slag were fed to immature swines, and CBAs were found to be 78% and 42% respectively. Our CBA results, relative to sodium arsenate (60.0±32.4%), are in relatively good agreement with those reported using more expensive animals such as the rabbit, swine and monkey. This supports the use of rats for the estimation of bioavailability of this element in human exposure.

Our chemical speciation results showed that there were significant levels of arsenite in soils used in the animal experiment. The arsenite was probably in the form of calcium arsenite, since it has been a general practice in the past to treat the exhausted dip solution with lime to cause precipitation prior to disposal. The half-live of arsenite in the soil has been reported to be 6.5±0.4 years, and the relatively high concentrations of arsenite in these soils are therefore not entirely surprising given this half-life and the relative insolubility of arsenic in water. It is reasonable to believe that the overall bioavailability of dip soils is 31.8±14.8% as shown in Figure 2B.

Our results show that the CBA varies significantly for different forms of arsenic (Figures 1); namely there is a decreasing CBA in the order of sodium arsenate, calcium arsenite, and sodium arsenite. Where CBA values were greater than 100% relative to sodium arsenate in the case of cattle dip soils number 2 and 4 (Figure 1), it can be explained by the probable presence of arsenite in the soils. In the absence of speciation results, CBA values are likely to be overestimated if one assumes that the arsenic in the soil is totally in the arsenate form (Figure 1C). Conversely, the results are likely to be underestimated if one assumes that the arsenic is in the arsenite form, Figure 1 (A and B). The overall bioavailability data can only be accurately determined when the chemical speciation of arsenic in the soil is available as illustrated by Figure 2 (A and B).

It is obvious from this study that the solubility and speciation of arsenic have a significant impact on the bioavailability from the soil. Therefore, the existing standards of health-based investigation levels for inorganic arsenic in soils are conservative given that they are based on the assumption that in humans the bioavailability of arsenic from ingested soil is equal to that from an aqueous solution.

4.2 NATURALLY OCCURRING MINERALISATION SITE

Similar to the cattle dip soils, the bioavailability of arsenic in mineralised soils varied widely from site to site but was generally much lower than that of anthropogenic contamination such as soils at dip sites.

For this study, the dosage given to experimental rats was standardised on the concentrations of arsenic found in the soils. Other heavy metals including Cd, Cr, Cu and Zn in the biological specimens were also measured (data not shown). Not surprisingly, the bioavailability of Pb in these mineralised soils was also relatively low.

4.3 REHABILITATED MINED LAND

None of the metals analysed in our studies, including arsenic, cadmium and zinc, caused product contamination (meat or organ) (i.e. all < ML) during the trial period under the 'worst-case' grazing scenario designed, hence, no recommendations relating to short-term exposures such as in the trials were needed. However, there were a number of important considerations, as long-term product contamination is possible unless various management considerations are noted. Such considerations relate to the exposure pathways for which exposure can be limited to reduce the potential for product contamination.

Firstly, it must be made clear that a proportion of the metal dose is received from the plant material. Even though the bioavailability of specific metals from plant material may be low, plant species chosen for remediation of mine waste materials should be those that don't accumulate metals in excessive concentrations. The adaptability of the grass species to these waste materials, their suitability to the environment and pasture needs are important considerations. However, their ability, or inability to accumulate metals should also play a role in the design stage of tailing dam revegetation.

Secondly, the percent ground cover over the waste materials is an important consideration when attempting to limit the risk of contaminant exposure. The areas of known acid condition in TP1 reduced the standing dry matter and associated percent ground cover consistently over the trial period. This may potentially have increased the risk of direct ingestion of tailing material within this paddock. Tailings in areas of known acid condition are often associated with elevated metal and metalloid levels due to the presence of soluble "bioavailable" constituents. These areas of exposed waste materials are likely to increase the potential for direct soil ingestion whilst contributing to an increase in adherent dust on adjacent plant materials.

Thirdly, the dust adhered to plant materials potentially contributes a major percentage of metals to the diet. This further highlights the importance of maximising ground cover across the tailing facility to minimise the potential for tailing dust to accumulate on the plant material. This is especially important in the first few years after development, before accumulation of leaf litter on the surface of the waste storage areas is significant.

Also, a major consideration relevant to the above considerations is stocking rate. Having used a high grazing intensity, these trials have highlighted the importance of testing metal and metalloid accumulation and associated risk and contamination under such conditions. Steps should be taken to prevent grazing intensity continuing at such rates for extensive periods of time.

Finally, an important consideration, coupled with grazing intensity, is the incidence at which the pasture of a particular waste storage area will be grazed. By including a much larger 'normal' grazing paddock, the incidence of mining waste exposure would be markedly decreased. However, if the area is to be grazed exclusively, the grazing intensity would have to be lower than used here and limiting the grazing period would need to be considered.

4.4 CRITERIA FOR MINED LAND

Criteria for environmental management and rehabilitation of mine sites have become more stringent in recent years with the increasing awareness of the potential for

detrimental effects on the environment and human health from exposure to metals liberated in tailings post closure. Also, the legacy of past uncontrolled mining activities and poor if not nil rehabilitation has resulted in the need for costly remediation.

The Strategic Framework for Mine Closure (ANZMECC/MCA, 2000) identifies six key areas of principles for mine closure development. These are:

- Stakeholder involvement
- Planning
- Financial provision
- Implementation
- Standards
- Relinquishment.

The framework identifies the need for the development of guidelines or standards for mine closure purpose which demonstrate successful completion of the process and enable sites to be returned to the State on an equitable and cost-efficient basis while ensuring long-term protection of the environment (ANZMECC/MCA, 2000).

A set of specific performance indicators should be developed to measure progress in meeting the completion criteria (ANZMECC/MCA, 2000), encompassing ecological processes leading to successful rehabilitation.

Targeted research is identified by ANZMECC/MCA (2000) as a necessary tool to assist both government and industry in making better and more informed decisions. Another specific item under 'principles' is the use of a risk-based approach to planning to reduce cost and uncertainty. The key issues are detailed in Table 6.

Table 6. Key issues.

• Risk assessment process underpins the risk management strategies
• Assume 100% bioavailability in absence of any data
• Bioavailability data is lacking for mine wastes under Australian conditions and data is therefore needed
• Utilisation of mined land for future pastoral activity
• More stringent criteria for mined land
• Bioavailability for lead and arsenic from mine materials to permit risk assessment of rehabilitated mined land under Australian conditions
• Development of physiologically-based pharmokinetic model in animals to give bioavailability of lead and arsenic

A risk assessment process underpins the risk management strategies selected. One of the key aspects of a risk assessment is the potential bioavailability of contaminants. In the absence of specific bioavailability data, this is assumed to be 100%. Data on bioavailability of toxic elements, particularly arsenic and lead, from tailing in Australian conditions are lacking.

It is obvious that any risk assessment that assumes 100% bioavailability could be overly conservative and may result in unnecessary and expensive remediation. It is equally important not to assume that the bioavailability of toxic metals in mine tailings is insignificant to impact upon environmental health of plants, animals and humans. It is therefore, essential to obtain site-specific bioavailability data to evaluate the risks under Australian conditions.

Table 7. Outcomes from risk assessment procedure.

- More accurate design of mine covers
- Minimisation of transfer of toxic elements to environment
- This study presents a unique approach which will help to provide quantitative information for succesful completion of the closure process

Costs are minimised when progressive rehabilitation is incorporated into the mine project design (Table 7). The specific strength of these studies is to generate bioavailability data for animal uptake of toxic elements from mine tailing for the risk assessment process. This will enable a more accurate design of mine soil covers based on the bioavailability data for tailing and other mine waste to be rehabilitated prior to closure. The transfer of toxic elements via the food chain can be minimised to levels considered to be of low consequence to the environment and the likely impacted species including humans.

The National Environment Protection Measure for the Assessment of Site Contamination (NEPC, 1999) uses a risk-based approach to identify soil criteria for various contaminants which are indicative of adverse site-specific health and ecological effects if investigation levels are exceeded.

The NEPC (1999) uses a number of generic settings upon which Health Investigation Levels (HILs) and Ecological Investigation Levels (EILs) can be based. The HILs and EILs are intended for assessment of existing contamination only and are intended to prompt an appropriate site-specific assessment when they are exceeded.

Mineralised areas are identified as locations that may have soils containing elevated levels of metals and metalloids due to natural processes. Generally the bioavailability of metals and metalloids in naturally occurring materials is much lower than that of an anthropogenic source (see Case Studies 1 and 2). Mining and mineral processing of ores can assist the solubilisation of compounds leading to potentially increased bioavailability compared with natural background occurrence.

A key point of relevance to this study is that there is no specific investigation levels for mined land which are applicable for mine closure purposes (Table 8). Thus it is appropriate to consider how site-specific health and ecological risk assessments can be developed to apply where there is a likelihood of adverse effects on human health or ecological values for that site. Using this approach the information expected to be generated is as detailed in Table 9.

Table 8. Risk-based criteria for mine closure.

- National Environment Protection Measure uses risk-based approach to identify soil criteria for contaminants indicating site-specific health and ecological assessments if investigation levels are exceeded
- Currently no specific investigation levels exist which are dedicated to mined land (although current ILs can be applied to mined land)

Table 9. Information gained from *in situ* risk assessment of a mine.

- Bioavailability estimate – depending on chemical species
- Age of tailings
- Exposure estimate (in situ dose rate)
- Accumulation prediction (grazing period)

Following mining of material, extraction or separation of the economic item is required. Usually crushing and grinding is necessary to improve surface area and to allow efficient extraction by reagents or separation e.g. if flotation is used.

The resulting waste products on a mine site, based on open cut mining, are waste rock and tailings, usually held in separately constructed landforms or structures which minimise their erosion. Key contaminants present in waste from base metal mining and other sulfidic deposits are metals, (e.g. Cd, Cu, Pb, Zn) and metalloids (As, Se, Sb, Bi). Because processed mine minerals are finely divided, there is a potential risk that such materials may find their way through the environment and food chain to animals/humans.

In Queensland, considering a sub-set of Australian conditions, the most preferred land use following mining is pastoral, followed by natural bushland, recreational and urban uses. Cattle are therefore identified as a key species of biota for assessing the uptake study of toxic metals/metalloids from rehabilitated mine sites. Studies have shown that arsenic transferred from mine tailings in waste dumps is attenuated after travelling a short distance but remains trapped with evaporites near the exit part of seepage from mine dumps. Cattle are attracted to evaporites and will ingest salt deposits if present on the surface (Noller *et al.,* 1997).

Some details identified as being of importance to mine rehabilitation have been identified from the work described and are summarised in the following table (Table 10).

Table 10. Details applicable to rehabilitating mined land.

- Species chosen for revegetation should not accumulate excessive metal levels
- Grazing trials show very little uptake from grass itself
- Dust adhering to plant material and direct soil ingestion contributes a major % to diet
- % Ground cover is an important consideration in limiting risk of contaminant transfer
- Stocking rates of cattle influence grazing intensity
- Acid generation may increase bioavailability of waste material

5 CONCLUSION

There is a demonstrated need to develop specific soil criteria for contaminated mined land and naturally-occurring mineralisation. It is clear that anthropogenic contaminants are more bioavailable than natural mineralisation sources and that bioavailability is dependent on chemical species.

For the rehabilitated mined sites tested, no significant, immediate risk was observed during the trial period. However, there may be risks of exceeding Maximum Levels (ANZFA) for cadmium from long term exposures under these 'worst-case' trial conditions. Long-term contamination was not considered to be likely, however, under more realistic grazing practices. Management strategies for the benefit of future grazing practice should focus on the importance of maximising percent ground cover and standing dry matter, and reducing grazing pressure including the incidence of grazing exclusively on the tailing material, in order to minimise the potential metal exposure.

As no safety guidelines exist for rehabilitated mined land, such trials are imperative if we are to better understand the environmental risks associated with mine closure, and enhance the associated regulatory processes. Investigation beyond bioavailability and

accumulation, including specific measurements of metal species (Thornton, 1997), geochemical factors (Davis *et al*., 1992) and mixture effects may also be necessary to generate a more comprehensive risk characterisation.

The bioavailability of wider spectrum of mine tailings from several mine sites is being determined under controlled laboratory feeding trial conditions using rodents, pigs and cattle. The rodent model will be used to calibrate against the larger animal species with the aim of developing a less expensive rodent model to predict uptake of metals from mine wastes (and wastes of other sources) and also to provide more realistic health risk assessments in humans.

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