ASSESSING IMPACT TO WILDLIFE AT BIOSOLIDS REMEDIATED SITES

Researchers focused on earthworm consumers as the highly exposed species that must be protected by the remediation technology used for a contaminated site.

Part II

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AS PART of the development of alternatives for the remediation of a Superfund site in Jasper County, Missouri, an evaluation was done of the ecosystem impacts from using biosolids plus alkaline by-products to revegetate the lead and zinc contaminated soils. A risk assessment was undertaken to see what available data indicate about the potential impact to the ecosystem, and limits to protect wildlife on biosolids-amended soils were developed. For the risk assessment process, we used an approach similar to what the U.S. Environmental Protection Agency employed in development of the 40 CFR 503 regulations for biosolids, which was based on pathways where elements in land applied biosolids could cause harm to highly exposed and sensitive species. In Jasper County — where application of biosolids and limestone can restore a vigorous plant cover to the sites — two potential pathways were evaluated. The findings of the first assessment — Soil to Plant to Animal (herbivore exposure) — were reviewed in Part I of this article (see "Soil Remediation Using Biosolids," June 2002). Part II examines the pathway, remediated soil to animal.

This pathway generally refers to animals that directly consume soil. For the Part 503 regulations, Pathway 3 was the most limiting pathway for lead (Pb) concentrations in biosolids. The animal in this case was a child and the IEUBK model was used in developing an appropriate biosolids Pb limit. In the case of ecosystem pathways and consideration of the potential for contaminant transfer, there are two potential approaches involving inadvertent soil ingestion by grazing animals or by animals which consume soil biota. One can consider earthworms to be the highly exposed sensitive species as their diet consists exclusively of organic material in soil. Before purging internal soil, earthworms contain a high level of soil, therefore it may be more appropriate to consider animals that feed on earthworms as the highly exposed species that must be protected by the remediation technology used for a contaminated site (Beyer and Stafford 1992).

The first question to address is whether earthworms can survive in mine tailings remediated with biosolids plus limestone (being tilled in above).

Table 1. Survival and biomass of earthworms in control and biosolids plus limestone amended soils[1] from alluvial tailings deposits in Leadville, CO (Compton et al., 2001)

	Untreated		Treated	
Location of Sample	*Survival(%)*	*Biomass (mg)*	*Survival (%)*	*Biomass (mg)*
BCL tailings	0	-	100	329
CO tailings	0	-	98.9	323
MB/ME tailings	0	-	90	372
RA/RB tailings	0	-	10	280
Upstream control			96.7	196

[1]Soils are a mix of 100 tons/acre of anaerobically-treated biosolids plus 100 tons/acre of agricultural limestone

SOIL TO EARTHWORM TO ANIMAL

There is a relatively large body of data on the concentration of metals in earthworms both from biosolids amended soils and from zinc (Zn), Pb and cadmium (Cd) mine spoils. Some basic patterns are common for most reports. Earthworms do not bioconcentrate Zn to levels higher in the earthworm than are present in the soil. They do bioconcentrate both Pb and Cd. Concentration of Cd in earthworms is generally greater than soil concentration while Pb concentrations in earthworms are generally similar or lower than soil concentrations (Beyer and Stafford 1992, Pietz *et. al,* 1984). These studies generally report the metal concentrations of worms after they have been treated to remove internal soil (purged worms). There is no discussion of partitioning of metal into different worm organs, except for Cd which is accumulated in a protein bound form. However, there is little evidence that earthworm-consuming animals can avoid ingesting the internal soil along with the tissues of the earthworms. For the purposes of risk assessment, we conclude that it is more pertinent to consider the transfer risk posed by worms that have not been purged.

The earthworm's diet consists of organic debris from plants and soil organisms in soil. At any given time, the earthworm consists of 20 percent earthworm solids, nine percent soil solids and 71 percent water. Approximately 45 percent of the dry weight (DW) of the unpurged earthworm is soil (Beyer and Stafford 1992). Total soil in the worm on a wet weight basis is nine percent. Animals that eat worms generally do not remove the soil from the worm before consumption. The potential for soil metal risk transfer to the vermivore's diet thus consists largely of soil in the ingested earthworm. If worms can survive in remediated soil, it is then more pertinent to examine metal risk to animals whose diet consists largely of earthworms rather than the toxicity to the earthworms themselves.

EARTHWORM SURVIVABILITY IN AMENDED SOILS

The first question to address is whether earthworms can survive in biosolids plus limestone remediated mine tailings. There are two cases where earthworm survivability has been measured in biosolids remediated Zn, Pb and Cd contaminated soil. Condor *et al.* (2000) amended tailings with a range of soil amendments and evaluated survival of earthworms exposed to the unamended and amended soils. Lime stabilized biosolids were able to eliminate earthworm mortality while other amendments were not.

Biosolids plus limestone amended soils (alluvial mine tailings deposits) in Leadville, Colorado also reduced earthworm mortality over unamended tailings. Survival in amended tailings was comparable to control soils (Table 1).

These studies suggest that earthworms can survive in biosolids-limestone amended mine waste soils. If the earthworms can survive, then animals which ingest earthworms can be expected to return to remediated areas. For this case, the common shrew can be used as the highly exposed species. The shrew has a lifespan of approximately 16 months. It has a very limited range, suggesting that it would not venture far from the remediated areas. Its diet consists of worms and insects. One study analyzed the gut contents of shrews trapped and found that 70 percent of the gut samples included at least some earthworm chaetae (Read and Martin 1993).

There have been no studies of shrews from biosolids-lime remediated sites. There are a number of studies that have examined metal uptake by shrews on contaminated sites. In addition, there are two studies of metal uptake by shrews on biosolids amended soils.

ESTIMATED ZINC, LEAD, CADMIUM RISKS TO VERMIVORE WILDLIFE: CASE OF THE SHREW

For shrews found on contaminated sites, tissue Zn is generally only mildly increased. As noted in Part I, homeostasis controls Zn uptake and excretion. For example, in one study

Table 2. Uptake slopes of kidney Pb in relation to total soil Pb for shrews based on values[1] reported in specific studies

Study	*Uptake Slope*	*Safe Soil Pb Concentration*
Hegstrom and West shrew	6/61.2 = 0.098	1224
Read and Martin young	36.5/(1142-33)*2 = 0.014	8,570
" " mature	380/(1142-33)*2 = 0.156	770
Ma	251/15000*2 = 0.008	15,000
Beyer *et al.*1985, healthy	199.5/1900*2 = 0.0525	2286
" " sick	1217/3800 = 0.32	375
Johnson *et al.*, Mouse at Y fan	20/13900*2 = 7.19 x 10^{-4}	167,000
Mouse at Minera	34/8330* 2 = 0.002	60,000

[1]These values were then extrapolated to determine the soil Pb concentrations that would be associated with 120 mg Pb kg-1 kidney dry weight for shrews.

a 700 percent increase in dietary Zn resulted in a 48 percent increase in body Zn. The majority of this increased Zn was stored in bone tissue (Andrews *et al.*, 1989).

Both Pb and Cd tend to accumulate in shrew tissues. For both metals, the organ that is commonly used to assess potential harm is the kidney although Pb also is accumulated in bone (where most of the carcass Pb accumulates). When supplied in dietary excess, Pb and Cd will accumulate in the kidney. Negative effects associated with these elements include kidney inclusion bodies from excessive Pb and renal tubular dysfunction from excessive Cd (Goyer *et al.* 1970, Roberts and Johnson, 1978). Kidney weight also can increase. When bioavailable dietary Pb is excessive, Pb interferes with heme formation and a heme precursor, delta-amino-levulinic acid (ALAD) levels in blood can also decrease (Beyer *et al.* 1985). There is also some discussion in the literature of weight loss or gain as a result of excess Cd and/or Pb. Relative weights of the kidney to the whole body also have been used to assess potential for damage. Results from these measures have yielded conflicting data. Measures of total kidney Pb and total Cd in the kidney cortex appear to be more reliable measures of potential for harm from excessive absorption of Pb and Cd by mammals and birds.

CALCULATION TO DETERMINE LEAD CONTENT

One approach to determining a permissible Pb content in remediated mine wastes that will not induce chronic kidney damage to shrews is to calculate the uptake slope or translocation slope for kidney Pb (divided by soil Pb) from the studies that have been published. This uptake slope then can be used with a reference value above which damage may occur. There are two numbers in the literature for the dry weight Pb concentration in the kidney where damage may occur, 25 mg kg^{-1}(National Research Council, 1980) and 120 mg kg^{-1} Pb (Goyer *et al*. 1970). These numbers are very different. Studying the literature helped to determine which value better approximates field observations. From the reported values, it seems likely that the 120 mg kg^{-1} Pb kidney DW (equivalent to about 25 mg Pb kg^{-1} fresh weight) is closer to an actual concentration that is associated with adverse health effects than 25 mg kg^{-1} Pb kidney DW. Adverse health effects were not observed in the field for kidney Pb up to 269 mg kg^{-1} DW.

The uptake slopes — defined as change in kidney Pb (mg kg^{-1})/change in soil Pb (kg Pb ha) — were calculated for the studies where soil Pb concentrations were provided. These slope values then can be used to determine a maximum loading value of Pb in soil that would be protective of the shrew (120 mg kg^{-1} kidney DW is used as the threshold organ concentration). This is similar to the process used in developing the metal loading limits for the EPA 503 biosolids regulations. For the uptake slopes generated from field data (Table 2), the soil Pb limit at which kidney Pb would reach 120 mg kg^{-1} DW range from 166,900 (Johnson *et al.* Minera site) to 375 (Beyer *et al.*, sick animal).

It is possible that animals that experience stress as a result of Pb exposure have different uptake slopes from healthy animals. For this reason, it is not necessarily relevant to use those uptake slopes when attempting to determine a soil Pb concentration that will be protective of healthy animals. The average permissible soil Pb level for shrews with no symptoms of Pb stress was 6,700 mg kg^{-1} Pb. We believe that this limit would be appropriate for soils treated with the biosolids plus alkaline amendment remediation methods to limit Pb bioavailability and aid plant cover persistence.

Further, the biosolids plus limestone treatment has been shown to reduce the bioavailability of soil Pb to rats in several tests of treating smelter contaminated soils or urban soils high in Pb. Both the phosphate precipitation of Pb as pyromorphite and increased adsorption of Pb in Fe and Mn oxides are effective in reducing bioavailability of soil Pb (Brown *et al.*, 1997; Brown *et al.*, unpublished data from Joplin plots).

CADMIUM RISK ANALYSIS

There is considerable discussion in the literature regarding earthworm absorption of soil metals, which in turn makes it less conclusive about a risk assessment for earthworm-Cd (see sidebar). Thus, we used an approach for Cd risk estimation similar to the one followed for Pb above. There is more information in the literature on Cd concentrations in wildlife than for Pb, which strongly support the low risk to wildlife of soil Cd through the earthworm pathway.

Research in the United Kingdom has actually observed kidney disease from excessive Cd exposure to shrews in the field (Hunter *et al.* 1984). Readers need to understand the unique aspects at one site where kidney harm to shrews has been repeatedly reported, and other sites where harm was not observed. At the Cu-Cd alloy factory in Prescott, UK, electrical switchgear is manufactured. Cd is included both to strengthen the metal without reducing electrical conductivity, and to plate the contact points to prevent corrosion from making it difficult to open switches. The contamination at this location includes little Zn, so high Cd uptake by soil organisms occurs compared to other sites. In addition, food-chain transfer risk is much greater than at mine sites where the normal geological Cd:Zn (1:100) ratio occurs.

One research study (Shore and Douben 1994) states that Cd concentrations in shrews collected from contaminated sites have been as high as 140 to 250 mg kg^{-1} DW in the kidney without any evidence of ill effect. Kidney cadmium concentrations have been reported in shrews from controlled feeding studies with as high as 990 to 1200 mg kg^{-1} DW whole kidney without evidence of adverse effects (Dodds Smith *et al.* 1992b). This is in contrast to lab studies where mice sometimes show potential evidence of adverse renal effects at kidney Cd concentrations as low as 105 mg kg^{-1} DW (much higher dose rates have been used in short-term testing and acute toxic effects on the liver were believed to have occurred). Further, mice and voles collected from contaminated sites always had Cd concentrations lower than the 105 mg kg^{-1} level (Shore and Douben 1994; Beyer 2000).

Existing data indicate that plant concentrations of metals at remediated sites are within acceptable limits for chronic lifetime livestock forages.

METAL SALTS IN DIETS VS. CONTAMINATED SOILS

The discrepancy between results found in mice in lab studies and actual accumulation patterns of mice in the field and the significantly higher concentrations found in shrews may be related to the nature of the dosing and of self-selected diets which include earthworms and other soil fauna. In lab studies, animals are generally given Cd as salts. In a field situation, uptake of Cd is influenced by the presence of other cations, particularly Zn. The adsorption of Cd by soil components such as hydrous iron oxides and humic acids also limits soil Cd bioavailability. These affect both the absorption of Cd as well as the concentration required for adverse effects to occur (Beyer 2000). This is similar to the results observed in humans when Cd was present as a portion of a balanced diet rather than in a diet marginal in Fe, Zn, or Ca supply (Reeves and Chaney, 2002). An example of the difference in behavior when Cd is present in the environment with or without Zn was found in a case (Chaney *et al.* 1993) where Cd was a cocontaminant with Zn. Plant uptake of Cd was reduced by an order of magnitude. While this relates to plant uptake, studies of human Cd absorption under similar circumstances suggest that the findings may be applicable to wildlife as well. Extensive studies by Fox *et al.* 1984 and others (Jacobs *et al.* 1983) illustrate that concurrent Zn ingestion inhibits Cd absorption by animals. In the Zn-Pb mine tailings in Jasper County, Zn is always found as a cocontaminant with Cd. This should be taken into account when setting a "low observed adverse effect level" or LOAEL.

Once the LOAEL kidney concentration has been agreed on, the soil concentration necessary to reach the LOAEL can be determined. The ratio of change in kidney Cd to change in soil Cd can be used to set an acceptable soil concentration. While there are no studies of Cd uptake by shrews on biosolids amended mine sites, there are three studies of Cd accumulation in shrew kidneys on biosolids amended sites (Table 3). By determining the uptake slope for the shrews on these soils, it may be possible to get an approximation of what Cd uptake

Table 3. Cadmium accumulation in kidneys of shrews living in environments where biosolids raised soil Cd concentration above background, and estimated safe soil Cd concentration for earthworm-consuming wildlife.

Study	Species	Uptake Slope[1]	Safe Soil Cd Concentration Based On Kidney Cortex Cd (mg kg^{-1} dry wt.) 105	200	632
Hegstrom and West, 1989	Shrews	24/2.55 = 9.41	11.2	21.3	67.2
Nickelson and West, 1996	Shrews	25.3/12.8 = 2.0	52.0	100.0	316.0

[1]Uptake slope is calculated from the increase in kidney Cd (mg kg-1 dry weight) above the control divided by the increase in soil Cd concentration (mg kg-1 dry weight) above the control soil.

The average permissible soil Pb level for shrews with no symptoms of Pb stress is 6,700 mg/kg-1 Pb.

might be on mine waste sites remediated with biosolids plus limestone.

Alternatively, the concentration of Cd in kidney cortex widely observed to be associated with the first evidence of renal tubular dysfunction is 200 mg kg^{-1} cortex fresh weight. This concentration must be converted to whole kidney Cd concentration; using the factor relating Cd in cortex to Cd in whole kidney (1.25) (Svartengren *et al*. 1986), the whole kidney fresh weight would contain 160 mg Cd kg^{-1} when 200 mg Cd kg^{-1} kidney cortex fresh weight was reached. Converting this figure to dry weight for comparison with wildlife research results is achieved by use of 25.3 percent dry matter in kidney of shrews (Ma 1989). The result is 632 mg Cd kg^{-1} whole kidney DW as the threshold for renal tubular dysfunction, which is three to six times higher than used in this estimate.

It should not be a surprise that soil Cd ingested by shrews which consume unpurged earthworms is of relatively low risk to shrews. The presence of Zn, Fe, and Ca in such diets prevent deficiencies in the animals, and the presence of Cd-adsorbing soil components such as hydrous iron oxides and humic acids should reduce bioavailability of Cd compared to Cd-salt additions to purified

DETERMINING ACCUMULATION OF CADMIUM IN EARTHWORMS

A NATURAL process causes earthworms to accumulate Cd in tissues. Whether this is accidental accumulation, or an evolved protection mechanism, all earthworms accumulate some Cd, and some species reach up to ten-fold higher Cd than the soil (dry weight basis). One approach to evaluate potential food chain soil Cd risk to earthworm consumers would be to estimate the "bioaccumulation" factor (worm-Cd/soil-Cd). Evaluation of the data summarized in a review of recent research (Sample *et al*. 1999) clearly shows that the bioaccumulation factor is much higher in low Cd (uncontaminated) soils than in Cd enriched soils (e.g., Beyer *et al.* 1982; Lock and Jansen 2001); thus, when the bulk of the data on Cd bioaccumulation in earthworms are for control soils, using the average of all results is not appropriate for contaminated soils (predicts ten-times higher worm Cd than found for unpurged worms from contaminated soils). When Zn accompanied the Cd of contaminated soils, bioaccumulation of Cd was reduced; and wildlife consume worms with soil present in their digestive tracts such that whole unpurged *Eisenia fetida* contain only about 70 percent as high Cd concentration as purged worms living in the same soil.

During review of these documents and reading and discussions about how such tests are best conducted, several points have been noted. One issue is the variation among worm species as to where they feed and what they feed on. Some worms feed on fresh leaves on the soil surface, but hardly consume subsurface materials. *Eisenia fetida*, the species used in many tests because they are easy to study, is likely to feed mostly on animal excreta or decaying plant materials deposited locally on the soil surface. Other earthworms, such as the widely occurring *Lumbricus terrestris,* come to the surface when soils are wet, but mostly consume plant residues in or on the soil. For *E. fetida*, this raises the question of whether the concentration of Cd in the soil *per se* has any effect on the worm Cd, or if it is the Cd concentration in some food item that was present in the mixed soil being supplied repeatedly to these worms. These issues appear to be "unknown" in risk assessment for soil Cd in the vermivore pathway.

Risk assessment for earthworm-Cd has been clarified somewhat by recent research by two groups in the Netherlands that examined how earthworms absorb soil metals. In one group (Saxe, *et al.*), the natural variance among 20 Dutch soils in Cd accumulation by earthworms in controlled tests were reported. Further evaluation of the results was concluded to indicate that earthworms accumulate Cd and other metals predominantly through their skin (dermal absorption) rather than their intestine (Peijnenburg *et al*.1999). We are uncomfortable with this conclusion based on the data they used. Correlation does not prove causality.

The other group used a direct experimental approach in which two treatments were applied in a factorial design to see what affect they had on Cd bioaccumulation in earthworms. Oste *et al.*(2001a; 2001b) added lime and/or MnO_2 (birnessite, a high sorbing form of MnO_2) to an aged Cd-amended soil. The added MnO_2 sharply reduced water soluble Cd without raising pH, while adding lime reduced Cd solubility for both treatments. But the 25-fold reduction in pore water Cd concentration of the acidic soil due to birnessite addition was very large compared to the small reduction in earthworm Cd. This group concluded that the bulk of Cd absorption by earthworms occurs in the intestine where pH is near neutral, not through the skin. This finding is in agreement with a large number of field and laboratory studies in which increasing soil pH had a small effect on Cd bioaccumulation in earthworms. The Oste *et al.* test with lime and MnO_2 is also a very direct soil Cd remediation test, essentially an evaluation of the potential of adding Cd-sorbent to soil. Keeping soil pH high aided in lowering potential adverse effects of the soil Cd, but the sorbent was more important than the pH shift in Cd bioaccumulation in the earthworms.

Other soil metal remediation studies are relevant to the present consideration. Pearson *et al.* (2000) examined remediation of Pb risk to earthworms in toxic mine soils using phosphate treatments; because phosphates often cause soil acidification, toxicity of the mine soil (from high Zn) was increased by phosphorus rather than reduced. The phosphate treatment — which is promising to reduce soil Pb bioavailability to humans — is not capable of remediating other metals such as Zn and Cd unless soil pH is raised or made calcareous to keep the bioavailability of these metals low. Conder *et al.* (2000) used limed biosolids to remediate Zn mine wastes which were strongly phytotoxic; this treatment allowed *E. fetida* to survive and reproduce rather than die as they did on the untreated soil. The unpublished results of Compton *et al.* in study of *E. fetida* tolerance of mine waste contaminated soils near Leadville, Colorado found similar remediation for the earthworms when soil had been treated to prevent Zn phytotoxicity.

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diets. One soil feeding study reported in the literature (Schilderman *et al.* 1997) found that soil Cd bioavailability to rats was 43 percent that of Cd salts (the test involved feeding $CdCl_2$-amended OECD synthetic soil at 4,400 mg Cd kg^{-1}, and used toxicological methods rather than chronic animal nutrition testing methods). In other tests in which recommended quality biosolids were fed to livestock, no increase in kidney or liver Cd was observed after one to four year feeding periods with three to ten percent biosolids in diets (Decker *et al.* 1980; Smith *et al.* 1985). So the Cd in soil inside earthworms comprises less potential risk than Cd in purged earthworms or from toxicological type feeding tests.

CONCLUSIONS

Existing data indicate that it is possible to restore a persistent vegetative cover to metal contaminated sites using biosolids plus alkaline amendments. Plant concentrations of metals at remediated sites are within acceptable limits for chronic lifetime livestock forages. Although full scale ecosystem monitoring has not been done at remediated sites, it should be appropriate to predict ecosystem impact by examining pertinent studies done on unremediated sites. These studies indicate that guidelines can be established as to what sites are suitable for restoration using biosolids plus limestone amendments. By taking into account existing information, these guidelines should adequately protect the highly exposed vermivore species attracted to the remediated, revegetated environments. ■

If the earthworms can survive, then animals which ingest earthworms can be expected to return to remediated areas. For this case, the common shrew can be used as the highly exposed species.

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In a field situation, uptake of Cd is influenced by the presence of other cations, particularly Zn.

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