ACCUMULATION OF ECO-TOXIC METALS IN POTATO PLANTS UNDER GREENHOUSE CONDITIONS IRRIGATED WITH SYNTHETIC ACID MINE DRAINAGE IMPACTED WATER AND HEALTH RISK EVALUATION[1]

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Abstract. Active mining operations and abandoned mines around the world discharge acid mine drainage (AMD) to surface water resources. These impacted streams are sometimes used in agricultural areas to irrigate crops for human consumption. Long-term use of impacted irrigation water contaminates food crops grown in these areas which in turn may expose humans to undue risks caused by chronic intake of metal-contaminated food. In Andean mining regions, like those near Potosí, Bolivia, the main staple crops -potato tubers- have been exposed long-term to AMD impacted irrigation water. To quantitatively investigate potential risks from contaminated irrigation water, two greenhouse studies (Summer and Fall) were performed to evaluate the distribution and accumulation of eco-toxic metals in potato plants and resultant possible human health risks. Soil, water and potato samples were collected from four synthetic AMD irrigation systems and one reference irrigation system in the Summer study, and three synthetic AMD irrigation treatments and one reference for the Fall study. The synthetic AMD irrigation waters were representative of those encountered in Potosí, Bolivia and had concentrations of 37 mg/L Fe, 3.4-14 mg/L Cd, 490-1980 mg/L Zn, 12 mg/L Al, 0.1-16 mg/L As, and 0.4-3.4 mg/L Pb. These waters had pH of 3.5-6.8 and specific conductance of 0.8–3.2 mS/cm, 26–44 mg/L Cl^-, 0.5–8.4 mg/L NO_2^-, 3–13 mg/L NO_3^-, 46–2730 mg/L SO_4^{2-}. After 4 months of exposure, soils and potato tubers were analyzed for total metal concentrations. Soils contained total metal concentrations in the range of <0.009-18 mg/kg As, 0.3-14 mg/kg Cd, 10.5–24.2 mg/kg Pb, and 44-680 mg/kg Zn. Metal concentrations were also analyzed in potato plant leaves, stems, and roots. Metal accumulation from soil to plant was determined using the enrichment factor and mobility of metals from root to shoots was determined using the translocation factor. In order to evaluate the risk posed by the consumption of those tubers, Environmental Protection Agency minimum risk levels (MRL) were used as the reference dose. Calculated ingested average daily dose and MRL were used to determine the hazard quotient (HQ) from potatoes. HQ>1 were found in Cd, Ni, and Pb indicating potential concern for non-carcinogenic effects.

Additional key words: farming, toxicology, trace metals, availability, desorption.

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Introduction

Uncontrolled discharge of AMD from active and abandoned mines to surface water resources is a major cause of environmental pollution in mining regions. In arid areas such as Potosí, Bolivia AMD-impacted streams may be used to irrigate agricultural food crops. The metals added to the soil from the AMD may eventually end up in the food chain or cause contamination of groundwater resources (Sayyad et al., 2010, Yang et al., 2009, Datta and Young. 2005). Contaminated soil often presents an unacceptable risk to human and ecological health and must be remediated (Liu et al., 2009).

Elevated concentrations of heavy metals in soils, accompanied with acidic pH, are likely to enhance uptake of metals by plants (Chen et al., 2010). Several factors influencing metal concentration on and within plants are climate, atmospheric deposition, nature of the soil, degree of maturity of the plant, application of fertilizer, sewage sludge or irrigation with wastewater (Muchuweti et al., 2006). High rate transfers from soil to roots and from roots to shoots have been determined in rushes (*juncus* and *salix* sp.) (Weigand et al., 2009). Concentrations of Cd, Zn, and Cu were found to be larger in the above ground portion of spinach grown in contaminated soils than those grown in agricultural soils with no foliar symptom of metal toxicity (kisku et al., 1999). Leaves and stems are main organs accumulating metals (Wei et al., 2008).

The accumulation of metals in food is of special concern around the world. The role of trace elements in the metabolism of humans is very important; deficiency of trace metals may cause diseases and excess may result in toxicity (Hashmi et al., 2007). Food health guidelines have been developed in different countries in order to assess human health risk by food consumption (e.g. Miller et al., 2004, and EC, 2006). The United States developed reference doses to estimate the daily exposure of humans to a hazardous substance, known as minimum risk levels (MRL). MRLs are likely to be without an appreciable risk of adverse noncancerous health effects over a specified route and duration (e.g., acute, intermediate, chronic) of exposure (e.g., inhalation, ingestion) (ATSDR, 2008).

ASTDR (2008) described in detail each of the effects to human health linked by chronic ingestion of eco-toxic metals. Some eco-toxic metals cannot be metabolized by humans and may accumulate, leading to heath impacts depending on concentrations. The main threats posed to human health due to chronic ingestion of eco-toxic metals are related to death, cancer, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, body weight, metabolic, neurological, and reproductive problems (ATSDR, 2008).

Figure 1 Local native inhabitant consuming locally grown potatoes and fish.

Different techniques have been used in order to evaluate the possible risks that contaminated food may pose to humans. Miller et al. (2004) compared Cd, Cu, Pb and Zn concentrations with commercially sold vegetable guidelines of different vegetables in rural communities from the Bolivian Andes. Yang et al. (2009) proposed that a more pragmatic solution is to assess the metal concentration exceeding the national limits in different vegetables and to advise farmers accordingly. Lim et al. (2006) assessed the risk of health effects determined by a hazard quotient (HQ) value on the residence of the abandoned Songcheon Au-Ag mine in Korea.

To quantitatively investigate potential risks, a greenhouse study was performed to evaluate the distribution and accumulation of eco-toxic metals in potato plants and resultant possible human health risks. Objectives of this paper focused on (1) determination of concentrations of eco-toxic metals in potato plants and tubers, soils, irrigation water, and leaching water, (2) identification of metal competition affecting metal bioavailability and bioaccumulation in potato plants, (3) identification of potential health risk that may be of concern at mining sites by comparing potato tuber concentrations with existing international health guidelines, and (4) to evaluate the potential risk to human health as a result of irrigation of staple crops with mining-polluted waters by determining the HQ.

Methods

All the experiments were conducted under greenhouse conditions at the Aquatic Research Facility (ARF) at the University of Oklahoma, Norman, OK. Irrigation water composition simulated impacted AMD surface water mean metal concentrations from previous studies in Potosí, Bolivia (Garrido et al., 2009). The experiment realized from May to August was designated “Summer study” and that from September to December as “Fall Study”.

In the summer study a five container greenhouse experiment was conducted with four (As, Cd, Pb and Zn treatments) synthetic AMD irrigation and one reference treatment from May to August 2009. Synthetic irrigation was prepared with nano-pure water by adding ten different compounds. Total concentrations of targeted elements (Fig. 2) were 1982 mg/L Zn, 3.4 mg/L Pb, 16 mg/L As and 14.4 mg/L Cd for Zn, Pb, As and Cd treatments, respectively, in the Summer study. Well water was applied in the reference treatment. Irrigation water samples were taken before synthetic AMD irrigation water was applied to the planted potatoes. An Irrigation frequency of approximately two weeks was used during the experiment.

The Fall study irrigation water preparation reduced the maximum concentrations of the salts by half. The salt concentration reduction was made due to the low development of potato plants from the Summer study. No Pb treatment was performed in the Fall study.

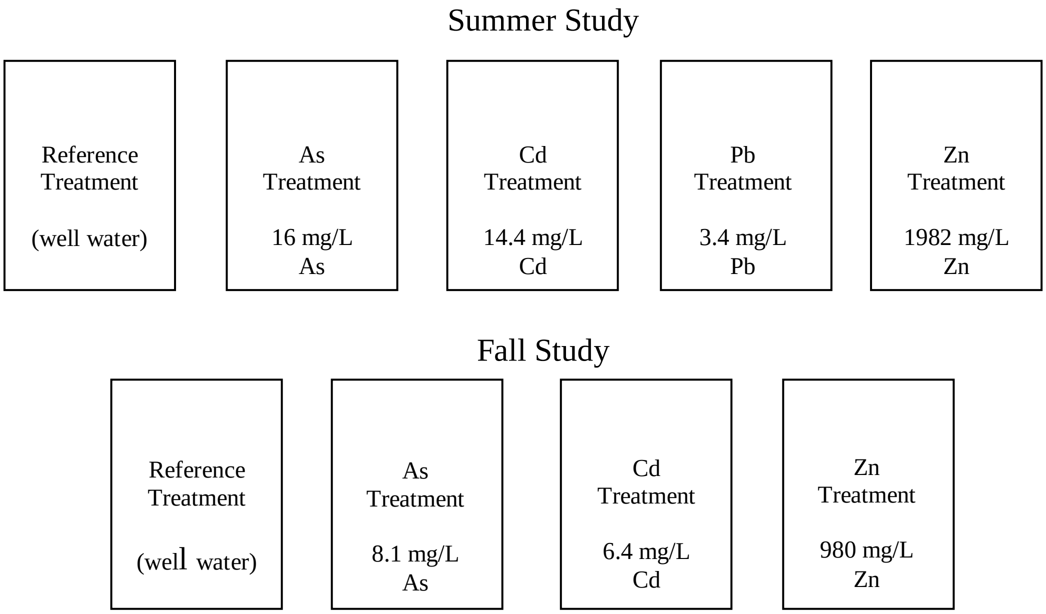

Figure 2 Schematic representation of the greenhouse studies with its respective targeted concentrations.

Synthetic AMD was mixed in cubitainers of 18-L HDPE in the laboratory every two weeks. Water samples were collected before irrigation and 24 h after irrigation (leaching water). Total metals analyses were conducted for water samples (irrigation and leaching water) stored in 60-mL HDPE containers and preserved with trace-metal grade HNO_3. Dissolved metal analyses were conducted to water samples collected via filtering (0.45µm) before acidification and anions analyses were performed to water sample stored at 4°C with zero headspace. Specific conductance (SC), pH, temperature (T), and dissolved oxygen (DO) were measured immediately after synthetic AMD irrigation water preparation and leaching water collection. Alkalinity was measured following standard methods using a Hach digital titrator method 8203(Hach 2004). Anion samples were filtered through Dionex OnGuard II H cartridge and 0.2 µm nylon filters, and concentrations measured on a MetrOhm 761 compact ion chromatograph following EPA method 300 (USEPA 1993). Acid microwave digestions of total and dissolved metal samples followed EPA method 3015.

Five treatments in the Summer study and four treatments in the Fall study were set up in containers of 85-L volume filled with “Gardeners Secret” top soil composed of compost, perlite, and pine bark. The top soil was mixed with 14.2g of muriate of potash used as an amendment. Muriate of potash was applied every month on the soil surface as fertilizer. Ten potato seeds were planted per container in each study and irrigated with 11 L of the synthetic irrigation water based on the potato water requirement of 500 mm/120d. Each pot that produced potato tubers in both studies received its own synthetic irrigation water composition over a period of 4 months.

Soil samples were taken at the beginning and at the end of each experiment. Initial soil samples before any irrigation water application were denoted as initial soils. Soil samples from treatment containers were collected at the end of the experiment a week after last irrigation. A drop of 10% HCl acid added to the soil confirmed the presence of free $CaCO_3$ in the initial soil. These soils were dried, sieved (<2mm) and homogenized, and then 0.5g of dry sample was digested following EPA method 3051A (USEPA, 2006). Dried soil also was used to determine soil pH (pH_w) following the method described by Thomas (1996).

Potato plant measurements were taken after harvested. Stem and root lengths and potato weights were recorded. Leaves, stems, roots, and potato tubers were collected from each pot, separated, weighed, and cleaned. Potato tubers were cut unpeeled into small chips. Leaves, stems, roots, and potato tubers were dried, crushed, homogenized, and a known mass (0.1-0.25g) was digested using microwave acid digestions following EPA method 3051A. Digested water, soil, and potato samples were analyzed by a Varian Vista-Pro simultaneous inductively coupled plasma-optical emission spectrometer (ICP-OES) following EPA method 6010 (USEPA 2006).

In order to determine accumulation of metals in potato leaves, stems, roots and tubers, enrichment factors (EF) were calculated. Parameters used in Equation 1 to determine EF were total metal potato tuber concentration (C_p) and total metal Soil concentration (C_s).

$$EF = C_p/C_s \qquad \text{Equation 1}$$

Translocation factors (TF) were determined from the ration of the total metal concentrations (C_t) of Potato leaves, stems and tubers with respect total metal concentrations of the roots (C_r) (Equation 2).

$$TF = C_t/C_r \qquad \text{Equation 2}$$

To quantitatively investigate potential health risk posed by consumption of contaminated potatoes, reference doses (RfD) were derived from MRLs (Table 1). RfDs are the estimated amount of the daily exposure level for the population that is likely to occur without an appreciable risk of deleterious effects during a life time (Lim et al., 2008). The estimated intake (dose) in the human body through contaminant ingestion was named as the average daily dose (ADD_i). ADD_i is the amount of chemical substance ingested per kilogram of body weight per day (mg/kg/day). The parameters used to determine the ADD_i (Equation 3) were compiled in Table 2 and were addressed to the specific conditions at the rural communities of Potosí, Bolivia.

Table 1 Summary of MRLs for eco-toxic metals by chronic ingestion (ATSDR, 2008).

Name	Route	MRL (RFD) (mg/kg/day)
Arsenic	Oral	0.0003
Cadmium	Oral	0.0001
Zinc	Oral	0.3
Aluminum	Oral	1
Cobalt	Oral	0.01
Copper	Oral	0.01
Nickel	Oral	0.00009

Table 2 Input parameters to determine ADD_i values (Lim et al., 2008) adapted to the Potosí, Bolivia ingestion rate.

Parameters	Description	Unit	Value
C	Contamination concentration in media	mg/kg	
IR	Ingestion rate per unit time		
	Soil	mg/day	
	Plant	g/day	80[1]
	Water	L/day	
EF	Exposure frequency	days/year	365
ED	Exposure duration	years	30
BW	Body weight	kg	70
AT	Average time	days	1564
CF	Conversion factor		0.000001

[1] Estimated for rural communities at the Bolivian Andes by Miller et al. (2004)

$$ADD_i = \frac{C * CF * IR * ED * EF}{BW * AT}$$

Equation 3 (Lim et al., 2008)

There is not an established MRL for Pb because some of the health effects associated with exposure to Pb occur at blood Pb-levels so low as to be essentially without a threshold (ATSDR, 2008). Pb RfD was derived from a base line that estimated potential human exposure for an intake of 0.001 mg/kg/day Pb (ATSDR, 2008).

The potential for non-carcinogenic effects to occur is evaluated by comparing exposure or average intake of hazardous substances with corresponding MRL (Lim et al., 2008). HQ (Equation 4) represents the non-cancerous risk effects for a single substance. ADD_i that exceeded its corresponding MRL (HQ > 1) indicated that there must be concern for potential non-carcinogenic effects.

$$HQ = \frac{\text{ADDi}}{\text{MRL}}$$ Equation 4 (Lim et al., 2008)

Average values, standard deviations and significant differences were calculated with Microsoft Excel®. Statistical tests were performed assuming normality, two-tailed two sample homoscedastic Student's *t*-tests ($\alpha = 0.05$) between treatments, treatments and references, and between studies.

Results

Irrigation water

Synthetic irrigation water had significant lower pH compared with the reference which had circum-neutral pH values (Table 3). Synthetic AMD irrigation water had water pH below 4 and no alkalinity. Dissolved oxygen was greater than one, indicating that all treatments presented oxic conditions. Anion concentrations (Table 4) between synthetic irrigation and reference were significantly different ($p<0.05$); no PO_4^{3-} was detected in the irrigation water.

Total metal concentrations (Table 5) in AMD-impacted treatments from the Summer and Fall study exceeded Canadian, Australian, United Nations Food and Agriculture Organization (UNFAO) long-term irrigation water guidelines for As, Cd, Pb and Zn as assessed in previous research (Miller et al., 2004; Strosnider et al., 2008; Garrido et al., 2009). Reference treatments in both studies only exceeded Canadian irrigation water guidelines for Pb. In general, reference metal concentrations were significantly lower than synthetic AMD irrigation treatments except for Na and Ca.

Table 3 Physical parameters measured in irrigation water.

	pH	**Alkalinity (mg/L as $CaCO_3$)**	**DO (mg/L)**	**SC (mS/cm)**	**T (°C)**
Summer Study					
Zn trtmt. (n=6)	3.8	0	8.5	3.2	22.1
Std. dev.	*0.09*		*0.3*	*0.2*	*0.8*
Pb trtmt. (n=11)	3.9	0	8.5	1.7	21.8
Std. dev.	*0.2*		*0.3*	*0.08*	*0.6*
As trtmt. (n=8)	3.9	0	7.9	1.8	19.5
Std. dev.	*0.2*		*0.8*	*0.1*	*0.7*
Cd trtmt. (n=6)	3.8	0	7.8	1.8	21.9
Std. dev.	*0.2*		*0.6*	*0.05*	*0.4*
Reference trtmt. (n=11)	6.6	290	7.8	0.9	25.2
Std. dev.	*0.3*		*0.7*	*0.07*	*2.5*
Fall Study					
Zn trtmt. (n=6)	3.5	0	8	2.3	22.5
Std. dev.	*0.1*		*0.2*	*0.1*	*0.3*
As trtmt. (n=6)	3.6	0	8.4	1.7	22.3
Std. dev.	*0.3*		*0.4*	*0.06*	*0.2*
Cd trtmt. (n=6)	3.6	0	8.2	1.7	22.4
Std. dev.	*0.2*		*0.3*	*0.08*	*0.3*
Reference trtmt. (n=6)	6.8	300	8.5	0.8	21.9
Std. dev.	*0.2*		*0.3*	*0.07*	*2.7*

Table 4 Anion results from irrigation water all values in mg/L

	F^-	**Cl^-**	**NO_2^-**	**NO_3^-**	**PO_4^{3-}**	**SO_4^{2-}**
Summer study						
Zn trtmt. (n=6)	<0.1	28.7	6.2	4.7	<0.75	2730
Std. dev.		*0.9*	*4*	*0.9*		*110*
Pb trtmt. (n=11)	<0.1	27.9	4.4	5.5	<0.75	1130
Std. dev.		*1.4*	*4.7*	*1*		*140*
As trtmt. (n=8)	<0.1	28.5	6	3.3	<0.75	1110
Std. dev.		*1.5*	*4.5*	*0.3*		*60.3*
Cd trtmt. (n=6)	<0.1	28.7	4.2	3.2	<0.75	1140
Std. dev.		*1.1*	*5.3*	*0.4*		*57*
Reference trt. (n=11)	0.3	44	0.5	13.4	<0.75	49.1
Std. dev.	*0.03*	*2.5*	*0.3*	*3.7*		*2.3*
Fall Study						
Zn trtmt. (n=6)	<0.1	25.6	8.4	4.3	<0.75	1640
Std. dev.		*0.7*	*3.3*	*1.1*		*76*
As trtmt. (n=6)	<0.1	26.1	5.1	3	<0.75	1060
Std. dev.		*1.5*	*5.5*	*0.3*		*18.8*
Cd trtmt. (n=6)	<0.1	26.2	6.1	3.1	<0.75	1070
Std. dev.		*0.5*	*3.7*	*0.2*		*14.2*
Reference trtmt. (n=6)	0.4	41.2	1.7	11.5	<0.75	46.3
Std. dev.	*0.06*	*2.8*	*3.9*	*0.4*		*0.4*

Table 5 Mean total metals irrigation water (mg/L). Bold values > guidelines.

	Al	**As**	**Ca**	**Cd**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Summer study											
Zn trtmt. (n=6)	11	**0.2**	113	**3.3**	35.5	2.5	38.5	26.1	2	**0.3**	**1980**
Std. dev.	*1.4*	*0.1*	*4.7*	*0.2*	*2*	*1.9*	*1.6*	*2.5*	*0.8*	*0.1*	*110*
Pb trtmt. (n=11)	10.7	**0.2**	112	**3.2**	35.7	1.9	38.5	24.8	1.9	**3.4**	**460**
Std. dev.	*1.6*	*0.1*	*4.8*	*0.2*	*1.9*	*0.9*	*2.1*	*3*	*0.7*	*0.3*	*31.6*
As trtmt. (n=8)	11.2	**16**	113	**3.4**	36.5	12.5	39.1	25.3	2	**0.3**	**470**
Std. dev.	*1.4*	*0.8*	*4.1*	*0.1*	*1.5*	*2.5*	*1.6*	*2.3*	*0.6*	*0.1*	*24.9*
Cd trtmt. (n=5)	11.5	**0.1**	117	**14.4**	36.9	2.3	39.5	27.2	1.6	**0.4**	**490**
Std. dev.	*1.5*	*0.2*	*3.2*	*2*	*1.3*	*1.6*	*1.5*	*2.4*	*0.2*	*0.1*	*11*
Reference trt. (n=11)	0.03	<0.009	129	<0.0002	0.02	2.5	25.9	0.002	41.4	**0.02**	0.1
Std. dev.	*0*	*0*	*4.8*	*0*	*0.03*	*0.3*	*0.9*	*0.002*	*2.7*	*0.03*	*0.1*
Fall study											
Zn trtmt. (n=6)	8.8	**0.2**	105	**3**	34.3	0.9	36.7	23.8	1.5	**1.2**	**980**
Std. dev.	*0.5*	*0.1*	*7.3*	*0.2*	*1.3*	*0.2*	*1.7*	*1*	*0.1*	*1.5*	*29.4*
As trtmt. (n=6)	8.7	**8.1**	104	**3**	35.2	6.1	36.4	22.9	2.1	**0.4**	**450**
Std. dev.	*0.5*	*2.6*	*7.2*	*0.1*	*1.9*	*2.3*	*1.6*	*1.3*	*0.7*	*0.1*	*19.9*
Cd trtmt. (n=6)	8.6	**0.1**	105	**6.4**	34.2	1.3	36.2	22.7	1.7	**0.3**	**460**
Std. dev.	*0.5*	*0.06*	*7.5*	*1.1*	*1*	*1.3*	*1.6*	*0.4*	*0.6*	*0.07*	*9.3*
Reference trt. (n=6)	0.04	<0.009	118	<0.0002	0.6	2.1	24	0.002	36.7	**0.01**	0.1
Std. dev.	*0*	*0*	*5.3*	*0*	*1.2*	*0.05*	*1*	*0.04*	*0.6*	*0*	*0.06*

Dissolved metal concentrations (Table 6) from Summer and Fall experiments indicated high metal solubility except for As. Low pH seemed to be the major factor affecting metal solubility in AMD-impacted irrigation waters. Low As solubility has probably occurred because As is strongly adsorbed from acidic and neutral waters on to sediments, clay, iron oxides, organic material (Tseng et al., 2009, Huang et al., 2005). Pentavalent As (As^{+5}) is the most common specie of As in surface water but in anaerobic groundwater the trivalent arsenite (As^{+3}) is the predominant species (Tseng et al., 2009). Pentavalent As (As^{+5}) was used in these studies. Low concentration of dissolved Fe and As may have been occurred to the sorption of As^{+5} on positively charged iron-oxide surfaces (Schlottmann and Breit., 1992).

Synthetic AMD-irrigation water was representative from those AMD-impacted irrigation water used in Potosí, Bolivia. Eco-toxic metals of concern due to the elevated concentrations exceeding long term irrigation water guidelines were Cd, Pb and Zn. These eco-toxic metals were of concern due to their high solubility indicating high potential for potato plant absorption.

Table 6 Mean dissolved metals irrigation water (mg/L). Bold values > guidelines.

	Al	**As**	**Ca**	**Cd**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Pb**	**Zn**
Summer study											
Zn trtmt. (n=6)	10.5	**0.1**	115	**3.3**	21.6	2.5	38.7	23.8	2.6	**0.3**	**1930**
Std. dev.	*2.3*	*0*	*4.6*	*0.2*	*6.2*	*1.4*	*2.4*	*0.5*	*1.2*	*0.1*	*68.5*
Pb trtmt. (n=11)	10.1	0.02	111	**3.2**	24.8	2	38.4	23.5	2.3	**2.8**	**460**
Std. dev.	*1.7*	*0.02*	*3.2*	*0.2*	*4.2*	*0.9*	*2.2*	*1.2*	*0.8*	*0.2*	*39.3*
As trtmt. (n=8)	10.6	0.5	112	**3.3**	11.1	11.3	38.9	23.8	1.9	**0.2**	**480**
Std. dev.	*0.4*	*0.01*	*1.0*	*0.01*	*1.4*	*0.4*	*0.1*	*0.5*	*0.3*	*0.09*	*20.7*
Cd trtmt. (n=5)	10.3	<0.009	114	**13.7**	18.3	1.5	38.3	23.7	1.6	**0.4**	**470**
Std. dev.	*2.3*	*0*	*5.0*	*2.8*	*0.9*	*0.8*	*2.5*	*1.4*	*0.1*	*0.2*	*33.7*
Reference trt. (n=11)	0.03	<0.009	130	<0.0002	0.03	2.8	25.5	8E-5	40.4	**0.04**	0.2
Std. dev.	*0.01*	*0*	*4.4*	*0*	*0.04*	*0.5*	*1.0*	*0.002*	*2.6*	*0.02*	*0.1*
Fall study											
Zn trtmt. (n=6)	8.7	<0.009	105	**3**	18.7	2.6	36.6	23	2.5	**1**	**950**
Std. dev.	*0.4*	*0*	*6.2*	*0.1*	*1.0*	*3.3*	*1.5*	*1.4*	*2.3*	*1.2*	*50*
As trtmt. (n=6)	8.7	**0.9**	104	**2.5**	20.9	6.6	36.4	23.2	2.3	**0.3**	**460**
Std. dev.	*0.5*	*0.3*	*7.4*	*0.2*	*2.6*	*2.9*	*1.7*	*1.7*	*0.9*	*0.09*	*29.5*
Cd trtmt. (n=6)	8.6	<0.009	105	**6.4**	19.8	1.9	36.2	23.3	1.9	**0.3**	**470**
Std. dev.	*0.5*	*0*	*7.2*	*1.1*	*2.1*	*1.2*	*1.6*	*1.2*	*0.6*	*0.06*	*20.3*
Reference trt. (n=6)	0.04	<0.009	120	<0.0002	0.6	2.2	23.9	0.02	36.6	**0.01**	0.1
Std. dev.	*0.02*	*0*	*5.9*	*0*	*1.5*	*0.3*	*1.0*	*0.05*	*0.8*	*0.00*	*0.05*

Soils

Low pH in soil water increases metal bioavailability for plants (Kirkham, 2006; Wei et al., 2008). Synthetic AMD irrigation treatments applied on soils ranged between pH values of 3.5-4. Soil pH_w for both experiments ranged from 6.5 to 7.7. The summer study had pH of 7.46 for the initial soil, 6.51 for the Pb treatment, 7.33 for the As treatment and 7.67 for the reference treatment. The fall study had pH values of 7.43 for the initial soil, 7.36 for Zn treatment, 7.47 for Cd treatment, 7.52 for As treatment and 7.77 for reference treatment. There were not significant differences between the initial soil pH_w and the final measured soil pH_w at each treatment except for the Pb treatment which is more acidic by an entire pH unit. It was probable that after applied on the treatments, synthetic AMD-irrigation water reacted with soil particles and most of the trace metals changed to insoluble forms due to the formation of precipitates. This interaction with soil organic and inorganic matter, and the adsorption to surface soil particles affected soil metal bioavailability. Huang et al. (2005), identify sorption of As to iron oxides as a main cause of unavailability of As to lettuce. In a soil study Tsadilas et al., (2009), concluded that adsorption reactions were mainly affected by pH as revealed by the significant correlation coefficients of Zn and Cu with soil pH.

Soil metal concentrations can be found in Table 7. Significant differences were found between the synthetic AMD-impacted soil treatments and the initial soil. Concentrations of Cd

Table 7 Soil total metal concentrations (mg/kg) and standard deviations at the greenhouse.

	Al	**As**	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**Pb**	**Zn**
Summer study															
Initial	3690	<1.6	10050	0.3	4.7	6.8	8.3	5800	2970	2960	190	340	7.7	10.7	33
Std. dev.	*170*		*940*	*0.04*	*0.2*	*0.4*	*1.4*	*380*	*89.9*	*130*	*8.5*	*8.6*	*0.5*	*0.3*	*1.9*
Zn trtmt.	5730	<1.6	4720	1.6	6.7	9	8.1	8990	2520	3010	240	<0.11	9.5	13.7	680
Std. dev.	*170*		*51.4*	*0.1*	*0.2*	*0.2*	*0.2*	*202*	*37.3*	*30.6*	*5.0*		*0.04*	*1.1*	*28.6*
Cd trtmt.	4030	<1.6	7330	13.5	5.2	7	7.5	6390	2530	2710	220	<0.11	8	11.4	420
Std. dev.	*120*		*404*	*1.1*	*0.06*	*0.3*	*0.2*	*248*	*40.8*	*67.7*	*4.9*		*0.3*	*0.0*	*29.5*
As trtmt.	3450	18.2	12890	4.1	5	8.4	10.8	6200	4290	3120	230	430	9.5	17.8	420
Std. dev.	*290*	*4.7*	*2640*	*2.0*	*0.9*	*2.7*	*2.7*	*1400*	*150*	*300*	*42.7*	*6.7*	*2.7*	*11.0*	*110*
Pb trtmt.	8410	<1.6	7230	5.4	6.9	12.1	9	11230	3960	3550	260	240	10.1	24.2	610
Std. dev.	*4090*	*9.6*	*5130*	*1.7*	*3.1*	*4.8*	*4.3*	*4920*	*2300*	*1760*	*120*	*210*	*4.1*	*6.6*	*250*
Reference trtmt.	7750	3.5	13020	0.4	6.1	12.7	8.5	10450	4580	3900	210	360	10.5	12.6	44
Std. dev.	*461*	*1.0*	*1980*	*0.0*	*0.3*	*0.3*	*0.5*	*520*	*33.2*	*190*	*10.1*	*13.7*	*1.2*	*1.0*	*3.9*
Fall study															
Initial	6640	<1.6	10320	0.4	5.6	10	8.3	8890	5040	3530	190	410	8.1	10.5	38.3
Std. dev.	*470*		*510*	*0.03*	*0.1*	*0.6*	*0.2*	*310*	*97.7*	*58.8*	*3.6*	*4.8*	*0.1*	*0.2*	*0.4*
Zn trtmt.	6070	5.8	13450	1.8	6.2	10.9	8	10890	4870	3950	220	330	11.2	13.6	450
Std. dev.	*1190*	*0.7*	*710*	*0.2*	*0.5*	*1.3*	*0.2*	*1980*	*450*	*340*	*6.8*	*20.8*	*0.7*	*1.2*	*58.2*
Cd trtmt.	5180	3.3	12000	3.6	5.3	9.4	7.5	8220	4410	3670	220	360	10.1	10.9	250
Std. dev.	*300*	*0.1*	*690*	*0.1*	*0.1*	*0.5*	*0.3*	*210*	*110*	*72.9*	*2.5*	*7.0*	*0.3*	*0.9*	*4.3*
As trtmt.	4960	4.9	10670	1	5.1	8.9	7.4	7920	4400	3400	220	330	9.5	11.9	140
Std. dev.	*340*	*0.1*	*470*	*0.1*	*0.3*	*0.6*	*1.1*	*470*	*110*	*100*	*0.9*	*5.9*	*0.3*	*0.5*	*6.4*
Reference trtmt.	5330	5.8	11990	0.5	5.6	10	7.7	8590	4220	3390	210	290	10	12.1	47.9
Std. dev.	*510*	*0.3*	*1750*	*0.0*	*0.1*	*0.3*	*0.2*	*560*	*170*	*110*	*3.0*	*9.3*	*0.2*	*0.9*	*1.8*

n = 3

and Zn were higher ($p<0.05$) than initial soils for all the impacted treatments. In general, significantly elevated As concentration from impacted treatments with respect to their initial soil were found in As and reference treatments for the Summer and all the impacted treatments in the Fall study. Cd, and As treatments from the Summer study and Zn and reference treatments from the Fall studies had significant elevated Fe concentrations in contrast with their initial soil concentrations. Pb had significant elevated concentrations in all the treatments with respect to their initial soil except for As treatment in Summer and Cd treatment in Fall.

A simple HCl test proved the presence of free calcium carbonate in the initial soil. It could explain the lack of difference in soil pH after irrigation with water of pH below 4, indicating the high buffer capacity of the soil (Brady and Weil, 2002). Elevated concentrations of $CaCO_3$ also affected the release and adsorption of ions for the treatments.

Adsorption and release of ions in a natural soil are related to the inherent variability of soil properties as well as its cationic exchange capacity. Sumner and Miller, (1996) defined the cationic exchange capacity as the attraction to cations from negative charge for neutralization and the remainder is involved in the repelling anions, which is equivalent to attracting cations. In this study, organic matter and circum-neutral pH favored adsorption of cations reducing metal concentrations in leaching water.

Potato Plants

A week after seed planting, shoots emerged from the soil in all the containers in the Summer study. It was not like that for the Fall study where just two plants in Zn treatment, and one in each Cd and As treatment germinated, and no plants in the reference. Summer study plants reached stem lengths (Table 8) of 59 cm and root lengths of 12 cm on average for Pb treatment, and reference treatment had length of 51.2 and 13.4 cm on average for stems and roots, respectively. Plants from the reference treatment were more vigorous than the plants from the Pb treatment. The numbers of final plants at the harvested time were 3 in the Pb versus 9 from the reference container. Fall study had plants with 60 and 24 cm lengths for stems and roots, respectively for the Cd treatment, 19.3 and 13 cm stem and root average lengths in the Zn treatment, and 13 and 11.5 cm stem and root length in the As treatment.

Table 8 Mean elongation of potato steams and roots from the greenhouse study.

	Steam (cm)	Root (cm)
Summer study		
Pb treatment	58.7	11.7
Reference treatment	51.2	13.4
Fall study		
Cd treatment	60	24
Zn treatment	29	19.3
As treatment	13	11.5

Decline of potato plants in the Cd and Zn treatments in the Summer study were mainly due to toxicity. Dead biomass (DB) contained significant elevated concentrations (Fig. 3 to 6) of eco-toxic metals at the As, Cd and Zn treatments compared with the plants from Pb and reference treatments. Three weeks after germination, potato shoots had yellowish colors and were shriveled (Fig. 7). Low root and shoot development was observed in elevated eco-toxic metal treatments in the Summer study.

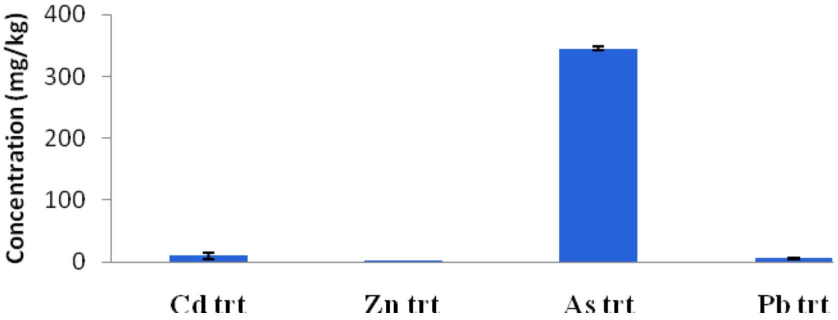

Figure 3 Dead Biomass As concentrations in AMD-impacted Treatments (Summer).

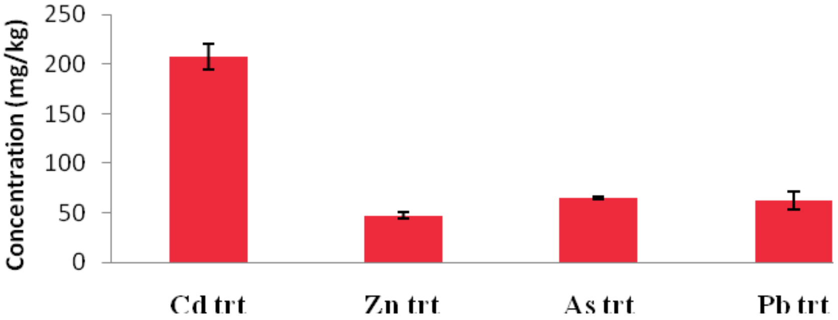

Figure 4 Dead Biomass Cd concentrations in AMD impacted treatments (Summer).

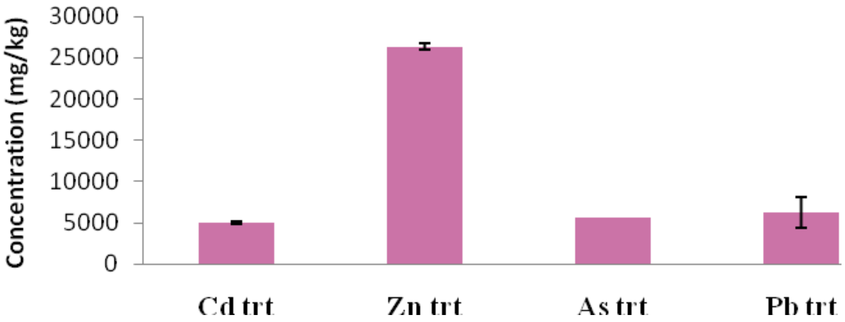

Figure 5 Dead biomass Zn concentrations in AMD-impacted treatments (Summer).

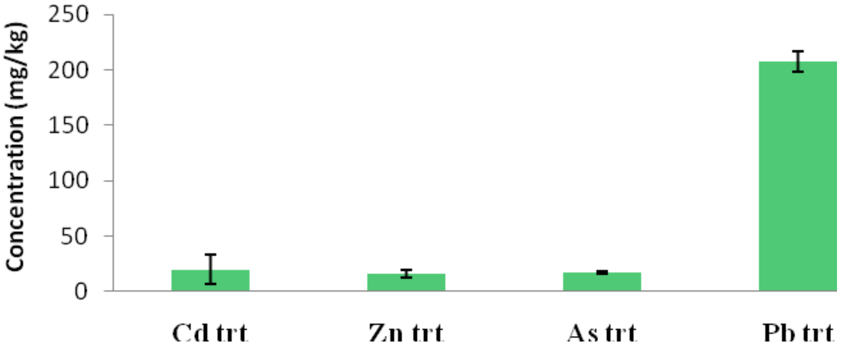

Figure 6 Dead biomass Pb concentrations in AMD-impacted treatments (Summer).

Metal toxicity was not the only cause of plant decline. The second main cause of plant loss was the constant and indiscriminate attack of insects (Fig. 8) on the potato plants. Pb and reference treatments survived but not the As treatment. Grasshoppers were identified affecting plant's photosynthesis and respiration by consumption of leaves; aphids also affected potato plant development. These insects caused observable mottled leaves, browning and death.

Metals absorbed by plants may be available in soil solution in the form of free ions or chelating ions (Wei et al., 2008). Once absorbed by the plant, metals are easily accumulated in tissue and organs by integration with proteins and polypeptides. Increased availability of metals concentrations in the soil water occurred due to the acidic conditions of the applied synthetic AMD irrigation water on these soils supporting metal plant uptake. Elevated concentration of metals in potato plants also were influenced by the total metal concentration added to the treatments and stored in the soil.

Figure 7 Summer study set up and chronological plant development for all the treatments.

Figure 8 Insects affecting the normal potato plant growth at the aquatic research facility (ARF) University of Oklahoma.

Enrichment factor (EF) > 1 was determined in leaves, stems, roots and tubers. Enrichment factors were calculated as the ratio of total metal concentration in leaves, stems, roots and tubers to total metal concentrations in soil. In leaves eco-toxic metals exceeding the EF were found in As for the As treatment in the Fall study, Cd for all the treatments including the reference in both studies, Cu for Pb and reference treatments in the Summer and Zn and As treatments in the Fall studies, Ni at the Pb treatment in the Summer and Cd and As treatments in the Fall studies, Pb for As and Pb treatments in the Summer and study and finally Zn for all the treatments in both studies except for the reference. Stems accumulated Cd and Zn in all the treatments, and As in the As treatment in the Summer. Stems analyzed at the reference treatment also had EF > 1 for Cu and Ni; Pb is accumulated in the stems from the Pb treatment. Eco-toxic metal concentrations in roots with EF > 1 were found in Cd, Cu and Zn for all the treatments in both studies, As and Pb exceeded the condition for As and Pb treatments respectively, and Ni was exceeded in all the treatments except for the reference in the Summer and Cd in the Fall studies.

Translocation factor (TF) determined the ability of plants to move eco-toxic metals from root to shoots in Cd and Zn for all the treatments in the Fall study, and Pb for the As treatment in the Summer. Translocation factors were calculated as the ratio of total metal concentrations of leaves, stems and tuber to total metal concentrations of roots in the potato plant. Ni and Cr are the main eco-toxic metal translocated from roots to potato tubers. In general essential elements were determined to have larger TF values compared with eco-toxic metals.

Essential elements (Ca, Mg, K, Fe, Mg, Cu, Ni, Zn) concentrations were found distributed in leaves, stems and roots, as required for a normal plant life cycle (Brandy and Weil, 2002). High concentrations of essential trace elements may be toxic for most living organisms. Eco-toxic metals concentrations in roots, stems and leaves are presented in Fig. 9-14. Zn concentrations were an order of magnitude lower in plants from the reference container than those plants irrigated with synthetic AMD. Fertilization studies realized by Hamlin et al., (2006) concluded that nitrate enhanced shoot biomass and stimulated Zn accumulation. Pb treatment significantly promoted Cd and Zn accumulations in leaves, stems and roots.

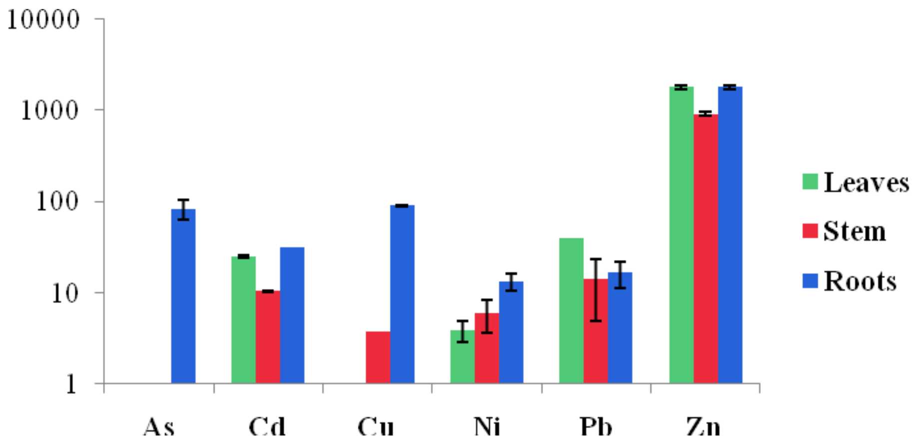

Figure 9 Eco-toxic metal concentrations (mg/kg) in As treatment-Summer Study.

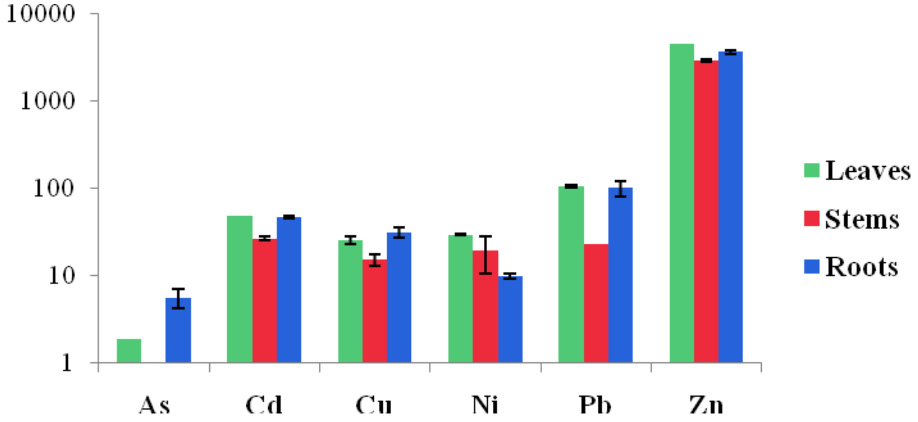

Figure 10 Eco-toxic metal concentrations (mg/kg) in Pb treatment-Summer Study.

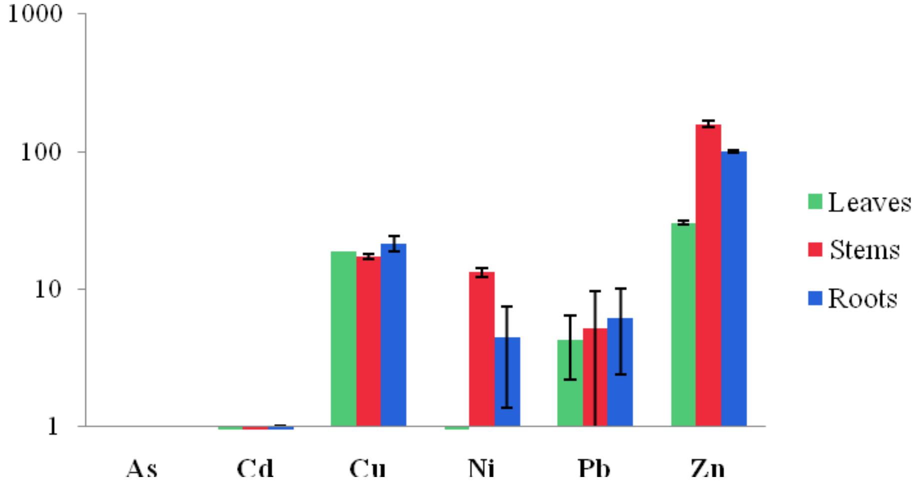

Figure 11 Eco-toxic metal concentrations (mg/kg) in reference treatment-Summer study.

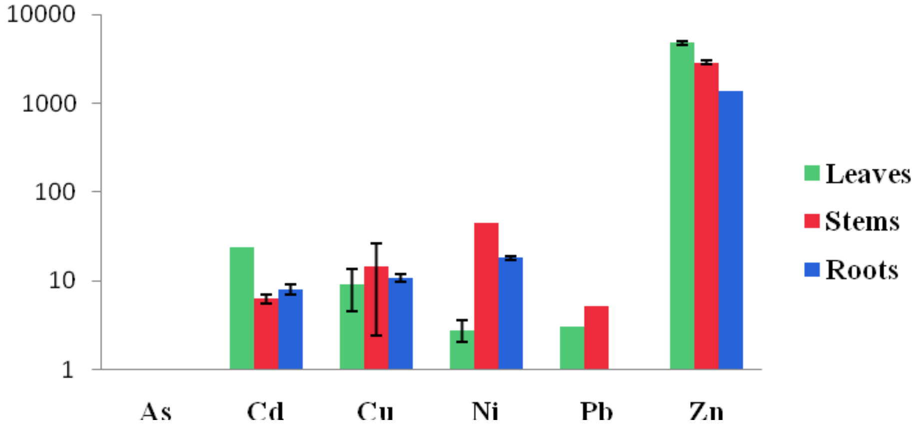

Figure 12 Eco-toxic metal concentrations (mg/kg) in Zn treatment-Fall study.

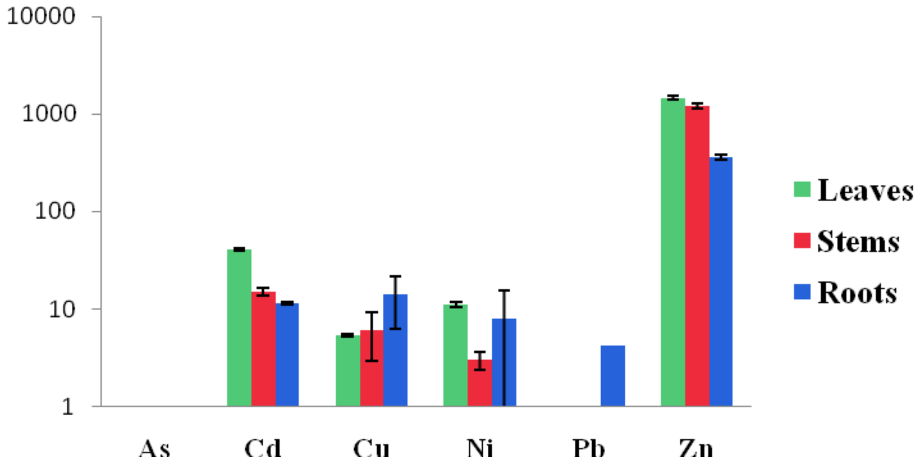

Figure 13 Eco-toxic metal concentrations (mg/kg) in Cd treatment-Fall study.

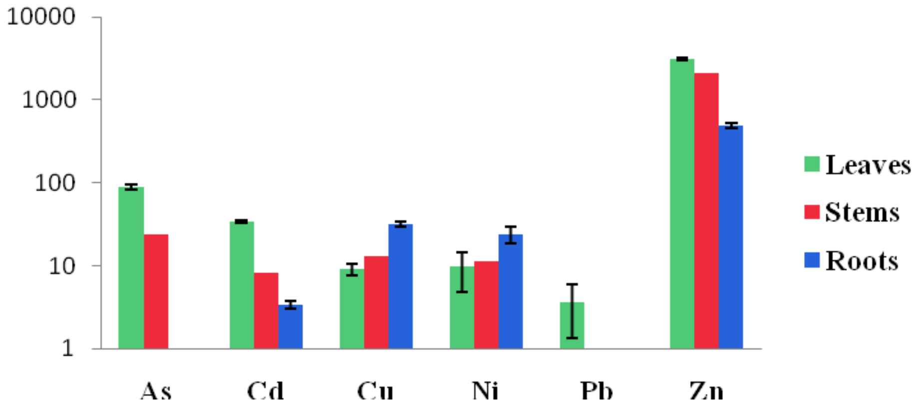

Figure 14 Eco-toxic metal concentrations (mg/kg) in As treatment-Fall study.

Yang et al. (2009), found larger accumulations of Cd in carrots and leafy vegetables in their edible parts than in radish and fruit vegetables. Observing Fig. 9 through 14, it is possible to appreciate the greater concentrations of Cd and Zn accumulated in leaves in the AMD-impacted treatments. Zheljazkov et al. (2008), found metal concentrations in plant parts in the order of: roots>leaves>flowers>stems for Cd, Pb, and Cu; and leaves>roots >flowers >stems for Mn and Zn. This study found concentrations of metals in the order of leaves>roots>stems for Cd, Mn, Pb and Zn; and roots>leaves>stems for Al, Cu, Fe, and Na. Auda et al. (2010) found that bioaccumulation in roots and leaves of Cd was greater at low metal application rates of Cd and Zn in combination than higher rates. This study concluded that total concentrations for targeted eco-toxic metals were found in significantly elevated concentrations in potato plants corresponding to its metal treatment.

Potato tubers

In the Summer study (Table 9), potatoes were collected only from the Pb and reference containers. Seed potatoes contained an unexpectedly high concentration of Pb. “Seed” refers to results from potato seeds planted in these experiments. Plants from the Zn, Cd, and As treatments died and did not produce potatoes. This mortality was attributed to the stress from metals applied in the irrigation water principally for Cd and Zn treatments. Plants from As treatments were fragile but the insect attack was catastrophic to them. Plants from the reference and Pb containers survived and produced a few tubers for the Summer study analysis.

Potato tubers were larger and more developed in the Fall than the Summer experiment. It was found that reference treatment grew potato tubers faster than the other treatments. Pb and reference potatoes from the Summer experiment reached diameters between 1.5 and 2 cm and the mass of potato collected in the Summer study from Pb treatment was 6.6 g, while the reference mass was 40.6 g. Potato masses harvested in the fall experiment were 257.6 g, 21.8 g, and 193.2 g for Cd, As, and Zn treatments, respectively. No potato tubers were collected in the Fall study. The mass of potato in the Fall study was at least twice the mass of potato harvested in the summer study.

In general, low eco-toxic metal concentrations were found in potato tubers compared with roots and shoots ($p<0.05$). Commercially sold vegetable guidelines were exceeded for all treatments in Summer and Fall study but not for the As guideline. Cd was exceeded in the Fall for the Cd treatment. Seeds had concentrations around the guideline edge (0.1 mg/kg Cd-0.08 mg/kg Cd) and also exceeded Ni and Pb guidelines for both studies. Cu concentrations at the reference treatment of the Summer study had concentrations close to the guideline (20 mg/kg Cu) and it exceeded Pb at the same treatment. Pb treatment exceeded Pb, Ni and Zn guidelines. Zn guidelines were also exceeded in Cd, Zn, and As treatments in the Fall study.

Table 9 Total metal concentrations (mg/kg) in potato tubers grown at the greenhouse.

	Al	**As**	**Ca**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**Ni**	**Pb**	**Zn**
Summer study															
Seed	11.3	<1.6	230	0.08	<0.13	0.2	<0.03	27.3	18100	960	5.8	<0.22	**3.0**	**4.8**	15.3
Std. dev	*0.7*	*0*	*7.3*	*0.05*	*0*	*0.06*	*0*	*1.1*	*133*	*10.7*	*0.1*	*0*	*1.0*	*0*	*0.4*
Pb trt.	28.2	<1.6	5140	**2.7**	<0.13	<0.1	<0.03	21.9	44.5	50390	2940	15.1	**1940**	**18.3**	**260**
Std. dev	*3.8*	*0*	*117*	*0.2*	*0*	*0*	*0*	*3.5*	*6.6*	*330*	*95.8*	*1.0*	*33.1*	*0*	*14.4*
Reference trt.	150	<1.6	5470	<0.0002	<0.13	<0.1	19.8	94.5	83560	3590	9.6	7620	0.7	**4.0**	36.4
Std. dev	*7.4*	*0*	*190*	*0.02*	*0*	*0*	*1.6*	*7.0*	*750*	*96.1*	*0.1*	*210*	*0*	*1.5*	*2.2*
Fall study															
Seed	10.4	<1.6	890	**0.1**	<0.13	0.4	<0.03	32.6	28340	1280	7.5	610	**20.7**	**14.9**	21.1
Std. dev	*1.7*	*0*	*42.4*	*0.1*	*0*	*0.6*	*0*	*7.4*	*150*	*19.8*	*0.2*	*6.3*	*12.3*	*5.3*	*0.9*
Cd trt.	80.3	<1.6	720	**0.6**	<0.13	4.2	2.8	490	26760	1370	8.2	560	**2.2**	<1.2	**54.7**
Std. dev	*10.6*	*0*	*150*	*0.1*	*0*	*2.3*	*0.3*	*680*	*2580*	*100*	*2.6*	*120*	*0.4*	*0*	*2.5*
Zn trt.	34	<1.6	570	<0.0002	<0.13	2.4	5.2	95.2	25980	1250	5.4	540	1.6	<1.2	**69.3**
Std. dev	*0.3*	*0*	*28.7*	*0.1*	*0*	*0.3*	*2.3*	*73.9*	*68.8*	*22.1*	*0.5*	*42.3*	*0.2*	*0*	*4.3*
As trt.	4.2	<1.6	950	<0.0002	<0.13	0.1	3.4	22.5	32980	1350	5.5	870	**5.6**	**20.6**	**50.7**
Std. dev	*0.7*	*0*	*26.8*	*0*	*0*	*0.01*	*0.1*	*3.7*	*1100*	*38.6*	*0.2*	*32.4*	*9.4*	*31.3*	*0.6*

n = 3

Significant differences were found in potato tuber metal concentrations between treatments and their seeds. For the Summer study Pb treatment. Significantly elevated concentrations than the seed, were found for Al, Pb, Ni, Pb and Zn. The reference treatment had significantly elevated concentrations compared with their seed for Al, Cu and Zn. Significant eco-toxic metal concentrations were found between Pb and reference treatments except for As and Pb.

In the Fall study significant differences were found between seed and Cd treatment for Al, Cd, Pb and Zn. In the Zn treatment elevated ($p<0.05$) concentrations with respect the reference treatment were found in Al and Zn. No significant differences were found in As treatment compared with the seed except for Al and Zn. Between AMD-impacted treatments significant differences were found in Al; Zn also had significant differences except for the comparisons Cd-Zn, and Cd-As treatments.

In general, total concentrations of eco-toxic metals (Zn, Cd, As and Pb) irrigated in organic top soil increased total concentrations of eco-toxic metals in the soil. High concentrations found in the potato leaves, stems and roots corresponded to those concentrations found in their soil. Potato tubers did not accumulate high concentrations of eco-toxic metals as found in leaves, stem and roots. Potato tuber concentrations of Cd, Pb, and Zn in their respective treatments were significantly elevated but not for As.

Heath risk evaluation

It was assumed that potato tubers grown at the greenhouse will have the same input parameters for ADD_i calculations as those at Potosí, Bolivia. Determined ADD_i values are compiled in Table 10. Highest values of ADD_i found in this study were 0.01 mg/kg/day Al, 0.0002 mg/kg/day Cd, 0.002 mg/kg/day Cu, 0.2 mg/kg/day Ni, 0.002 mg/kg/day Pb and 0.02 mg/kg/day Zn.

HQ values were summarized in Table 11. Potato tubers that posed potential human health risk were found in the Pb treatment for Cd, Ni, and Pb in the summer study. Potato tubers posing threats on human health were identified for Ni for all the treatments and Pb for the As treatment in the Fall Study.

Table 10 ADD$_i$ determined in the greenhouse study assuming Potosí, Bolivia input parameters.

Summer study	Al	Cd	Cu	Ni	Pb	Zn
Seed	0.0009	6E-06		0.0002	0.0004	0.001
Pb pot	0.002	0.0002		0.2	0.001	0.02
Reference pot	0.01		0.002	0.00006	0.0003	0.003
Fall study						
Seed	0.0008	0.000008		0.002	0.001	0.002
Cd pot	0.006	0.00005	0.0002	0.0002		0.004
Zn pot	0.003		0.0004	0.0001		0.006
As pot	0.0003		0.0003	0.0004	0.002	0.004

Table 11 HQ posed by consumption of contaminated tubers from the greenhouse study.

Summer study	Al	Cd	Cu	Ni	Pb	Zn
Seed	0.0009	0.06		2.7	0.4	0.004
Pb pot	0.002	2.2		1720	1.5	0.07
Reference pot	0.01		0.2	0.6	0.3	0.01
Fall study						
Seed	0.0008	0.08		18.4	1.2	0.006
Cd pot	0.006	0.5	0.02	2		0.02
Zn pot	0.003		0.04	1.4		0.02
As pot	0.0003		0.03	5	1.6	0.01

Datta and Young (2004) measured the relationship between HQ and pH for Cd in spinach leaves and found that increasing pH decreased HQ values. In agricultural fields, soil pH could vary accordingly to the addition of fertilizers and amendments. Good soil management practices such us humus additions could be a promising technique in the control of bioavailability of metals in AMD-impacted crops.

Conclusions

Concentration of metals accumulated in each treatment was estimated from removal of total metals in irrigation and leaching water. Elevated eco-toxic metal removal percentages were reached in all the AMD-impacted treatments. The reference treatments irrigated with well water leached from their soils essential nutrients and eco-toxic metals. Clean water irrigation on contaminated soils could be an important reclamation technique to decrease eco-toxic metals from AMD-impacted agricultural soils.

It was concluded that the metal burden in agricultural soils and potato tubers were directly related to metal concentrations and metal bioavailability. Metal concentrations were higher in potato plant shoots than in potato tubers. Plant metal toxicity was shown in the summer study and it was the principal cause of mortality at the Cd and Zn treatment. Grasshoppers and aphids fed on contaminated biomass; more research is needed to determine how it may impact their ecological niche. Potato tubers grown in AMD-impacted agricultural fields accumulated metals that posed potential non-cancerous human health risk to consumers. Potato tubers at the greenhouse study were a potential health hazard for non-cancerous effects concerning to the ingestion of eco-toxic metals such as Cd, Ni, and Pb.

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