

# Spatial and seasonal variations in lead content of plants colonizing the Bo Ngam lead mine, Thailand

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**ABSTRACT:** Seasonal and spatial variations in lead concentrations in soil and plants and lead-tolerant plant species diversity were studied in a closed open-pit Bo Ngam lead mine area. Two different sampling sites at the open pit mine area, the pond site and land site, were observed. Lead content in soil and plants was seasonally dependent. The lowest lead concentrations in soils were found in July during the wet season (0.6%) and highest in October during the dry season (> 11%). Most plants had the highest lead content during the wet season (May to September) and the lowest during the dry season (October to April). Most plants examined were perennials (16 species) with some annuals (6 species). Twelve species were common to both sampling sites. There were a total of 17 plant species that had lead accumulation in shoots > 1 g/kg, though only six species (*Ageratum conyzoides*, *Buddleja asiatica*, *Chromolaena odoratum*, *Conyza sumatrensis*, *Mimosa pudica*, and *Sonchus arvensis*) showed a translocation factor > 1. These plant species have a high potential for remediating the lead mine area.

**KEYWORDS:** lead contaminated soil, contaminant seasonal variation, contaminant spatial variation, translocation factor, lead-tolerant plants, phytoremediation of lead

## INTRODUCTION

Mining was once one of the biggest revenue-earners in Thailand. Surface mining activity constitutes a major environmental disturbance since vegetation and the underlying soil mantle have to be removed to obtain ore. Some mining activities have damaged the ecosystems that many rural populations (including ethnic minority groups) depend on for their livelihood and health.

Metalliferous mining and processing usually produce the most severe cases of heavy metal pollution. Mining activities result in mine spoils and other degraded land materials that typically lack organic matter including nitrogen and other primary nutrients<sup>4</sup>. Metal uptake by plants growing in mined areas is dependent on both nutrients and metal bioavailability in the soil.

Metal-accumulating plants are often the pioneer species growing on mine spoils, and serve to slowly condition the soil for other species requiring higher quality soil. A few species of metal tolerant higher plant have adaptations that enable them to survive and to reproduce in soils heavily contaminated with heavy metals. Metal tolerance may result from two basic

processes: metal exclusion and metal accumulation<sup>3</sup>. The exclusion strategy comprises avoidance of metal uptake and restriction of metal transport to the shoots. The accumulation strategy consists of accumulating high concentrations of metals in plant shoots<sup>9</sup>.

Several field studies have found that metal accumulation varies seasonally. Martin and Coughtrey<sup>15</sup> found the highest metal foliar levels during the north temperate spring and the lowest levels during north temperate winter, whereas Brekken and Steinnes<sup>7</sup> identified the highest metal content (Cd, Cu, Ni, Pb, Sn, Zn) during autumn and relatively low levels during spring in north temperate latitudes. They concluded that the lower metal concentrations in the rainy season may be due to the dilution effect of heavy rain. Deram *et al*<sup>10</sup>, studying at the Pb-Zn mine in the north of France, found significant seasonal variations of Zn and Cd concentrations in shoots of *Arrhenatherum elatius*. Shoot concentrations were high at the end of winter, but decreased until late spring. The recorded concentration decrease during spring is generally referred to as a dilution effect due to growth increase since it occurs without an increase in translocation<sup>10</sup>.

Because mined-out land contains relatively



**Fig. 1** Map of Thailand showing Kanchanaburi province and the Bo Ngam lead mine study site.

less topsoil with low nutrients, the colonizing pioneer plants must be able to withstand drought conditions to adapt and to grow in a stressed environment. Botanical survey and plant screenings are necessary because they can help to identify the plant species which have potential for phytoremediation. Phytoremediation can be defined as the clean up of pollutants primarily mediated by photosynthetic plants and their associated microflora<sup>12</sup>. The aims of this study were to investigate and to compare the seasonal variations in lead concentrations in plant and soil samples from the Bo Ngam lead mine, Kanchanaburi, a badly polluted site in western Thailand, and to search for potential lead hyperaccumulators among plants presently growing at the site.

## MATERIALS AND METHODS

### Site description

The study area, Bo Ngam lead mine (47.8 km<sup>2</sup>), is located in Klity village, Thong Pha Phum district, Kanchanaburi, Thailand (14°55'–14°60'N, 98°55'–98°60' E; Fig. 1). The average annual temperature is 26.7 °C and the average annual rainfall is 1,744 mm (Thai Meteorological Department). In general, there are two seasons in Thailand: dry and wet seasons. The dry season (with the average monthly rainfall of 10–50 mm and the relative humidity of 60–70 %) is from October to April. The wet season (with an average monthly rainfall of 300–400 mm and a relative humidity of 70–80%) is from May to September. Bo Ngam lead mine was an open pit mine that ceased operation in 1996, and since 2005 has been under going a restoration. Plant diversity in Bo Ngam lead mine is high and the secondary succession process in

disturbed areas seems to be very rapid. The restoration project started in 2003, when several plants had started to colonize the vegetation-free mine-spoiled land.

The mineral deposits in Thong Pha Phum area consist mainly of lead and zinc ores. The primary ore is lead sulphide, and the secondary ores are lead carbonate (cerussite) and lead sulphate due to chemical alteration of the host rock and oxidation processes along a contact zone with limestone.

### Sampling sites

In order to compare the characteristics of the various plant communities, quadrat sample grids were set up in two different sampling sites at the open pit mine area which we will refer to as the pond site and the land site. The pond site was on the bank of the open pit pond where soil with lead carbonate was dug up for mining. The land site was about 500 m away from the pond on an approximately 30° slope. Two sampling plots of 10x10 m, one for each sampling site, were examined at 3-month intervals (January, April, July, and October 2004). Each plot was divided into 100 quadrats of 1x1m each. Ten quadrats from each plot were randomly selected to study for plant diversity, evenness and frequency, and lead concentrations in plants and soil.

### Soil sampling and analysis

At both sampling sites, soil samples around the plant roots were collected. After collection, they were dried at 60 °C for 48 h, then ground into fine powder and sieved through a 0.28 mm nylon sieve. Soil samples were sent to the Department of Soil Science, Faculty of Agriculture, Kasetsart University for characterization of pH, electrical conductivity, organic matter content, total N, available P, available K, and texture. For analysis of soil lead content, 0.5 g of the soil sample was digested with 5 ml of 69% nitric acid (BDH)<sup>1</sup>. After digestion, the lead concentration was determined by a flame atomic absorption spectrophotometer (FAAS, Varian SpectraA 55 B).

### Plant sampling and analysis

Three individuals of each plant species were randomly collected within the 10 sampling quadrats of each sampling site. Plant samples for identification were kept in a plant press and plant classification followed that of Smitinand<sup>19</sup>. Plant identification was confirmed by the Department of Botany, Kasetsart University, Bangkok, Thailand.

After collection, soil around the plant roots was

separated and analysed for lead concentration, while each plant sample was thoroughly washed with a solution of phosphate-free detergent for 15 s followed by tap water for another 15 s, rinsed with deionized water, and separated into shoots and roots. These samples were then treated and analysed in the same way as the soil samples.

### Translocation factor and phytoextraction coefficient

The translocation factor (TF) is defined as the ratio of the heavy metals concentration in plant shoot to that in plant root. A TF value > 1 indicates preferential partitioning of metals to the shoot<sup>6</sup>.

The phytoextraction coefficient is defined as the ratio of the lead concentration in plant shoot to lead concentration in the soil. The phytoextraction coefficient can be used to evaluate the ability of plants to accumulate heavy metals<sup>16</sup>.

### Plant community study

The Shannon index<sup>17</sup> was used to quantify the diversity of plants at the site. The Shannon-Wiener heterogeneity index is given by

$$H' = -\sum p_i \ln p_i$$

where  $p_i$  is the proportion of individuals of the  $i^{\text{th}}$  species.  $H'$  indicates the relationship between species richness and the abundance of each species for any given plant community. For normal communities it typically falls between 1.5 and 3.5.

### Statistical analysis

Statistical analyses consisting of analysis of variance (One-Way ANOVA) of the data were performed to assess the seasonal variation of lead soil concentration, lead accumulation in shoots, roots, and soil of each plant species.

## RESULTS

### Soil characterization and soil lead concentration

The soil textures of both sampling sites were sandy loam except for the subsurface soils at the pond site which were silt loam and loam. The pH of soil was slightly higher at the land site (pH 7.7) than the pond site (pH 6.8). The highest electrical conductivity values at the pond and land sites were 0.16 and 0.22 dS/m, respectively, and the lowest values were 0.15 and 0.2 dS/m, respectively. The percentage of total nitrogen from both sites did not differ (0.11–0.12), though the soil at the pond site had more available phosphorus (7–14 ppm at the pond site, 2–3 ppm at the land site). The organic matter (0.1–0.2) of soils from both sites was quite similar. The soil from the land site

had higher lead concentration (about 110 g/kg) than that from the pond site (about 12 g/kg).

The relative humidity was stable, ranging from 60% in March to about 85% in July (Fig. 2). Lowest lead concentrations in soils from both sampling sites were found in July during the wet season (6.26 g/kg and 79.61 g/kg in the pond and land sites, respectively) and highest in October during the dry season (8.09 g/kg and 111.72 g/kg in the pond and land sites, respectively) (Fig. 2). The fluctuations of lead concentrations in soil at both sampling sites were inversely related to the monthly rainfall but directly affected by the relative humidity all year.

### Seasonal variation in plant diversity

A total of 22 species of plants, from 12 families were recorded on both study plots combined, including herbs, shrubs, and grasses (Table 1). In both sites, the highest number of individual plant species was found in October, whereas the lowest was found in April. The numbers of plant species was directly related to the monthly rainfall and to the relative humidity. At the pond site, 17 plant species (10 families) were found, including some wetland and fern species. Ten species were found all year. At the land site, 17 plant species (8 families) were found with 9 species present all year. Twelve species were common at both sampling sites (*Ageratum conyzoides*, *Buddleja asiatica*, *Conyza sumatrensis*, *Equisetum debile*, *Imperata cylindrica*, *Mimosa pudica*, *Neyraudia reynaudiana*, *Paspalum conjugatum*, *Phragmites karka*, *Sonchus arvensis*, *Thysanolaena maxima* and *Vigna umbellata*). *E. debile* had the higher number of individuals at both sites. Most of these plants were perennials (16 species) while the remainders were annuals (6 species).

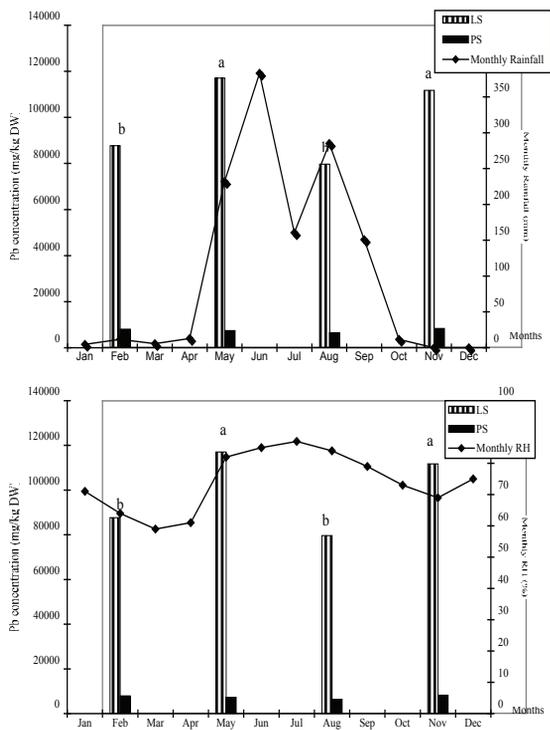
The Shannon diversity index (Fig. 3) was not significantly different all year for both sampling sites ( $P > 0.05$ ) except for April. In April, the diversity index at the land site was lower than that at the pond site (2.27 and 2.64, respectively). The rather lower  $H'$  values found in January and April at both sampling sites may be related to the monthly rainfall and the relative humidity in this area (Fig. 2). The highest  $H'$  was found in October (2.75 and 2.67 at the pond site and land site, respectively).

### Seasonal variation of lead accumulation in plants

The seasonal variation of lead concentrations in plants in the land and pond sites were different, even for the same species and the same time of collection (Tables 2–4). The results clearly demonstrate a seasonal variation in lead accumulation.

Thirteen plant species at both sites could be found all year. Lead concentration in whole plants varied between sampling times with remarkably high concentrations in the dry season (October to April) and lower concentrations in the wet season. For example,  $5.5 \pm 1.4$  g/kg DW in shoots of *I. cylindrica* at the

land site and  $0.28 \pm 0.16$  g/kg DW in shoots of *N. reynaudiana* at the pond site during the dry season as compared to  $0.30 \pm 0.12$  g/kg DW in shoots of *I. cylindrica* at the land site and  $42 \pm 10$  mg/kg DW in shoots of *N. reynaudiana* at the pond site during the wet season (Table 2).



**Fig. 2** Seasonal variation in soil lead concentration in relation to monthly rainfall and relative humidity.

**Choice of lead accumulator**

The phytoextraction coefficient of plant species at both sampling sites varied between 0.01 and 0.12. The *B. asiatica* at the pond site had the highest phytoextraction coefficient of 0.12. When comparing the same plant species at the pond site and the land site, the plant species at the pond site had a higher phytoextraction coefficient than at the land site (Table 5).

There were a total of 17 plant species that had a lead accumulation in shoots > 1 g/kg at one or more sampling times (Table 1). Among these species, only six species (*A. conyzoides*, *B. asiatica*, *C. odoratum*, *C. sumatrensis*, *M. pudica* and *S. arvensis*) showed a TF > 1 (Table 5). Thirteen of 16 perennial plant species and four out of six annual plant species had lead accumulation in their shoots > 1 g/kg and/or TF more than 0.8 (Tables 1 and 5).

**DISCUSSION**

The soil characteristics of both sampling sites were typical of mined degraded soils. They exhibited low nutrients, low organic matter, and low electrical

**Table 1.** Seasonal variation in number of plant species found at pond site (PS) and land site (LS)

Family	Plant species	Duration	January		April		July		October	
			PS	LS	PS	LS	PS	LS	PS	LS
Buddlejaceae	<i>Buddleja asiatica</i>	P	2	8	0	4	0	9	2	6
Commelinaceae	<i>Commelina diffusa</i>	A	0	0	0	0	0	2	0	2
Asteraceae	<i>Ageratum conyzoides</i>	A	11	0	4	21	10	13	3	3
	<i>Chromolaena odoratum</i>	P	0	2	0	0	0	9	0	26
Cyperaceae	<i>Conyza sumatrensis</i>	A	15	15	3	22	7	22	9	18
	<i>Sonchus arvensis</i>	P	21	22	31	21	35	88	14	62
	<i>Cyperus iria</i>	A	76	0	69	0	72	0	66	0
Equisetaceae	<i>C. flavidus</i>	P	0	0	0	0	12	0	9	0
	<i>Equisetum debile</i>	P	77	153	162	250	237	196	212	277
Poaceae	<i>Crassocephalum crepidioides</i>	P	0	3	0	0	0	11	0	9
	<i>Imperata cylindrica</i>	P	24	12	210	16	39	23	58	7
	<i>Neyraudia reynaudiana</i>	P	66	48	87	124	56	80	54	59
	<i>Paspalum conjugatum</i>	P	0	49	0	45	102	61	90	91
	<i>Pennisetum polystachyon</i>	P	0	0	0	4	0	1	0	3
	<i>Phragmites karka</i>	P	45	19	68	45	60	46	52	39
Mimosaceae	<i>Thysanolaena maxima</i>	P	16	1	29	0	22	4	32	6
Mimosaceae	<i>Mimosa pudica</i>	P	6	18	27	49	26	90	22	89
Ophioglossaceae	<i>Ophioglossum</i> sp.	A	12	0	20	0	52	0	153	0
Papilionaceae	<i>Vigna umbellata</i>	A	2	1	76	0	24	1	28	1
Parkeriaceae	<i>Pityrogramma calomelanos</i>	P	5	0	6	0	9	0	7	0
Pteridaceae	<i>Pteris vittata</i>	P	8	0	7	0	4	0	4	0
Orchidaceae	<i>Eulophia</i> sp.	P	0	0	0	0	0	16	0	9

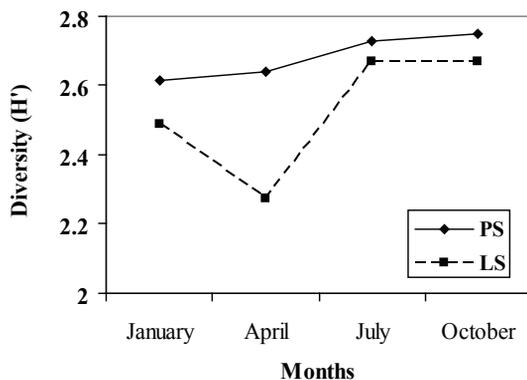
A = Annual plant; P = Perennial plant

**Table 2.** Seasonal variation of lead accumulation (mg/kg DW) (mean + SE; n = 3) in plant shoot collected from land site (LS) and pond site (PS)

Plant species	Site	Duration	Lead accumulation in shoot (mg/kg DW)			
			January	April	July	October
<i>A. conyzoides</i>	LS	A	-	4850±3944	1375±566	-
	PS		400±57	346±168	738±210	-
<i>B. asiatica</i>	LS	P	1128±84b	858±188b	3023±1835a	1153±537b
	PS		293±97	-	-	730±680
<i>C. odoratum</i>	LS	P	1595±13	-	1983±404	2783±29
<i>C. diffusa</i>	LS	A	-	-	3533±2 872	1700±1083
<i>C. sumatrensis</i>	LS	A	1505±9a	7017±6605a	2700±854a	7033±3364a
	PS		509±269	-	1030±467	458±41
<i>C. crepidioides</i>	LS	P	2369±293	-	1292±342	688±445
	PS		-	-	-	62±8
<i>C. flavidus</i>	PS	P	-	-	128±92	192±19
<i>C. iria</i>	PS	A	208±43a	323±173a	223±153a	227±84a
<i>E. debile</i>	LS	P	1585±10b	6133±2 136a	497±141b	432±128b
	PS		160±40a	127±86a	208±102a	73±41a
<i>Eulophia</i> sp.	LS	P	-	-	3288±2 591	2050±300
<i>I. cylindrica</i>	LS	P	1395±351bc	5467±1436a	297±119c	1967±778b
	PS		182±104a	63±8b	33±28b	45±9b
<i>M. pudica</i>	LS	P	1542±14b	1087±1008b	2021±1308b	5267±1620a
	PS		193±59a	80±30b	118±36b	97±20b
<i>N. reynaudiana</i>	LS	P	612±172b	618±266b	674±138b	8033±4389a
	PS		130±9ab	278±163a	137±76ab	42±10b
<i>Ophioglossum</i> sp.	PS	A	-	576±217	682±195	963±159
<i>P. conjugatum</i>	LS	P	1068±469b	5400±3601a	1305±378b	3367±1985ab
	PS		-	-	160±35	1517±1294
<i>P. polystachyon</i>	LS	P	-	1600±229	-	412±182
<i>P. karka</i>	LS	P	695±365ab	643±270ab	203±60b	1030±467a
	PS		92±54b	333±231a	55±9b	95±27b
<i>P. calomelanos</i>	PS	P	400±40b	987±475a	287±203b	323±188b
<i>P. vittata</i>	PS	P	439±202a	377±65a	238±80a	275±103a
<i>S. arvensis</i>	LS	P	1592±6b	2900±2594ab	8967±6181a	7133±3116ab
	PS		520±232b	252±60b	218±180b	920±123a
<i>T. maxima</i>	LS	P	805±438	-	1827±1625	6017±5360
	PS		197±54a	213±19a	65±15b	92±24b
<i>V. umbellata</i>	LS	A	-	-	475±104	7467±6593
	PS		92±18	652±519	396±169	-

A = Annual plant; P = Perennial plant

Means followed by a common letter in the same row for each metal are not significantly different from each other using LSD test ( $P > 0.05$ ).



**Fig. 3** Variation in homogeneity index over one year.

conductivity, features which tend to inhibit soil-forming processes and plant growth<sup>22</sup>.

Lead concentration in the soil from the land site was about ten times higher than that from the pond site. The pond site was located near the open pit, where ore digging was performed extensively. After the mine was closed, the pit became filled with water, receiving run-off from the nearby slopes. The soluble Pb would have been washed into the pond and the soil Pb content was consequently much lower, especially in the pond site soil. However, these soil concentrations were both extremely high (10–100 g/kg) as compared to other mining areas. For example, studies have found 0.1–12.0 g/kg in soil from Lanping Pb/Zn mine area, China<sup>23</sup>, 2.6–13.3 g/kg from Pb/Zn mining

**Table 3.** Seasonal variation of lead accumulation (mg/kg DW) (mean + SE; n = 3) in plant root collected from land site (LS) and pond site (PS)

Plant species	Site	Duration	Lead accumulation in root (mg/kg DW)			
			January	April	July	October
<i>A. conyzoides</i>	LS	A	-	3204±697	2405±935	-
	PS		2125±1237	1944±1425	1250±250	-
<i>B. asiatica</i>	LS	P	1117±398b	3229±839a	1700±654b	1917±1077ab
	PS		575±136	-	-	1465±384
<i>C. odoratum</i>	LS	P	3708±1841	-	1404±609	806±222
<i>C. diffusa</i>	LS	A	-	-	23479±8071	5950±3054
<i>C. sumatrensis</i>	LS	A	2368±886b	10421±1736a	2746±1364b	4750±2179b
	PS		2400±736	-	2833±2073	418±45
<i>C. crepidioides</i>	LS	P	8250±2833	-	1363±456	873±581
	PS		-	-	-	150±0
<i>C. flavidus</i>	PS	P	-	-	2361±743	3700±1735
<i>C. iria</i>	PS	A	3300±826a	4333±382a	1652±180b	3583±722a
<i>E. debile</i>	LS	P	8050±25a	41750±9331b	7542±1438a	8567±2725a
	PS		6366±3096a	4443±1164ab	1377±209b	3383±1651ab
<i>Eulophia</i> sp.	LS	P	-	-	5200±1998	11833±4980
<i>I. cylindrica</i>	LS	P	4708±2872b	14028±8461a	1400±922b	2358±101b
	PS		2458±1778a	1958±191ab	1048±356ab	237±187b
<i>M. pudica</i>	LS	P	7421±2040a	3778±440b	3269±1411b	5039±2055ab
	PS		917±217b	2583±1010a	375±139b	857±643b
<i>N. reynaudiana</i>	LS	P	7150±1301b	22375±6140a	13100±2020b	12950±6414b
	PS		813±249b	1667±181a	612±265bc	287±38c
<i>Ophioglossum</i> sp.	PS	A	-	1270±167	1581±181	2456±42
<i>P. conjugatum</i>	LS	P	4101±119b	24083±12475a	2583±236b	9550±5370b
	PS		-	-	1957±327	8833±4680
<i>P. polystachyon</i>	LS	P	-	23333±5481	-	1235±989
<i>P. karka</i>	LS	P	7850±284a	20972±12763a	7356±6614a	10778±6893a
	PS		1139±409b	7942±4692a	1208±539b	1285±452b
<i>P. calomelanos</i>	PS	P	6767±1038a	3386±1292bc	2356±830c	4861±1578ab
<i>P. vittata</i>	PS	P	3683±772a	2956±1267a	5520±3384a	4233±2599a
<i>S. arvensis</i>	LS	P	4392±2477a	2067±752a	4312.5±764a	4917±3547a
	PS		600±205a	500±250ab	283±63b	764±61a
<i>T. maxima</i>	LS	P	4414±3041	-	13517±11053	11183±8193
	PS		1142±597ab	1694±458a	340±123b	955±457ab
<i>V. umbellata</i>	LS	A	-	-	700±239	18400±2828
	PS		107±18	2575±1531	1088±589	-

A = Annual plant, P = Perennial plant

Means followed by a common letter in the same row for each metal are not significantly different from each other using LSD test ( $P > 0.05$ ).

area in Yunnan, China<sup>24</sup>, 4.5 g/kg in Au-Ag-Pb-Zn mines in Daduk, Korea<sup>14</sup>, and 2.3–5.7 g/kg from Pb/Zn mine tailing in Guangdong province of China<sup>18</sup>. Similar seasonal variation in metal content was found elsewhere. During winter months with lower flow, less mineral surface area was exposed, and less water was available to flush weathering products<sup>2</sup>. Lee *et al*<sup>14</sup> reported seasonal variations of metals in stream sediments near an Au-Ag-Pb-Zn mine. The results showed a relatively high concentrations

of metals (Cd, Cu, Pb, Zn) in water and sediment samples in the dry season. Lower Pb concentration in soil during the wet season may be due to a dilution effect by heavy rain in the early wet season (May–June).

The annual plant diversity of the pond site was more stable than that of the land site. This is probably due to the higher moisture contents and the lower Pb concentration of the soil. Variation of Pb accumulation was more pronounced in plants collected from the

**Table 4.** Seasonal variation of lead concentration (mg/kg DW) (mean + SE; n = 3) in soil around plant root system collected from the land site (LS) and pond site (PS)

Plant species	Site	Duration	Lead concentration in soil (mg/kg DW)			
			January	April	July	October
<i>A. conyzoides</i>	LS	A	-	12333+5346	63500+29193	-
	PS		46500+13141	5883+2487	4700+2209	-
<i>B. asiatica</i>	LS	P	101111+16563a	4667+764b	74167+24111a	120000+46209b
	PS		6533+104	-	-	3833+1258
<i>C. odoratum</i>	LS	P	122167+15003	-	94333+17467	105167+23929
<i>C. diffusa</i>	LS	A	-	-	49333+2021	96000+49267
<i>C. sumatrensis</i>	LS	A	62500+9659b	14500+3905c	67833+17786b	112333+20251a
	PS		10917+775	-	32833+19504	6750+3733
<i>C. crepidioides</i>	LS	P	95833+3215	-	83167+15695	42333+9385
	PS		-	-	-	4667+2021
<i>C. flavidus</i>	PS	P	-	-	10667+7256	3333+878
<i>C. iria</i>	PS	A	15017+1969a	5267+448b	8000+4131b	3667+1283b
<i>E. debile</i>	LS	P	101167+30184a	13167+3329c	69167+7251ab	66500+15322b
	PS		4558+774bc	9233+2417a	6083+1665c	2667+764b
<i>Eulophia sp.</i>	LS	P	-	-	84000+26187	105000+40844
<i>I. cylindrica</i>	LS	P	89333+11251a	14667+7943b	91500+25239a	116500+39652a
	PS		6278+428a	4067+1069a	3750+1392a	4500+2500a
<i>M. pudica</i>	LS	P	82833+0c	9667+3055b	75667+18936c	131167+20251a
	PS		12983+10082a	11117+925a	2000+0a	12667+10531a
<i>N. reynaudiana</i>	LS	P	81500+6000b	157167+289a	83167+15695b	158500+4770a
	PS		3683+462b	7433+2570ab	10583+5364a	4000+3041b
<i>Ophioglossum sp.</i>	PS	A	-	9133+1390	8250+1572	10333+289
<i>P. conjugatum</i>	LS	P	77167+5008a	12500+3606b	77667+9005a	69667+22745a
	PS		-	-	2500+433	52583+13853
<i>P. polystachyon</i>	LS	P	-	7833+2887	-	88000+8231
<i>P. karka</i>	LS	P	74167+2843a	11500+2179b	80333+28108a	98500+11269a
	PS		7433+1450a	7100+1381a	3917+1465a	11500+8012a
<i>P. calomelanos</i>	PS	P	7467+407a	7867+2223a	7217+3467a	13000+8231a
<i>P. vittata</i>	PS	P	7217+225b	7867+2900b	6750+433b	39000+21868a
<i>S. arvensis</i>	LS	P	119333+4272a	7333+4042b	97000+19346a	132333+21050a
	PS		6550+150a	6700+781a	12250+9148a	8000+2598a
<i>T. maxima</i>	LS	P	83666+6110	-	80833+19763	159167+2887
	PS		5850+50b	5500+507b	3667+1809b	31500+15597a
<i>V. umbellata</i>	LS	A	-	-	77667+20642	98833+33168
	PS		6917+226	8117+3073	6167+1422	-

A = Annual plant; P = Perennial plant

Means followed by a common letter in the same row for each metal are not significantly different from each other using LSD test ( $P > 0.05$ ).

land site, whose soil contained extremely high concentrations of Pb. This suggests that plant growth was responsible for the higher uptake of Pb. Larsen and Schierup<sup>13</sup> also found a sharp increase of Pb in leaves of *Phragmites australis* during and after the growth season. Leaves produced earlier in the growing season had higher concentration of Hg than leaves produced later. Caçador *et al*<sup>8</sup> also noted that root concentrations of Zn, Pb, Cu, and Cd in *Spartina maritima* and *Halimione portulacoides* were lowest in January (the north temperate winter) and they increased during the growth period. Shoots of *Spartina alteriflora*, however, accumulated Mn, Cu, and Zn rapidly in the spring,

and then the levels decreased<sup>11</sup>. The decrease was attributed to a growth-dilution effect due to growth increase, whereby changes in the amount of plant biomass bring about corresponding changes in plant metal content<sup>10</sup>.

Different metals and different plant species show different patterns of seasonal variation of growth and metal accumulation. While several studies showed seasonal changes in metal content in plants (such as Zn, Cu, Pb, Cr, Hg), others found no seasonal changes in these metals<sup>8,11,13,21</sup>. Hence, it is very difficult to generalize about seasonal changes in metal levels, since they appear to vary greatly with the metal and the species of plants studied<sup>22</sup>. Most plant species

**Table 5.** Plant species that showed phytoextraction coefficient, lead accumulation in shoot > 1g/kg and translocation factor between 0.8 to 1 (underlined numbers)

Plant species	Site	Phytoextraction coefficient	Lead accumulation in shoot (mg/kg DW)				Translocation factor (TF)			
			Jan	Apr	Jul	Oct	Jan	Apr	Jul	Oct
<i>A. conyzoides</i>	LS	0.04	-	4850	1375	-	-	1.5	0.6	-
	PS	0.08	400	346	738	-	0.2	0.2	0.6	-
<i>B. asiatica</i>	LS	0.02	720	858	3023	1153	0.6	0.3	1.8	0.6
	PS	0.12	293	-	-	730	0.5	-	-	0.5
<i>C. odoratum</i>	LS	0.02	1595	-	1983	2783	0.4	-	1.4	3.5
	PS	-	-	-	-	-	-	-	-	-
<i>C. diffusa</i>	LS	0.05	-	-	3533	1700	-	-	0.2	0.3
	PS	-	-	-	-	-	-	-	-	-
<i>C. sumatrensis</i>	LS	0.05	1505	7017	2700	7033	0.6	0.7	1	1.5
	PS	0.05	509	-	1030	458	0.2	-	0.4	1.1
<i>C. crepidioides</i>	LS	0.02	2369	-	1292	688	0.3	-	1	0.8
	PS	0.01	-	-	-	62	-	-	-	0.4
<i>E. debile</i>	LS	0.03	1585	6133	497	432	0.2	0.2	0.1	0.1
	PS	0.03	160	127	208	73	0.03	0.03	0.2	0.02
<i>Eulophia sp.</i>	LS	0.03	-	-	3288	2050	-	-	0.6	0.2
	PS	-	-	-	-	-	-	-	-	-
<i>I. cylindrica</i>	LS	0.02	1395	5467	297	1967	0.3	0.4	0.2	0.8
	PS	0.02	182	63	33	45	0.1	0.03	0.03	0.2
<i>M. pudica</i>	LS	0.03	1542	1087	2021	5267	0.2	0.3	0.6	1.1
	PS	0.02	193	80	118	97	0.2	0.03	0.3	0.1
<i>N. reynaudiana</i>	LS	0.02	612	618	674	8033	0.1	0.03	0.1	0.6
	PS	0.02	130	278	137	42	0.2	0.2	0.2	0.2
<i>P. conjugatum</i>	LS	0.03	1068	5400	1305	3367	0.3	0.2	0.5	0.4
	PS	0.05	-	-	160	1517	-	-	0.1	0.2
<i>P. polystachyon</i>	LS	0.01	-	1600	-	412	-	0.1	-	0.3
	PS	-	-	-	-	-	-	-	-	-
<i>P. karka</i>	LS	0.01	695	643	203	1030	0.1	0.03	0.03	0.1
	PS	0.02	91	333	55	95	0.1	0.04	0.1	0.1
<i>S. arvensis</i>	LS	0.06	1592	2900	8967	7133	0.4	1.4	2.1	1.5
	PS	0.06	520	252	218	920	0.9	0.5	0.8	1.2
<i>T. maxima</i>	LS	0.02	805	-	1827	6017	0.2	-	0.1	0.5
	PS	0.02	197	213	65	92	0.2	0.1	0.2	0.1
<i>V. umbellata</i>	LS	0.04	-	-	475	7467	-	-	0.7	0.4
	PS	0.06	92	652	396	-	0.9	0.3	0.4	-

found in the mine area were perennials, which favours phytostabilization since perennial plants can stabilize Pb in their shoots or roots for a longer time than annual plants which have a shorter life-cycle.

*B. asiatica* at the pond site had the highest phytoextraction coefficient, making it suitable for phytoremediation, if this concept is applied. From the TF results, six plant species from this study could be considered Pb hyperaccumulators with an extremely high capacity to take up metals by roots<sup>5</sup>. Some of these plant species were previously identified<sup>16</sup>.

In conclusion, the patterns of metal accumulation and distribution in the plant parts were significantly influenced by the plant species and season. The different soil moisture content of the land site and the pond site affected plant diversity, plant metal uptake, and lead concentration in the soil. Lead concentrations in soil and plants were seasonally dependent. Lead concentrations in plants differed even

within the same species and at the same collection time. Most plants had the highest lead concentrations during the wet season, and lowest during the dry season. Six herbaceous plant species (*A. conyzoides*, *B. asiatica*, *C. odoratum*, *C. sumatrensis*, *M. pudica* and *S. arvensis*) showed a TF > 1. However, if both the phytoextraction coefficient and translocation factor are taken into account, *B. asiatica* is the best candidate for a phytoremediation project.

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## REFERENCES

1. APHA, AWWA, WEF (1998) Standard Methods for the Examination of Water and Wastewater. The Association. Washington DC.
2. August EE, McKnight DM, Hrcir DC, Garhart KS (2002) Seasonal variability of metals transport through a wetland impacted by mine drainage in the Rocky Mountains. *Environ. Sci. Technol.* **36**, 3779–86.
3. Baker AJM (1987) Metal tolerance. *New Phytol.* **106**, 93–111.
4. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of *in situ* heavy metal decontamination of polluted soils using crops of metal-accumulating plants. *Resour. Conserv. Recycl.* **11**, 41–9.
5. Baker AJM, McGrath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: *Phytoremediation of Contaminated Soil and Water* (Terry N, Banuelos G, Eds), pp 85–107, Lewis Publishers, Florida.
6. Baker AJM, Whiting SN (2002) In search of the holy grail: a further step in the understanding of metal hyperaccumulation. *New Phytol.* **155**, 1–4.
7. Brekken A, Steinnes E (2004) Seasonal concentrations of cadmium and zinc in native pasture plants: consequences for grazing animals. *Sci. Total Environ.* **326**, 181–95.
8. Caçador I, Vale C, Catarino F (2000) Seasonal variation of Zn, Pb, Cu, and Cd concentrations in the root-sediment system of *Spartina maritima* and *Halimione portulacoides* from *Tagus* estuary salt marshes. *Mar. Environ. Res.* **49**, 279–90.
9. Dahmani-Muller H, Van Oort F, Gelie B, Balabane M (2000) Strategies of heavy metal smelter. *Environ. Pollut.* **109**, 231–8.
10. Deram A, Denayer FO, Petit D, Van Haluwyn C (2006) Seasonal variations of cadmium and zinc in *Arrhenatherum elatius*, a perennial grass species from highly contaminated soils. *Environ. Pollut.* **140**, 62–70.
11. Gleason ML, Drifmeyer JE, Zieman JC (1979) Seasonal and environmental variation in Mn, Fe, Cu, and Zn content of *Spartina alterniflora*. *Aquat. Bot.* **7**, 385–92.
12. Horne JA (2000) Phytoremediation by constructed Wetlands. In: *Phytoremediation of contaminated soil and water* (Terry N, Banuelos G eds), pp 14–37. Lewis publishers, Washington D.C.
13. Larsen VJ, Schierup HH (1981) Macrophyte cycling of zinc, copper, lead, and cadmium in the littoral zone of a polluted and a non-polluted lake: II. Seasonal changes in heavy metal content of above-ground biomass and decomposing leaves of *Phragmites australis* (Cav.) Trin. *Aquat. Bot.* **11**, 211–30.
14. Lee CG, Chon HT, Jung MC (2001) Heavy metal contamination in the vicinity of the Daduk Au-Ag-Pb-Zn mine in Korea. *Appl. Geochem.* **16**, 1377–86.
15. Martin M, Coughtrey P (1982) Biological monitoring of heavy metal pollution. Applied Sciences Publications, London/New York.
16. Rotkittikhun P, Kruatrachue M, Chaiyarat R, Ngermsaengsaruy C, Pokethitiyook P, Pajitpraporn A and Baker AJM (2006) Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand. *Environ. Pollut.* **144**, 681–8.
17. Shannon CE, Weaver W (1949) *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.
18. Shu WS, Lan CY, Zhang ZQ, Wong MH (2000) Use of vetiver and other three grasses for revegetation of Pb/Zn mine tailings at Lechang, Guangdong Province: field Experiment. In: 2<sup>nd</sup> Int. Vetiver Conference Bangkok, Thailand, January.
19. Smitinand, T. (1980) *Thai Plant Names*. Funny Publishing Limited Partnership, Bangkok, Thailand.
20. United States Environmental Protection Agency (USEPA) (2000) *Electrokinetic and phytoremediation in situ treatment of metal-contaminated soil: State-of-the-practice*. Draft for final review. EPA/542/R-00/XXX. US Environmental Protection Agency, Office of Solid Waste and Emergency Response Technology Innovation Office, Washington, DC.
21. Weis JS, Windham L, Weis P (2003) Patterns of metal accumulation in leaves of the tidal marsh plants *Spartina alterniflora* Loisel and *Phragmites australis* Cav. *Trin ex Steud.* **23**, 459–65.
22. Weis JS, Weis P (2004) Metal uptake, transport, and release by wetland plants: implications for phytoremediation and restoration. *Environ. Int.* **30**, 685–700.
23. Yanqun Z, Yuan L, Schwartz C, Langlade L, Fan L (2004) Accumulation of Pb, Cd, Cu, and Zn in

- plants and hyperaccumulator choice in Lanping lead-zinc mine area, China. *Environ. Int.* **30**, 567–76.
24. Yanqun Z, Yuan L, Jianjun C, Haiyan C, Li Q, Schwartz C (2005) Hyper-accumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. *Environ. Int.* **31**, 755–62.