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# Heavy Metals Contaminated Soils and Phytoremediation Strategies in Taiwan

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## 1. Introduction

Heavy metals (HMs) in soils primarily result from the weathering of parent materials and from human activities, which including mining, smelting, application of sludges, and discharge of wastewaters, etc. (Kabata-Pendias & Pendias, 2001). Soil contaminated with HMs has become a worldwide problem and pose a serious threat to the environment (Anwar et al., 2009), leading to losses in agricultural yield and hazardous health effects as they enter the food chain (Salt et al., 1995). Cropping lands contaminated by HMs were mostly resulted from the use of polluted irrigated water in the downstream of discharged water of industrial parks of Taiwan. In 2007, approximate 400 ha of rural soils in Taiwan were contaminated with single or combined HMs and they are fallow according to Soil and Groundwater Pollution Remediation Act (SGWPR Act) announced in 2000 by Taiwan Environmental Protection Administration (Taiwan EPA). There were many techniques been used to treat the HMs-contaminated soils which including isolation, mechanical separation, chemical treatment, electrokinetics, soil washing, and phytoremediation (Mulligan et al., 2001). Various soil amendments were applied to the HMs-contaminated soils to reduce the mobility of HMs in the contaminated soils and thus to decrease their further uptake by crops (Chen & Lee, 1997; Kabata-Pendias and Pendias, 2001; Lee et al., 2004; Mench et al., 1994).

In Taiwan, most of these contaminated sites were restored with turnover/dilution and acid washing methods to reduce the total concentration of HM to conform the regulation announced. Besides the two techniques, phytoremediation was demonstrated to be a feasible method in treating these contaminated lands, which have large areas and low to medium level of HM concentration (Lai et al., 2005). It also accompanies with other environmental agenda, such as biomass energy, biodiversity, carbon sequestration, and soil quality (Dickinson et al., 2009). However, most hyperaccumulators used in removing these HMs have lower biomass and growing rate and thus extending the period needed in decontamination. The application of chemical agents has significant effect on increasing the phytoavailability and accumulation of HMs of plants (Chen & Cutright, 2001; Meers et al.,

2004; Meers et al., 2005). However, results of most previous studies showed that chemical agents have negative effect on the growth of Indian mustard, sunflower, or corn and thus decreased the total removal of HMs by plants (Blaylock et al., 2007; Madrid et al., 2003; Turgut et al., 2004). After the application of chemical chelating agents, the risk of groundwater contamination may be increased because the mobility of HMs increased (Jiang et al., 2003; Lai & Chen, 2004; Lai et al., 2005). For those lands with sandy texture or high level of groundwater table, chemical agents should be carefully applied to decrease the health risk of groundwater quality (Lai & Chen, 2006; Wu et al., 2004).

Rice dominates the daily intake of cereals in most Asian countries. In Taiwan, about half of arable land is used as rice-growing field and two rice varieties including Indica and Japonica varieties are cultivated, but the latter is the major one (90%) because of taste preferences. Cadmium (Cd), normally occurs in low concentrations in soils (Wagner, 1993), is a non-essential element for plants and potentially toxic pollutant all over the world. The toxicity of Cd to plant growth, photosynthesis, carbohydrate metabolism, and enzyme activities is well documented (Javed & Greger, 2011; Sanita di Toppi & Gabrielli, 1999). Elevated levels of arsenic (As) in soils may potentially enter food chain (Meharg & Hartley-Whitaker, 2002) and increase the risk of cancer development (Anderson et al., 2011). According to the SGWPR Act, the cropping land with total soil Cd concentration (aqua regia soluble) exceeding  $5 \text{ mg kg}^{-1}$  will be announced as Soil Pollution Control Site (SPCS) and all farming activities are not allowed. However, many previous field surveys showed Cd-contaminated rice can still be produced from fields with total soil Cd levels lower than  $5 \text{ mg kg}^{-1}$ . The Standard for the Tolerance of Cd in rice has been reduced from  $0.5 \text{ mg kg}^{-1}$  to  $0.4 \text{ mg kg}^{-1}$  in 2007. Many studies were also subsidized by governments to assess the food safety of rice cultivated in Cd-contaminated soil. In this paper, we reviewed some previous researches regarding the accumulation of Cd and As of different rice varieties. Its safety after growing in As- or Cd-contaminated soils was also evaluated. For those contaminated lands not suitable for planting crops, the use of phytoremediation and planting non-edible plants may be a candidate for solving this problem. Experimental results of phytoremediation were also illustrated in this paper.

## 2. Phytoremediation for potted Cd-contaminated soils

The selection of suitable plants is the first and the critical step in conducting a successful phytoremediation. These plants should grow well and accumulate higher concentration of HMs in the harvestable parts when growing in HM-contaminated soils. There were approximately 420 species of plants that can be regarded as hyperaccumulators (Baker et al., 2000). A pot experiment was conducted to test the accumulation capacity of five garden flower species, which was regarded as a potential hyperaccumulator previously (Chen & Lee, 1997). Seedlings of them were planted in the artificially Cd-contaminated loamy soils to assess their Cd accumulation when growing in control (Cd-CK) ( $0.43 \pm 0.15 \text{ mg kg}^{-1}$ ), Cd-10 ( $9.73 \pm 0.05 \text{ mg kg}^{-1}$ ), and Cd-20 ( $17.6 \pm 0.8 \text{ mg kg}^{-1}$ ) (Lin et al., 2010). One seedling of Star cluster (*Pentas lanceolata* Defflers.), French marigold (*Tagetes patula* L.), Impatiens (*Impatiens walleriana* Hook. f.), Garden verbena (*Verbena bipinnatifida* Nutt.), or Scarlet sage (*Salvia splendens* Ker-Gawl.) was planted in each pot contained three kilograms of artificially Cd-contaminated soils. The pot experiment was conducted in a 30/25°C (day/night) phytotron in three replicates, controlled the soil moisture content at 50-70% water-holding capacity

(WHC) by adding deionized water, and harvested after growing for 35 days. After pretreatment, the Cd concentrations in the tissues were determined.

The Cd concentrations in initial seedlings of five plants were not detectable ( $\text{Cd} < 0.38 \text{ mg kg}^{-1}$ ). After growing in the artificially Cd-contaminated soils for 35 days, five tested plants can significantly accumulate much higher Cd concentrations in their shoots relative to Cd-CK. Among the five plants, Impatiens grown in the Cd-20 treatment had the highest shoot Cd concentration near  $100 \pm 11 \text{ mg kg}^{-1}$ , which was more than the threshold of a Cd hyperaccumulator ( $100 \text{ mg kg}^{-1}$ ) reported (Baker et al., 2000). French marigold grown in Cd-10 and Cd-20 treatments accumulated  $44.9 \pm 0.7$  and  $66.3 \pm 6.5 \text{ mg kg}^{-1}$  in their shoots and no toxic symptoms were observed in the appearance during pot experiment. Chen & Lee (1997) reported that Star cluster, Scarlet sage, and Impatiens can accumulate 44, 12, and  $42 \text{ mg kg}^{-1}$ , respectively, in their leaves when *in-situ* growing in a Cd-contaminated site (Tatan village) in northern Taiwan. For another *in-situ* experiment carried out in Chungfu village in northern Taiwan, the final Cd concentration in their leaves was  $247 \text{ mg kg}^{-1}$  in French marigold,  $52 \text{ mg kg}^{-1}$  in Garden verbena,  $12 \text{ mg kg}^{-1}$  in Impatiens, and  $11 \text{ mg kg}^{-1}$  in Scarlet sage, respectively. French marigold and Impatiens used in this study accumulated higher Cd concentration in their shoots compared with the result of previous study possibly resulted from the higher phytoavailability of Cd in artificially Cd-contaminated soils.

Besides the accumulated concentration, bioconcentration factor ( $\text{BCF} = \text{shoot HM concentration}/\text{soil HM concentration}$ ) and translocation factor ( $\text{TF} = \text{shoot HM concentration}/\text{root HM concentration}$ ) were two indexes most used to evaluate the accumulating capacity of HMs by plants. For a Cd hyperaccumulator (Baker et al., 2000; Mattina et al., 2003), the BCF and TF should more than one besides the high concentration accumulated ( $100 \text{ mg kg}^{-1}$ ) (Sun et al., 2009). Experimental result of this study showed that the BCF values of French marigold, Impatiens, Garden verbena, and Scarlet sage were all more than one and ranged from 1.75 to 5.68 (Table 1). However, Impatiens was the only one that its TF was in the levels of 1.01-1.66. According to the standards summarized by Sun et al. (2009) for a Cd hyperaccumulator, Impatiens was a potential Cd hyperaccumulator when growing in the artificially Cd-contaminated soils. The pot experimental result was against with the *in-situ* selection experiment, possible resulted from the special variation and interaction of HMs in the field.

The total removal of Cd by plants determines the duration needed in decontamination. Although root of plants accumulated higher concentration of Cd in compartment with shoot, the total removal of Cd by shoot was larger because of its larger biomass (Fig. 1). Among the five tested plants, the shoots of French marigold and Impatiens removed 380-510 and 790-820 g Cd plant<sup>-1</sup> from Cd-10 and Cd-20. One can calculate the period needed for phytoremediation in an ideal situation, i.e. if the removal of plants of each harvest is a constant and the phytoavailability of Cd will not change with time, etc. It will take approximately 4.6-8.0 years for continuous planting (six times year<sup>-1</sup>) French marigold and Impatiens to decrease the current Cd concentration (Cd-10 and Cd-20) to below the SPCS for cropping lands ( $5 \text{ mg kg}^{-1}$ ). The major drawback of phytoremediation is that it always consumes longer period compared with other techniques. Experimental results show that planting French marigold and Impatiens in Cd-contaminated soils seems to be a feasible method to remove Cd from contaminated soil and the period needed for decontamination is acceptable.

Plants	Treatments*	Shoot	Root	BCF	TF
		----- mg kg <sup>-1</sup> -----			
Star cluster	Cd-CK	ND b	ND a	----	----
	Cd-10	8.20±0.94 a <sup>#</sup>	22.7±2.02 a	0.84	0.36
	Cd-20	10.7±2.8 a	18.9±17.2 a	0.61	0.57
French marigold	Cd-CK	ND c	ND c	----	----
	Cd-10	44.9±0.7 b	65.0±17.8 b	4.61	0.69
	Cd-20	66.3±6.5 a	113±21 a	3.77	0.59
Impatiens	Cd-CK	ND c	ND b	----	----
	Cd-10	48.9±11.7 b	29.5±9.6 ab	5.02	1.66
	Cd-20	100±11 a	99.0±8.4 a	5.68	1.01
Garden verbena	Cd-CK	ND b	ND b	----	----
	Cd-10	21.5±5.5 a	39.3±13.5 a	2.21	0.55
	Cd-20	7.63±1.75 b	49.5±11.2 a	0.43	0.15
Scarlet sage	Cd-CK	ND b	ND c	----	----
	Cd-10	21.8±7.6 a	45.9±8.2 b	2.24	0.47
	Cd-20	30.8±5.3 a	71.0±15.5 a	1.75	0.43

\* Total Cd concentration digested by aqua regia: Cd-CK = 0.43±0.15 mg Cd kg<sup>-1</sup>; Cd-10 = 9.73±0.05 mg Cd kg<sup>-1</sup>; Cd-20 = 17.6±0.8 mg Cd kg<sup>-1</sup>

<sup>#</sup> means ± standard deviation (n = 3); ND: not detectable; The different small letters within same column of same plant tissue stand for statistical significance (*p* < 0.05)

Table 1. The Cd concentration, bioconcentration factor (BCF), and translocation factor (TF) of Star cluster, French marigold, Impatiens, Garden verbena, and Scarlet sage grown in the different Cd-treated soil for 35 days (Lin et al., 2010).

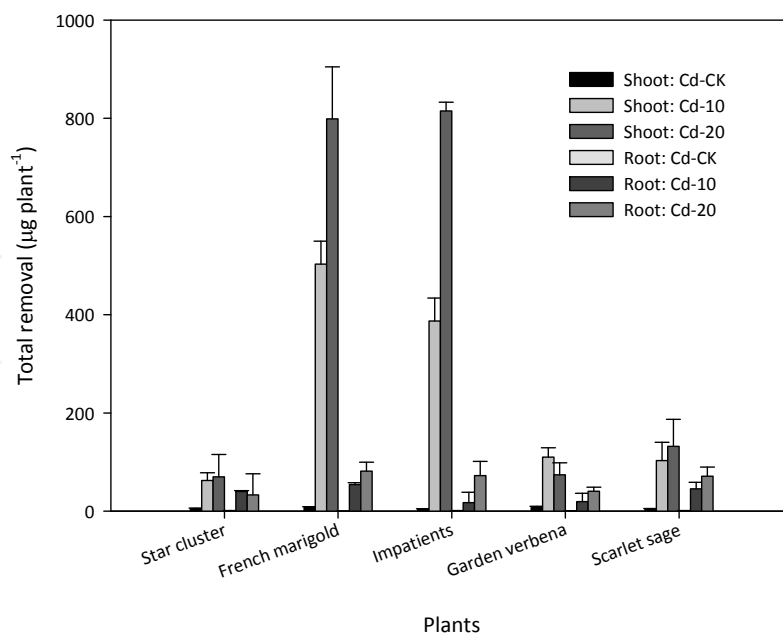


Fig. 1. The total removal of Cd concentration of Star cluster, French marigold, Impatiens, Garden verbena, and Scarlet sage grown in the different Cd-treated soil for 35 days (Lin et al., 2010).

### 3. Phytoremediation for Cr, Cu, Ni, and Zn-contaminated soils

Eight blocks (11 m by 4 m) located in central Taiwan (Fig. 2), which were relatively higher in Cr (chromium), Cu (copper), Ni (nickel), and Zn (zinc) concentrations were used for *in-situ* selection experiment (Lai & Chen, 2009). After making a market survey, 33 plant species of garden flower species (Table 2) were selected for this *in-situ* experiment and two seedlings per plant species were planted in each of the eight blocks. The interval space between two plants was controlled at about 50-70 cm to avoid interference. The concentration of Cu, Cr, Ni, and Zn in the 33 plant species before planting was determined. We recorded the growth condition of plants, whether toxic symptom occurred or not, two weeks after planting in the site and they were harvested after planting for 33 days. After pretreatment, the Cu, Cr, Ni, and Zn concentration in the digest solution of initial and harvested plants were determined. After *in-situ* growing in the contaminated soils for two weeks, we observed withered and yellow color in the leaves of Bougainvillea and some of them fell off. The flowers of Cockscomb were damaged and their color changed from red (before planting) to yellow. Except for the two plants, there were no observed injures for the other 31 plants grown in the 8 blocks. Before planting, the initial concentration of HMs in the shoot were in the levels of ND ( $< 1.71 \text{ mg kg}^{-1}$ ) to  $37.2 \text{ mg Cu kg}^{-1}$ , ND ( $< 4.65 \text{ mg kg}^{-1}$ ) to  $21.7 \text{ mg Cr kg}^{-1}$ , and  $8.07$  to  $103 \text{ mg Zn kg}^{-1}$ . These 33 plant species have low Ni concentration ( $< 10.1 \text{ mg kg}^{-1}$ ) in their tissues before planting, except for Bougainvillea, Common melastoma, and Garden Canna. Although Cockscomb showed yellow flowers at second week after planting, it accumulated the highest Cu, Cr, Ni, and Zn concentration in its shoots in relative to other plant species. Cockscomb and rainbow pink accumulated  $317 \pm 117$  and  $231 \pm 73 \text{ mg Cr kg}^{-1}$  in their shoots, respectively, after *in-situ* growing for 33 days in the site. Their BCF was 1.7 in cockscomb and 1.3 in rainbow pink.

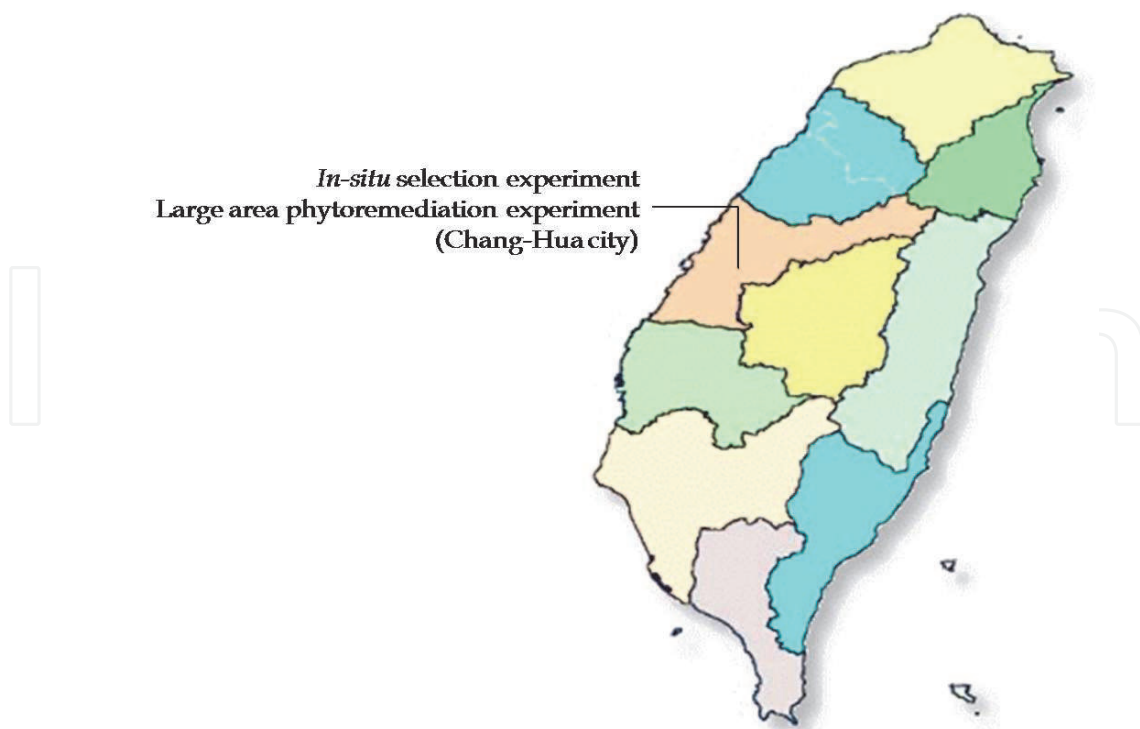


Fig. 2. Map of Taiwan and location where the phytoremediation experiments were conducted (Lai & Chen, 2009; Lai et al., 2010a).

No.	Plant species	Scientific name	Property
1	Bougainvillea	<i>Bougainvillea spp.</i>	woody
2	Rainbow pink	<i>Dianthus chinensis</i>	herbaceous
3	Serissa	<i>Serissa japonica</i>	woody
4	French marigold	<i>Tagetes patula</i>	herbaceous
5	Rose of Sharon	<i>Hibiscus syriacus</i> L.	woody
6	Water willow	<i>Salix warburgu</i>	woody
7	Chinese ixora	<i>Ixora chinensis</i> Lam.	woody
8	Sunflower	<i>Helianthus annuus</i>	herbaceous
9	Chinese hibiscus	<i>Hibiscus rosa-sinensis</i>	woody
10	Gold dewdrop	<i>Duranta repens</i>	woody
11	Kalanchoe	<i>Kalanchoe blossfeldiana</i>	herbaceous
12	Creeping Trilobata	<i>Wedelia trilobata</i>	herbaceous
13	Garden Canna	<i>Canna generalis spp.</i>	herbaceous
14	Garden verbena	<i>Verbena hybrida</i>	herbaceous
15	Malabar chestnut	<i>Pachira macrocarpa</i> Walp	woody
16	Purslane	<i>Portulaca oloraua</i> Linn	herbaceous
17	Common Lantana	<i>Lantana camara</i> L.	woody
18	Fancy leaf caladium	<i>Caladium hortulanun</i> Birdsey	herbaceous
19	Coleus	<i>Coleus blumei</i>	herbaceous
20	Golden trumpet	<i>Allamanda cathartica</i> Linn	woody
21	Common melastoma	<i>Melastoma candidum</i>	woody
22	Carland flower	<i>Hedychium coronarium</i>	herbaceous
23	Manaca raintree	<i>Brunfelsia uniflora</i>	woody
24	Yellow Cosmos	<i>Cosmos sulphureus</i>	herbaceous
25	Sliver apricot	<i>Ginkgo biloba</i> L.	woody
26	Temple tree	<i>Plumeria acutifolia</i> Poir.	herbaceous
27	Orchid tree	<i>Aglaia odorata</i> Lour.	woody
28	Star cluster	<i>Pentas lanceolata</i>	herbaceous
29	Blue daza	<i>Evolvulus nuttallianus</i>	herbaceous
30	Cockscomb	<i>Celosia cristata</i>	herbaceous
31	Scandent Schefflera	<i>Schefflera arboricola</i> Hayata.	woody
32	Bojers spurge	<i>Euphorbia splendens</i>	woody
33	Croton	<i>Codialum variegatum.</i>	woody

Table 2. The 33 plant species planted in the central Taiwan used for *in-situ* selection experiment.

For Cu, the accumulation capacity of various tested plants was in the order of Cockscomb ( $117 \pm 40$  mg kg<sup>-1</sup>), Garden verbena ( $84.7 \pm 46.6$  mg kg<sup>-1</sup>), and Star cluster ( $80.4 \pm 80.6$  mg kg<sup>-1</sup>). The average Cu concentration of corn and food grains of China was 2.67 and 6.46 mg kg<sup>-1</sup> (Chen et al., 1994) and the Cu concentration for foodstuff crops was less than 10 mg kg<sup>-1</sup> (Kabata-Pendias & Pendias, 2001). The Cu concentration in the brown rice of Japan and Indonesia was 2.16-4.4, 2.9, and 3.41 mg kg<sup>-1</sup>, respectively (Iimura, 1981; Masironi, 1977; Suzuki et al., 1980). Although the accumulated Cu concentration of these 33 plants increased after 33 days, the BCF were less than 1.1 because the surface soil has low Cu concentration, ranged from 112 to 122 mg kg<sup>-1</sup>. Because of the low Ni concentration in the initial plants, the Ni concentration of shoot in the 33 plants increased after *in-situ* planting

in the contaminated site for 33 days. The Ni concentration of shoot was in the order of Cockscomb ( $145 \pm 38 \text{ mg kg}^{-1}$ ), French marigold ( $90.9 \pm 42.4 \text{ mg kg}^{-1}$ ), and Garden verbena ( $88.0 \pm 36.8 \text{ mg kg}^{-1}$ ). Because of the high Ni concentration in the surface soil of the 8 blocks (ranged from 314 to  $412 \text{ mg kg}^{-1}$ ), the accumulated Ni concentration in the shoots of plants increased after *in-situ* planting for 33 days. After 33 days, the accumulated Zn concentration in the shoots of plants was in the order of Cockscomb ( $435 \pm 127 \text{ mg kg}^{-1}$ ), Garden verbena ( $339 \pm 210 \text{ mg kg}^{-1}$ ), and Yellow Cosmos ( $328 \pm 157 \text{ mg kg}^{-1}$ ). However, the BCF of Zn of Cockscomb was only 0.7 which revealed that the accumulation capacity of these 33 plants was weak.

Experimental results also show that the accumulation of HMs of woody and herbaceous plants after growing for 33 days was quite different (Table 2). Similar to the results of Chen & Lee (1997), herbaceous plants have accumulated higher concentration of HMs in comparison to woody plants. Except for the low Ni concentration in initial plants, the increase for HMs concentration in woody plants was about  $3.1 \pm 2.9$  fold for Cu,  $2.5 \pm 1.5$  fold for Cr, and  $4.3 \pm 3.1$  fold for Zn, respectively. Herbaceous plants have higher uptake of HMs in relative to woody plants and their increase on the concentration of HMs are  $9.4 \pm 6.5$  fold for Cu,  $5.1 \pm 2.7$  fold for Cr, and  $8.9 \pm 6.1$  fold for Zn.

#### 4. Large area phytoremediation experiments of 12 plant species in HMs-contaminated site

Twelve plants species (Table 3) were selected from 33 plant species testing in a site contaminated by combined HMs in central Taiwan (Fig. 2) to study the feasibility of *in-situ* phytoremediation (Lai et al., 2010a). Soil samples of topsoil (0-15 cm) and subsoil (15-30 cm) were collected and analyzed for the total concentration of eight HMs (As, Cd, Cr, Cu, Hg, Pb, Ni, and Zn). The studied site was mainly contaminated by Cr, Cu, Ni, and Zn and some of the concentrations were much higher than the SPMS (Soil Pollution Monitoring Standard) or SPCS. The total area for each plant species was approximately 0.1 ha and their planting density was  $10,000 \text{ seedlings ha}^{-1}$ . Plants were harvested after growing for one and two months and their concentration of Cu, Cr, Ni, and Zn in the shoot were determined.

Results of two times of large area experiments after foregoing 12 plant species were growing for one month and two months showed that they can grow well in this combined HMs-contaminated site. The concentrations of Cr, Cu, Ni, and Zn in the shoots increased after growing for 31 days compared with those of it before planting. The extension of their time of growth, from one month to two months in the contaminated site, has positive effects on increasing their accumulation of HMs. However, the 12 tested plant species could not accumulated higher concentrations of Cr, Cu, Ni, and Zn possibly resulted from the lower concentrations of foregoing HMs. None of the plant species can regard as a hyperaccumulator according to the definition of Baker et al. (2000). After *in-situ* growing in the contaminated site for one month, the roots of most of the 12 plant species accumulated higher concentrations of HMs compared with the shoots. Among the four HMs, the TF of Zn was highest in comparison to others. The TF of some tested plant species were more than one from the result of *in-situ* experiment (Table 4). In an ideal situation, it will take about 4.3 to 66 years by planting foregoing plant species to reduce the concentrations of HMs to conform the SPMSs.



No.	Plant species	Scientific name	Property
1	Chinese ixora	<i>Ixora chinensis</i> Lam.	woody
2	Garden verbena	<i>Verbena hybrida</i>	herbaceous
3	Rainbow pink	<i>Dianthus chinensis</i>	herbaceous
4	Bojers spurge	<i>Euphorbia splendens</i>	woody
7	Kalanchoe	<i>Kalanchoe blossfeldiana</i>	herbaceous
5	Scandent Scheffera	<i>Schefflera arboricola</i> Hayata.	woody
6	Purslane	<i>Portulaca oloraua</i> Linn	herbaceous
7	Croton	<i>Codialum variegatum.</i>	woody
8	Serissa	<i>Serissa japonica</i>	woody
9	Garden Canna	<i>Canna generalis</i> spp.	herbaceous
11	French marigold	<i>Tagetes patula</i>	herbaceous
12	Sunflower	<i>Helianthus annuus</i>	herbaceous

Table 3. The 12 plant species planted in the central Taiwan used for large area phytoremediation experiment.

Plant species	Translocation factor (TF) <sup>#</sup>			
	Cr	Cu	Ni	Zn
Chinese ixora	0.41	1.28	0.22	1.40
Garden verbena	0.35	0.64	0.33	2.13
Rainbow pink	0.58	0.78	0.89	1.17
Bojers spurge	0.84	1.44	0.75	1.98
Kalanchoe	0.15	1.31	0.28	--
Scandent Scheffera	0.07	0.84	0.61	--
Purslane	5.06	0.84	0.68	0.72
Croton	0.05	0.38	0.25	--
Serissa	0.40	0.90	0.57	--
Garden Canna	0.61	0.54	0.43	1.35
French marigold	0.18	0.56	0.30	--
Sunflower	0.21	1.39	0.41	3.15

<sup>#</sup> TF = The ratio of shoot HM concentration to root HM concentration

Table 4. The translocation factor (TF) of 12 plant species after *in-situ* growing in a combined HMs-contaminated site for one month.

The median and maximum concentrations of Cr, Cu, Ni, and Zn in the topsoil were used in this study to calculate the mean and maximum effect of contaminants. The exposure risk was resulting from the ingestion of contaminated soils ( $EXP_{ing}$ ), inhalation of air containing contaminated soil particles ( $EXP_{inh}$ ), and absorption by skin ( $EXP_{abs}$ ). Different equations were used to calculate the carcinogenic and non-carcinogenic risks of contaminants to the health of humans (Lai et al., 2011). Where HQ is the hazard quotient and  $EXP_{total}$  is the sum of total exposure. There are carcinogenic and non-carcinogenic risks when the values of HQ and TR are less than unitary and  $10^{-6}$ , respectively. The concentrations of Cr, Cu, Ni, and Zn in the topsoil after phytoremediation were estimated by considering the removal of plants. The results showed that although the study site was contaminated with combined HMs, there are no carcinogenic and non-carcinogenic risks (Tables 5 and 6) although some of the total concentrations of Cr, Cu, Ni, and Zn were higher than the SPMSs or SPCSs.

	Zn		Cr		Cu		Ni	
	Med.	Max.	Med.	Max.	Med.	Max.	Med.	Max.
Before phytoremediation								
EXP <sub>inh</sub>	5.9×10 <sup>-6</sup>	1.1×10 <sup>-5</sup>	1.4×10 <sup>-6</sup>	3.5×10 <sup>-6</sup>	1.5×10 <sup>-6</sup>	2.1×10 <sup>-6</sup>	3.7×10 <sup>-6</sup>	7.0×10 <sup>-6</sup>
EXP <sub>ing</sub>	2.7×10 <sup>-4</sup>	5.2×10 <sup>-4</sup>	6.5×10 <sup>-5</sup>	1.6×10 <sup>-4</sup>	7.1×10 <sup>-5</sup>	9.6×10 <sup>-5</sup>	1.7×10 <sup>-4</sup>	3.2×10 <sup>-4</sup>
EXP <sub>abs</sub>	4.7×10 <sup>-5</sup>	8.9×10 <sup>-5</sup>	1.1×10 <sup>-5</sup>	2.8×10 <sup>-5</sup>	1.2×10 <sup>-5</sup>	1.6×10 <sup>-5</sup>	3.0×10 <sup>-5</sup>	5.6×10 <sup>-5</sup>
HQ	1.0×10 <sup>-3</sup>	2.0×10 <sup>-3</sup>	2.5×10 <sup>-2</sup>	6.2×10 <sup>-2</sup>	3.0×10 <sup>-3</sup>	4.1×10 <sup>-3</sup>	9.8×10 <sup>-3</sup>	1.9×10 <sup>-2</sup>
After phytoremediation								
EXP <sub>inh</sub>	5.8×10 <sup>-6</sup>	1.1×10 <sup>-6</sup>	1.4×10 <sup>-6</sup>	3.5×10 <sup>-6</sup>	1.5×10 <sup>-6</sup>	2.0×10 <sup>-6</sup>	3.7×10 <sup>-6</sup>	7.0×10 <sup>-6</sup>
EXP <sub>ing</sub>	2.6×10 <sup>-4</sup>	5.1×10 <sup>-4</sup>	6.4×10 <sup>-5</sup>	1.6×10 <sup>-4</sup>	7.0×10 <sup>-5</sup>	9.5×10 <sup>-5</sup>	1.7×10 <sup>-4</sup>	3.2×10 <sup>-4</sup>
EXP <sub>abs</sub>	4.5×10 <sup>-5</sup>	8.8×10 <sup>-5</sup>	1.1×10 <sup>-5</sup>	2.8×10 <sup>-5</sup>	1.2×10 <sup>-5</sup>	1.6×10 <sup>-5</sup>	2.9×10 <sup>-5</sup>	5.5×10 <sup>-5</sup>
HQ	1.0×10 <sup>-3</sup>	1.9×10 <sup>-3</sup>	2.4×10 <sup>-2</sup>	6.1×10 <sup>-2</sup>	2.9×10 <sup>-3</sup>	4.0×10 <sup>-3</sup>	9.7×10 <sup>-3</sup>	1.8×10 <sup>-2</sup>

Med.: median value; Max.: maximum value

Table 5. The hazard quotient (HQ) and the exposure risk of the three pathways before and after phytoremediation.

	Cr		Ni	
	Med.	Max.	Med.	Max.
Before phytoremediation				
EXP <sub>inh</sub> (mg kg <sup>-1</sup> day <sup>-1</sup> )	2.1×10 <sup>-8</sup>	5.1×10 <sup>-8</sup>	5.3×10 <sup>-8</sup>	1.0×10 <sup>-7</sup>
TR	7.0×10 <sup>-7</sup>	1.8×10 <sup>-6</sup>	3.7×10 <sup>-8</sup>	7.0×10 <sup>-8</sup>
After phytoremediation				
EXP <sub>inh</sub> (mg kg <sup>-1</sup> day <sup>-1</sup> )	2.0×10 <sup>-8</sup>	5.0×10 <sup>-8</sup>	5.3×10 <sup>-8</sup>	1.0×10 <sup>-7</sup>
TR	6.9×10 <sup>-7</sup>	1.7×10 <sup>-6</sup>	3.7×10 <sup>-8</sup>	7.0×10 <sup>-8</sup>

Med.: median value; Max.: maximum value

Table 6. The exposure and carcinogenic risk before and after phytoremediation if only the inhalation was considered.

## 5. Uptake characteristics of different rice varieties growing in Cd-contaminated soils

In 2005 and 2006, field studies were conducted in Taiwan to investigate the uptake characteristics of rice varieties growing in 19 different paddy fields in three counties across the western plains in Taiwan (Römken et al., 2009). The studied fields were located at the towns of Chang-hua (three fields), Ho-Mei (three fields), Lu-Kang (two fields), Hsin-Chu (three fields), and Pa-Deh (eight fields) (Fig. 3). Twelve rice cultivars of Indica or Japonica varieties were planted in each field with 5-9 replicates for each cultivar depends on the field size. Samples of topsoil (0-25 cm) and rice plants at full maturity were collected together at the same location in studied fields in May (harvest 1) and November (harvest 2) of the two years. Total numbers of soil and rice plant samples in this study were both 3,198. The total soil Cd concentration in studied fields ranged from 0.06 mg kg<sup>-1</sup> to as high as 27.8 mg kg<sup>-1</sup>, the maximum level is about 6-fold higher than the SPCS (5 mg kg<sup>-1</sup>) enacted in Taiwan. Around 27% of the studied field area was defined as Cd-contaminated soil according to the SGWPR Act.

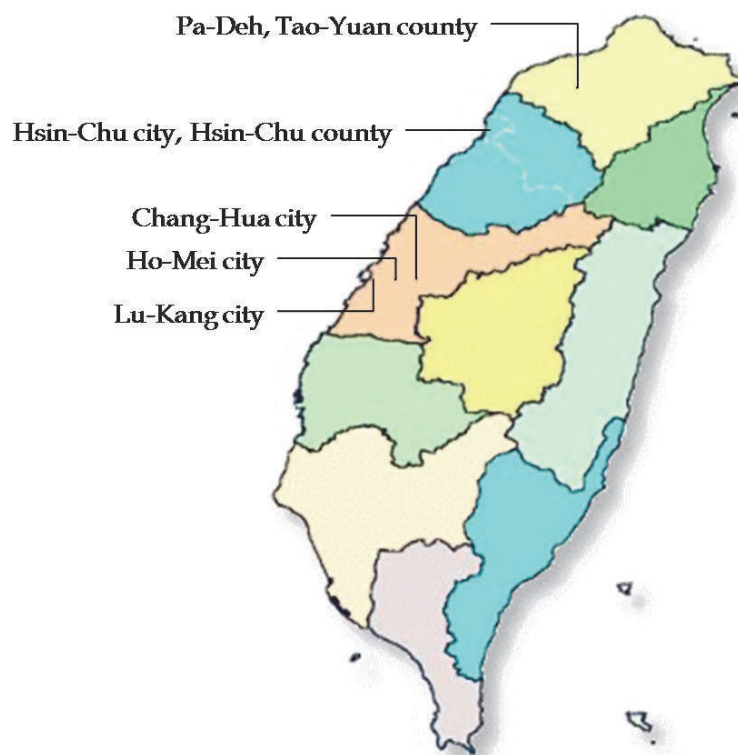


Fig. 3. Map of Taiwan and location of communities where the paddy fields are located (Römken et al., 2009).

Soil pH, CEC (Cation Exchange Capacity), and soil organic matter (SOM) varied widely in the 19 paddy fields (Table 7). Cadmium concentrations in rice grains were quite different among cultivars even though they were planted in soils with comparable soil properties and total soil Cd levels. Overall, median Cd concentrations in rice grains of Indica variety were 2-3 times higher than that of Japonica variety no matter the rice is planted in low or high Cd-contaminated fields or in different climates (Fig. 4). Higher variation was found in the concentration of Cd in Indica brown rice compared with that in Japonica brown rice. Some studies also found that Cd accumulation in brown rice of Indica was 1.54 times higher than that of Japonica. This uptake characteristic of rice varieties is important for selecting rice cultivars with low Cd accumulating ability in rice grain planted in slightly Cd-contaminated soil.

	pH	CEC (cmol <sub>+</sub> kg <sup>-1</sup> )	SOM (%)	Total Cd (mg kg <sup>-1</sup> )
Japonica	3.8-7.2	2.6-24.2	1.4-9.5	0.06-27.8
Indica	4.1-7.0	2.6-25.1	1.3-10.2	0.08-25.9

Table 7. The pH, CEC, and SOM of the soils growing for two rice varieties.

Liu et al. (2007) reported that Cd was not evenly distributed in different parts of rice grain. The results of their pot experiments planting six rice cultivars (include Indica, Japonica, hybrid Indica, and New Plant type) in artificially Cd-contaminated soil showed that the average percentage of Cd quantity accumulated in chaff, cortex (embryo), and polished rice were about 15%, 40%, and 45%, respectively. The cortex occupied only 9% of the grain dry weight in average but the polished rice occupied 71%, so Cd concentration in cortex is significantly higher than that in polished rice. They suggested that polished rice produced

from Cd-contaminated fields may be safer for consumers than brown rice. However, Moriyama et al. (2003) reported that Cd concentration in six Japonica rice cultivars reduced only 3% in average after milling process. A study using *in-situ* synchrotron X-ray fluorescence to identify Cd distribution in brown rice produced from Bangladesh, China, and U.S. also showed that Cd is evenly distributed in brown rice (Meharg et al., 2008). The inconsistent findings among these studies may be caused by errors from rice polishing process or inherent differences of Cd distribution in rice grain among rice cultivars. More careful studies are required to clarify the inconsistent results.

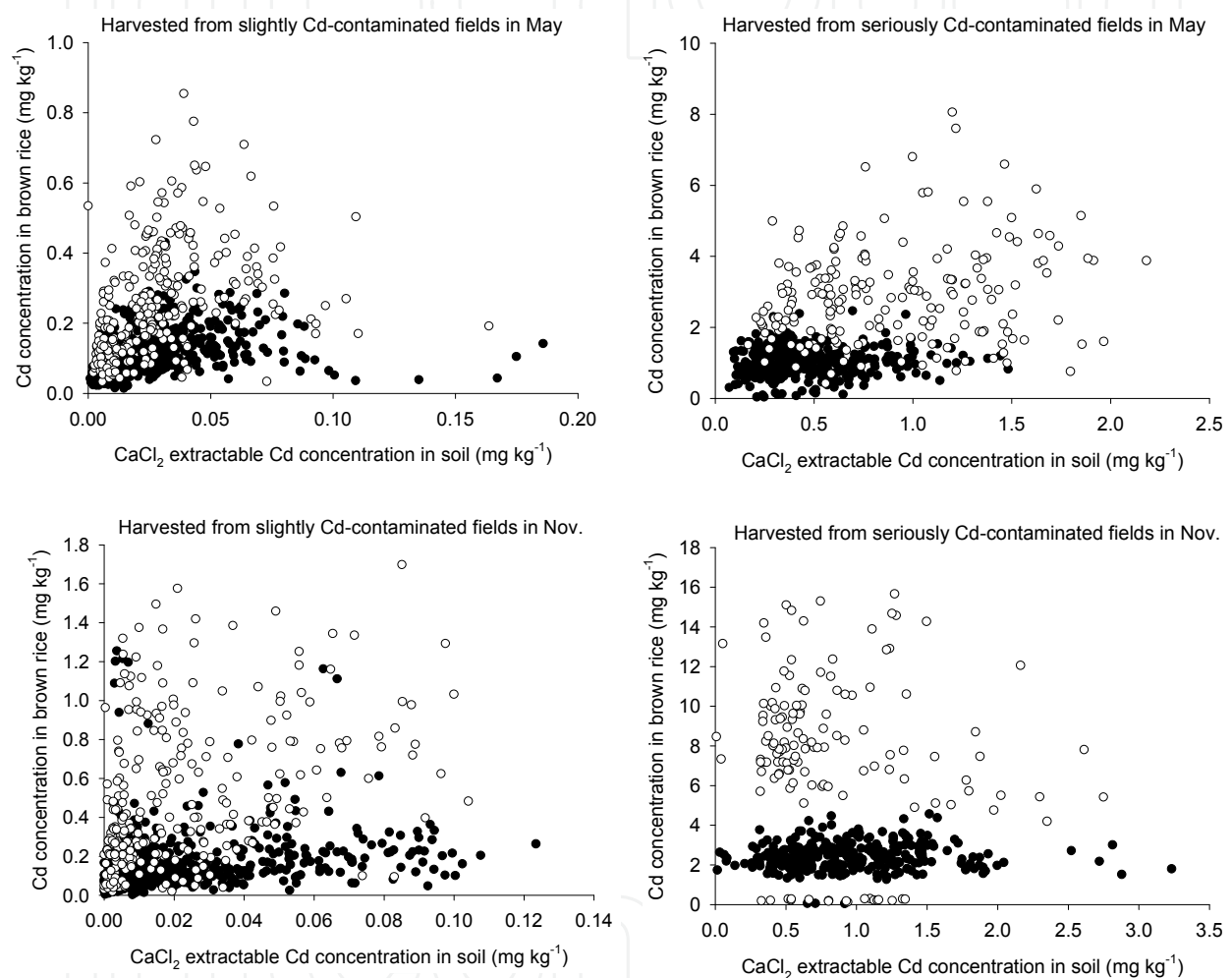


Fig. 4. The relationship between  $\text{CaCl}_2$  extractable Cd concentration in soil and Cd concentration in brown rice ( $\bullet$ : Japonica species,  $\circ$ : Indica species) harvested in May and November.

## 6. Various Cd uptake models were used to efficiently predict their accumulation

Total Cd concentration in soil is not a reliable index to determine whether rice grain is safe for consumers. Rice varieties and soil characteristics such as soil pH, Eh (redox potential), CEC, texture, and SOM are important factors affecting Cd concentration in rice grain. To determine whether a rice-growing field can produce safe rice grains with Cd levels lower

than FQS (food quality standard), it is necessary to develop a simple and reliable soil tests to predict available Cd concentration in rice grains.

Previous studies indicated that 0.01M CaCl<sub>2</sub>, 0.1M HCl, 0.43M HNO<sub>3</sub>, and 0.05M EDTA (Na<sub>2</sub>-EDTA 2H<sub>2</sub>O) are ideal extractants to estimate soil available Cd concentration (Houba et al., 1997; Nelson et al., 1959; Tiwari & Kumar, 1982). This study compared these methods to assess which method is better for predicting Cd levels in rice grains. The best well-performed regression equation to predict Cd levels in rice grain was presented here using soil available Cd and Zn concentrations determined by 0.01M CaCl<sub>2</sub>:

$$\log[\text{Cd-grain}] = 0.94 + 0.78 \times \log[\text{Cd-CaCl}_2] - 0.30 \times \log[\text{Zn-CaCl}_2], r^2 = 0.73 \dots \text{for Indica} \quad (1)$$

$$\log[\text{Cd-grain}] = 0.60 + 0.82 \times \log[\text{Cd-CaCl}_2] - 0.28 \times \log[\text{Zn-CaCl}_2], r^2 = 0.86 \dots \text{for Japonica} \quad (2)$$

The CaCl<sub>2</sub> extractable Zn is also included in the equation because it is able to compete with Cd for plant uptake and reduce toxic effects of Cd. The critical concentrations of CaCl<sub>2</sub>-extractable Cd in soil under different levels of soil CaCl<sub>2</sub>-extractable Zn are constructed for farmers and authorities in Taiwan to prevent the production of Cd-contaminated rice by using above equations.

The concentration of CaCl<sub>2</sub>-extractable Zn in soil ranged usually from 0.1 to 50 mg kg<sup>-1</sup> when the total soil Zn concentration is less than 600 mg kg<sup>-1</sup>, the SPCS for cropping lands enacted in Taiwan. According to the equations, less Cd will be accumulated in rice grain if the soil CaCl<sub>2</sub>-extractable Zn is getting higher, therefore, only the critical concentrations of CaCl<sub>2</sub>-extractable Cd in soil under the soil CaCl<sub>2</sub>-extractable Zn lower than 50 mg kg<sup>-1</sup> are presented. If the measured soil CaCl<sub>2</sub>-extractable Cd is higher than the critical value, it is possible to produce rice grain with Cd concentration exceeding the Standard for the Tolerance of Cd in rice (0.4 mg kg<sup>-1</sup>) (Table 8). Further studies are required to validate the practicability of regression equations.

Rice variety	CaCl <sub>2</sub> -extractable Zn in soil (mg kg <sup>-1</sup> )							
Indica	0.007	0.019	0.035	0.046	0.060	0.071	0.079	0.086
Japonica	0.027	0.060	0.105	0.133	0.168	0.193	0.213	0.230

Table 8. Critical concentrations of CaCl<sub>2</sub>-extractable Cd (mg kg<sup>-1</sup>) in soil under different levels of soil CaCl<sub>2</sub>-extractable Zn (mg kg<sup>-1</sup>) for the two rice varieties. Cadmium concentration in rice grain will exceed 0.4 mg kg<sup>-1</sup> if the measured soil CaCl<sub>2</sub>-extractable Cd is higher than the critical concentration.

To predict Cd concentration in rice grain, Simmons et al. (2008) also developed a regression equation using soil pH (1:5) and CaCl<sub>2</sub> extractable Cd determined on field-moist samples collected during the grain-filling period. The equation can predict Cd concentrations in unpolished rice grain with an r<sup>2</sup> value of 0.638. If air-dried soil samples were used for Cd-CaCl<sub>2</sub> and pH determination, the regression equation cannot explain the variability of Cd levels in rice grain. Air-drying may affect soil sample conditions to an extent that CaCl<sub>2</sub> extractable Cd cannot represent Cd availability in soil compared to extracts collected from

field-moist soil. However, the soil samples used for developing regression equations in the study of Taiwan as mentioned above were air-dried and collected during rice harvest period, an easier pretreatment for soil samples and more suitable for routine monitoring. Brus et al. (2009) recently developed a multiple regression model using 0.43M HNO<sub>3</sub> extractable Cd, pH, clay, and SOM as predictors to predict Cd levels in rice grain harvested from the paddy fields in Fuyang, Zhejiang province, China. The model performed much better ( $r^2_{\text{adj}} = 0.661$ ) than the linear model using only 0.01M CaCl<sub>2</sub> extractable Cd as a predictor ( $r^2_{\text{adj}} = 0.281$ ). The field study in Taiwan as mentioned above also developed a multiple regression model using 0.43M HNO<sub>3</sub> extractable Cd, pH, and CEC to predict Cd levels in rice grain. Although the model using more predictors to reflect the effects of pH and CEC on the availability of Cd, it did not perform much better ( $r^2 = 0.81$  and  $0.74$  for Japonica and Indica, respectively) than the model using 0.01M CaCl<sub>2</sub> extractable Cd and Zn as predictors ( $r^2 = 0.86$  and  $0.73$  for Japonica and Indica, respectively). Therefore, the latter simpler model is preferred to be validated and used in Taiwan. Since different environmental and soil factors affect the accumulation of Cd in rice grain in different ways and extents, the predicting models developed by using local data will be more reliable to be used for the specific area.

## 7. As-contaminated soils in Guandu plain

Arsenic is a contaminant of public concern since it is highly toxic and carcinogenic. It may be accumulated in plants and eventually be transferred to humans through the food chain. A regular monitoring for HM concentrations in soil conducted by Taipei government found that some soil samples in Guandu Plain were contaminated by As. Further comprehensive survey conducted in 2006 showed that more than 60 ha of rice-growing soils located in that area were contaminated by As. The maximum As concentration in topsoil (0-15 cm) reached 535 mg kg<sup>-1</sup> in this area, which was almost 9 times of the SPCS (60 mg kg<sup>-1</sup>) enacted in Taiwan. The contamination source of As in this area may come from the hot spring water of Thermal Valley. The hot spring water flowed out and mixed with the stream water which was used as irrigation water for the As-contaminated area of the Guandu Plain (Chang et al., 2007). Some studies indicated that the soil parent materials may also contribute to the high levels of As in soils of Guandu Plain (Su & Chen, 2008; Wu, 2007).

Arsenic in soils occurs mainly as inorganic species (Huang, 1994). In well-aerated soils, arsenate (As(V)) is the predominate form, whereas in reduced environment such as paddy soils, arsenite (As(III)) species prevails. Previous studies showed that As(V) in aerated soils will be reduced to more mobile and toxic As(III) in paddy soils and transferred to rice (Huang, 1994; Masscheleyn et al., 1991). Since As(III) is much more toxic, more soluble, and more mobile than As(V), it is a big chance that arsenic in rice-growing soils in Guandu Plain may transfer to rice and reduce rice yield or even impairs food safety. Meharg & Rahman (2003) indicated that As levels of paddy soils in Bangladesh irrigated with As-contaminated groundwater reached only 46 mg kg<sup>-1</sup> but the As concentration in rice grains were as high as 1.7 mg kg<sup>-1</sup> DW. Liao et al. (2005) also reported high levels of As in rice (0.5-7.5 mg kg<sup>-1</sup> DW) grown on As-contaminated soils in China. Whether the rice produced in highly As-contaminated soil in Guandu Plain is safe for human consumption or not is an emergent and important issue of local residents and government agency.

## 8. Uptake characteristics of two rice varieties growing in highly As-contaminated soils

In 2007, thirteen topsoil (0-15 cm) and rice (*Oryza sativa* L.) samples were collected together in 13 paddy fields (Fig. 5) with various levels of total As, ranging from 12 to 535 mg kg<sup>-1</sup>, in soil according to previous survey. Two Japonica rice cultivars, Taikeng No. 8 and Tainan No. 11, were planted in the 13 paddy fields. The 13 collected soil samples were acidic (pH 4.6-5.9) and fine textured (clay content 38-58%).

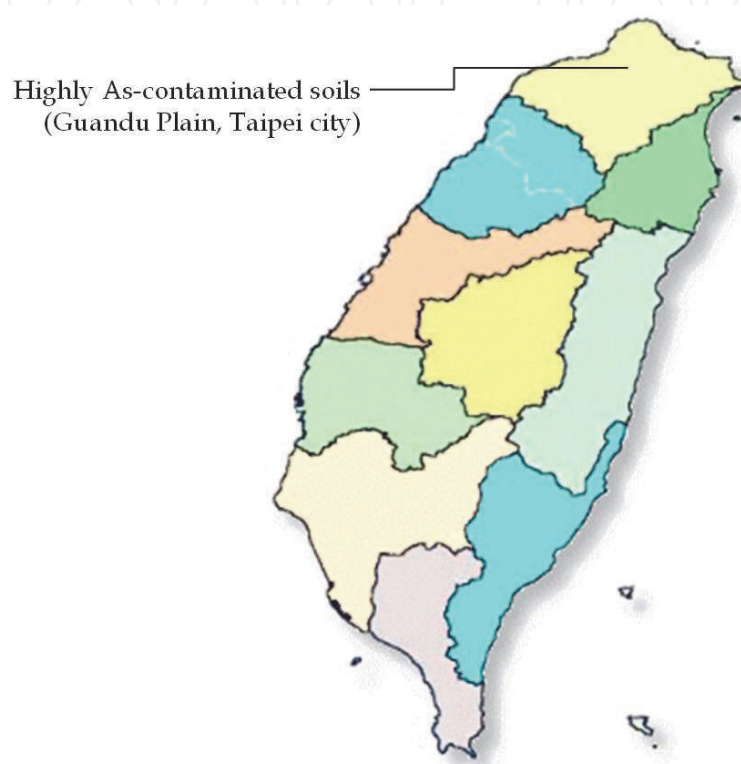


Fig. 5. Map of Taiwan and location where As-contaminated soil samples were collected (Su & Chen, 2008).

Although total soil As concentrations varied widely from 12.4 to 535 mg kg<sup>-1</sup>, As concentrations in brown rice were all below 0.35 mg kg<sup>-1</sup> DW and no adverse effects were shown on rice growth (Fig. 6). The Standards for the Tolerance of HMs in rice enacted in Taiwan does not include As. According to the statutory limits of As concentration in cereals or food crops constructed in different countries, the rice harvested from the As-contaminated soils in Guandu Plain was still safe for consumers.

Zavala & Duxbury (2007) suggested a global "normal" range of As concentration in rice as 0.08-0.20 mg kg<sup>-1</sup>, according to the combination of data set (n = 411) from their study and literatures. They also found that As levels in rice produced from Asia were significantly lower than that from U.S. or EU (Table 9). The As concentration in the majority of rice samples from Asia were lower than 0.098 mg kg<sup>-1</sup>. Compared with their findings, the As levels in rice grain produced in Guandu Plain were higher than the suggested global normal range even though they did not exceed the statutory limits. However, a pot experiment conducted in Taiwan also showed that As concentrations in brown rice ranged from 0.1 mg kg<sup>-1</sup> to as high as 0.4 mg kg<sup>-1</sup>, even the rice was cultivated in soils not seriously contaminated

by As (total As < 25 mg kg<sup>-1</sup>) (Simmons et al., 2008). In the study of Zavala & Duxbury (2007), the rice samples collected from many countries may not be representative of major rice consumption in those countries, it is necessary to conduct a comprehensive survey for As concentrations in different rice cultivars produced in Taiwan to estimate the normal levels of As in rice and compared with the data from Guandu Plain.

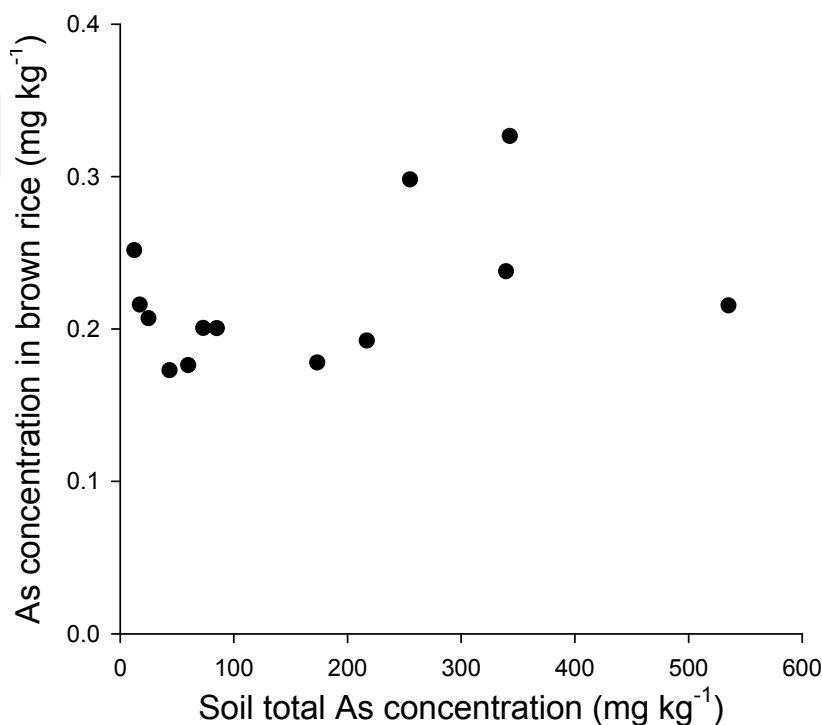


Fig. 6. The relationship between As concentration in soil and in brown rice collected together from 13 paddy fields in Guandu-Plain, north Taiwan.

Country/Institute	Regulation item	Statutory limit	Reference
Australia	cereals	1 mg kg <sup>-1</sup> FW	Brus et al., 2009
Canada	food crops	1 mg kg <sup>-1</sup> FW	Zandstra & Kryger, 2007
China	rice	0.15 mg kg <sup>-1</sup> DW*	URL, 2005
New Zealand	cereals	1 mg kg <sup>-1</sup> FW	Brus et al., 2009
Switzerland	food crops	4 mg kg <sup>-1</sup> DW	Gulz et al., 2005
United Kingdom	food in sale	1 mg kg <sup>-1</sup> FW	Warren et al., 2003

FW: fresh weight; DW: dry weight; \*: limit for inorganic As

Table 9. The statutory limits of arsenic concentration in cereals or food corps announced by WHO or different countries.

Many studies found that the arsenic concentration in rice grain harvested from As-contaminated soil could reach above 0.7 mg kg<sup>-1</sup>. However, rice produced in Guandu Plain is not apparently affected by As-contaminated soil. The availability of As in soil may be very low. To investigate the distribution of As forms associated with soil solid phases, an As-specific sequential extraction procedure proposed by Wenzel et al. (2001) was conducted for the collected 13 soil samples.



The results showed that relative portions of all As fractions were similar in 13 collected soil samples even the total soil As levels varied widely. The level of non-specifically-bound As in soil samples were all below 0.7% of total arsenic concentration in soils. Since the non-specifically-bound As represented the bioavailable As in soils and correlated well with As concentrations in soil solution collected in fields, the extremely low concentration of this As fraction may explain the facts that arsenic concentration in brown rice cultivated in highly As-contaminated soils of Guandu Plain were all below 0.35 mg kg<sup>-1</sup> (Fig. 6) and no adverse effects on rice growth.

Abedin et al. (2002) conducted a pot experiment using As-contaminated irrigation water to grow rice and suggested that As can be readily transferred from root to shoot if As levels in root exceeded the As storage capacity. However, a possible protection mechanism may exist in rice straw and husk to inhibit As accumulation in rice grain because the ratio of As concentration in grain/husk/straw is around 1/10/100 at the highest arsenate treatment (As levels in irrigation water = 8 mg L<sup>-1</sup>). This suggested that the suppression of As transfer from rice husk to grain may play a key role in reducing As concentration in rice grain. Since the primary As forms in soil environments are As(III) and As(V), As uptake by rice in paddy fields may mostly accumulate in rice roots. These transferring characteristics of As in rice may also contribute to the fact that low As levels in rice grain was found in Guandu Plain.

The amorphous hydrous Fe and Al oxide-bound As was the major fraction in soils (>50% of total As). This suggested that the amorphous materials in soils may play a central role in limiting the availability of arsenic in soils. However, the levels of specifically-bound As were around 10% of total arsenic in soils. Since the total arsenic concentration in some soils were very high and the application of lime materials or phosphorus fertilizer may potentially mobilize the specifically-bound As, further studies on these potential risks to agroecosystems were absolutely required.

Each fraction of arsenic in soil had significant linear relationship with total arsenic concentration in soil. This suggested that a single source of As contamination in Guandu Plain and the soil properties affected As adsorption in this area were similar. A significant linear relationship was found between Al<sub>o</sub> + 1/2Fe<sub>o</sub> (%) in soil and total soil As concentration (mg kg<sup>-1</sup>) ( $r^2 = 0.89$ ,  $P < 0.001$ ). This result indicated the source of As contamination may rich in amorphous Fe and Al. Since the Al<sub>o</sub> + 1/2Fe<sub>o</sub> (%) was good indicator of andic soil properties (Soil Survey Staff, 2006) and andesite is the parent material of soils in Guandu Plain, this findings suggested that the parent material may also contribute to the high levels of As in soils of Guandu Plain.

## 9. Conclusion

According to pot experiments and *in-situ* field scale experimental results, many plants can accumulate high concentration of HMs in their tissues and phytoremediation is feasible in removing HMs using suitable plant species. The critical concentrations of CaCl<sub>2</sub>-extractable Cd in soil under different levels of soil CaCl<sub>2</sub>-extractable Zn are constructed for farmers and authorities in Taiwan to prevent the production of Cd-contaminated rice by using the two equations developed in this study for Indica and Japonica rice cultivars. A single source of As contamination in Guandu Plain and a significant linear relationship was found between Al<sub>o</sub> + 1/2Fe<sub>o</sub> content in soil and total soil As concentration. Although total soil As concentrations varied widely from 12.4 to 535 mg kg<sup>-1</sup>, As concentrations in brown rice were all below 0.35 mg kg<sup>-1</sup> (DW) even the As regulation for rice was not announced in Taiwan.

According to the statutory limits of As concentration in cereals or food crops constructed in different countries, the rice harvested from the As-contaminated soils in Guandu Plain was still safe for consumers.

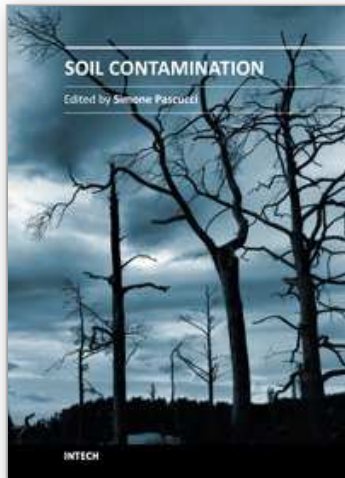
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## **Soil Contamination**

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Soil contamination has severely increased over the last decades, mainly due to petroleum hydrocarbons, solvents, pesticides, lead and other heavy metals from industrial wastes and human activities. The critical point regarding contaminated soil monitoring is the intrinsic difficulty in defining fixed monitoring variables and indicators as the establishment of any a priori criterion and threshold for soil quality can be still considered subjective. This book is organized into eight chapters and presents the state-of-the art and new research highlights in the context of contaminated soil monitoring and remediation strategies, including examples from South America, Europe and Asia. The chapters deal with the following topics: - monitoring of dioxin, furan, hydrocarbons and heavy metals level in soils - bioindicators and biomarkers for the assessment of soil toxicity - use of reflectance spectroscopy for soil contaminants and waste material detection - remediation technologies and strategies.

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