PHYTOREMEDIATION AND THE UPTAKE CHARACTERISTICS OF DIFFERENT RICE VARIETIES GROWING IN Cd- OR As- CONTAMINATED SOILS IN TAIWAN

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ABSTRACT

Mostly due to the announcement of Soil and Groundwater Pollution Remediation Act (SAGPRA) in 2000, many heavy metals-contaminated cropping lands were announced in Taiwan. Besides the application of soil remediation techniques, such as turnover or dilution and acid washing after the discovery of soil contamination, phytoremediation is another candidate for soil contamination because it is an environmental friendly technique. Rice would possibly accumulate cadmium (Cd) from Cd-contaminated soils, but the Cd uptake was quite different between different rice species and in different soil situations. Various Cd uptake models were used to efficiently predict their accumulation when it was grown in the Cd-contaminated soils with different Cd extraction concentrations and soil characteristics. Similar phenomenon was found for arsenic (As) and a survey investigated As concentration in brown rice harvested from contaminated soils with different levels of total arsenic concentrations will also be introduced in this paper.

Keywords: Cadmium, Phytoremediation, Rice varieties, Cd uptake models, Arsenic

INTRODUCTION

Even if the consumption of wheat products increased in recent years, rice still dominates the daily intake of cereals in Asian countries. In Taiwan, half of arable land is used as rice-growing field and two rice varieties including Indica and Japonica species are cultivated, but the latter is the major one (90%) because of taste preferences. Cropping lands contaminated by heavy metals were found by using the polluted irrigated water in the downstream of discharged water of industrial parks of Taiwan. The Soil and Groundwater Pollution Remediation Act (SAGPRA) announced in 2000 through the Taiwan Environmental Protection Administration (Taiwan EPA), that the cropping land with total soil Cd concentration exceeding 5 mg kg⁻¹ will be announced as Pollution Control Site and all farming activities will not be allowed. However, many field surveys in previous years showed Cd-contaminated rice can still be produced from fields with total soil Cd levels lower than 5 mg kg⁻¹. The government’s biggest challenge was the Cd pollution in rice grain since it was the major source of dietary intake of toxic Cd. The Standard for the Tolerance of Cd in rice has been reduced from 0.5 mg kg⁻¹ to 0.4 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 2007, approximate 400 ha of rural soils
in Taiwan were contaminated with single or combined heavy metals, according to SAGPRA of Taiwan. These contaminated sites were restored with turnover/dilution and acid washing methods to reduce the concentration of heavy metal 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 heavy metal concentration [1]. However, most hyperaccumulators used in removing these heavy metals have lower biomass and growing rate. The application of chemical agents has significant effect on increasing the phytoavailability and accumulation of heavy metals of plants [2, 3, 4, 5]. 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 heavy metals by plants [6, 7, 8, 9]. After the application of chemical chelating agents, the risk of groundwater contamination may be increased because the availability and mobility of metals increased [1, 10, 11]. 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 [9, 12].

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 heavy metal concentrations in soil conducted by the 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 has. of rice-growing soils located in that area were contaminated by arsenic ($\geq 60$ mg kg$^{-1}$). The maximum As concentration in topsoil (0-15 cm) reached 535 mg/kg in this area, which was almost 9 times of the Soil Pollution Control Standards (60 mg kg$^{-1}$) enacted in Taiwan. The contamination source of arsenic 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 [13]. Some studies indicated that the soil parent materials may also contribute to the high levels of As in soils of Guandu Plain [14, 15].

Arsenic in soils occurs mainly as inorganic species [16]. In well-aerated 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 [16, 17]. Since As(III) is much more toxic, more soluble, and more mobile than As(V), there 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 and Rahman [18] indicated that As levels of paddy soils in Bangladesh irrigated with As-contaminated groundwater reached only 46 mg kg$^{-1}$ but the As concentration in rice grains were as high as 1.7 mg kg$^{-1}$ DW. Liao et al. [19] also reported high levels of As in rice (0.5-7.5 mg kg$^{-1}$ 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.

**PHYTOREMEDIATION OF Cd-CONTAMINATED RURAL SOILS IN TAIWAN**

**In-Situ Selection of suitable phytoextraction plants in a Cd-contaminated site**

Two selection experiments were conducted where in-situ was carried out in a Cd-contaminated site in northern Taiwan. In the first experiment [20], eight flower species were planted to assess their accumulating capacity which include Azalea (Rhododendron spp.), Osmanthus (Osmanthus spp.), Star cluster (Pentas lanceolata), Scarlet sage (Salvia splendens), Common Cosmos (Cosmos bipinnatus), Zinnia (Zinnia elegans), Cockscamb (Celosia argentea), and Garden Verbena (Verbena tenera). Ten seedlings of each species were planted in a 2 m × 1.5 m area with three replicates.

The tested plants accumulated higher Cd concentration including Cockscamb, Star cluster, and Garden Verbena and the increases were about 30 to 40-fold compared with the initial Cd concentration in plant tissues. Although Cockscamb could accumulate high Cd concentration (86 mg kg$^{-1}$), but we saw brown and yellow colors in their leaves and veins, which probably resulted from the soil Cd’s toxicity. The increases of Cd in Common Cosmos, Scarlet sage, and Zinnia were about 10-fold compared with the initial Cd concentration in plant tissues. The color of leaves of Zinnia changed to yellow color when the Cd concentration was higher than 10 mg kg$^{-1}$. For those plants that...
accumulated lower Cd concentration (Osmanthus and Azalea), the Cd concentration ranged from 2 to 4 mg kg⁻¹ only.

A similar selection experiment was also conducted in a Cd-contaminated site in northern Taiwan. The tested plant species included Vinca rosea (*Catharanthus roseus*), Impatiens (*Impatiens wallerana*), Rainbow pink (*Dianthus chinensis*), Common Cosmos (*Cosmos bipinnatus*), Common Lantana (*Lantana camara*), Crepe myrtle (*Lagerstroemia indica*), Gardenia (*Gardenia jasminoides*), Cape Plumbago Blue (*Plumbago auriculata* Lamm), Cigar flower (*Cuphea ignea*), Scarlet sage (*Salvia splendens*), Wax Begonia (*Begonia Semperflorens-culturum*), and Parlor Palm (*Collinia elegans*). Ten seedlings of each species were planted in a 2 m × 1.5 m area with three replicates.

Experimental result shows that the most suitable and recommended garden flowers were Rainbow pink, Common Cosmos, Cigar flower, Scarlet sage, and Wax Begonia. Vinca rosea, Crepe myrtle, Gardenia, Wax Begonia, and Parlor Palm were not suitable to be recommended grown in Cd-contaminated soils.

**Selection of suitable phytoextraction plants using artificially Cd-contaminated soils**

Seedlings of five garden flower species were planted in the artificially Cd-contaminated loamy soils to assess their Cd accumulation when growing in control (CK) (0.43±0.15 mg kg⁻¹), Cd-10 (9.73±0.05 mg kg⁻¹), and Cd-20 (17.6±0.8 mg kg⁻¹) [21]. One seedling of Star cluster, French marigold (*Tagetes patula* L.), Impatiens, Garden verbena, or Scarlet sage 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 for 35 days in three replicates and plants were 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 (Cd < 0.375 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 control treatment (Table 1). Among the five plants, Impatiens grown in the Cd-20 treatment had the highest shoot Cd concentration near 100±11 mg kg⁻¹, which was more than the threshold of a Cd hyperaccumulator (100 mg kg⁻¹) reported by Baker et al. [22]. French marigold grown in Cd-10 and Cd-20 treatments accumulated 44.9±0.7 and 66.3±6.5 mg kg⁻¹ in their shoots and no toxic symptoms were observed in the appearance during pot experiment. Chen and Lee [20] reported that Star cluster, Scarlet sage, and Impatiens can accumulate 44, 12, and 42 mg kg⁻¹, respectively, in their leaves when in-situ growing in a Cd-contaminated site (Tatan village) in northern

<table>
<thead>
<tr>
<th>Plants</th>
<th>Treatments*</th>
<th>Shoot</th>
<th>Root</th>
<th>BCF</th>
<th>TF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star cluster</td>
<td>CK</td>
<td>ND b</td>
<td>ND a</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Cd-10</td>
<td>8.20±0.94 a &amp;</td>
<td>22.7±2.02 a</td>
<td>0.84</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Cd-20</td>
<td>10.7±2.8 a</td>
<td>18.9±17.2 a</td>
<td>0.61</td>
<td>0.57</td>
</tr>
<tr>
<td>French marigold</td>
<td>CK</td>
<td>ND c</td>
<td>ND c</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Cd-10</td>
<td>44.9±0.7 b</td>
<td>65.0±17.8 b</td>
<td>4.61</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Cd-20</td>
<td>66.3±6.5 a</td>
<td>113±21 a</td>
<td>3.77</td>
<td>0.59</td>
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<tr>
<td>Impatiens</td>
<td>CK</td>
<td>ND c</td>
<td>ND b</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Cd-10</td>
<td>48.9±11.7 b</td>
<td>29.5±9.6 ab</td>
<td>5.02</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>Cd-20</td>
<td>100±11 a</td>
<td>99.0±8.4 a</td>
<td>5.68</td>
<td>1.01</td>
</tr>
<tr>
<td>Garden verbena</td>
<td>CK</td>
<td>ND b</td>
<td>ND b</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Cd-10</td>
<td>21.5±5.5 a</td>
<td>39.3±13.5 a</td>
<td>2.21</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Cd-20</td>
<td>7.63±1.7 b</td>
<td>48.5±11.2 a</td>
<td>0.43</td>
<td>0.15</td>
</tr>
<tr>
<td>Scarlet sage</td>
<td>CK</td>
<td>ND b</td>
<td>ND c</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Cd-10</td>
<td>21.8±7.6 a</td>
<td>45.9±8.2 b</td>
<td>2.24</td>
<td>0.47</td>
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<tr>
<td></td>
<td>Cd-20</td>
<td>30.8±5.3 a</td>
<td>71.0±15.5 a</td>
<td>1.75</td>
<td>0.43</td>
</tr>
</tbody>
</table>

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

# means ± standard deviation (mg Cd kg⁻¹) (n = 3); ND: not detectable

The different small letters within same column of same plant tissue stand for statistical significance (p < 0.05)
Taiwan. For another in-situ experiment carried out in Chungfu village in northern Taiwan, the final Cd concentration in their leaves was 247 mg kg\(^{-1}\) in French marigold, 52 mg kg\(^{-1}\) in Garden verbena, 12 mg kg\(^{-1}\) in Impatiens, and 11 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.

Bioconcentration factor (BCF = shoot heavy metal concentration/soil heavy metal concentration) and translocation factor (TF = shoot heavy metal concentration/root heavy metal concentration) were two indexes mostly used to evaluate the accumulating capacity of heavy metals by plants. Besides the high concentration accumulated (100 mg kg\(^{-1}\)) [22], the BCF and TF should be more than one for a Cd hyperaccumulator [23, 24]. 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 less than one and ranged from 1.01-1.66. According to the standards summarized by Sun et al. [24] for a Cd hyperaccumulator, Impatiens accumulated more than 100 mg kg\(^{-1}\) in its shoot and its BCF and TF were all more than one. Impatiens was a potential Cd hyperaccumulator when growing in the artificially Cd-contaminated soils. The pot experimental result was against the in-situ selection experiment, which was possibly caused by the special variation and interaction of heavy metals in the field.

**In-Situ Selection of suitable phytoextraction plants in a combined metals-contaminated site**

Eight blocks (11 m by 4 m) located in central Taiwan, were used for in situ selection experiment. These areas were found to be relatively higher in Cr, Cu, Ni, and Zn concentrations [25]. After making a market survey, we selected 33 plant species of garden flower species for this in-situ experiment and two seedlings per plant species were planted in each of the 8 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 14 days, we observed withered and yellow color in the leaves of Bougainvillea while 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 injuries for the other 31 plants after growing in the 8 blocks for 14 days. Before planting, the initial concentration of metals in the shoot were in the levels of ND (<1.71 mg kg\(^{-1}\)) to 37.2 mg Cu kg\(^{-1}\), ND (<4.65 mg kg\(^{-1}\)) to 21.7 mg Cr kg\(^{-1}\), and 8.07 to 103 mg Zn kg\(^{-1}\), respectively. These 33 plant species have low Ni concentration (<10.1 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 relation to other plant species. Cockscomb and rainbow pink accumulated 317±117 and 231±73 mg Cr kg\(^{-1}\) in their shoots after in-situ growing for 33 days in the site. Their BCF was 1.7 in cockscomb and 1.3 in rainbow pink.

For Cu, the accumulation capacity of various tested plants was in the order of Cockscomb (117±40 mg kg\(^{-1}\)), Garden verbena (84.7±46.6 mg kg\(^{-1}\)), and Star cluster (80.4±80.6 mg kg\(^{-1}\)). The average Cu concentration of corn and food grains of China was 2.67 and 6.46 mg kg\(^{-1}\) [26] and the Cu concentration for foodstuff crops was less than 10 mg kg\(^{-1}\) [27]. The Cu concentration in the brown rice of Japan and Indonesia was 2.16-4.4, 2.9, and 3.41 mg kg\(^{-1}\), respectively [28, 29, 30]. 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\(^{-1}\). 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±38 mg kg\(^{-1}\)), French marigold (90.9±42.4 mg kg\(^{-1}\)), and Garden verbena (88.0±36.8 mg kg\(^{-1}\)). Because of the high Ni concentration in the surface soil of the 8 blocks (ranged from 314 to 412 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±127 mg kg\(^{-1}\)), Garden verbena (339±210 mg kg\(^{-1}\)), and Yellow Cosmos (328±157 mg kg\(^{-1}\)). However, the
BCF of Zn of Cockscomb was only 0.7 which revealed that the accumulation capacity of these 33 plants were weak.

Experimental result shows that the accumulation of heavy metals of woody and herbaceous plants after growing for 33 days was quite different. Similar to the result of Chen and Lee [20], herbaceous plants accumulated higher concentration of heavy metals in relation to woody plants. Except for the low Ni concentration in initial plants, the increase for heavy metals concentration in woody plants after they were growing for 33 days was about 3.1±2.9 fold for Cu, 2.5±1.5 fold for Cr, and 4.3±3.1 fold for Zn, respectively. Herbaceous plants have higher uptake of heavy metals in relation to woody plants and their increase on the concentration of metals are 9.4±6.5 fold for Cu, 5.1±2.7 fold for Cr, and 8.9±6.1 fold for Zn, respectively.

Large area in-situ phytoremediation in a combined metals-contaminated site

Twelve plants species were selected from 33 plant species testing in a site contaminated by combined heavy metals (Cu, Cr, Ni, and Zn) in central Taiwan to study the feasibility of in-situ phytoremediation [31]. The total area for each plant species was 0.1 ha and their planting density was 10,000 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 large area experiments which were conducted twice after it was growing for one month and two months, showed that these 12 plant species can grow well in this combined heavy metals-contaminated site. The concentrations of Cr, Cu, Ni, and Zn in the shoots increased after growing for 31 days compared with those before planting. The extension of their growth time, from one month to two months in the contaminated site, has positive effect on increasing their accumulation of heavy metals. Except for Zn, these 12 plant species can accumulate higher concentrations of heavy metals in their shoots than those of roots (Table 2). However, TF of only some of the tested plant species were more than one from the result of in-situ experiment, especially for Zn.

THE UPTAKE CHARACTERISTICS OF DIFFERENT RICE VARIETIES GROWING IN Co-CONTAMINATED SOILS IN TAIWAN

Experimental design of Cd-contaminated field studies

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 [32]. Twelve rice cultivars of Indica or Japonica varieties were planted in each field with 5-9 replicates for each cultivar depending 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)

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

# TF = shoot heavy metal concentration / root heavy metal concentration
and November (harvest 2) of the two years. Total numbers of soil and rice plant samples in this study were both 3198. The total soil Cd concentration in studied fields ranged from 0.06 mg kg\(^{-1}\) to as high as 27.8 mg kg\(^{-1}\), the maximum level is about 6-fold higher than the Soil Pollution Control Standards (5 mg kg\(^{-1}\)) enacted in Taiwan. Around 27% of the studied field area was defined as Cd-contaminated soil according to the regulation standards.

**Cd concentration in rice grains of different species**

Soil pH, CEC, and soil organic matter (SOM) varied widely in the 19 paddy fields. 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 if the rice is planted in low or high Cd-contaminated fields or in different climates (Fig. 1). Higher variation was found in the concentration of Cd in Indica brown rice compared with that in Japonica brown rice. The majority of brown rice harvested from seriously Cd-contaminated fields was not safe for consumers. He et al. [33] 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.

Liu et al. [34] reported that Cd was not evenly distributed in different parts of rice grain. The results of their pot experiments planting six rice

![Graphs showing the relationship between CaCl\(_2\) extractable Cd concentration in soil and Cd concentration in brown rice (•: Japonica species, ○: Indica species) harvested in May and November.](image-url)
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. [35] reported that Cd concentration in 6 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 [36]. 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.

Prediction of Cd concentration in rice grains

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, 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, it is necessary to develop a simple and reliable soil test to predict Cd concentration in rice grains.

Previous studies indicated that 0.01M CaCl₂ [37], 0.1M HCl [38], 0.43M HNO₃ [39], and 0.05M EDTA (Na₂-EDTA·2H₂O) [40] are ideal extractants to estimate soil available Cd. 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 CaCl₂:

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

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

The CaCl₂ 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 [41, 42]. The regression equations may be used in Taiwan as a screening tool to predict Cd concentrations in rice grains before rice cultivation. As shown in Table 3, the critical concentrations of CaCl₂-extractable Cd in soil under different levels of soil CaCl₂-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 CaCl₂-extractable Zn in soil ranged usually from 0.1 to 50 mg kg⁻¹ when the total soil Zn concentration is less than 600 mg kg⁻¹, which is the Pollution Control Standards for cropping lands enacted in Taiwan. According to the equations, less Cd will be accumulated in rice grain if the soil CaCl₂-extractable Zn is getting higher, therefore, only the critical concentrations of CaCl₂-extractable Cd in soil under the soil CaCl₂-extractable Zn lower than 50 mg kg⁻¹ are presented in Table 3. If the measured soil CaCl₂-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⁻¹). Further studies are required to validate the practicability of regression equations.

Table 3. Critical concentrations of CaCl₂-extractable Cd (mg kg⁻¹) in soil under different levels of soil CaCl₂-extractable Zn (mg kg⁻¹) for the two rice varieties. Cadmium concentration in rice grain will exceed 0.4 mg/kg if the measured soil CaCl₂-extractable Cd is higher than the critical concentration.

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>CaCl₂-extractable Zn in soil (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Indica</td>
<td>0.007</td>
</tr>
<tr>
<td>Japonica</td>
<td>0.027</td>
</tr>
</tbody>
</table>
To predict Cd concentration in rice grain, Simmons et al. [43] also developed a regression equation using soil pH (1:5) and CaCl₂ 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 $r^2$ value of 0.638. If air-dried soil samples were used for Cd–CaCl₂ 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 CaCl₂ 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. [44] recently developed a multiple regression model using 0.43M HNO₃ extractable Cd, pH, clay, and soil organic matter as predictors to predict Cd levels in rice grain harvested from the paddy fields in Fuyang, Zhejiang province, China. The model performed much better ($r^2_{adj} = 0.661$) than the linear model using only 0.01M CaCl₂ extractable Cd as a predictor ($r^2_{adj} = 0.281$). The field study in Taiwan as mentioned above also developed a multiple regression model using 0.43M HNO₃ 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 phytoavailability 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 CaCl₂ 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.

**THE UPTAKE CHARACTERISTICS OF RICE GROWING IN As-CONTAMINATED SOILS IN TAIWAN**

**Field survey for As concentration in brown rice**

In 2007, 13 topsoil (0-15 cm) and rice (*Oryza sativa* L.) samples were collected together in 13 paddy fields with various levels of total As, ranging from 12 to 535 mg kg⁻¹, 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%).

**Food safety of brown rice harvested from Guandu Plain**

Although total soil As concentrations varied widely from 12.4 to 535 mg kg⁻¹, As concentrations in brown rice were all below 0.35 mg kg⁻¹ DW and no adverse effects were shown on rice growth (Fig. 2). The Standards for the tolerance of heavy metals 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 (Table 4), the rice harvested from the As-contaminated soils in Guandu Plain was still safe for consumers.

Zavala and Duxbury [45] suggested a global “normal” range of As concentration in rice as 0.08-0.20 mg kg⁻¹, 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. The As concentration in the majority of rice samples from Asia were lower than 0.098 mg kg⁻¹. 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\(^{-1}\) to as high as 0.4 mg kg\(^{-1}\), even the rice was cultivated in soils not seriously contaminated by As (total As < 25 mg kg\(^{-1}\)) [46]. In the study of Zavala and Duxbury [45], 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.

Factors limiting As transfer from soil into brown rice

Many studies found that the arsenic concentration in rice grain harvested from As-contaminated soil could reach above 0.7 mg kg\(^{-1}\) [18, 52]. 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. [53] 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 if the total 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 [53, 54], 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\(^{-1}\) and no adverse effects on rice growth. Abedin et al. [55] 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). Williams et al. [56] found a highly significant linear relationship between As levels in rice shoot and husk, but the relationship between As levels in shoot and polished rice was not linear where the As levels in polished rice was curtailed at higher As levels in shoot. 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. Marin et al. [57] reported that DMAA (dimethylarsinic acid) was more readily transferred to rice shoot compared with MMAA (monomethylarsonic acid), As(V), and As(III) when rice seedlings were grown in nutrient solution amended with different As compounds. On the other hand, MMAA, As(V), and As(III) preferred to accumulated in rice roots. Since the primary As forms in soil environments are As(III) and As(V) [17], 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 [58, 59], 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 $A_l + \frac{1}{2}Fe_o$ (%) in soil and total soil As concentration (mg kg$^{-1}$) ($r^2 = 0.89$, $P < 0.001$). This result indicated the source of As contamination may rich in amorphous Fe and Al. Since the $A_l + \frac{1}{2}Fe_o$ (%) was good indicator of andic soil properties [60] 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.

CONCLUSION

According to pot experiments and in-situ field scale experimental results, many plants can accumulate high concentration of heavy metals in their tissues and phytoremediation is feasible in removing contaminants using suitable plant species. The critical concentrations of CaCl$_2$-extractable Cd in soil under different levels of soil CaCl$_2$-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 $A_l + \frac{1}{2}Fe_o$ content in soil and total soil As concentration. Although total soil As concentrations varied widely from 12.4 to 535 mg kg$^{-1}$, As concentrations in brown rice were all below 0.35 mg kg$^{-1}$ (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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