Cd and As contamination of agricultural products and countermeasures in Japan

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Abstract: Many heavy metals exist in minute amounts in natural agricultural soil. However, when their amounts exceed a certain level due to pollutants brought from outside, soil contamination occurs and agricultural products become contaminated. There have been many cases in Japan of heavy metal contamination originating from old mines and smelters, and soil contamination of agricultural land has become a social issue. In particular, cadmium (Cd) is one of the most harmful heavy metals. If agricultural products absorb an excessive amount of Cd, they may adversely affect people’s health, and therefore allowable concentrations are regulated by law. If agricultural land has become contaminated with Cd, measures for minimizing the absorption of Cd by agricultural crops are necessary; these include: (1) soil dressing, (2) water management (paddy field), (3) chemical cleaning of soil, (4) phytoextraction, and (5) use of different varieties and rootstock.

Rice consumption is not only a major source of Cd but also that of arsenic (As) for the population of Asia. Flooding of paddy fields is effective in reducing grain levels of Cd; however, anaerobic conditions in paddy soil lead to As mobilization and, therefore, As uptake by rice could increase. A new study has been launched investigating whether As and Cd concentration in rice grains can be lowered simultaneously by controlling irrigation water and by using a rice cultivar with low Cd uptake, along with agricultural materials.

Key Words: Cd, As, rice, chemical washing, phytoextraction

1. Introduction

After the first metal mine in Japan began operating in the early eighth century, metal mining became an established industry between the late sixteenth and early seventeenth centuries, and especially during the Edo period (1603–1867) when many metal mines were developed (Arao et al., 2010). However, it was not until the Meiji Era (1868–1912) when Japanese metal mines started to be run as modern businesses; major mines were scaled up through direct management by the national government. Among various metals mined, there was a remarkable increase in the demand for copper (Cu) and zinc (Zn) as raw materials for general and military industries, and so the production outputs of mines increased dramatically. After the end of World War II, further demand for metals was driven by rapid economic growth in the 1960s. Enormous quantities of metal ore were mined and smelted to meet this demand, yet domestic output alone could not keep pace and increasing quantities had to be imported from overseas. During this historical process, various heavy metals were released into the environment, causing extensive soil contamination by toxic metals such as cadmium (Cd).

The three heavy metals designated by the Agricultural Land Soil Pollution Prevention Law (1970) of Japan as specified toxic substances are Cu, Cd, and arsenic (As). Past incidents of acute soil contamination with these three metals include the following. The Ashio Copper Mine Mineral Pollution incident—in which Cu spilled from the Ashio Copper Mine and contaminated the soils of paddy and upland fields along the Watarase River in the middle of the Meiji Era—is regarded as the beginning of Japan’s problems with pollution.

Another example was itai-itai disease, which occurred in the lower reaches of the Jinzu River and was caused by Cd that had leaked into the river from the Kamioka Copper Mine upstream (Toyama Prefectural Itai-itai Disease Museum, 2012). Thereafter, Cd contamination of agricultural land along rivers with a mine upstream caused problems at several locations in Japan. Furthermore, smoke emitted by Zn and Cu smelters into the atmosphere contaminated neighboring agricultural land with Cd. In another case, As compounds that spilled from some mines in Kyushu and the northern part of the Chugoku region contaminated nearby agricultural land, damaging the paddy rice and the health of the local population that relied on contaminated well water.

The Japanese government has been investigating the quantities of these metals present in agricultural soil and their concentrations in agricultural crops, and has been implementing
countermeasures using the soil dressing method through the Special Land Improvement Project for Pollution Prevention, etc. The heavy metal responsible for the most serious contamination of agricultural land is Cd. Today, there are few new cases of soil contamination by Cd from mines and smelters because of strict regulations and management by law. Countermeasures have been completed in most of the Cd polluted sites. But low Cd polluted field exits around these sites.

Cd is toxic to humans at concentrations lower than those at which it is toxic to plants because its effects on humans are cumulative. A health-based guidance value for Cd of 25 μg kg\(^{-1}\) bodyweight per month was established by the JECFA (2010), and a ML of 0.4 mg kg\(^{-1}\) for Cd in polished rice has been adopted by the Codex Alimentarius Commission. Rice is a staple crop in Asia and is also the principal source of dietary intake of Cd in the Japanese population; therefore, minimizing the intake of Cd from rice is an important health goal. Although flooding of paddy fields effectively reduces grain levels of Cd (Masui et al. 1971; Otake 1992; Ogawa 1994; Yamada et al. 1971), anaerobic conditions in paddy soil lead to As mobilization which could consequently increase the uptake of As by rice (Koyama, 1975).

The Ministry of Agriculture, Forestry, and Fisheries of Japan analyzed the As content of staple crops in Japan and found that an average value of total As and inorganic As concentration in polished rice were 0.14 mg kg\(^{-1}\) and 0.12 mg kg\(^{-1}\), respectively (2014). As concentration in most other agricultural products was below the detection limit. Although sea food is a common source of total As, most of As in sea food are organic form which is less toxic than inorganic As. A market-basket survey, with As-speciation analysis, indicated that rice is a major source of dietary intake of inorganic As in the Japanese population (Oguri et al. 2014). The intake of inorganic As in rice carries a significant risk for cancer in populations for whom rice is a staple food. In some cases, human intake of inorganic As from the consumption of rice exceeds that from drinking water. A ML for inorganic As (0.2 mg kg\(^{-1}\)) in polished rice has been adopted by the 37th Session of the Codex Commission (2014).

A new study has been launched investigating whether As and Cd concentration in rice grains can be lowered simultaneously by controlling irrigation water and by using a rice cultivar with low Cd uptake, along with agricultural materials.

2. Countermeasures against Cd contamination of agricultural crops

2.1. Soil dressing

Soil dressing is a very effective soil improvement method that prevents agricultural products from being contaminated with Cd. It involves covering the contaminated agricultural soil with uncontaminated soil (Yanagizawa et al. 1984; Yamada 2007). There are several ways of improving polluted soils by soil dressing. As of 2012, 91.0% of the total polluted land (7,592 ha), designated by the Agricultural Land Soil Pollution Prevention, was remedied by applying uncontaminated soil and/or replacing the soil (MOE 2013).

However, if the soil dressing is not sufficiently thick, the roots of rice will reach the contaminated soil, decreasing the effect of the countermeasure. Experiments conducted to date show that the required thickness of soil dressing is 20–40 cm, depending on the soil, method, and environment. If the land is cultivated too deeply, causing mixing with contaminated soil, or if the Cd content in irrigation water is high, the effect of soil dressing will decrease over time.

While soil dressing is very effective for reducing Cd absorption by paddy rice, many problems have been pointed out with the method. The biggest problem is the cost: the unit cost of soil dressing is very high, and so the method is impractical without imposing a large penalty on the responsible party or without a sufficiently large federal subsidy (Otake 1992). Second, soil dressing materials are difficult to procure: it is no longer easy to obtain large quantities of high-quality mountain soil that contains an appropriate amount of clay. In addition, soil dressing causes a decline in soil fertility on paddy fields, because the mountain soil used for soil dressing is often sterile, containing little humus. Therefore, it is necessary to restore soil fertility by applying large quantities of organic fertilizers for many years. In addition, soil dressing raises the paddy surface, making it necessary to construct levees and improve irrigation and drainage facilities.
2.2. Water management and control of Cd absorption by paddy rice using a combination of materials

When a paddy field is flooded and the soil is under reducing conditions, hydrogen sulfide will be generated, which equilibrates with sulfate ions. The hydrogen sulfide is dissociated into sulfide ions depending on the pH and precipitates with cadmium ions as cadmium sulfide (CdS), which is less soluble in water as shown by its low solubility product $(K_{sp} = 5.0 \times 10^{-28})$:

However, when the field is drained and the soil is under oxidative conditions, CdS is ionized to form cadmium sulfate (CdSO₄), which is soluble in water (Ito and Iimura 1976). This means that the solubility of Cd changes depending on the redox potential (Eh) of the soil (Iimura and Ito 1978), and the Eh of paddy field soil can be controlled through water management. Consequently, it is possible to control Cd absorption by paddy rice through water management. Therefore, Cd absorption by paddy rice is suppressed by filling the paddy field with water to prevent the soil from drying and thus reducing the amount of Cd released into the water. Several studies on the effects of water management on Cd absorption by paddy rice confirm that it is possible to control the Cd content in brown rice through water management during the growing period (Masui et al. 1971; Otake 1992; Ogawa 1994; Yamada et al. 1971).

Furthermore, if the soil pH changes to neutral, Cd bonds with phosphate ions or carbonate ions and becomes less soluble in water. An increase in pH enhances the cation exchange capacity (CEC) of the soil, which has a variable charge and increases the Cd ion adsorption to clay. Therefore, Cd absorption by paddy rice can be suppressed by applying a material that increases the pH of the soil, such as calcium carbonate, calcium silicate, autoclaved lightweight concrete (ALC), or fused silicate phosphate (Otake 1992; Ogawa 1994; Hasegawa et al. 1995; Yamada et al. 1973). However, if the soil acts as a strong buffer, application of materials may not be sufficient to increase the pH of the soil. For such soil, the control of Cd absorption by paddy rice through the application of materials may be limited and sometimes close to zero. Thus, a combination of material application and the aforementioned water management technique is used to enhance the control of Cd absorption by paddy rice (Otake 1992).

2.3. Chemical washing method for contaminated soil

Soil washing is a remediation technology that involves mixing contaminated soil with a washing material in liquid form, extracting contaminant from the soil, and then processing the extract containing Cd in a purification system. This chemical method can be used to remove the contaminant effectively and restore the soil for a relatively short period. Although studies on washing-based soil remediation have been conducted by several private companies, many of these studies targeted old factory sites, etc., and involve transporting contaminated soil to processing facilities for purification, where both the volume of contaminated soil and its Cd content are reduced by separating the clay fraction, which has a higher concentration of heavy metals. However, it is difficult to apply these methods to paddy fields.

In order to apply the washing method to paddy fields, the following need to be completed: (1) selection of washing materials that have a low environmental burden, high efficiency, and low-cost; (2) development of an onsite washing and drainage treatment system; (3) ensuring good soil fertility and crop growth; and (4) maintenance of the washing effect. A washing method that addresses these issues and is applicable to paddy fields is described below.

Examination of optimum washing conditions in a preliminary test, ferric chloride was selected as a washing agent and the optimum washing conditions using this material were examined (Makino et al. 2006; Makino et al. 2008). When ferric iron chloride is added to the water in a paddy field, the dissociated iron ions that may form hydroxide precipitate with the release of protons, thus reducing the pH of the water and eluting Cd:

In order to optimize soil washing for paddy fields, it is necessary to examine various conditions such as the amount of washing agent applied, soil–water ratio, agitation time, material washing cycles,
and water washing cycles. As for the soil–water ratio, the larger is the liquid phase fraction, the more efficient is the removal of Cd. However, due to the height of paddy field levees and the structural limitations of the tractors used in the washing process, the soil–water ratio should be approximately 1:2. Although the Cd extraction rate increases with the number of material washing cycles, there should be only one material washing cycle in consideration of the amount applied on-site and the Cd removal effect. In order to reduce the residual chlorine concentration to a nontoxic level (500 mg l-1 or lower), there should be three or more water washing cycles and the chlorine concentration should be monitored on-site during the work.

In previous studies on washing contaminated soil, tests were conducted on local paddy fields using hydrochloric acid (Takijima et al. 1973) or EDTA (Nakasima and Ono 1979) and the effect was confirmed. However, the on-site processing of the wastewater left after washing had not been examined for a Cd-contaminated paddy field. In order to apply washing technology to paddy fields, on-site treatment of wastewater from the washing process is required. In other words, after the on-site washing process is completed using the aforementioned optimum washing conditions, Cd in the wastewater should be collected and removed using on-site wastewater processing facilities.

The procedure was as follows (Fig. 1):

(1) the local Cd-contaminated paddy fields were materialwashed (to extract Cd from the soil) and then (2) waterwashed (to remove residual Cd and chlorine), and finally, (3) the wastewater was processed (to collect and remove Cd from the wastewater using an on-site wastewater treatment system employing a chelating agent).

After water-wash cycles, the concentration of residual chlorine in the paddy field water was lower than 500 mg l⁻¹, the level at which chlorine is considered to begin affecting the growth of crops. The Cd concentration in the wastewater after washing with ferric chloride and water can be reduced to lower than the effluent standard (0.1 mg l⁻¹) and the environmental standard for water quality (0.003 mg l⁻¹) by collecting and removing Cd using an on-site wastewater treatment system (Takano and Makino 2006; Makino et al. 2007).

The Cd content in the washed soil in the area measured using the 0.1 mol l⁻¹ hydrochloric acid extraction process was reduced to 20–40% of that in the unwashed area (Cd reduction rate of 60–80%), confirming that this washing method effectively removes Cd.

As some soil properties, such as exchangeable potassium, exchangeable magnesium and pH, had been changed after the soil washing, they can be corrected by applying fertilizers.

The washing process had very little effect on the growth and yield of paddy rice, and although certain changes in soil fertility do occur, fertilizers can correct them. Thus, we conclude that this
washing method does not adversely affect soil fertility or the growth of paddy rice to a significant degree (Takano and Makino 2006; Makino et al. 2007).

The Cd concentration in brown rice dramatically reduced after the washing treatment to 30–40% of that in the nonwashed area (Takano and Makino 2006; Makino et al. 2007). The total cost of the chemical washing method depends on the Cd concentration of soil and other factors, and is estimated to be approximately 60% of that of soil dressing.

Akahane et al. (2013) evaluated the effects of soil washing with ferric chloride (FeCl₃) on Cd concentrations in soil solutions and Cd absorption by two spinach cultivars in pot experiments. Soil washing with FeCl₃ affected the exchangeable cations (i.e. calcium increased and magnesium decreased). The Cd concentration in the soil solution from washed plot was lower than that in the solution from the unwashed plot throughout the spinach growth period, which was attributed to the exchangeable Cd content in both soils, because the fraction equilibrated with the Cd concentration in the soil solution. The exchangeable cation composition was affected by soil washing, but no significant difference in spinach yield was observed between the washed and unwashed plots. The leaf Cd concentration in the two spinach cultivars was up to 70% lower in the washed soils. This study suggested that soil washing in rice paddy fields with FeCl₃ was effective for controlling the Cd absorption risk of upland crops such as spinach.

2.4. Purification technology based on phytoextraction

Phytoextraction has been studied in the US and in Europe as a method of preventing soil and groundwater pollution. This technology aims to purify the environment by utilizing the functions of plants. For some time now, it has been known that some plants can efficiently absorb Cd in soil. For example, tall goldenrod (Solidago altissima L.) in the composite family and pennycress (Thlaspi caerulescens L.) in the brassica family (Brown et al. 1995; Hammer and Keller 2003) are considered to be able to absorb large amounts of Cd. However, these plants may not be suitable for large-scale phytoextraction because they are small and grow slowly, and has basal rosettes of leaves, making them difficult to harvest mechanically.

To Select plants to be used in phytoextraction, Ishikawa et al. (2006) grew leaf mustard (Brassica juncea L.), maize (Zea mays L.), sugar beet (Beta vulgaris L.), and rice (Oryza sativa L.) in pots filled with two kinds of Cd-contaminated soil (gray lowland soil and ando soil) for 1 month. The rice and sugar beet planted in both soils showed the highest level of Cd absorption in their aboveground parts. Since sugar beet is a cold-climate crop, they concluded that rice was the most appropriate purifying plant.

Murakami et al. (2007) selected maize (Zea mays L.), soybean (Glycine max (L.) Merr.), and rice (Oryza sativa L.) as the major crops grown in paddy fields and upland fields converted from paddy fields in Japan. These plants were grown in pots filled with three kinds of Cd-contaminated soil (two kinds of gray lowland soil and one kind of ando soil) for 2 months. Soybean and rice showed the highest level of Cd absorption. Since soybean defoliates after the blooming stage, rice was the most promising purifying plant in Japan.

As rice does not have replant failure and has an established growing system and mechanized harvesting system, rice is considered to be the most appropriate plant to be used for the phytoextraction of paddy fields. Moreover, it has been discovered that not only the Japonica-Indica hybridized variety but also certain kinds of the Indica variety have a high-Cd-absorption ability (Arao and Ae 2003). The variety Cho-ko-koku has been selected as a candidate phytoaccumulator for paddy fields contaminated with low to moderate levels of Cd, and a field trial of phytoextraction by using this cultivar has been launched. (Murakami et al. 2009). The heavy metal ATPase 3 (OsHMA3) was identified as the gene that controls root-to-shoot Cd translocation rates in Cho-ko-koku (Miyadate, et al., 2011).

In order to achieve commercialization of phytoextraction technology, the aforementioned varieties of rice with a high-Cd-absorption ability as well as efficient harvesting of Cd-containing rice plants, on-site drying, packaging, storage, and transportation of such rice plants, and safe treatment of absorbed Cd are important. To address these challenges, Murakami et al. (2010) have (1) established
an integrated mechanized system of harvesting, on-site drying, and packaging of Cd-containing rice, and (2) developed an efficient system for collecting Cd involving the incineration of the harvested rice plants. The integrated system, from harvesting grown rice to incineration, is shown in Fig. 2.

In a paddy field with good drainage conditions, to ensure on-site drying, rice plants should be harvested using a combine harvester that separates rough rice and straw. The straw should be sun-dried and rolled for collection. In contrast, in a paddy field with poor drainage, the rough rice and straw should be harvested together using a self-propelled whole crop harvester, and then rolled, collected, and stored under a moisture-permeable waterproof sheet (sheet that lets internal moisture pass through to the outside but does not allow rainwater from the outside through to the inside) in the field for about 2 months (Taniguchi 2006; Murakami 2007; Ibaraki and Taniguchi 2007).

Next, it is necessary to efficiently and safely collect the Cd contained in the harvested rice. For this purpose, an incineration system has been developed that incinerates dried rice, volatilizes most of the Cd in the harvest as metallic ions, captures them with a bug filter, collects nearly the whole amount as fly ash, and leaves no Cd in the main ash (incinerated ash), preventing the discharge of effluent gas.

In an incineration test, when harvested rice was incinerated at higher than 900ºC inside the incinerator, there was no unburned matter, and the Cd collection rate from fly ash was 99.6%. The Cd concentration in the effluent gas was lower than 0.01 mg kg⁻¹, with almost no release into the atmosphere (Taniguchi 2006; Ibaraki and Taniguchi 2007).

While phytoextraction is a low-cost, environment-friendly method, it is time-consuming. Since it is difficult to recover Cd-contaminated soil in a single step by phytoextraction, two to four applications (harvests) may be necessary.

In a test conducted in three local fields, phytoextraction by the Indica rice varieties grown for 2–3 years without irrigation after drainage reduced the soil Cd content by 18–38%, and reduced the grain Cd content in subsequently grown Japonica food rice by 39–50% (Murakami et al. 2009; Honma et al. 2009; Ibaraki et al. 2009). The total cost of the phytoextraction method depends on the Cd concentration of soil and other factors, and is estimated to be less than one-seventh of that of soil dressing.

The world rice core collection (WRC), consisting of 69 accessions which covers the genetic diversity of almost 32 000 accessions of cultivated rice, was also used to select candidate for phytoremediator, and Jarjan (WRC28), Anjana Dhan (WRC30) were found (Uraguchi et al. 2009).
Abe et al. (2013) have developed new high-Cd-accumulating practical rice lines with non-shattering derived from gamma ray mutation from Jarjan, and Anjana Dhan.

2.5. Use of different varieties and rootstock

It has been known for some time now that the Cd-absorption capability of paddy rice differs from variety to variety. If grown in the same environment, the Japonica variety such as Koshihikari generally has a lower Cd concentration than the Indica variety, which is a long-grain rice (Arao and Ae, 2003).

Energetic heavy-ion beams have been recently used to generate mutants in higher plants because they induce mutations with high frequency at a relatively low dose, and they induce a broad spectrum of phenotypes without affecting other plant characteristics. Carbon ion-beam irradiation produced three rice mutants with <0.05 mg Cd·kg\(^{-1}\) in the grain compared with a mean of 1.73mg Cd·kg\(^{-1}\) in the parent, Koshihikari (Ishikawa et al. 2012). There were no apparent differences in plant or grain morphologies between Koshihikari and low Cd mutants, and there were no significant differences in grain and straw yield and even in eating quality. Mutants produced by ion-beam radiation are not transgenic, so they are more likely to be accepted by consumers. Low cadmium Koshihikari mutant-2 is applied for the registration of commercial cultivar as Koshihikari-kan-1 (Fig. 3).

It has also been found that with soybean, the Cd absorption ability and Cd concentration in the fruit body differ depending on the variety (Arao et al. 2003; Ishikawa et al. 2005; Sugiyama 2007), and this research is expected to result in the development of a new low-Cd-absorption variety. Significant inter-cultivar differences of soybean seed Cd concentrations arise from the inter-cultivar differences in root Cd accumulation ability. The Cd concentration in the shoots of plants at the vegetative stage is already controlled by the roots Cd concentration in the same way that it determines seed Cd concentration (Sugiyama et al. 2010).

Eggplant (Solanum melongena) fruits in Japan tend to have higher Cd concentrations than international maximum limits for fruiting vegetables. Grafting onto the Solanum torvum cultivars reduced Cd concentrations of eggplant fruits by about 1/2 to 3/4 compared with grafting onto eggplant or self roots (Takeda et al. 2007; Arao et al. 2008). For some vegetables including tomato, grafting is a useful tool to cope with problems of soil-borne diseases. If there is a rootstock, which translocates low Cd from root to shoot in commercially available rootstocks, it would be a practical method for reducing the Cd concentration of vegetables by grafting onto the rootstock.

Physiological properties involved in the differences in shoot Cd accumulation among rice cultivars (Uraguchi et al. 2009) and between Solanum species (Mori et al. 2009) were characterized. Cd was allocated in the central cylinder for Solanum melongena and localized around the endodermis.
for Solanum torvum (Yamaguchi et al. 2011a). The results demonstrated that the xylem loading process is a major factor in determining shoot Cd accumulation in both rice and Solanum species.

Ishikawa et al. (2011) have visualized and quantitatively analysed the real-time Cd dynamics from roots to grains in typical rice cultivars that differed in grain Cd concentrations. by the positron-emitting tracer imaging system (PETIS), and revealed that the high-Cd accumulating rice cultivars were characterized by rapid and abundant Cd transfer to the shoots from the roots, a faster transport velocity of Cd to the panicles, and Cd accumulation at high levels in their panicles, passing through the nodal portions of the stems where the highest Cd intensities were observed.

In rice nodes, the diffuse vascular bundles, which enclose the enlarged elliptical vascular bundles, are connected to the panicle and have a morphological feature that facilitates xylem-to-phloem transfer. Elemental maps of Cd, Zn, Mn, and S in the vascular bundles of node I were obtained by synchrotron micro-X-ray fluorescence spectrometry and electron probe microanalysis (Yamaguchi et al. 2012). The results provide evidence that transport of Cd, Zn, and Mn is differentially controlled in rice nodes, where vascular bundles are functionally interconnected.

Genetic analyses of shoot Cd accumulation have been reported in rice (Ishikawa et al. 2010; Ishikawa et al. 2012) and Solanum species (Yamaguchi et al. 2010). Such genetic information will be useful in developing efficient new varieties with low Cd trait.

2.6. A simple and quick on-site test for trace levels of Cd in food

Measuring Cd concentrations in plants is costly and troublesome, because it requires expensive analytical instruments for such procedures as ICP-OES, ICP-MS, or flameless atomic absorption spectrophotometry. These methods also require the services of analytical experts and the use of exhaust systems that can remove the toxic gases produced during acid digestion.

Recently, an immunochromatographic assay kit for detecting Cd in rice was developed by Kansai Electric Power Co. of Japan (Tawarada et al., 2003; Sasaki et al., 2007). This method uses the antigen–antibody complex reaction between the Cd–EDTA complex and an anti-Cd–EDTA antibody that reacts specifically with this complex to detect Cd at concentrations of 0.01 mg L⁻¹ or higher; the results are read by the degree of color developed on a test paper. Cd in brown rice was extracted with HCl, and the extract was purified on a chelate silicagel column to remove other metals. The pretreated solution was then diluted with buffer for neutralization and tested with the immunochromatographic assay (Abe et al., 2006).

Cd extracted with HCl from wheat grain and fresh eggplant was purified sufficiently using an ion-exchange column treatment. Appropriate HCl extraction rates and dilution rates for the column eluate were selected; Cd concentrations in wheat grain and fresh eggplant were determined successfully by immunochromatography with respect to the international standards of 0.2 mg kg⁻¹ and 0.05 mg kg⁻¹ fresh weight, respectively (Abe K et al., 2011). Approximate Cd concentrations in wheat grain and fresh eggplant can be monitored easily and quickly by this method at locations where facilities for acid digestion and precision analysis are not available.

Abe et al. (2014) conducted an interlaboratory study to evaluate the kit for determining Cd in wheat, rice and soybeans and the results indicated that the kit was an inexpensive, reliable tool for quick and easy on-site determination of Cd in wheat, rice and soybeans.

3. Countermeasures against As contamination of agricultural crops

3.1. Bioavailability of arsenic in soils and its uptake by crops

It is well known that damage by As tends to occur more in paddy rice than other upland crops. In the 1970s and 1980s, the mechanism of As damage of paddy rice and countermeasures for paddy rice fields were clarified by Koyama (1975), Koyama et al. (1976), Koyama and Shibuya (1976), Yamane et al. (1976), and Yamane (1979, 1989). Koyama and Shibuya (1976) conducted a pot experiment of paddy rice with As-contaminated soils and found a significant, negative correlation between 1 M HCl-soluble As in soil and brown rice yield. Countermeasures for paddy rice to prevent As damage
were proposed by Yamane (1979), who suggested that the As-contaminated soils should be maintained in the oxidative state to suppress the dissolution of As.

Ishizuka and Tanaka (1962) reported that 60–80% of total As content accumulated in the roots of paddy rice. Subsequently, Yamane et al. (1976) and Yamane (1989) reported that 90% of the total content of As accumulated in the roots. In addition, X-ray microanalysis of root sections revealed that most As in the roots was distributed at the root surface with Fe (Yamane, 1989).

Elevated As concentrations in rice and the soil solution result from changes in soil redox conditions, influenced by the water management practices during rice cultivation. Microscale changes in redox conditions from rhizosphere to soil matrix affect the As speciation and Fe-plaque deposition. In order to focus on the rhizosphere environment, Yamaguchi et al. (2014) observed microscale distribution and speciation of As around the rhizosphere of paddy rice with h X-ray fluorescence mapping and X-ray absorption spectroscopy. When the soil matrix was anaerobic during rice growth, Fe-plaque did not cover the entire root, and As(III) was the dominant As species in the soil matrix and rhizosphere. Draining before harvest led the conditions to shift to aerobic. Oxidation of As(III) to As(V) occurred faster in the Fe-plaque than the soil matrix. As was scavenged by iron mottles originating from Fe-plaque around the roots. The ratio of As(V) to As(III) decreased toward the outer-rim of the subsurface Fe mottles where the soil matrix was not completely aerated. These results provide direct evidence that speciation of As near rice roots depends on spatial and temporal redox variations in the soil matrix.

3.2. Effects of water management on As and Cd content in rice grains

Flooding increased As concentrations in rice grains, whereas aerobic treatment increased the concentration of Cd. Flooding for 3 weeks before and after heading was most effective in reducing grain Cd concentrations, but this treatment increased the As concentration considerably, whereas aerobic treatment during the same period was effective in reducing As concentrations but increased the Cd concentration markedly (Arao et al., 2009). Concentrations of dimethylarsinic acid (DMA) in grain were very low under aerobic conditions but increased under flooded conditions. DMA accounted for 3-52% of the total As concentration in grain grown in soil with a lower As concentration and 10-80% in soil with a higher As concentration.

When rice was grown under flooded conditions after the heading stage, DMA amendment to the soil resulted in higher DMA concentration in brown rice and rice straw (Arao et al., 2011). In the solution culture, not only DMA amendment but also MMA or arsenite amendment increased the DMA concentration in brown rice and rice straw. DMA was detected in the solution amended by MMA or arsenite with young rice plants. When the solution included the antibacterial agent chloramphenicol, DMA concentration in the solution decreased dramatically. When only the soil was incubated with MMA or arsenite, only a slight amount of DMA was detected in the soil. These results suggest that rice rhizosphere associated bacteria would be involved in the formation of DMA in brown rice.

As is highly mobilized when paddy soil is flooded, causing increased uptake of As by rice. Yamaguchi et al. (2011b) investigated factors controlling soil-to-solution partitioning of As under anaerobic conditions. Changes in As and iron (Fe) speciation due to flooded incubation of two paddy soils (soils A and B) were investigated by HPLC/ICP-MS and XANES. The flooded incubation resulted in a decrease in Eh, a rise in pH, and an increase in the As(III) fraction in the soil solid phase up to 80% of the total As in the soils. The solution-to-soil ratio of As(III) and As(V) (R(L/S)) increased with pH due to the flooded incubation. The R(L/S) for As(III) was higher than that for As(V), indicating that As(III) was more readily released from soil to solution than was As(V). Despite the small differences in As concentrations between the two soils, the amount of As dissolved by anaerobic incubation was lower in soil A. With the development of anaerobic conditions, Fe(II) remained in the soil solid phase as the secondary mineral siderite, and a smaller amount of Fe was dissolved from soil A than from soil B. The dissolution of Fe minerals rather than redox reaction of As(V) to As(III) explained the different dissolution amounts of As in the two paddy soils. Anaerobic incubation for 30 d after the incomplete suppression of microbial activity caused a drop in Eh. However, this decline in Eh did not induce the transformation of As(V) to As(III) in either the soil.
solid or solution phases, and the dissolution of As was limited. Microbial activity was necessary for the reductive reaction of As(V) to As(III) even when Eh reached the condition necessary for the dominance of As(III). Ratios of released As to Fe from the soils were decreased with incubation time during both anaerobic incubation and abiotic dissolution by sodium ascorbate, suggesting that a larger amount of As was associated with an easily soluble fraction of Fe (hydr) oxide in amorphous phase and/or smaller particles.

3.3. Genetic diversity of arsenic accumulation in rice

As levels among Japanese cultivars may not influence dietary As exposure, because there was little genotypic difference in the accumulation of inorganic As (Kuramata et al. 2011).

The genetic diversity in As accumulation and As speciation in rice grains was investigated using WRC comprising 69 accessions grown over a 3-year period (Kuramata et al. 2013). There was a 3-fold difference in the grain As concentration of WRC. Concentrations of total-As, inorganic As, and DMA were significantly affected by genotype, year, and genotype-year interaction effects. Among the WRC accessions, Local Basmati and Tima (indica type) were identified as cultivars with the lowest stable total-As and inorganic As concentrations. Using an F2 population derived from Padi Perak (a high-DMA accession) and Koshihikari (a low-DMA cultivar), two QTLs on chromosome 6 and one QTL on chromosome 8 that were responsible for variations in the grain DMA concentration were identified. Approximately 73% of total phenotypic variance in DMA was explained by the three QTLs.

3.4. Chromatographic separation of As species in rice

Various chromatographic separation modes are used for As speciation analysis, including anion-exchange, cation-exchange, reversed phase, ion-pair reversed phase, ion exclusion, and size exclusion. Baba et al. (2014) investigated HPLC conditions for As speciation analysis using a simple volatile mobile phase, isocratic elution, and silica-based pentafluorophenyl (PFP) columns, which are less expensive than polymer columns, such as the PRP-X100. The Discovery HS F5 column with a PFP stationary phase gave sharp peaks and full separation of the As species in 5 min, and other PFP columns showed lower performance. This separation method was applied to As species analysis in rice. The extraction of As from rice samples was performed using 0.15 M nitric acid. The methodology was validated by use of certified reference materials, NMIJ CRM 7503-a and NIST SRM 1568a, and extremely low As rice samples as blank samples.

4. References


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quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. J. Exp. Bot. 61:923-934


