Partitioning of Lead Accumulation in Rice Plants

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Abstract

A greenhouse study was conducted for two consecutive years to examine lead accumulation in various parts of the rice plant cv 'Prathum Thani 1'. In the preliminary experiment, rice plants were grown in buckets and lead subacetate was supplied as a solution in distilled water at concentrations of 2500, 5000, 7500 and 10000 mg L^{-1} of lead subacetate (equivalent to 1365.6, 2731.1, 4096.7 and 5462.3 mg Pb L^{-1}, respectively), until the tillering stage of crop production began. The results indicated that a concentration of 2,500 mg L^{-1} resulted in the highest lead accumulation and was thus chosen as the reference concentration for the main experiment. The experiment comprised two concentrations of lead subacetate at 1,500 and 3,000 mg L^{-1}(equivalent to 1638.7 and 3277.4 mg Pb L^{-1}, respectively) applied once in the soil at tillering stage. The result of the experiment revealed that lead was accumulated to the highest concentrations of 400.5, 127.7, 5.3 and 0.2 mg kg^{-1} in roots, stems and leaves, bran, and husk, respectively. White rice had no detectable lead accumulation. The bran from the seed with the highest lead accumulation was observed on a transmission electron microscope which showed presumptive lead deposition in vacuoles, endoplasmic reticulum and cytoplasm. From our study, the concentration of lead in white rice did not exceed the food hygienic concentration limit. Thus, it is unlikely that lead can be accumulated to the toxic levels in rice grain. However, the concentration of lead in vegetative biomass (roots, stems and leaves) was rather high for use as animal feed. Although rice plants can accumulate a certain amount of lead, they do not appear to be suitable for use in cleaning up lead in soil.

Keywords: lead accumulation, *Oryza sativa*, rice, grain, bran**,** husk

Introduction

Currently, industry and agriculture in Thailand are co-evolving, often in the same area. Factories are established in the central region where there are paddy fields. Some electronic and computer, battery, printing, and house paint factories have released lead (Pb) and other metals and polluted the environment. Lead is a heavy metal considered highly toxic to human and animals.

The effects of lead are the same whether it enters the body through breathing or swallowing. The main target of lead toxicity is the nervous system, both in children and adults. Long-term exposure of adults to lead at work has resulted in decreased performance in some tests that measure functions of the nervous system. Lead exposure may also cause weakness in fingers, wrists, or ankles. Some studies in humans showed that lead exposure may increase blood pressure, but the evidence is still inconclusive. Lead exposure may also cause anemia, a symptom showing low number of red blood cells. High-level exposure in males can damage the organs responsible for sperm

production. In plants, lead inhibits chloroplast bio-generation and photosynthesis due to direct interference with the reaction caused by light or indirect interference with the synthesis of carbohydrates, resulting in flaccidity of stomatal guard cells, impairing transpiration and carbon dioxide exchange, and further reducing of carbohydrate synthesis. The toxicity of lead also inhibits DNA synthesis, cell division, and seed germination (Iqbal et al., 2000).

Rice (*Oryza sativa*) is one of the world's most important cereal crops, providing staple food for nearly a half of the world population. In many developing countries, rice is the main source of food security and is intimately associated with local lifestyles and culture. Rice may be contaminated by lead. So far, there was no report regarding harm from lead contaminated rice to consumers, but this does not mean that there is no lead contamination in rice. Food Control Division of Thailand (2009) announced that lead in rice grain must not exceed the food hygiene concentration limit (1.0 mg Pb per 1 kg dry weight (DW) of food). Lead may also be a contaminant in bran, the most widely-used rice by-product. Rice bran is a good source of vitamins for humans and animals and is valuable as food and feed. A basic knowledge of lead accumulation in rice would be beneficial and essential to rice marketing in the future. Thus, this experiment is aiming to study lead accumulation in roots, shoots/leaves and grains of rice grown in contaminated soils.

Materials and Methods

Plant and Soil Preparation

Rice seed cv. 'Pathum Thani 1' was obtained from the Rice Department, Bangkok. The soil was of Kamphaeng Saen series collected from Kamphaeng Saen district, Nakhon Pathom province. The soil was air–dried for 2 days, mixed and put in buckets. A total of 10 kg dry soil was put in each 15 liter experimental bucket, drinking water (no Pb contamination) was added and the soil mixed well until muddy. In the preliminary study, the soil was applied with lead subacetate at 2500, 5000, 7500 and 10000 mg lead subacetate L^{-1} (equivalent to 1365.6, 2731.1,4096.7 and 5462.3 mg Pb L^{-1}, respectively) to 5 buckets each treated as a replicate. Based on this test, the main experiment was designed to apply lead subacetate at concentrations of 1,500 and 3,000 mg PbL^{-1} once in the soil at the tillering stage of rice production, with 10 buckets per concentration, and with 10 additional lead-free buckets as control. The experimental design was a Completely Randomized Design (CRD) with 10 replications (buckets) per treatment. Soil pH was measured following Thomas (1996), organic matter content was measured following Walkley-Black method (1946), cation exchange capacity (CEC) was determined as described by Page et al. (1982), electrical conductivity was measured according to Rhoades (1996). Total soil lead was determined following Anonymous (1970). Briefly, the soil was air-dried and extracted in a mixture of 1 N HCl with 1 N H_2SO_4 at the rate of 2:1 in 20 mL, followed by shaking, filtering and analysis using a flame atomic absorption spectrophotometer. The physical and chemical properties of the soil used in the experiment are presented in Table 1.

Lead Concentration

Two stock solutions of lead subacetate at the concentration of 1,500 and 3,000 mg L^{-1}(equivalent to 1638.7 and 3277.4 mg Pb L^{-1}, respectively) were prepared for the experiment. The solutions were mixed with the soil in each experimental bucket at the tillering stage of rice production, with each concentration added to 10 buckets treated as replicates. Rice grown in ten more buckets without lead was treated as control. All plants were visually checked for phytotoxicity every 5 days until the end of the experiment.

Table 1 Physical and chemical characteristics of the soil used in the experiment.

Soil property	Analytical result
Texture	clay
Soil pH	6.61
Organic matter (%)	1.89
CEC (cmol kg^{-1})	18.73
EC (dS m^{-1})	1.22
Total soil Pb (mg kg^{-1})	0.17

Plant Organ Analysis

The lead-treated and control plants were harvested and washed thoroughly with running tap water. Plants were morphologically divided into 5 parts of roots, stems and leaves, bran, husk, and white rice (polished rice). Each part was cut into small pieces, ground and dried for 2 days at 80 ºC. Approximately 0.2 g of sample was dissolved in a 5 mL mixture of HNO_3 and $HClO_4$ at the ratio of 2:1. Samples were digested in a block digester at 200ºC, filtered and adjusted to a volume of 50 mL with distilled water. The lead content of the acid extract was determined with a flame atomic absorption spectrophotometer (FAAS).

Phytoextraction Coefficients

The amount of metal extractable from plant tissue can be presented as a phytoextraction coefficient, which is the ratio of metal concentration in the tissue (g metal per g dry weight of tissue) to the initial soil concentration of the metal (g metal per g dry weight soil). Plants which are considered to be hyperaccumulators should have a phytoextraction coefficient in the above-ground tissue greater than 10 (Environmental Protection Agency, 2000).

Transmission Electron Microscope Study

The bran samples from the lead-treated plants were fixed in 5 % glutaraldehyde in 0.1 M phosphate buffer and postfixed in 2 % osmium tetroxide. They were dehydrated through a graded series of ethanol concentrations (30, 50, 70, 80, 90, 95 and 100%) and embedded in Spurr's resin. Ultrathin sections were prepared by a grass knife and stained in 10 % uranyl acetate and lead citrate, then examined under a transmission electron microscope.

Data Analysis

The observed data were subjected to analysis of variance (ANOVA) and F-test using SPSS software. A probability of 0.05 or less was considered to be statistically significant. The contrast coefficient was calculated when the treatment difference was significant. Coefficient of variation was determined in each trait to indicate the reliability of the experiment.

Results and Discussion

Lead Accumulation

The rice grains from the experiment were analyzed for lead contamination prior to sowing and showed a small degree of natural lead contamination at 4.8 mg Pb kg^{-1}. The seeds were sown in the lead-contaminated soil of 0, 1500 and 3000 mg lead subacetate L^{-1} until harvesting, The removal process will be most practical, relatively timely, and effective if the plant has a high biomass and can accumulate lead in its above-ground tissue. The average lead concentration in various parts of rice plant and in the soil is shown in Table 2. Most lead was found accumulating in roots and less was transported to stems and leaves. Panich-pat et al. (2004) studied removal of lead from contaminated soils by *Typha angustifolia* and found the ability to accumulate high concentration of lead in roots. Accumulation by roots increased with the increase in soil lead concentration.

When two concentrations of lead subacetate at 1,500 and 3,000 mg L^{-1} were added once into the soil at tillering stage, lead accumulation was the highest in roots with 400.5 mg Pb kg^{-1} in the 3,000 mg L^{-1} bucket. Lead concentration in roots was significantly increased with the increase in concentration in the soil. Lead accumulation in stems and leaves was less than in roots, again depending on lead concentration in the soil. Lead in bran was not different among different lead concentrations. Lead was accumulated at a very low concentration in husk, while white rice had no detectable accumulation of lead.

Lead concentration in stems and leaves was in the same order of magnitude as found in roots, suggesting that lead can be readily translocated to rice shoot. Abedin et al. (2002) studied arsenic accumulation and metabolism in rice and found that high arsenic concentration may have adverse health effects on cattle and increase arsenic exposure in humans via the plant-animal-human pathway. Our study showed very low concentration of lead in rice bran and undetectable levels in white rice. However, a significant amount of lead was found in rice straw (stems and leaves), and thus may cause risk associated with feeding large amount of lead-contaminated straw to the cattle as well as using rice straw in fresh vegetable production.

Table 2 Lead concentration (mg kg^{-1} DW) in various parts of rice plant and in soil treated with different concentrations of lead subacetate.

Lead subacetate treatment (mg L^{-1})	Lead concentration in mg kg^{-1} DW					
	Root[1/]	Stem and leave[1/]	Bran	Husk	White rice[2/]	Soil[1/]
0	24.9±2.3	11.0±1.6	5.4±0.2	0.0±0.0	ND	1.6±0.1
1500	165.9±24.9	58.7±4.9	6.0±0.5	0.4±0.3	ND	12.6±3.5
3000	400.5±86.7	127.7±21.8	5.3±0.3	0.2±0.9	ND	16.1±4.5

[1/] Lead concentrations among different treatments were significantly different at $P \leq 0.05$
[2/] ND = not detectable

Table 3 Phytoextraction coefficients of rice plant in the soil treated with different concentrations of lead subacetate.

Lead subacetate treatment (mg L^{-1})	Phytoextraction coefficient				
	Root	Stem and leave	Bran	Husk	White rice
0	15.7	6.9	3.4	0	0
1500	13.1	4.7	0.5	0	0
3000	24.9	8	0.3	0	0

The soil used in our experiment did not readily absorb lead during the course of experiment. The total soil Pb (mg kg^{-1} DW) was found in low amounts in the experiment, i.e. lead concentration of 16.1 and 12.6 mg Pb kg^{-1} DW were found in the soil applied with 3,000 and 1,500 mg lead subacetate L^{-1}, respectively. Soil is a major sink for lead, which might be absorbed and bioaccumulated by plants and animals, and eventually become available for human consumption. Several soil parameters have been shown to influence the uptake of lead, and pH, CEC and organic matter were the soil properties involved in stabilization of lead. In our experiments, there was a significant difference in lead accumulation in soil, although the coefficient of variation (C.V.) was rather high, reflecting in high standard error associated with the mean lead concentration (Table 2).

Visual Observation of the Rice Plants

Most rice plants in the experiments looked healthy and grew well. Experimental plants in the experiment grew up to 1.4 m in height and 0.8 kg in average fresh weight per hill. Lead concentrations in this study did not physically cause toxicity to the rice plants, although certain rice diseases caused death in of some plants. Panich-pat et al. (2004) also reported that lead did not cause any visible phytotoxicity symptoms to treated *T. angustifolia* plants.

Phytoextraction Coefficients

Plants that can be used for phytoremediation purposes should be able to uptake a considerable amount of contaminants. Hyperaccumulator plant species should be able to bioconcentrate up to 1,000 mg kg^{-1} lead in their tissues (Baker and Brooks,

1989). On the other hand, hyperaccumulator plants actively translocate heavy metals into the shoots, presumably because of the existence of heavy metal tolerance mechanisms operating in the shoot. In our experiment, rice plants showed a higher phytoextraction coefficient in the roots than in stems and leaves, bran and husk, as shown in Table 3. Rice plants accumulated only up to 127.7 mg Pb kg^{-1} DW in stems and leaves. It can be concluded from the experiment that, although rice plants accumulate some lead, they are obviously not suitable for cleaning up lead contaminated soil.

Transmission Electron Microscope Study

Bran samples were taken from the treatments with highest lead accumulation and examined with a transmission electron microscope. Most of the accumulated lead granules appeared to be deposited in vacuoles (Figure 1A) and around the vacuoles (Figure 1B). The bran cell at 1,500 mg L^{-1} of lead subacetate showed presumptive lead granules distributing and accumulating around the endoplasmic reticular (Figure 1C). A few presumptive lead granules were also observed in the cytoplasm (Figure 1D). Although, we did not

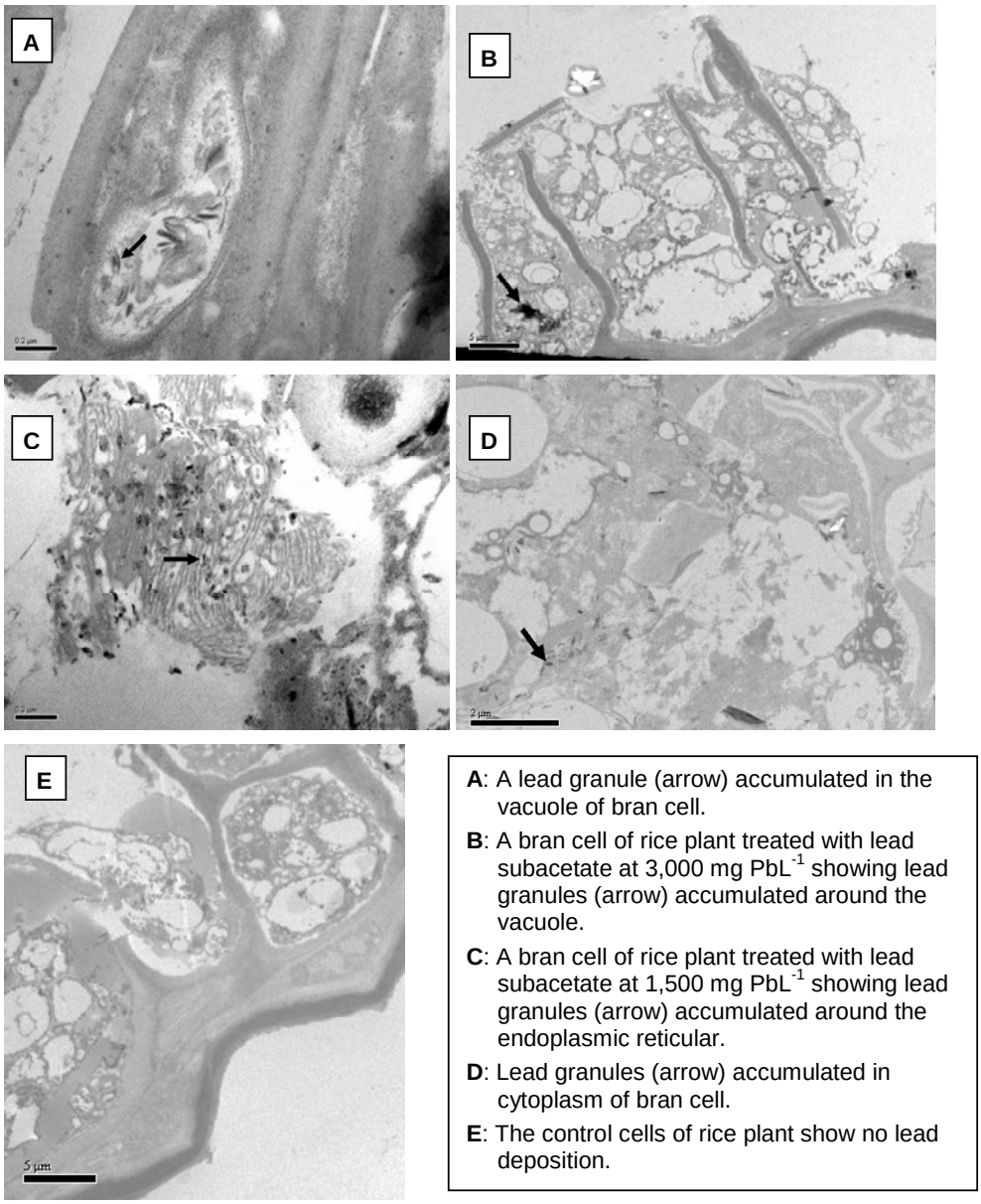

Figure 1 Transmission electron micrographs of bran samples.

measure lead in the specific plant tissue areas, i.e. the endoplasmic reticulum or cytoplasm, out TEM scans strongly suggest lead deposits in place. The normal cells without lead deposition is shown in Figure 1E.

Sharpe and Denny (1976) found lead in leaf tissue of *Potamogeton pectinatus* mostly localized near cell walls. Skaar et al. (1973) found lead accumulation in vacuoles, mitochondria, chloroplasts and nuclei of *Rhytidiadelphus squarrosus*. While Malone et al. (1974) used light and transmission electron microscopic techniques to find lead crystals accumulated most in vesicles cell wall of dictyosome of corn roots. Panich-pat et al. (2005) used electron microscopy and studied the localization of presumptive lead in organs of *Typha angustifolia* grown in contaminated soil and found presumptive lead deposition in cell walls, vacuoles and chloroplasts. The retention of lead in roots involves binding to the cell wall and extracellular precipitation. Metals and heavy metals can also be stored in vacuoles as free ions.

Conclusions

Rice plants accumulated lead in the respective order from high to low in roots, stems and leaves, bran, and husk, with no detectable amount in white rice. The concentration of lead in rice grain did not exceed the food hygiene concentration limit. Thus, our results indicate that we can consume white rice safely. However, lead deposited in stems and leaves and bran may enter the food chain as cattle feeds or mulch in vegetable production. The extent of this risk requires further investigation. Rice plants are obviously not suitable for use in cleaning up lead-contaminated areas.

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