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ACCUMULATION OF HEAVY METALS IN SOIL AND AEROBIC
RICE (ORYZA SATIVA L.) SAMPLES

Zakaria Zuraini (a)*, Mohd Yusob Yusmizawati (b), Amri Saidon Shamsul (c), Mohd Yusob Shajarutulwardah (d), Nasarudin Noor Syahira (e)

*Corresponding author

(a) Biology Programme, School of Distance Education, Universiti Sains Malaysia, 11800 Minden, Penang.
zuraini@usm.my

(b) Technology Transfer & Entrepreneur Development Center, MARDI Alor Setar, Km 5, Jalan Kuala Kedah, 06600 Alor Setar, Kedah. ymy@mardi.gov.my

(c) Paddy and Rice Research Center, MARDI Seberang Perai, 13200 Kepala Batas, Penang. shamri@mardi.gov.my

(d) Universiti Putra Malaysia Bintulu Campus, 97008 Bintulu, Sarawak. shajarutulwardah@gmail.com

(e) Horticulture Research Center, MARDI Headquarters, 43400 Serdang, Selangor. noor.syahira.nasa@gmail.com

Abstract

This study was conducted to determine the content of heavy metals in soil samples and in various parts of rice plant namely root, stem, leaf, rice husk, rice bran, rice grain, brown rice and white rice. Samples were collected from an aerobic rice cultivation area in Kampung Bukit Kura, Pedu, Kedah, Malaysia. The types of heavy metal studied were cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). The heavy metals present in paddy plants and soils were determined using reverse aqua regia digestive method analyse using Inductively Coupled Plasma Mass Spectrometry (ICPMS). Results showed that iron (Fe) was the most predominant metal ion in the soil ($6903.33-7314.66 \text{ mgkg}^{-1}$) and root ($2891.630 \pm 123.670 \text{ mgkg}^{-1}$), followed by stem ($2286.400 \pm 39.160 \text{ mgkg}^{-1}$), leaf ($765.710 \pm 16.185 \text{ mgkg}^{-1}$), rice husk ($292.510 \pm 0.090 \text{ mgkg}^{-1}$) and rice bran ($600.510 \pm 80.015 \text{d}$). Mn was the highest accumulated metal in the rice grain ($74.540 \pm 2.570 \text{ mgkg}^{-1}$) meanwhile Zn was the highest in brown rice ($30.580 \pm 3.130 \text{ mgkg}^{-1}$) and white rice ($22.785 \pm 0.025 \text{ mgkg}^{-1}$). Cd was the lowest concentrated metal and was evenly distributed in the plant parts. The heavy metal content in *Oryza sativa* in this study did not exceed the general permissible limit except for Fe concentration in the soil. The excessive accumulation of various heavy metals in the paddy plant parts and soil may pose a threatening threat to human health especially for Asians where rice is consumed daily.

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Keywords: *Oryza sativa*, heavy metal, soil.



1. Introduction

Heavy metals are a group of elements that can create inorganic chemical hazards when the concentrations are higher than the allowed requirement. Kim, Kim, and Seo (2015) reported that almost all heavy metals are considered as toxics and may become carcinogenic. Heavy metals such as cadmium (Cd), chromium (Cr), arsenic (As) and nickel (Ni) are utilized commercially even though there are classified as category 1 heavy metals according to the International Agency For Research On Cancer.

The accumulation of heavy metals in the soil and plants may come from natural sources or pollution as a result of human activities. High concentrations of heavy metals in soils may cause long term risks to ecosystems and humans. Once introduced into soils, heavy metals are difficult to remove or decompose and will remain in the soils for a prolonged period of time. Moreover, plants may have the potential to uptake these heavy metals and pose a possible health risk when consumed.

In agricultural fields, heavy metals may accumulate from the frequent use of fertilizers or pesticides (Malidareh, Mahvi, Yunesian, Alimohammadi, & Nazmara, 2014). Some of the common fertilizers or pesticides contain certain heavy metals (Wuana & Okieimen, 2011). Kumar Sharma, Agrawal, and Marshall (2005) reported that heavy metals derived from anthropogenic sources are believed to be easily accumulated in the top soil. Soils that accumulate heavy metals above the standard value may cause serious danger to the ecosystem including to plants, animals and humans.

Rice is widely cultivated and is consumed on a daily basis by Malaysians. Therefore, it is important to determine the content and distribution of heavy metals in paddy (*Oryza sativa*) plant parts as well as the cultivated paddy soil to ensure the safety of this staple food. The objective of this study was to determine the concentration of heavy metals in several paddy plant parts and the root zone soil in a cultivated paddy area of Kedah, Malaysia.

2. Problem Statement

Food security has become a national agenda with the implementation of Malaysia's Agrofood Policy 2011-2020. Factors such as global climate change, increasing prices of agricultural inputs, increasing food demands due to population growth and dietary changes have contributed to higher food import bill. The production of major agricultural commodities contracted only 3.4 % to 10,811 TMT (thousand metric tons) in 2017 compared to 11,597 TMT in 2015. According to the Ministry of Agriculture and Agro-based Industry, rice production declined 3.2 % in 2018 from the previous year. Although there are many factors that may affect rice production, reports have shown that the presence of heavy metals in the soil can reduce rice yields and may have negative effects to human health (Gramss & Voigt, 2016).

Crops have different abilities to absorb and accumulate metals in different plant parts. There are wide variations in the uptake and translocation of heavy metals between plant species and even between cultivars of the same species. Other factors that may influence the adsorption of heavy metals are soil contact time, soil concentration and pH. Cereal crops such as rice develops roots within 25 cm from the top soil. Soils with high concentration of heavy metals will increase the potential of being taken up by plants from the sub-surface of 25 cm depth zone of the soil. The heavy metals will be translocated from the roots to shoots then to the grains, which are consumed by humans.

3. Research Questions

Currently there is no information on the accumulation of heavy metals in the soil around the root zone and plant parts of aerobic rice cultivated in Kampung Bukit Kura, Pedu, Kedah. This area has yet to undergo any studies in terms of heavy metal uptake by the soil or paddy plant.

3.1 What are the type of heavy metals that accumulate in the soil and in paddy plant parts?

3.2 Are these heavy metals present in deficient, sufficient or above permissible limit?

4. Purpose of the Study

The purpose of the study is to determine the heavy metals uptake in soil and paddy plant parts in Kampung Bukit Kura, Pedu, Kedah.

5. Research Methods

This study was carried out using soil samples and paddy plants of a local aerobic rice variety MR1A1 obtained from Kg Bukit Kura, Pedu, Kedah which is located in the northern region of Peninsular Malaysia (Latitude: 6.242036/ Longitude: 100.657157).

5.1. Sample preparation

Samples of the paddy plant parts and the soil around the root zone area were selected randomly. The soil samples were taken using auger at a depth of 0-30 cm and oven-dried at 70°C in the laboratory before being ground and sieved through 0.01 mm mesh. The paddy plants were washed thoroughly with deionised water and separated into parts of root, stem, leaf and rice grain. The plant parts were also oven dried at 70°C for 24 hours. The rice grains were separated into rice husk, rice bran, brown rice and white rice, followed by hand pounding in a mortar to obtain powder form.

5.2. Heavy metal analysis

For the analysis of heavy metals, 1 g of soil and 1 g of each plant part were digested according to reverse aqua regia method with 10 mL nitric acid (65 % HNO₃) and 5 mL hydrochloric acid (37 % HCL) in the ratio 2:1 respectively. The clear digested liquid was filtered and made up to a volume of 100 mL. A clear solution with no residue will be obtained at this stage. Blank samples (samples without soil) and laboratory standard samples (SRM) were also subjected to the same procedure and the precision was assessed by replicate analysis. The filtrates of the plant parts and soil were detected for the presence of heavy metals using Inductively Coupled Plasma Mass Spectrometry (ICPMS) model PE nexION 300 brand Perkin Elmer. The observed accuracy (3%) was also examined by analyzing in duplicate Certified Reference Materials Project standard and the recovery test ranged from 95 % to 105 %.

5.3. Statistical analysis

The SAS version 9.4 was used to statistically evaluate all data. All experiments were conducted in three replicates to make allowances for experimental errors and results were reported as mean \pm standard

deviation (SD). A post-hoc multiple comparison of means was conducted using the Duncan test ($P < 0.05$), with statistical significance being defined as $p \leq 0.05$.

6. Findings

6.1. Heavy metals in parts of *Oryza sativa*

Table 01 shows mean concentrations of heavy metals Cd, Cr, Pb, Cu, Fe, Mn and Zn in different paddy plant parts.

Iron (Fe) was the highest metal concentrated in the root, stem, leaf, rice husk and rice bran. This finding was similar to Dobermann & Fairhurst (2000) where the concentration of iron was also found to be the highest and highly toxic in their grown rice. In plants, iron is required for electron transport in photosynthesis and is a constituent of iron porphyrins and ferredoxins, both of which are essential components in the light phase of photosynthesis. However, excessive iron uptake will result in the formation of oxygen radicals which are phytotoxic to plants. Iron toxicity is related to multiple nutritional stress, which leads to reduced root oxidation power, resulting in K, P, Mg and Ca deficiency (Dobermann & Fairhurst, 2000).

Within the whole paddy grain, manganese (Mn) was the highest, but the metal was highly concentrated in the root. Rice is more resistance to Mn toxicity but critical plant concentrations for the occurrence of Mn toxicity are poorly defined. In some cases, rice plants with 3000 mg kg^{-1} Mn did not show Mn toxicity symptoms and yield was not affected (Dobermann & Fairhurst, 2000).

In general, root contained the most highly concentrated metals except for Cr, Cu and Zn. Chromium (Cr) accumulated at its highest concentration in the stem whilst the lowest is in white rice. Distribution of Cr in *Oryza sativa* varied and was in the order of stem > root > leaf > rice husk > rice bran > brown rice > paddy > white rice.

Copper (Cu) concentrations varied in descending order from rice husk > paddy grain > stem > rice bran > leaf > brown rice > white rice. Copper is one of the nutrients needed to process a variety of enzyme activities in plants. In this study, Cu was found to be still under permissible limit allowance except in rice husk ($80.175 \text{ mg kg}^{-1}$). If the presence of copper metal exceeded the limit of 25 mg kg^{-1} , it may cause stunted growth of roots and the whole plant.

Table 01. Mean concentration of heavy metals ($\text{mg kg}^{-1} \pm$ standard deviation) in various paddy plant parts at Kampung Bukit Kura, Pedu, Kedah, Malaysia

Heavy metal	Cadmium (Cd)	Chromium (Cr)	Lead (Pb)	Copper (Cu)	Iron (Fe)	Manganese (Mn)	Zinc (Zn)
Plant part							
Root	0.247 ± 0.003 a	102.110 ± 101.794 b	2.266 ± 0.114 a	26.285 ± 0.065 b	2891.630 ± 123.670 a	325.260 ± 7.350 a	44.585 ± 1.555 b
Stem	0.052 ± 0.001 c	248.010 ± 2.879 a	1.040 ± 0.005 c	16.380 ± 0.170 d	2286.400 ± 39.160 b	171.280 ± 1.970 c	31.890 ± 4.780 c

Leaf	0.047 ± 0.002 d	73.140 ± 5.150 b	0.848 ± 0.040 d	10.395 ± 0.235 e	765.710 ± 16.185 c	170.005 ± 1.235 c	32.550 ± 0.630 c
Rice Husk	0.043 ± 0.0009 e	5.310 ± 0.116 c	1.192 ± 0.029 b	80.175 ± 2.435 a	292.510 ± 0.090 e	219.010 ± 0.110 b	47.515 ± 3.815 b
Rice Bran	0.063 ± 0.001 b	2.450 ± 0.231 c	0.659 ± 0.124 e	16.200 ± 0.020 d	600.510 ± 80.015 d	147.440 ± 2.230 d	81.330 ± 1.950 a
Paddy	0.048 ± 0.000 d	1.600 ± 0.078d	0.593 ± 0.044 e	24.255 ± 1.085 c	68.960 ± 6.625 f	74.540 ± 2.570 e	33.420 ± 4.070 c
Brown Rice	0.047 ± 0.000 d	1.640 ± 0.500 c	0.287 ± 0.060 f	5.330 ± 0.150 f	20.330 ± 1.110 f	24.125 ± 0.425 f	30.580 ± 3.130 c
White Rice	0.046 ± 0.000 d	1.420 ± 0.139 c	0.307 ± 0.061 f	4.370 ± 0.130 f	12.800 ± 0.660 f	8.670 ± 0.020 g	22.785 ± 0.025 d

*Note: Means within the same row followed by the same letter are not significantly different to each other at $p > 0.05$ based on Duncan Multiple Range Test (DMRT)

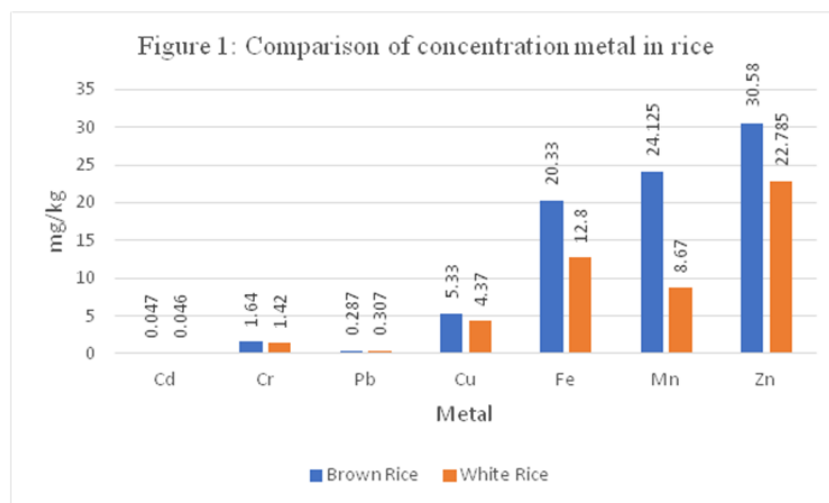


Figure 01. Comparison of heavy metal concentrations between brown rice and white rice

Zinc (Zn) was the highest accumulated metal found in both brown and white rice (Figure 01). The distribution of Zn accumulation occurred in the descending order of rice bran > rice husk > root > paddy grain > leaf > stem > brown rice > white rice (Table 01). Zinc is an important nutrient required by plants to synthesis enzymes and proteins, for growth hormone production and chlorophyll production. Zinc deficiency is seldom found in upland and aerobic soil conditions (Dobermann & Fairhurst, 2000). However, it may reach phytotoxic concentrations (more than 200 mgkg^{-1}) due to anthropic contaminations from the application of fertilisers, pesticides and manures.

Cadmium (Cd) was evenly distributed at low concentrations in various parts of the MR1A1 variety, and showed significant difference in root, stem, rice husk and rice bran. Cadmium and its compounds are the seventh most toxic heavy metal as determined by the Agency for Toxic Substances and Disease Ranking

(Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). Cadmium is released into the environment through natural activities such as volcanic eruptions, weathering, river transportation as well as from some human activities such as mining, smelting, tobacco smoking, incineration of municipal waste and manufacture of fertilizers. Once this heavy metal gets absorbed by humans, it will remain and accumulate inside the body throughout its life.

6.2. Heavy metals in parts of *Oryza sativa*

Table 02 shows that iron (Fe) maintained as the highest metal accumulated followed by Mn, Cr, Zn, Cu, Pb and Cd. Biologically, iron is the most important nutrient for most living organisms as it is the cofactor for many vital proteins and enzymes and mediates the respiration process. Excessively, iron can activate reactions involving the formation of radicals which may damage biomolecules, cells and tissues of an organism.

Table 02. Range of heavy metal concentration ($\text{mg/kg}^{-1} \pm$ standard deviation) of cultivated *Oryza sativa* soil samples at Kampung Bukit Kura, Pedu, Malaysia (N=30)

Cadmium (Cd)	Chromium (Cr)	Lead (Pb)	Copper (Cu)	Iron (Fe)	Manganese (Mn)	Zinc (Zn)
0.024 - 0.026	17.305 - 19.092	4.796 - 4.882	4.15 - 4.31	6903.33 - 7314.66	54.75 - 54.96	13.81 - 14.23

Results of the aerobic paddy soil analysis indicated that the concentration levels of all of the heavy were within allowable levels by the Malaysia National Water Quality Standard (NMQS), except for iron (Fe) which was beyond the tolerable limit. The soil samples contained high concentrations of Fe probably due to the heavy use of pesticides and insecticides in the cultivated paddy field.

7. Conclusion

Heavy metals of Cd, Pb, Fe and Mn in this study accumulated abundantly in root compared to other parts of the paddy (*Oryza sativa*) plant. Meanwhile, Cr, Cu and Zn showed the highest concentration in the stem, rice husk and rice bran, respectively. Each heavy metal was present in different concentrations in each part of the plant and did not exceed the permissible limit, and also the limits set by various other countries. Iron was under the critical level for high toxicity (>300-2000) for most parts of the plants such as root, stem, leaf, rice husk and rice bran. In the soil, only iron showed the concentration in excess to the permissible level and was considered as highly toxic and outside the safety level.

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