



HEAVY METAL (Cr, Mn, Fe) CONTENTS IN SOILS FROM THE MADA RICE FIELDS IN KEDAH, MALAYSIA

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ABSTRACT

The aim of the present study was to investigate the heavy metal (Cr, Mn, and Fe) contents in paddy soils of the Muda Agricultural Development Authority (MADA) in the state of Kedah, Malaysia. The study was conducted in fields using two different types of irrigation water namely, recycled (RCL) and non-recycled (N-RCL) water. The heavy metals in the soil were extracted using “the sequential extraction method”. The heavy metals were extracted from the four different soil fractions using different reagents. The results showed that the concentration of heavy metals in the studied area was in the following decreasing order: Fe > Mn > Cr. The total Fe concentration in the areas supplied with RCL water ranged from 21256.895 mg/Kg to 26915.28 mg/Kg, whereas in the areas with N-RCL water Fe concentration ranged from 16090.196 mg/Kg to 18420.457 mg/Kg. It was found that the total Mn concentrations in the areas supplied with RCL water ranged from 31.948 mg/Kg to 44.444 mg/Kg, whereas in areas with the N-RCL water, it ranged from 27.673 mg/Kg to 33.260 mg/Kg. The total Cr concentration in the areas supplied with the N-RCL water, it ranged from 14.595 mg/Kg to 19.073 mg/Kg. The results also showed that the heavy metals in the paddy soil fractions were concentrated mainly in the RR fraction. However, Mn was found to be at the highest level in the ELFE fraction. Fe was generally found to have the highest concentration in the RR fraction.

Key words: Heavy metal, Soil fractions, Paddy fields, Chromium, Iron, Manganese.

INTRODUCTION

Although heavy metals are present naturally in the soil; they occur at concentration that are non-toxic and some of these metals are required in small quantities by plants and

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animals¹. However, heavy metals are not easily metabolized, and they tend to accumulate in the soft tissues, making it toxic when they reach high concentrations¹. Heavy metals are chemical elements with specific gravity of at least five times that of water². According to the United States Environmental Protection Agency (USEPA) and state regulations, heavy metals are defined as trace elements that can be hazardous to the environment, humans, animals and plants. Heavy metals are extremely persistent in the environment. They are non-biodegradable and non-thermodegradable and can therefore readily accumulate to toxic levels³.

Heavy metals accumulated in soil, water and sediment contaminates crops⁴⁻⁶. Various factors affect metal transfer to different parts of food chain. The type of metals as well as the soil factors are important variables in all the pathways⁷. Transfer of non-essential metals, such as Cr and Mn can be very different from that of the essential metal transfer. This is due to effective mechanisms that regulate the cellular uptake and accumulation of essential metals⁸. Argillaceous layers, pH and total organic content are factors known to influence metal transfer soil to the plant⁹.

Paddy fields tend to receive large amounts of anthropogenic contaminants due to excessive applications of chemical pesticides and fertilizers, which eventually result in considerable levels of heavy metals, within the soil. According to Kara et al.¹⁰, several factors are involved in the contamination of the agricultural soils. Some of the major factors include atmospheric sedimentation, increasing mining activities, contamination from animal manure, and use of contaminated irrigation water¹¹. Heavy metal contamination due to human activities can have strong effect on their speciation and availability in soils¹². Besides oil palm and rubber, rice is regarded as one of the most important crops, which are cultivated in Malaysia. The rice bowl of the country is located in the northern part of Peninsula Malaysia, and it constitutes part of the Muda irrigation scheme. This area, which covers approximately 97,000 ha of paddy fields, is managed by the Muda Agricultural Development Authority (MADA) and consists of a massive network of irrigation canals and drainage systems that make it possible to implement rice double-cropping. In MADA, two types of irrigation water are used, namely non-recycled (N-RCL) and recycled (RCL) irrigation water. MADA covers the states of Kedah and Perlis, where paddy is grown twice a year (during the main season and off-season) using irrigation water from the Muda and Pedu Dams in Kedah. The present study aims at investigating the concentration and distribution status of selected heavy metals (eg. Cr, Mn, and Fe) in paddy soil fractions of the rice fields of MADA.

EXPERIMENTAL

Materials and methods

Sampling sites

The study was conducted on paddy soils, samples of which were obtained from the MADA agricultural area in the state of Kedah ($6^{\circ} 13' N$, $6^{\circ} 10' N$ and $100^{\circ} 14' E$, $100^{\circ} 18' E$). The selected study area, situated in the northwest of Peninsular Malaysia, is shown in Fig. 1. Soil samples (five replicates each) were collected from a depth of 0 cm to 30 cm using an auger measuring 7 cm in diameter. The soil samples were taken from areas receiving N-RCL and RCL water. The samples were collected in April and Dec. 2013. The samples of soil, which were collected for the study, were initially air-dried in the laboratory. The air-dried samples were then strained through 250 μm mesh, after being pulverized using a mortar and pestle, before analysis¹³.

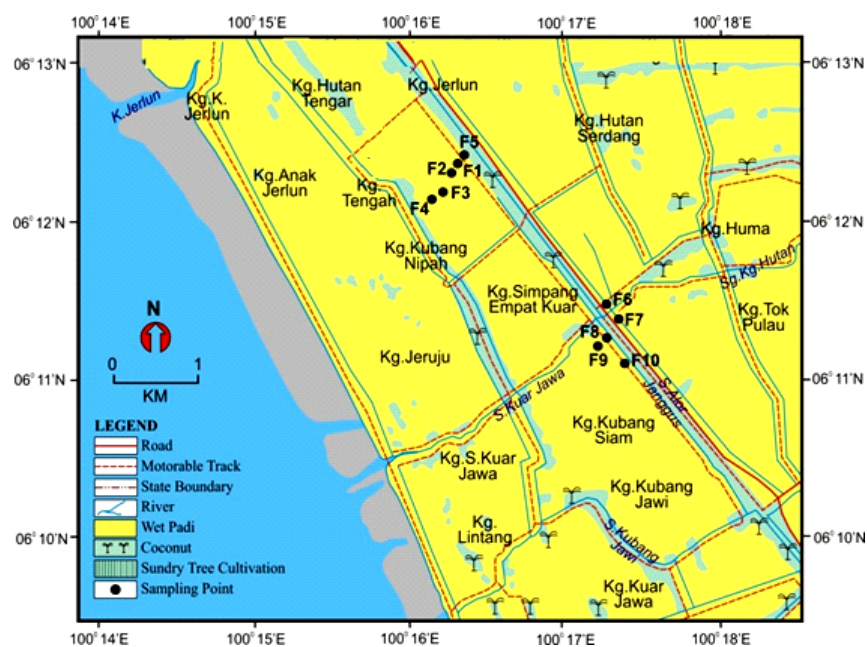


Fig. 1: Location of the sampling stations in the paddy fields of the study area in MADA, Kedah

Soil characteristics

The physio-chemical characteristics of soil was analyzed for pH, particle size, and total organic carbon content. Soil pH was measured using a modified version of the method

by Duddridge and Wainwright¹⁴. In this method, 20 g of the dried soil sample were diluted with 50 mL of deionized water, and mixed for 15 min. ADELTA 320 pH meter was used to read the pH value. The wet sieving method was used to determine the percentage soil particles that were below 63 μm ¹³. 10 g of the dried soil samples were washed and then sieved using 63 μm sieves under slow-flowing tap water. The samples of the soil, which remained in the filter were dried at 105°C in an oven until the mass of the soil samples stabilized. The percentage organic carbon in the soil samples were determined using the method introduced by Walkley and Black¹⁵. The organic carbon in the soil was oxidized using potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) as the reducing agent. About 1 g of the dried soil sample was placed in a clear, dry, 500 mL conical flask, and 10 mL of 1N $\text{K}_2\text{Cr}_2\text{O}_7$ were added to it, followed quickly by 20 mL of concentrated H_2SO_4 . The content of the conical flask were immediately combined by swirling gently at first, and then vigorously for up to one min. The mixture was then shaken on a hot plate for approximately thirty min, before being diluted with 200 mL of distilled water, followed by 10 mL of concentrated phosphoric acid and 1 mL of diphenylamine indicator. When a blue violet colour appeared, the mixture was titrated with (N/2) ferrous ammonium sulphate solution until the colour changed to green.

Extraction procedures

A sequential extraction method¹³ was used to extract the heavy metals from the soil samples. Here, 10 g soil samples from the four different fractions were used. During the process, the ELFE (easily leachable and freely exchangeable fraction), which is the first extraction fraction, utilized 50 mL of 1.0 M ammonium acetate at pH 7 as the extraction reagent at room temperature. All the collected samples were quavered at 150 rpm for approximately ninety min. The mixture was also centrifuged at 3000 rpm for 30 min at 20°C. The Millipore filter paper, with pore size 0.45 μm , was used to filter the solutions before metal analysis. The soil samples in the bottles were then washed by mixing fifty milligrams of distilled de-ionized water. A similar method of centrifugation and shaking was performed. In the second fraction of extraction, the acid reduction fraction (AR), 50 mL of 0.25 M hydroxylamine chloride at pH 2 was used as the extraction reagent at room temperature. On the other hand, for the OO (organic oxidation fraction), which is the third extraction fraction, the mixture of H_2O_2 (hydrogen peroxide) was used with 30 percent in the water bath at 94°C to 97°C for about 1-1.5 hrs. The released metals were shaken continually with 1.0 M ammonium acetate, which was acidified to pH 3.5 along with hydrochloric acid at normal room temperature. It should be noted that the entire process was done during the cooling of the mixture. For the resistant fraction (RR), which is the last fraction, concentrated HClO_4 (perchlorate acid) and HNO_3 (nitric acid) were used at a ratio of 5:2. The first three extraction fractions (ELFE, AR, and OO) were used for extracting anthropogenic metals

from the sediment, which originated from polluted sources. The final fraction of extraction was used for extraction of natural metals in particles that were strongly bound to each other. The mixture was shaken and centrifuged during each extraction process, and the solution was filtered before analysis for metals. The concentration of heavy metals was determined using the ICPMs (inductively coupled plasma mass spectrometry) method.

Quality control/Quality assurance (QC/QA)

The essential quality assurance precautions and procedures were also utilized, in order to assure the coherency and validity of the results. Double distilled water was utilized throughout the research. Moreover, all glass-made apparatus were cleaned thoroughly. Higher quality reagents were utilized, to ascertain the reliability of the results. In addition to this, blank determinations were done on the reagents, in order to get accurate readings from the instruments. In order to ensure the QC/QA, the concentrations of the heavy metals, specifically in the soil, was estimated by considering their dry weight.

Statistical analysis

The one-way ANOVA was applied to detect significant differences in heavy metal concentration among the paddy soil fractions as well as among the sampling times. The mean difference in metal concentration between the areas using RCL and N-RCLwater was determined using the T-test. All statistical analysis was performed using the SPSS 16.

RESULTS AND DISCUSSION

Soil parameters

Paddy soils are of specific interest because they are used for cultivation; these soils are kept submerged for long periods of time, and this may cause the reduction and subsequent mobilization of various metals such as Fe and Mn¹⁶. Paddy soils are of interest to scientists, whose aim have been to determine the levels and sources of heavy metals¹⁷.

Table 1 summarizes the average soil pH, organic carbon, and grain size percentages. The results from the present study showed that the paddy areas were slightly acidic (4.02 to 5.87 and 4.12 to 5.85 in areas using RCL and N-RCLwater, respectively). These results are similar to the soil pH values ranging from 4.36 to 6.36 reported in a previous study for Khairiah et al.¹⁸ pH is one of the most important factors that influence the bioavailability and transport of heavy metals in the soil. According to Smith and Giller¹⁹, heavy metal mobility decreases with increasing soil pH because of the precipitation of hydroxides and carbonates, as well as the formation of insoluble organic complexes.

The results showed that the percentage grain size less than 63 μm ranging from 74.80% to 87.70% with a mean value of 82.53% in the areas using recycled water and from 73.20% to 87.90% with a mean value of 82.73% in areas using non-recycled water. All samples contained high percentages of clay and silt fractions of size less than 63 μm . According to Chaney and Hornick²⁰ and Chaudri et al.²¹, clayey soils are responsible for lower metal bioavailability compared to sandy soils. Prabu²² reported that the concentration of heavy metals in soil is dependent on the clay content because clay particles have a large number of ionic binding sites (because of their large surface area).

In the current study, soil organic carbon content ranged from 5.92% to 8.94% and 6.38% to 8.94% in the fields using RCL and N-RCL water respectively. These values are higher than the organic carbon content values of 4.36% to 6.41% reported for states of Kedah and Perlis¹⁸ in a previous study. Soil organic matter content is the most important indicator of soil quality and productivity and it consists of a complex and varied mixture of organic substances. Soil organic matter content is commonly defined as the percentage of humus in the soil. Humus is the unidentifiable residue of plants, microorganisms, and fauna that become fairly resistant to further decay²³.

Table 1: Characteristics of the soils in the study areas

Areas using	pH		Percentage grain size less than 63 μm (%)		Organic carbon content (%)	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
n = 150						
Recycled water	5.07 \pm 0.18	4.02-5.87	82.53 \pm 1.46	74.80-87.70	7.54 \pm 0.50	5.92-8.94
Non-recycled water	4.91 \pm 0.20	4.12-5.85	82.73 \pm 0.39	73.20-87.90	7.61 \pm 0.35	6.38-8.94

Cr in the soil fractions

Cr is a toxic and carcinogenic element, and its toxicity is dependent mainly on its chemical state, (Cr(III) and Cr(VI)) rather than difference in properties. Cr(VI) is considered to be the more toxic form, whereas Cr(III) is an essential element for mammals. Cr is considered a non-essential trace metal for microorganisms and plants. The geochemical fractions of Cr in the paddy soils of MADA, Kedah are shown in Table 2. The total Cr concentration in the areas supplied with RCL water ranged from 17.35 mg/Kg to 20.660 mg/Kg, whereas that in the rice fields supplied with the N-RCL water ranged from 14.595 mg/Kg to 19.073 mg/Kg. Cr in the ELFE fraction in areas supplied with RCL and N-RCL

water ranged from 0.292 mg/Kg to 0.374 mg/Kg and 0.323 mg/Kg to 0.371 mg/Kg, with mean percentages of 1.64% and 2.03%, respectively.

Table 2: Concentration of Cr (mg/Kg) in paddy soils of MADA, Kedah

Cr		ELFE	AR	OO	RR	Non-resistant	Total	Non-resistant (%)	Resistant (%)	
Area	Fields	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD					
Areas using	Recycled water	F1	0.374 \pm 0.308 ^b	0.135 \pm 0.121 ^b	2.307 \pm 0.787 ^b	17.844 \pm 14.726 ^a	2.816	20.660	13.63	86.37
		F2	0.294 \pm 0.237 ^b	0.109 \pm 0.121 ^b	1.986 \pm 0.677 ^b	16.303 \pm 12.959 ^a	2.390	18.692	12.78	87.22
		F3	0.295 \pm 0.212 ^b	0.118 \pm 0.104 ^b	2.089 \pm 0.830 ^b	16.268 \pm 10.478 ^a	2.502	18.770	13.33	86.67
		F4	0.292 \pm 0.210 ^b	0.101 \pm 0.105 ^b	2.117 \pm 0.648 ^b	16.709 \pm 10.004 ^a	2.511	19.220	13.07	86.93
		F5	0.300 \pm 0.212 ^b	0.098 \pm 0.092 ^b	2.227 \pm 0.653 ^b	14.711 \pm 9.892 ^a	2.624	17.335	15.14	84.86
	Mean	0.311 (1.64)	0.112 (0.59)	2.145 (11.29)	16.367 (86.43)	2.569	18.935	13.59	86.41	
	Non-recycled water	F6	0.340 \pm 0.238 ^b	0.131 \pm 0.126 ^b	1.919 \pm 0.440 ^b	12.205 \pm 7.798 ^a	2.390	14.595	16.37	83.63
		F7	0.323 \pm 0.225 ^b	0.127 \pm 0.105 ^b	1.994 \pm 0.450 ^b	12.214 \pm 8.899 ^a	2.443	14.657	16.67	83.33
		F8	0.343 \pm 0.243 ^b	0.112 \pm 0.093 ^b	1.870 \pm 0.679 ^b	16.022 \pm 10.113 ^a	2.325	18.347	12.67	87.33
		F9	0.360 \pm 0.250 ^b	0.110 \pm 0.097 ^b	1.914 \pm 0.441 ^b	16.321 \pm 9.998 ^a	2.383	18.704	12.74	87.26
F10		0.371 \pm 0.264 ^b	0.114 \pm 0.087 ^b	1.968 \pm 0.399 ^b	16.620 \pm 11.220 ^a	2.453	19.073	12.86	87.14	
Mean	0.347 (2.03)	0.119 (0.70)	1.933 (11.32)	14.676 (85.95)	2.399	17.075	14.26	85.74		

The Cr in the AR fraction in the areas with recycled water ranged from 0.098 mg/Kg to 0.135 mg/Kg, with a mean percentage of 0.59%, whereas that in the areas with N-RCL water ranged from 0.110 mg/Kg to 0.131 mg/Kg, with a mean percentage of 0.70%. The Cr in the OO fraction ranged from 1.986 mg/Kg to 2.307 mg/Kg and 1.870 mg/Kg to 1.994 mg/Kg, with mean percentages of 11.29% and 11.32%, in the areas with RCL and N-RCL water, respectively. Cr in the RR fraction in the areas with RCL water ranged from 14.711 mg/Kg to 17.844 mg/Kg, with a mean percentage of 86.43%, whereas in areas with N-RCL water it ranged from 12.205 mg/Kg to 16.620 mg/Kg, with a mean percentage of 85.95%.

The fractionation of Cr in the paddy soils of MADA was in the order of RR > OO > ELFE > AR for areas supplied with both types of irrigation water (Figs. 2 and 3). The results indicated that the Cr concentrations were significantly ($p > 0.05$) higher in the RR fraction of the soils in rice fields supplied with both RCL and N-RCL water. The results also suggest that the Cr found in the soil samples was obtained from natural marine alluvial deposits. Among the non-resistant fractions in the areas with RCL water, the mean percentages of Cr in the ELFE, AR, and OO were 12.11%, 4.36%, and 83.50%, respectively, whereas the percentages in the areas with N-RCL water were 14.46%, 4.96%, and 80.58%, respectively. The present study indicates that a significant amount of Cr that is relatively stable in soils, was derived mainly from natural deposits. Cr concentration of the RR fraction of the studied area was low compared to that of agricultural soils in Thailand (91 mg/Kg)²⁴ and paddy soils of the Pearl River Delta (54.3 mg/Kg)¹⁷.

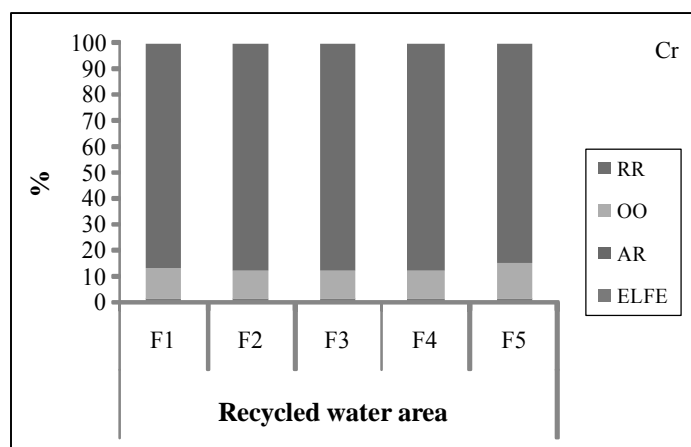


Fig. 2: Distribution of Cr in soil fractions of areas with recycled water

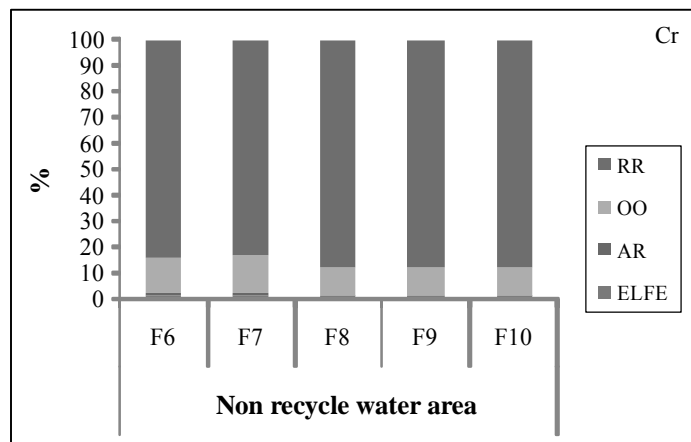


Fig. 3: Distribution of Cr in soil fractions of areas with non-recycled water

Mn in the soil fractions

Mn is one of the most important elements required for optimal plant growth. The geochemical fractions of Mn in the paddy soils of MADA, Kedah are tabulated in Table 3. The total Mn concentrations in the areas supplied with RCL water ranged from 31.948 mg/Kg to 44.444 mg/Kg, whereas in areas with the N-RCL water, it ranged from 27.673 mg/Kg to 33.260 mg/Kg. Mn in the ELFE fraction in the areas supplied with RCL and N-RCL water ranged from 12.84 mg/Kg to 16.88 mg/Kg and 8.69 mg/Kg to 17.46 mg/Kg, with mean percentages of 38.04% and 43.09%, respectively. Mn in the AR fraction of the paddy fields supplied with RCL water ranged from 8.73 mg/Kg to 13.25 mg/Kg, with a mean percentage of 28.23%, whereas that in the paddy fields supplied with N-RCL water, it ranged from 7.36 mg/Kg to 12.40 mg/Kg, with a mean percentage of 30.38%. Mn concentration in the OO fraction ranged from 1.71 mg/Kg to 2.86 mg/Kg and 0.90 mg/Kg to 1.09 mg/Kg, with a mean percentage of 6.37% and 3.26%, in the areas supplied with RCL and N-RCL water, respectively. The Mn in the RR fraction of the areas with recycled water ranged from 8.52 mg/Kg to 11.46 mg/Kg, with a mean percentage of 27.34%, whereas that in the areas supplied with N-RCL water ranged from 5.35 mg/Kg to 8.76 mg/Kg with a mean percentage of 23.28%.

The fractionation of Mn in the paddy soils of MADA was in the order of ELFE > AR > RR > OO for both areas with both types of irrigation water as (Figs. 4 and 5). In the present study, Mn concentration was found to be significantly highest ($P > 0.05$) in the ELFE soil fractions for all the study areas.

Table 3: Concentration of Mn (mg/Kg) in paddy soils of MADA, Kedah

Mn		ELFE	AR	OO	RR	Non resistant	Total	Non-resistant (%)	Resistant (%)	
Area	Fields	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD					
Areas using	Recycled water	F1	16.88 \pm 9.57 ^a	13.25 \pm 7.92 ^a	2.86 \pm 2.03 ^b	11.46 \pm 11.68 ^a	32.989	44.444	74.23	25.77
		F2	13.63 \pm 7.75 ^a	10.74 \pm 6.96 ^a	2.85 \pm 2.25 ^b	11.11 \pm 9.66 ^a	27.218	38.330	71.01	28.99
		F3	13.39 \pm 6.49 ^a	8.97 \pm 5.67 ^a	1.71 \pm 0.93 ^b	9.79 \pm 8.60 ^a	24.076	33.870	71.08	28.92
		F4	13.49 \pm 5.71 ^a	10.42 \pm 6.58 ^a	2.46 \pm 2.13 ^b	9.59 \pm 7.90 ^a	26.365	35.953	73.33	26.67
		F5	12.84 \pm 7.26 ^a	8.73 \pm 5.49 ^b	1.86 \pm 1.04 ^c	8.52 \pm 6.28 ^b	23.427	31.948	73.33	26.67
	Mean	14.04 (38.04)	10.42 (28.23)	2.35 (6.37)	10.09 (27.34)	26.816	36.909	72.60	27.40	
	Non recycled water	F6	8.69 \pm 6.90 ^a	12.40 \pm 15.81 ^a	1.09 \pm 0.65 ^b	6.47 \pm 3.66 ^{ab}	22.170	28.645	77.40	22.60
		F7	17.46 \pm 27.58 ^a	8.16 \pm 7.85 ^{ab}	0.98 \pm 0.74 ^b	5.35 \pm 4.55 ^b	26.602	31.953	83.25	16.75
		F8	11.66 \pm 9.49 ^a	7.36 \pm 7.17 ^a	0.90 \pm 0.41 ^b	7.75 \pm 5.18 ^a	19.922	27.673	71.99	28.01
		F9	12.03 \pm 6.32 ^a	8.87 \pm 8.15 ^a	1.05 \pm 0.58 ^b	8.76 \pm 4.24 ^a	21.945	30.706	71.47	28.53
F10		15.76 \pm 10.05 ^a	9.46 \pm 9.11 ^b	0.94 \pm 0.37 ^c	7.10 \pm 5.90 ^b	26.163	33.260	78.66	21.34	
Mean	13.12 (43.09)	9.25 (30.38)	0.992 (3.26)	7.087 (23.28)	23.360	30.447	76.55	23.45		

The high bioavailability of Mn can possibly be attributed to the application of agrochemical fertilizers and pesticides in these areas. Rock phosphate fertilizers have been reported to contain high amounts of Mn²⁵. Among the non-resistant fractions, the mean

percentages of Mn in the ELFE, AR, and OO in the areas supplied with recycled water were 52.36%, 38.86%, and 8.76%, respectively, whereas the percentages in the areas with the N-RCL water were 56.16%, 39.60%, and 4.25%, respectively. In Malaysia, NPK fertilizers (with different ratio combinations) are repeatedly used for paddy cultivation. The addition of fertilizers/micronutrients to agricultural soils may result in enhanced Fe and Mn levels²⁶. The repeated application of these fertilizers over several years could possibly be responsible for the accumulation of Mn in paddy soils. According to Wong et al.¹⁶, Mn in crops and natural soils is predominantly associated with Fe-Mn fractions. Manceau et al.²⁷ found that Mn in the clayey paddy soils of Vietnam was concentrated in Fe-Mn soft mottles.

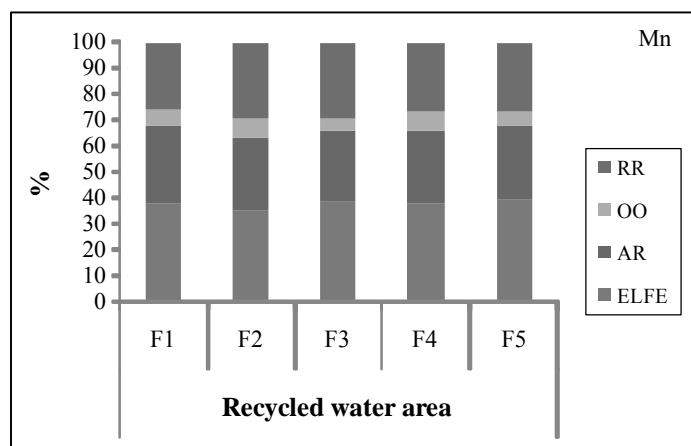


Fig. 4: Distribution of Mn in soil fractions of areas with recycled water

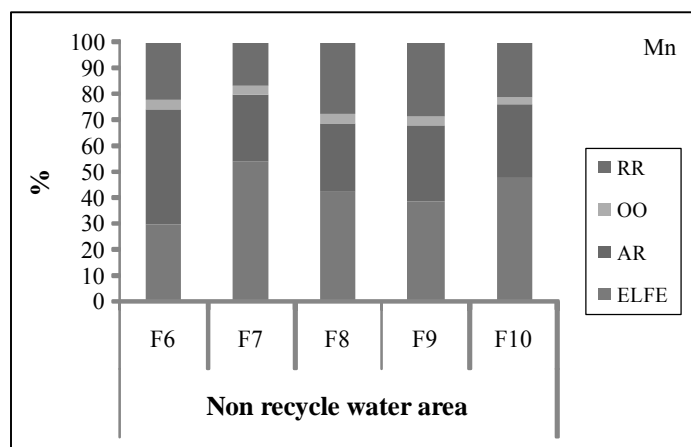


Fig. 5: Distribution of Mn in soil fractions of areas with non-recycled water

Fe in the soil fractions

Fe is an essential element for plants and other living organisms because of its importance in major biological mechanisms such as electron transport, nitrogen fixation, and DNA synthesis. The geochemical fractions of Fe in the paddy soils of MADA, Kedah are shown in Table 4. The total Fe concentration in the areas supplied with RCL water ranged from 21256.895 mg/kg to 26915.28 mg/kg, whereas in the areas with N-RCL water its concentration ranged from 16090.196 mg/kg to 18420.457 mg/kg. The concentration of Fe in the ELFE fraction in the areas supplied with RCL and N-RCL water ranged from 597.62 mg/kg to 911.38 mg/kg and 610.17 mg/kg to 900.25 mg/kg, with mean percentages of 3.17% and 4.56%, respectively. The concentration of Fe in the AR fraction in the areas supplied with RCL water ranged from 1037.31 mg/kg to 1954.97 mg/kg, with a mean percentage of 6.39%, whereas in the areas with N-RCL water, it ranged from 1102.94 mg/kg to 2091.85 mg/kg, with a mean percentage of 9.80%. Fe concentration in the OO fraction ranged from 2135.13 mg/kg to 2361.65 mg/kg and 852.07 mg/kg to 1607.50 (mg/kg), with mean percentages of 9.89% and 7.35%, for the areas supplied with RCL and N-RCL water, respectively. The Fe concentration in the RR fraction in the areas with RCL water ranged from 16028.9 mg/kg to 22057.5 mg/kg, with a mean percentage of 80.56%, whereas in the areas with N-RCL water, it ranged from 12427.80 mg/kg to 14855.2 mg/kg, with a mean percentage of 78.29%.

The fractionation of Fe in the paddy soils of MADA was in the order of RR > OO > AR > ELFE in the areas supplied with RCL water (Fig. 6), and in the order of RR > AR > OO > ELFE for areas with N-RCL water (Fig. 7). The iron concentration in paddy soils was significantly ($p > 0.05$) the highest in the RR fraction. The results suggest that the Fe found in the soil samples came from natural, marine alluvial deposits that are low in Fe. The increased levels, of soil Fe in the studied soils can possibly be attributed to the fertilizers applied. According to Jäckel and Schnell²⁸, ferric iron fertilization can suppress methane emission in paddy soils. In the current study, both types of fertilizers used (i.e., N: P: K 13:13:21 and N: P: K 12:12:17:2 + TE) contained Fe, and these fertilizers could have contributed to the increased levels of Fe in the paddy soils after several years of continuous usage. Soils in the study areas were also slightly acidic and exposed to the redox fluctuation condition. Under these conditions, the insoluble hydrated iron oxide (Fe (OH)₃) and the more stable dehydrated iron oxides, such as goethite and lepidocrocite, could have been developed^{27,29}. The high Fe concentration in the RR, OO, and AR fractions can thus be explained by the occurrence of these insoluble forms of Fe in the soils or by the low bioavailability of the Fe.

Table 4: Concentration of Fe (mg/Kg) in paddy soils of MADA, Kedah

Fe Area	Fields	ELFE		AR		OO		RR		Total	Non resistant (%)	Resistant (%)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Non resistant	Resistant			
Recycled water	F1	738.52 ± 1079.64 ^b	1037.31 ± 1550.00 ^b	2258.20 ± 2972.28 ^b	22057.5 ± 15427.16 ^a	4034.028	26091.528	15.46	84.54			
		597.62 ± 992.22 ^b	1111.47 ± 1656.08 ^b	2304.41 ± 3078.84 ^b	19596.3 ± 12296.53 ^a	4013.501	23609.801	17.00	83.00			
	F3	647.06 ± 965.83 ^b	1303.60 ± 1926.60 ^b	2135.13 ± 2703.15 ^b	17406.3 ± 10402.61 ^a	4085.789	21492.089	19.01	80.99			
		758.88 ± 1121.15 ^b	1952.99 ± 2884.23 ^b	2339.57 ± 3020.30 ^b	17755.4 ± 9981.97 ^a	5051.440	22806.840	22.15	77.85			
	F5	911.38 ± 1343.40 ^b	1954.97 ± 2891.91 ^b	2361.65 ± 3139.19 ^b	16028.9 ± 5896.64 ^a	5227.995	21256.895	24.59	75.41			
Mean		731 (3.17)	1472 (6.39)	2280 (9.89)	18569 (80.56)	4482.55	23051.43	19.64	80.36			
Non recycled water	F6	854.82 ± 1303.39 ^b	1102.94 ± 1653.66 ^b	1607.50 ± 2018.60 ^b	14855.2 ± 5160.56 ^a	3565.257	18420.457	19.35	80.65			
		900.25 ± 1317.52 ^b	1778.25 ± 2645.70 ^b	1444.45 ± 1689.14 ^b	12770.4 ± 5131.60 ^a	4122.948	16893.348	24.41	75.59			
	F8	745.62 ± 1101.64 ^b	1790.13 ± 2642.30 ^b	1493.78 ± 1923.36 ^b	13998.5 ± 5080.13 ^a	4029.531	18028.031	22.35	77.65			
		835.75 ± 1226.54 ^b	1713.81 ± 2535.13 ^b	852.07 ± 923.29 ^b	13662 ± 3599.13 ^a	3401.631	17063.631	19.93	80.07			
	F10	610.17 ± 888.96 ^b	2091.85 ± 3096.80 ^b	960.38 ± 1173.37 ^b	12427.80 ± 4854.53 ^a	3662.396	16090.196	22.76	77.24			
Mean		789 (4.56)	1695 (9.80)	1272 (7.35)	13542.78 (78.29)	3756.353	17299.132	21.76	78.24			

Areas using

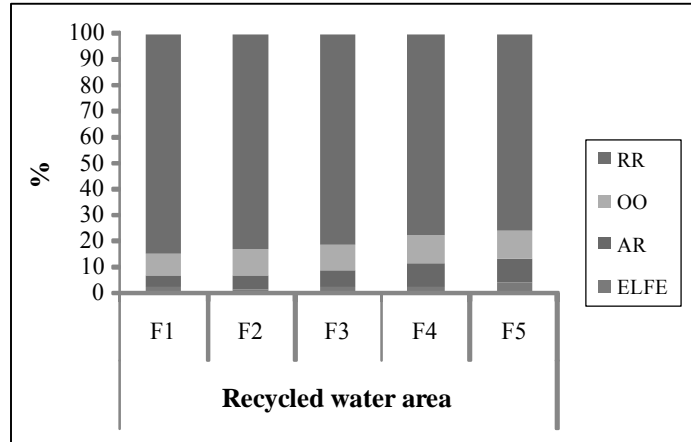


Fig. 6: Distribution of Fe in soil fractions of areas with recycle

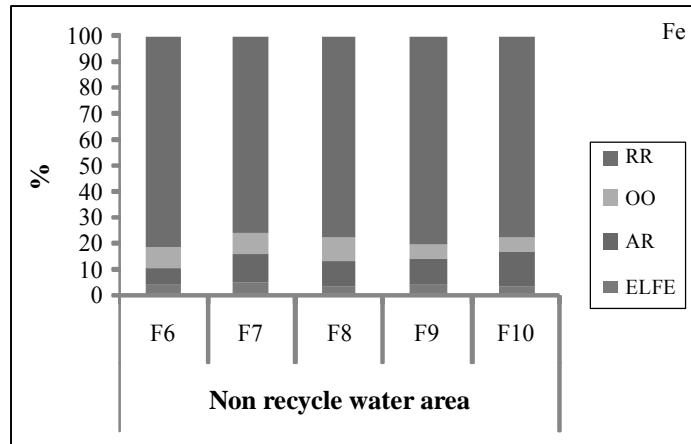


Fig. 7: Distribution of Fe in soil fractions of areas with non-recycled water

Table 5 summarizes the heavy metal concentration at different sampling times (seedling, mid season and harvesting stages). The Duncan test showed that most of the heavy metals were significantly ($p > 0.05$) at the highest concentration at harvesting time except for Pb, which had significantly ($p > 0.05$) higher concentration at the seedling stage. On the other hand, Mn concentration was not significantly different at all the sampling times.

The difference of heavy metal concentrations in paddy soils supplied with RCL with and N-RCL water was compared using the paired sample T-test (Table 6). The results of the statistical analysis suggested that the highest significant difference ($p > 0.05$) in the concentration of heavy metals was in the areas supplied with recycled water compared to

those with regular, irrigation water (non-recycled) for all metal ions, with the exception of Cr, where no significant difference was observed.

Table 5: Heavy metal concentration in paddy soils of MADA, Kedah at different sampling times

Metal	N	Sample 1 (seedling time)		Sample 2 (middle season)		Sample 3 (harvesting time)	
		Mean \pm S.D	Range	Mean \pm S.D	Range	Mean \pm S.D	Range
Cr	200	1.781 \pm	0.030-	4.360 \pm	0.006-	7.363 \pm	0.029-
		2.017 ^c	11.201	6.988 ^b	24.758	11.815 ^a	39.015
Mn	200	7.981 \pm	0.408-	8.159 \pm	0.004-	9.118 \pm	0.422-
		11.094 ^a	102.335	8.291 ^a	47.935	7.432 ^a	28.152
Fe	200	2229.41 \pm	0.031-	4203.910 \pm	-0.964-	8698.14 \pm	0.567-
		3794.835 ^c	15700	6257.687 ^b	25660	10738.46 ^a	44070

Table 6: The mean difference of heavy metal concentration in areas supplied with recycled and non-recycled water

Metal	N	Recycled water	Non recycled water	Mean Difference	Sig. (2-tailed)
		Mean \pm S.D	Mean \pm S.D		
Cr	300	4.734 \pm 8.872	4.269 \pm 7.729	0.465	0.494
Mn	300	9.227 \pm 7.918	7.612 \pm 10.046	1.615	0.029
Fe	300	5762.860 \pm 9457.154	4324.780 \pm 6051.586	1438.083	0.027

CONCLUSION

The repeated use and application of chemical fertilizers and pesticides in the paddy fields of MADA over a prolonged period will result in significant accumulation of heavy metals and other pollutants in the soils. The chemical fractionation of the studied metals in majority of stations were in the order of Fe in the area irrigated with recycled water; RR (80.55%) > OO (9.89) > AR (6.39%) > ELFE (3.17) and for the areas irrigated with N-RCL water in the order RR (78.29%) > AR (9.80%) > OO (7.35%) > ELFE (4.56%); Mn ELFE (38.05 & 43.09%) > AR (28.24 & 30.37%) > RR (27.35 & 23.28%) > OO (6.36 & 3.26%) for RCL and N-RCL irrigation water, respectively. Cr: RR (86.43%) > OO (11.33%) >

ELFE (1.64%) > AR (0.59%) for the areas irrigated with RCL water and RR (85.95%) > OO (11.32%) > ELFE (2.03%) > AR (0.69%) for the area with N-RCL water. The mean percentages of the anthropogenic portions of the investigated metals decreased in the order of Mn (72.60% and 76.55%) > Fe (19.64% and 21.76%) > Cr (13.59% and 14.26%) for the area irrigated with RCL and N-RCL water, respectively. The results showed that Fe and Cr in the paddy soils of MADA existed in the resistant fractions, whereas Mn existed in the non-resistant fractions. As the soils were clayey in nature, slightly acidic, rich in organic matter and exposed to the redox fluctuation conditions, most of these heavy metals tend to accumulate in the unavailable forms in the RR and OO fractions. An increase of Mn in non-resistant fractions, suggests that heavy usage of agrochemical materials for paddy cultivation could be the cause of the increase in the content of heavy metals in the soil. The results also showed that the main concentration of heavy metals in the paddy soil fractions was in the RR fraction with the exception of Mn, which was at the highest level in the ELFE fraction, followed by Mn in the ELFE fraction.

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REFERENCES

1. N. Rascio and F. Navari-Izzo, *Plant Sci.*, **180**, 169-181 (2011).
2. G. Kvesitadze, Khatisashvili, T. Sadunishvili and J. J. Ramsden, *Biochemical Mechanisms of Detoxification in Higher Plants: Basis of Phytoremediation*, Springer, New York (2006).
3. N. Akguc, I. Ozyigit and C. Yarci, *Pak. J. Bot.*, **40**, 1767-1776 (2008).
4. A. Kabata-Pendias and H. Pendias, *Trace Elements in Soils and Plants*, 2nd Ed. Boca Raton Fl; CRC Press (1992).
5. M. U. Etesin and N. U. Benson, Cadmium, Copper, Lead and Zinc Tissue Levels in Bonga Shad (*Ethmalosa Fimbriata*) and Tilapia (*Tilapia Guineensis*) caught from Imo River, Nigeria, *Am. J. Food Technol.*, **2**, 48-54 (2007).
6. M. A. Chukwujindu, S. O. N. Iwegbue, E. K. Ossai and G. E. Nwajei, *Amer. J. Food Tech.*, **3**, 220-223 (2008).

7. A. Tremel-Schaub and F. Isabelle, Contamination des Sols, EDP Sciences & ADEME Ed. Cedex (2005) p. 422.
8. Richard F. Shore and B. A. Rattner, Ecotoxicology of Wild Mammal, 1st Ed. Wiley (2001) p. 752.
9. N. A. Obasi, E. I. Akubugwo, O. C. Ugbogu and Glory Otuchristian, Asian J. Appl. Sci., **5**, 342-356 (2012).
10. A. B. Kara, Harley E. Hurlburt, Charlie N. Barron, Alan J. Wallcraft and E. Joseph Metzger, Tellus A, **62(1)**, 48-61 (2010).
11. N. Sridhara Chary, C. Kamala and D. Samuel Suman Raj, Ecotoxicol. Environ. Safe, **69**, 513-524 (2008).
12. A. Lu, S. Zhang and X -Q. Shan, Geoderma, **125**, 225-234 (2005).
13. M. Badri and S. Aston, Environ. Pollut. B, **6**, 181-193 (1983).
14. J. Duddridge and M. Wainwright, Environ. Pollut. B., **2**, 387-397 (1981).
15. A. Walkley and I. A. Black, Soil Sci., **37**, 29-38 (1934).
16. N. Prakongkep, A. Suddhiprakarn, I. Kheoruenromne and R. Gilkes, J.: Nat. Sci. **41**, 42-48 (2007).
17. S. Wong, X. Li, G. Zhang, S. Qi and Y. Min, Environ. Pollut., **119**, 33-44 (2002).
18. J. Khairiah, H. Habibah, I. Anizan, A. Maimon, A. Aminah and B. S. Ismail, J. Appl. Sci. Res., **5**, 2179-2188 (2009).
19. S. R. Smith and K. E. Giller, Soil Biol. Biochem., **24**, 781-788 (1992).
20. R. L. Chaney and S. B. Hornick, Accumulation and Effects of Cadmium on Crops, In: Cadmium 77, Proceeding of the 1st International Cadmium Conference, San Francisco (1978) pp. 125-140.
21. A. M. Chaudri, B. P. Knight, V. L. Barbosa-Jefferson, S. Preston, G. I. Paton, K. Killham, N. Coad, F. A. Nicholson, B. J. Chambers and S. P. McGrath, Environ. Sci. Technol., **33**, 1880-1885 (1999).
22. P. Prabu, Electron J. Environ. Agri. Food Chem., **8**, 818-827 (2009).
23. B. Jankauskas, G. Jankauskienė and M. A. Fullen, Ekologija, **53**, 21-28 (2007).
24. N. Prakongkep, A. Suddhiprakarn, I. Kheoruenromne, M. Smirk and R. J. Gilkes, Geoderma, **144**, 310-324 (2008).

25. V. Ramachandran, B. M. Bhujal and T. J. D'Souza, *Fresenius Environ. Bull.*, **7**, 551-556 (1998).
26. J. M. Hart, C. G. Landgren, R. A. Fletcher, M. C. Bondi, B. A. Whithrow-Robinson and G. A. Chastagner, *Christmas Tree Nutrient Management Guide: Western Oregon and Washington*, Corvallis, Or.: Extension Service, Oregon State University (2009).
27. A. Manceau, C. Tommaseo, S. Rihs, N. Geoffroy, D. Chateigner, M. Schlegel, D. Tisserand, M. A. Marcus, N. Tamura and Z. S. Chen, *Geochim. Cosmochim. Acta*, **69**, 4007-4034 (2005).
28. U. Jäckel and S. Schnell, *Soil Biol. Biochem.*, **32**, 1811-1814 (2000).
29. B. J. Alloway, Introduction, In: Alloway B. J. (Ed.) *Heavy Metals in Soils*, Blackie Academic & Professional, New York, 3-9 (1995).

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