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THE DISTRIBUTION AND ACCUMULATION STATUS OF HEAVY METALS (Pb, Cd, Cu AND Zn) IN SOILS FROM THE RICE FIELDS OF MADA IN KEDAH, MALAYSIA

NASSER ALRAWIQ[a,b], J. KHAIRIAH[a], M. T. LATIF[a] and B. S. ISMAIL[a*]

[a]School of Environmental & Natural Resource Sciences, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, MALAYSIA
[b]Department of Biology, Faculty of Arts & Science, Ubari, Sebha University, LIBYA

ABSTRACT

This study was conducted to determine the accumulation and distribution status of heavy metals (Pb, Cd, Cu, and Zn) in the soil fractions of the Muda Agricultural Development Authority (MADA) paddy fields situated in Jitra, in the state of Kedah, Malaysia. The study includes two areas that used different irrigation systems, namely non-recycled (N-RCL) and recycled (RCL) water. Five soil samples were collected from each area. The heavy metals in the soil were extracted using the sequential extraction method. The heavy metals were extracted from the four different fractions in the soil using different reagents. For the extraction from the first fraction, namely the easily leachable and ion exchange fraction (ELFE), ammonium acetate (NH_4CH_3COO) was used. For the second fraction, the acid reduction fraction (AR), hydroxylamine chloride ($NH_2OH.HCl$) was used, whereas for the third fraction, the organic oxidation fraction (OO), a combination of hydrogen peroxide (H_2O_2) and CH_3COONH_4 was used. For the last fraction, the resistant fraction (RR), concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) were used. The results showed that the heavy metals in the paddy soil fractions were mainly concentrated in the RR fraction. The exceptions were Pb, which was at the highest level in the OO fraction viz. 3.635 and 2.65 mg/kg in RCL and N-RCL irrigation water system. It was found that Cd was more evenly distributed throughout all the soil fractions. The results showed that the concentration of heavy metals in the studied area was in the following decreasing order: Zn > Pb > Cu > Cd.

Key words: Heavy metals, Soil fractions, Paddy fields.

INTRODUCTION

Heavy metals are critical environmental pollutants, and many of these metals are toxic even at very low concentrations. Pollution of the biosphere with toxic metals has

*Author for correspondence; E-mail: ismail@ukm.edu.my

increased dramatically since the beginning of the industrial revolution[1]. Heavy metal pollution is mainly the result of human activities such as agriculture, mining, construction, and industrial processes[2,3]. According to Kabata-Pendias and Pendias[4], improper waste disposal activities and overuse of pesticides are among the most significant sources of heavy metal pollution in the environment. Heavy metals in the environment are health hazards because of their persistence, bioaccumulation, and toxicity to plants, animals, and human beings[3,5].

Contamination and subsequent pollution of the environment by toxic, heavy metals have become issues of global concern because of their sources, widespread distribution, and multiple effects on ecosystems[6]. Heavy metals are generally present in agricultural soils at low levels[7]. Such metals have been reported to accumulate in the soil at toxic levels because of long-term application of wastewater[5].

Paddy fields are likely to receive large amounts of anthropogenic pollutants owing to the over use of chemical fertilizers and pesticides. These applications will eventually contribute to a significant accumulation of heavy metals in paddy field soils. The exploitation of polluted water for irrigation as well as pollution from animal manure, mining activities, and atmospheric depositions are factors that contribute to heavy metal contamination in agricultural areas. According to Chary et al.[8], heavy metals derived from anthropogenic sources can highly influence speciation and availability in soils. Most heavy metals derived from anthropogenic sources are likely to accumulate in the top soil[9].

In Malaysia, rice is one of the most important agricultural crops besides the commercially grown oil palm and rubber. North Malaysia has the largest paddy planting area in the country.The Muda plain, under the Muda Agricultural Development Authority (MADA) is considered as the rice bowl of Malaysia.The area covers the states of Kedah and Perlis where paddy is grown twice a year, during the wet and dry seasons, using irrigation water from the Muda and Pedu Dams in the state of Kedah, Malaysia.The present study aims at investigating the concentration and distribution status of selected heavy metals (Pb, Cd, Cu and Zn) in the paddy soil fractions of the rice fields of MADA.

EXPERIMENTAL

Materials and methods

The study was conducted on soils collected from the MADA agricultural area in the state of Kedah (6° 13’ N, 6° 10’ N and 100° 14’ E, 100° 18’ E) located northwest of Peninsular Malaysia. The study included two areas that used different irrigation systems,

namely non-recycled (N-RCL) and recycled (RCL) water. The soil samples were collected from a depth of 0 cm to 30 cm using an auger measuring 7 cm in diameter. Five soil samples were taken from each of the two irrigation systems (N-RCL and RCL). The soil samples were collected in April and Dec 2013. They were placed in polyethylene plastic bags and air-dried in the laboratory before being ground and sieved (using a 250 μm mesh)[10].

The soil physical characteristics analyzed were pH, particle size, and total organic carbon content. The soil pH was measured following the reported method[11]. In this method, 50 mL of diionized water was added to 20 g dried soil sample, and mixed for 30 min before the pH was measured using a pH meter (Model DELTA 320). The particle size was measured following the reported method[10], where a 10 g of the dried soil sample was passed through a 63 μm mesh using slow-flowing water. The organic content in the soil was determined using the reported method[12], where a 1.0 g dried soil sample was placed into a flask containing 10 mL of 1N potassium dichromate ($K_2Cr_2O_7$), then 20 mL of concentrated sulfuric acid (H_2SO_4) was added and the mixture heated on a hot plate for 30 min. Approximately 200 mL of deionized water was then added to the mixture, followed by 10 mL of concentrated phosphoric acid. Finally, 1 mL of diphenylamine was used as an indicator, and titration was carried out using ferrous ammonium sulfate 1.0 N (FAS).

A sequential extraction method[10] was used to extract the heavy metals from the soil samples. In this method, 10 g soil samples were extracted from the four different fractions using different reagents. For the first fraction, namely the easily leachable and iron exchange fraction (ELFE), the extraction reagent used was 50 mL of 1.0 M ammonium acetate (CH_3COONH_4), pH 7, at room temperature. The samples were shaken for 90 min at 150 rpm and centrifuged at 3,000 rpm for 30 min at 20°C. Each solution was then filtered using Millipore filter paper, (0.45 μm pore size) prior to metal analysis. The soil samples in the bottles were then washed with 50 mL distilled deionized water. The same procedure of shaking and centrifuging was adopted. For the second fraction, the acid reduction fraction (AR), 50 mL of 0.25 M hydroxylamine chloride ($NH_2OH.HCl$), pH 2 was used as the extraction reagent, at room temperature. For the third fraction, the organic oxidation fraction (OO), extraction was carried out using using 30% hydrogen peroxide (H_2O_2) in a water bath at 94°C to 97°C for approximately 1 h to 1.5 h. After cooling, the metal released from the organic complexes was continuously shaken with 1.0 M ammonium acetate (CH_3COONH_4) acidified to pH 3.5 with HCl at room temperature. For the last fraction, the resistant fraction (RR), concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) were used in the ratio of 5:2. From the first three extraction fractions (EFLE, AR, and OO) anthropogenic metals from sediments known to be from pollution sources were extracted. From the final fraction

of extraction (RR) natural metals strongly bonded to particles were extracted. The mixture was shaken and centrifuged during each extraction, and the solution was filtered prior to metal analysis. The heavy metal concentration was analyzed using inductively coupled plasma mass spectrometry.

The one-way ANOVA test was applied to detect significant differences in heavy metal concentrations within the paddy soil fractions and between the two irrigation systems. The mean difference in metal concentration between the areas using RCL and N-RCL water was determined using the T-test. All statistical analysis was performed using the SPSS 16.

RESULTS AND DISCUSSION

Paddy soils are of specific interest because they are used for rice 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 Mn[13]. Paddy soils are of interest to scientists whose aim has been to determine the levels and sources of the heavy metals[14].

Table 1 shows 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 recycled and non-recycled water, respectively). These results are similar to the soil pH values ranging from 4.36 to 6.36 reported for Kedah and Perlis[15]. pH is one of the most important factors that influence the bioavailability and transport of heavy metals in the soil. According to Smith and Giller[16], 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.

Table 1: Characteristics of the soils in the study areas

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

The results showed that the percentage soil grain size less than 63 µm ranged from 74.80% to 87.70% with a mean value of 82.53% in the areas using RCL water and from 73.20% to 87.90% with a mean value of 82.73% in areas using N-RCL water. All samples

contained high percentages of clay and silt grain of size less than 63 µm. According to Chaney and Hornick[17] and Chaudri et al.[18], clayey soils are responsible for lower metal bioavailability compared to sandy soils. Prabu[19] reported that the concentration of heavy metals in the soil is dependent on the clay content because clay particles have a large number of ionic binding sites due to their large surface area.

In the present study, the 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 the states of Kedah and Perlis, Malaysia in a previous study[15]. Soil organic matter content is the most important indicator of soil quality and productivity and 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 decay[20].

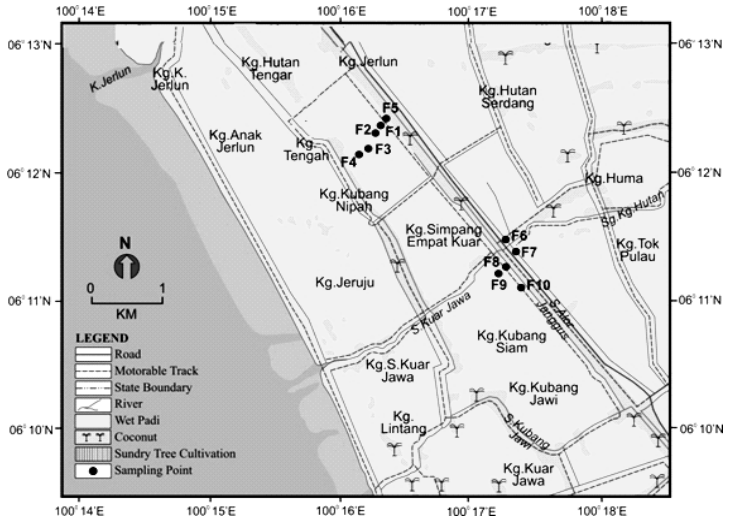

Fig. 1: Location of the sampling stations in the paddy fields of the study area in MADA, Kedah

Pb has toxic effects on living organisms and is often considered a contaminant. The mean concentration and percentage Pb from the sequential extractions at each sampling station, is shown in Table 2. The total Pb concentration ranged from 7.432 mg/kg to 7.697 mg/kg in the areas supplied with RCL water and from 5.671 mg/kg to 6.588 mg/kg in the areas with N-RCL water. Pb in the ELFE fraction ranged from 0.26 mg/kg to 0.38 mg/kg and 0.28 mg/kg to 0.50 mg/kg, with mean percentages of 4.50% and 6.43%, in the areas with RCL and N-RCL water, respectively. Pb concentration in the AR fraction of

the areas with RCL water ranged from 0.42 mg/kg to 0.57 mg/kg, with a mean percentage of 6.68%, whereas that in the areas with N-RCL water ranged from 0.46 mg/kg to 0.61 mg/kg, with a mean percentage of 8.71%. The Pb concentration in the OO fraction ranged from 3.17 mg/kg to 3.85 mg/kg and 2.08 mg/kg to 2.89 mg/kg, with mean percentages of 47.92% and 42.08%, for areas with RCL and N-RCL water, respectively. For the RR fraction Pb concentration ranged from 2.86 mg/kg to 3.43 mg/kg and 2.44 mg/kg to 2.83 mg/kg for areas with RCL and N-RCL water, respectively. In the present study, averages values of 40.91% and 42.77% were computed for Pb concentration in the RR fraction for areas supplied with RCL and N-RCL water, respectively. Fig. 2 shows the fractionation of Pb in the paddy areas supplied with RCL water at MADA in the descending order of OO > RR > AR > ELFE, and that in the areas with N-RCL water in the descending order of RR > OO > AR > ELFE (Fig. 3).

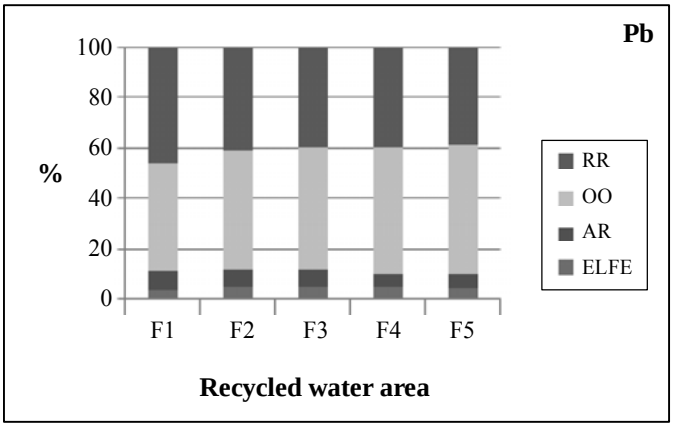

Fig. 2: Distribution of Pb in soil fractions of areas with recycled water

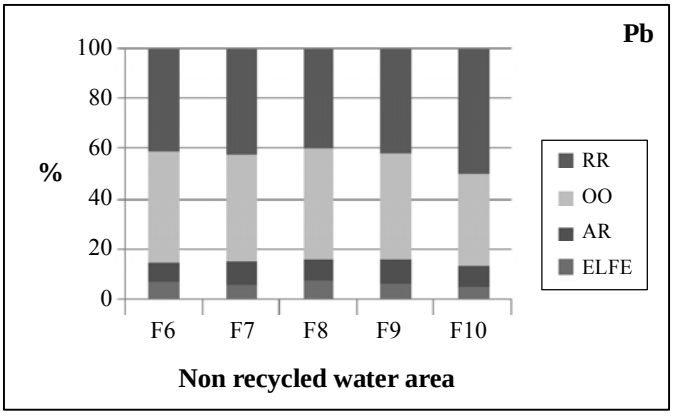

Fig. 3: Distribution of Pb in soil fractions of areas with non recycled water

The highest significant ($p > 0.05$) concentration of Pb in the areas with RCL water was in the OO fraction, where it accumulated and was strongly bonded with organic matter. According to Adriano[21], organic matter and clay are the dominant constituents contributing to Pb adsorption. Meanwhile, the highest significant ($p > 0.05$) concentration of Pb in the areas supplied with N-RCL water was in the RR fraction. Pb was largely associated in the resistant fraction, where it was strongly bonded to silicate minerals and was unavailable to plants grown in those areas. The first three fractions, namely the ELFE, AR, and OO constitute the non-resistant fractions[10]. Pb in the non-resistant fractions of the areas supplied with RCL and N-RCL water ranged from 0.341 mg/kg to 3.635 mg/kg, with mean values of 59.08% and 57.08%, respectively. The Pb concentrations at most sampling stations were dominant in the OO fraction. Morin et al.[22] showed that 60% of the total Pb in the soil is present as Pb–organic matter complexes, with 30% bound to hydrous manganese oxides and 10% to hydrous ferric oxides and goethite. In the paddy soil of the Pearl River Delta China, a large fraction of the Pb was also found to be bound in the Fe-Mn oxide phase, and the second largest fraction was residual Pb. Pb in agricultural soils is largely associated with Fe-Mn oxides, followed by organic/sulfide and residual fractions[14].

Cd, a toxic metal released into agricultural ecosystems, induces numerous changes in plant growth and physiology. The geochemical distribution and mean concentration of Cd are shown in Table 3. The total concentration of Cd ranged from 0.045 mg/kg to 0.060 mg/kg in the areas supplied with RCL water and from 0.044 mg/kg to 0.050 mg/kg in the areas with N-RCL water. Cd in the ELFE fraction ranged from 0.012 mg/kg to 0.016 mg/kg and 0.009 mg/kg to 0.013 mg/kg, with mean percentages of 26.31% and 23.40%, in the areas supplied with RCL and N-RCL water, respectively. Cd in the AR fraction in the area with RCL water ranged from 0.016 mg/kg to 0.022 mg/kg, with a mean percentage of 33.33%, whereas that in the area with the N-RCL water Cd concentration ranged from 0.016 mg/kg to 0.021 mg/kg, with a mean percentage of 38.30%. Cd in the OO fraction ranged from 0.008 mg/kg to 0.011 mg/kg and 0.006 mg/kg to 0.007 mg/kg, with mean percentages of 15.79% and 12.77%, in areas with RCL and N-RCL water, respectively. Cd in the RR fraction ranged from 0.009 mg/kg to 0.016 mg/kg and 0.009 mg/kg to 0.012 mg/kg, with mean percentages of 21.05% and 23.40%, in areas with RCL and N-RCL water, respectively.

The fractionation of Cd in the paddy soil was distributed in the descending order of AR > ELFE > RR > OO for both areas, as shown in Figs. 4 and 5. In the non-resistant fraction Cd concentration in the areas with RCL and N-RCL water ranged from 73.27% to 82.42%, with mean values of 78.48% and 77.18%, respectively. The total concentration of Cd was highest in the AR fraction of the paddy soils of MADA. This result complements

that reported by Khairiah et al.[23] for stations 4 and 6 in the KETARA area of Besut, in the state of Terengganu, Malaysia. Cd is largely associated in the AR fraction and its presence can be attributed to the continuous application of fertilizers and pesticides for paddy cultivation. Khairiah et al.[15] also reported that the bioavailability of Cd in paddy soils of Kedah can be attributed to the continuous application of agrochemical fertilizers and pesticides over several years.

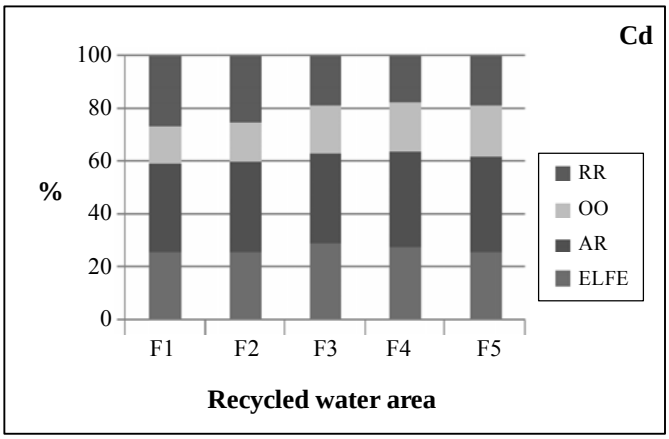

Fig. 4: Distribution of Cd in soil fractions of areas with recycled water

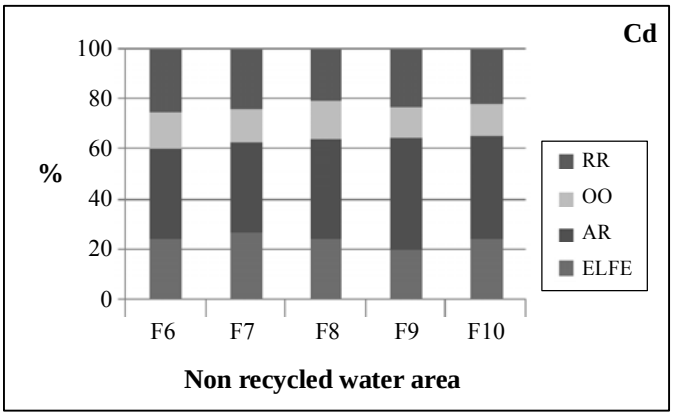

Fig. 5: Distribution of Cd in soil fractions of areas with non recycled water

Cu is one of the most important micronutrients essential for plant growth; it is an integral component of numerous enzymes and is actively involved in lignification[24]. The geochemical fractions of Cu in the paddy soils of MADA, Kedah, Malaysia are shown in Table 4. The total Cu concentration in the areas supplied with RCL water ranged from 1.243 mg/kg to 1.587 mg/kg, whereas in areas without N-RCL water it ranged from

0.734 mg/kg to 1.037 mg/kg. The Cu in the ELFE fraction for areas with RCL and N-RCL water ranged from 0.038 mg/kg to 0.081 mg/kg and 0.035 mg/kg to 0.037 mg/kg, with mean percentages of 3.84% and 4.15%, respectively. The Cu in the AR fraction in the areas supplied with RCL water ranged from 0.021 mg/kg to 0.044 mg/kg, with a mean percentage of 2.21%, whereas in areas with N-RCL water it ranged from 0.025 mg/kg to 0.038 mg/kg, with a mean percentage of 3.35%. The Cu in OO fraction ranged from 0.040 mg/kg to 0.159 mg/kg and 0.032 mg/kg to 0.044 mg/kg, with mean percentages of 5.20% and 4.50%, in the area supplied with RCL and N-RCL water, respectively. Cu in the RR fraction ranged from 1.108 mg/kg to 1.361 mg/kg, with a mean percentage of 88.75% in areas supplied with the RCL water, whereas in areas with N-RCL water it ranged from 0.615 mg/kg to 0.933 mg/kg, with a mean percentage of 88.01%.

The fractionation of Cu in the paddy soils of MADA was in the order of RR > OO > ELFE > AR for both areas, as shown in Figs. 6 and 7. The study showed that more than 85% of the Cu was significantly ($p > 0.05$) associated with the RR fraction. The results suggest that the Cu found in the soil samples could have been obtained from natural, marine alluvial deposits. Low Cu levels in marine alluvial soils were also reported by Khairiah et al.[25] for Sitiawan, in the state of Perak, Malaysia. Among the non-resistant fractions of the areas supplied with RCL water, the mean percentages of Cu in the ELFE, AR, and OO were 34.18%, 19.62%, and 46.20%, respectively, whereas the percentages in the areas with N-RCL water were 34.62%, 27.89%, and 37.5%, respectively. However, Cu in the RR fraction of the studied area was low compared to that of the agricultural soils of Cameron Highlands (77.39 mg/kg)[25] and the paddy soils of the Pearl River Delta China (14.1 mg/kg)[14].

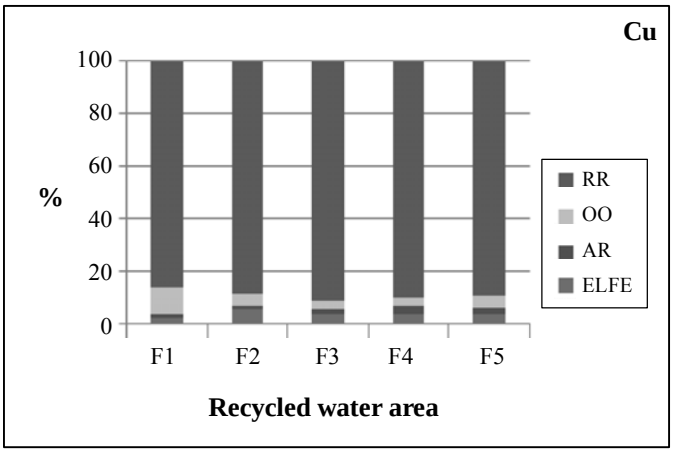

Fig. 6: Distribution of Cu in soil fractions of areas with recycled water

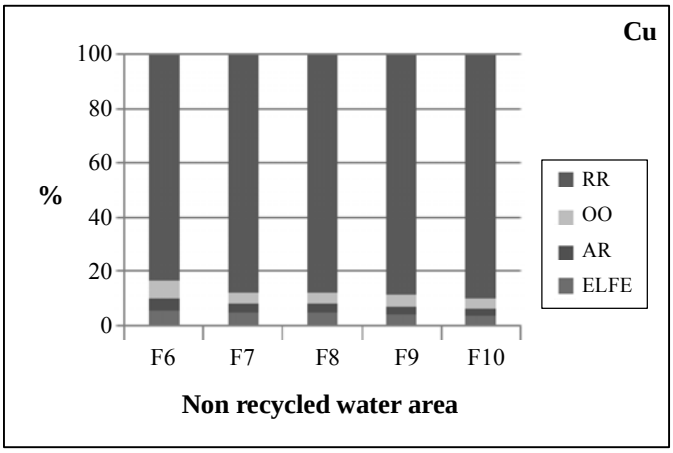

Fig. 7: Distribution of Cu in soil fractions of areas with non recycled water

Zn, in the form of micronutrients, is required by humans and other organisms in small quantities throughout life to orchestrate a range of physiological functions. The geochemical fractions of Zn in the paddy soils of MADA, Kedah are shown in Table 5. The total Zn concentration in the area supplied with RCL and N-RCL water ranged from 18.670 mg/kg to 20.888 mg/kg and 13.316 mg/kg to 21.845 mg/kg, respectively. Zn in the ELFE fraction for the areas receiving the RCL water ranged from 1.03 mg/kg to 1.32 mg/kg, with a mean percentage of 5.85%, whereas in areas with the N-RCL water it ranged from 0.55 mg/kg to 1.14 mg/kg, with a mean percentage of 4.24%. Zn in the AR fraction of the areas supplied with RCL and N-RCL water ranged from 1.86 mg/kg to 2.12 mg/kg and 1.31 mg/kg to 5.60 mg/kg, with mean percentages of 9.82% and 17.08%, respectively. Zn in the OO fraction ranged from 2.52 mg/kg to 2.82 mg/kg and 1.84 mg/kg to 2.39 mg/kg, with mean percentages of 13.07% to 12.02%, for the areas supplied with RCL and N-RCL water, respectively. Zn in the RR fraction ranged from 12.96 mg/kg to 15.14 mg/kg, with a mean percentage of 71.28% forareas supplied with RCL water, and it ranged from 9.46 mg/kg to 13.14 mg/kg, with a mean percentage of 66.63%, for the areas with N-RCL water.

The fractionation of Zn in the paddy soils of MADA was distributed in the order of RR > OO > AR > ELFE in the areas with RCL water and in the order of RR > AR > OO > ELFE in the areas with N-RCL water, as shown in Figs. 8 and 9, respectively. This study showed that most of the Zn was associated with the RR fraction rather than with the other fractions. The results suggest that Zn found in the RR fraction indicate the occurrence of Zn in the unavailable form. The Zn concentrations in the ELFE fraction of both areas were very

low, suggesting low bioavailability. The results suggest that paddy soils in the studied areas were deficient in Zn because of their physio-chemical characteristics and soil conditions. According to Adriano[21], Zn deficiency has been detected in many paddy soils throughout Asia, that are neutral to alkaline calcareous soils, especially in soils containing more than 1% organic matter. Among the non-resistant fractions, the mean percentages of Zn in the ELFE, AR, and OO constituted 20.38%, 34.20%, and 45.60% in the areas with RCL water and 12.72%, 51.22%, and 36.03% in the areas with N-RCL water, respectively. Sharma et al.[5] reported soil Zn concentration of 43.56 $\mu g g^{-1}$, which is lower than the concentration recorded in the present study. The high Zn concentration in the Kedah soils may be attributed to fertilizers and metal-based pesticides used in paddy cultivation.

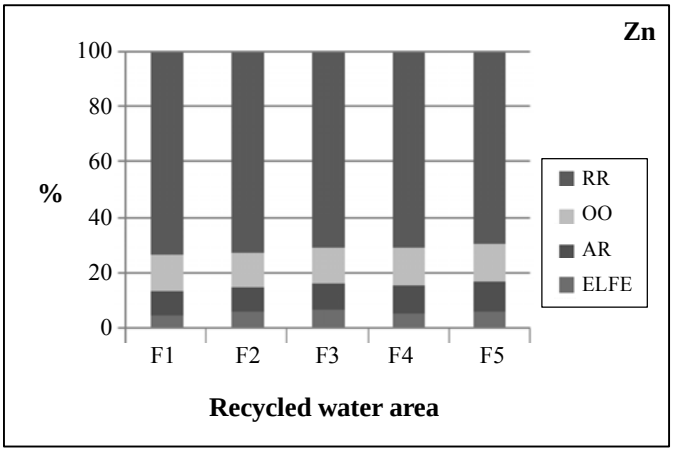

Fig. 8: Distribution of Zn in soil fractions of areas with recycled water

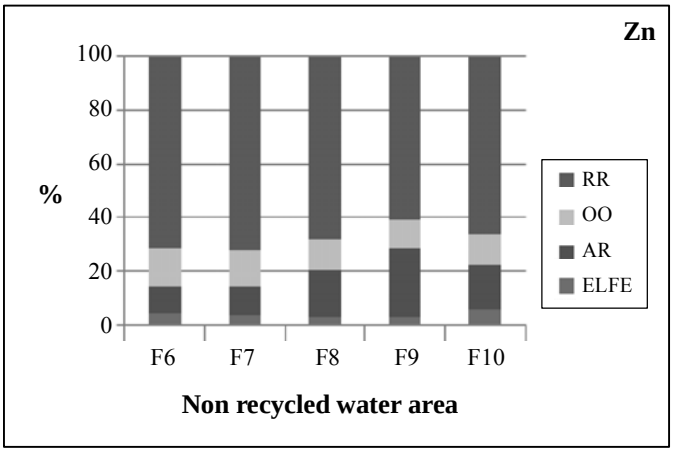

Fig. 9: Distribution of Zn in soil fractions of areas with non recycled water

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

Pb		ELFE	AR	OO	RR	Non resistant	Total	Nonresistant (%)	Resistant (%)
Areas	**Fields**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**				
Recycled water	F1	0.26 ± 0.21[b]	0.57 ± 0.52[b]	3.17 ± 1.86[a]	3.43 ± 1.54[a]	4.001	**7.432**	53.825	46.175
	F2	0.38 ± 0.31[b]	0.56 ± 0.50[b]	3.61 ± 2.28[a]	3.14 ± 1.22[a]	4.546	**7.688**	59.135	40.865
	F3	0.37 ± 0.29[b]	0.53 ± 0.45[b]	3.72 ± 2.74[a]	3.02 ± 1.16[a]	4.633	**7.650**	60.559	39.441
	F4	0.37 ± 0.28[b]	0.42 ± 0.30[b]	3.85 ± 2.59[a]	3.06 ± 1.06[a]	4.635	**7.697**	60.218	39.782
	F5	0.32 ± 0.26[b]	0.44 ± 0.33[b]	3.83 ± 2.53[a]	2.86 ± 0.77[a]	4.600	**7.460**	61.664	38.336
	Mean (%)	**0.341 (4.50)**	**0.507 (6.68)**	**3.635 (47.92)**	**3.103 (40.91)**	**4.483**	**7.585**	**59.080**	**40.920**
Non recycled water	F6	0.42 ± 0.36[b]	0.46 ± 0.33[b]	2.66 ± 2.55[a]	2.44 ± 0.47[a]	3.534	**5.971**	59.191	40.809
	F7	0.36 ± 0.30[b]	0.53 ± 0.35[b]	2.48 ± 2.26[a]	2.48 ± 0.66[a]	3.371	**5.854**	57.583	42.417
	F8	0.50 ± 0.44[b]	0.57 ± 0.36[b]	2.89 ± 2.75[a]	2.63 ± 0.72[a]	3.954	**6.588**	60.021	39.979
	F9	0.41 ± 0.33[b]	0.61 ± 0.40[b]	2.72 ± 2.27[a]	2.65 ± 0.73[a]	3.742	**6.389**	58.566	41.434
	F10	0.28 ± 0.23[c]	0.48 ± 0.31[c]	2.08 ± 1.68[b]	2.83 ± 0.95[a]	2.837	**5.671**	50.035	49.965
	Mean (%)	**0.392 (6.43)**	**0.531 (8.71)**	**2.565 (42.08)**	**2.607 (42.77)**	**3.488**	**6.095**	**57.079**	**42.921**

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

Cd		ELFE	AR	OO	RR	Non resistant	Total	Non resistant (%)	Resistant (%)
Area	**Fields**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**				
Recycled water	F1	0.015 ± 0.008^{ab}	0.020 ± 0.01^{a}	0.008 ± 0.003^{b}	0.016 ± 0.010^{a}	0.043	**0.059**	73.265	26.735
	F2	0.014 ± 0.009^{ab}	0.020 ± 0.017^{ab}	0.008 ± 0.004^{b}	0.014 ± 0.012^{a}	0.042	**0.057**	74.779	25.221
	F3	0.016 ± 0.012^{ab}	0.020 ± 0.015^{a}	0.010 ± 0.006^{b}	0.011 ± 0.009^{b}	0.046	**0.057**	81.064	18.936
	F4	0.016 ± 0.011^{ab}	0.022 ± 0.016^{a}	0.011 ± 0.006^{b}	0.011 ± 0.008^{b}	0.049	**0.060**	82.416	17.584
	F5	0.012 ± 0.007^{ab}	0.017 ± 0.012^{a}	0.009 ± 0.005^{b}	0.009 ± 0.004^{b}	0.037	**0.045**	80.891	19.109
	Mean (%)	**0.015 (26.31)**	**0.019 (33.33)**	**0.009 (15.79)**	**0.012 (21.05)**	**0.043**	**0.057**	**78.483**	**21.517**
Non Recycled water	F6	0.011 ± 0.006^{ab}	0.016 ± 0.010^{a}	0.007 ± 0.001^{b}	0.011 ± 0.008^{ab}	0.033	**0.044**	74.774	25.226
	F7	0.013 ± 0.009^{ab}	0.018 ± 0.015^{a}	0.007 ± 0.002^{b}	0.012 ± 0.009^{ab}	0.038	**0.049**	76.338	23.662
	F8	0.011 ± 0.005^{b}	0.018 ± 0.016^{a}	0.007 ± 0.002^{b}	0.009 ± 0.007^{b}	0.035	**0.045**	79.408	20.592
	F9	0.009 ± 0.005^{b}	0.020 ± 0.017^{a}	0.006 ± 0.002^{b}	0.010 ± 0.007^{b}	0.035	**0.045**	77.181	22.819
	F10	0.012 ± 0.007^{b}	0.021 ± 0.015^{a}	0.007 ± 0.002^{b}	0.011 ± 0.010^{b}	0.039	**0.050**	78.179	21.821
	Mean (%)	**0.011 (23.40)**	**0.018 (38.30)**	**0.006 (12.77)**	**0.011 (23.40)**	**0.036**	**0.047**	**77.176**	**22.824**

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

Cu		ELFE	AR	OO	RR	Non resistant	Total	Non resistant %	Resistant %
Area	Fields	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD				
Recycledwater	F1	0.038 ± 0.048[b]	0.028 ± 0.023[b]	0.159 ± 0.230[b]	1.361 ± 0.746[a]	0.226	**1.587**	14.22	85.78
	F2	0.081 ± 0.130[b]	0.021 ± 0.018[b]	0.066 ± 0.089[b]	1.262 ± 0.541[a]	0.168	**1.429**	11.74	88.26
	F3	0.056 ± 0.067[b]	0.025 ± 0.030[b]	0.040 ± 0.028[b]	1.248 ± 0.452[a]	0.121	**1.369**	8.82	91.18
	F4	0.051 ± 0.059[b]	0.044 ± 0.062[b]	0.047 ± 0.042[b]	1.255 ± 0.393[a]	0.141	**1.397**	10.13	89.87
	F5	0.045 ± 0.054[b]	0.037 ± 0.047[b]	0.054 ± 0.044[b]	1.108 ± 0.346[a]	0.135	**1.243**	10.90	89.10
	Mean	**0.054 (3.84)**	**0.031 (2.21)**	**0.073 (5.20)**	**1.247 (88.75)**	**0.158**	**1.405**	**11.16**	**88.84**
Non recycled water	F6	0.037 ± 0.047[b]	0.038 ± 0.050[b]	0.044 ± 0.040[b]	0.615 ± 0.256[a]	0.119	**0.734**	16.17	83.83
	F7	0.036 ± 0.046[b]	0.028 ± 0.028[b]	0.032 ± 0.020[b]	0.708 ± 0.243[a]	0.096	**0.804**	11.92	88.08
	F8	0.037 ± 0.041[b]	0.029 ± 0.033[b]	0.035 ± 0.041[b]	0.751 ± 0.301[a]	0.101	**0.852**	11.91	88.09
	F9	0.035 ± 0.036[b]	0.026 ± 0.029[b]	0.040 ± 0.046[b]	0.806 ± 0.343[a]	0.100	**0.906**	11.06	88.94
	F10	0.036 ± 0.038[b]	0.025 ± 0.026[b]	0.043 ± 0.039[b]	0.933 ± 0.362[a]	0.104	**1.037**	10.06	89.94
	Mean	**0.036 (4.15)**	**0.029 (3.35)**	**0.039 (4.50)**	**0.763 (88.01)**	**0.104**	**0.867**	**12.23**	**87.77**

Table 5: Concentration of Zn (mg/Kg) in paddy soils of MADA, Kedah

Zn		ELFE	AR	OO	RR	Non resistant	Total	Non resistant %	Resistant %
Area	**Fields**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**				
Recycled water	F1	1.03 ± 0.40^{b}	1.88 ± 1.50^{b}	2.68 ± 1.12^{b}	15.14 ± 10.33^{a}	5.588	**20.731**	26.95	73.05
	F2	1.23 ± 0.75^{b}	1.86 ± 1.36^{b}	2.52 ± 0.95^{b}	14.79 ± 9.21^{a}	5.607	**20.395**	27.49	72.51
	F3	1.32 ± 0.87^{b}	1.99 ± 1.49^{b}	2.61 ± 1.02^{b}	14.26 ± 7.87^{a}	5.923	**20.185**	29.34	70.66
	F4	1.18 ± 0.79^{b}	2.12 ± 1.91^{b}	2.82 ± 1.12^{b}	14.77 ± 8.03^{a}	6.119	**20.888**	29.30	70.70
	F5	1.14 ± 0.72^{b}	2.04 ± 2.03^{b}	2.54 ± 1.04^{b}	12.96 ± 7.32^{a}	5.714	**18.670**	30.61	69.39
	Mean	**1.18 (5.85)**	**1.98 (9.82)**	**2.64 (13.07)**	**14.38 (71.28)**	**5.790**	**20.174**	**28.74**	**71.26**
Non recycled water	F6	0.61 ± 0.52^{b}	1.31 ± 1.28^{b}	1.94 ± 1.35^{b}	9.46 ± 4.71^{a}	3.860	**13.316**	28.99	71.01
	F7	0.55 ± 0.38^{b}	1.47 ± 1.40^{b}	1.84 ± 1.07^{b}	9.95 ± 5.76^{a}	3.864	**13.810**	27.98	72.02
	F8	0.59 ± 0.38^{b}	3.12 ± 3.97^{b}	2.04 ± 1.14^{b}	12.12 ± 6.97^{a}	5.747	**17.867**	32.17	67.83
	F9	0.72 ± 0.43^{c}	5.60 ± 7.72^{b}	2.39 ± 1.31^{bc}	13.14 ± 7.52^{a}	8.707	**21.845**	39.86	60.14
	F10	1.14 ± 0.89^{b}	3.01 ± 3.71^{b}	1.98 ± 1.09^{b}	11.91 ± 7.19^{a}	6.132	**18.038**	34.00	66.00
	Mean	**0.72 (4.24)**	**2.90 (17.08)**	**2.04 (12.02)**	**11.31 (66.63)**	**5.662**	**16.975**	**32.60**	**67.40**

Table 6 shows 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 ($p > 0.05$) the highest concentration at the seedling stage. The difference of heavy metal concentrations in paddy soils supplied with RCL and N-RCL water were compared using the paired sample T-test (Table 7). 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 RCL water compared to those with N-RCL water for all the metal ions with the ion except of Zn where no significant difference was observed.

Table 6: Heavy metal concentration in paddy soils of MADA, Kedah at different sampling times

Metal	Sample 1 (Seedling stage)			Sample 2 (mid season)		Sample 3 (harvesting time)	
	N	Mean ± S.D	Range	Mean ± S.D.	Range	Mean ± S.D.	Range
Pb	200	2.382 ± 2.338[a]	0.123-8.448	1.119 ± 1.110[c]	-0.008-4.016	1.628 ± 1.588[b]	0.003-5.777
Cd	200	0.015 ± 0.011[a]	0.001-0.055	0.009 ± 0.006[b]	-0.002-0.028	0.014 ± 0.013[a]	0.001-0.063
Cu	200	0.188 ± 0.269[b]	0.005-0.980	0.265 ± 0.450[b]	-0.003-1.560	0.399 ± 0.642[a]	0.003-2.613
Zn	200	1.649 ± 1.180[c]	0.112-5.765	5.233 ± 6.339[b]	-0.251-22.608	7.049 ± 8.101[a]	0.113-28.999

Table 7: 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 ± S.D	Mean ± S.D		
Pb	300	1.896 ± 1.999	1.524 ± 1.615	0.373	0.012
Cd	300	0.014 ± 0.011	0.012 ± 0.010	0.002	0.010
Cu	300	0.351 ± 0.581	0.217 ± 0.354	0.135	0.001
Zn	300	5.043 ± 6.931	4.244 ± 5.753	0.800	0.125

CONCLUSION

The repeated use and application of chemical fertilizers and pesticides in the paddy fields of MADA over a period of time will eventually result in a significant accumulation of heavy metals and other pollutants in the soils. The chemical fractionation of the studied metals (except for Cd) in the majority of stations were in the order of resistant > oxidisable-organic > acid-reducible > exchangeable. The mean percentages of the anthropogenic portions of the investigated metals decreased in the order of Cd (78.48% and 77.18%) > Pb (59.08% and 57.08%) > Zn (28.74% and 32.60%) > Cu (11.16% and 12.23%) in the areas irrigated with RCL and N-RCL water, respectively. The results showed that Zn and Cu in the paddy soils of MADA existed in the resistant fractions, whereas Cd and Pb existed in the non-resistant fractions. Likewise, more than 45% of the Pb was associated with oxidisable-organic. Being clayey in nature, slightly acidic, rich in organic matter and exposed to the redox fluctuation conditions, most of these heavy metals tended to accumulate in the unavailable forms in the RR and OO fractions. An increase of Cd, and Pb in the non-resistant fractions, suggests that the heavy use of agrochemical materials for paddy planting activities could cause increase in the content of heavy metals in the soil. The results showed that the total concentration of heavy metals in the studied areas was in the following decreasing order: Zn > Pb > Cu > Cd. The results also showed that the heavy metals in paddy soil fractions were concentrated mainly in the RR fraction. The exceptions were Pb, which was highest in the OO fraction. Cd which was evenly distributed throughout the soil fractions.

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