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RESEARCH ARTICLE

Geofractionation of heavy metals and application of indices for pollution prediction in paddy field soil of Tumpat, Malaysia

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Abstract The present study investigates the concentration of Pb, Cd, Ni, Zn, and Cu in the paddy field soils collected from Tumpat, Kelantan. Soil samples were treated with sequential extraction to distinguish the anthropogenic and lithogenic origin of Pb, Cd, Ni, Zn, and Cu. ELFE and oxidizable-organic fractions were detected as the lowest accumulation of Pb, Cd, Ni, Zn, and Cu. Therefore, all the heavy metals examined were concentrated, particularly in resistant fraction, indicating that those heavy metals occurred and accumulated in an unavailable form. The utilization of agrochemical fertilizers and pesticides might not elevate the levels of heavy metals in the paddy field soils. In comparison, the enrichment factor and geoaccumulation index for Pb, Cd, Ni, Zn, and Cu suggest that these heavy metals have the potential to cause environmental risk, although they present abundance in resistant fraction. Therefore, a complete study should be conducted based on the paddy cycle, which in turn could provide a clear picture of heavy metals distribution in the paddy field soils.

Keyword Heavy metals · Paddy field soil · Sequential extraction procedure · Enrichment factor · Geoaccumulation index

Introduction

Kelantan is well-known as one of the major rice producers in Malaysia, after the Kedah State. In Kelantan, some of the paddy fields have been identified as actively functioning as rice producers, while others are not suitable at all. The majority of paddy fields located in Kelantan are under Kemubu Agricultural Development Authority (Kemubu), and this is also includes the current study area, located in Tumpat. Paddy fields are one of the habitats for aquatic organisms, particularly small fish, invertebrate species, and paddy eels (Sow et al. 2013). Generally, cultivated rice (*Oryza sativa*) is a common and annual grass that evolves from a semiaquatic ancestor, and due to its specific characteristics, rice is highly sensitive to water shortage (Kögel-Knabe et al. 2010). In Malaysia, the traditional way of growing the paddy rice that is being used nowadays by farmers involves the tillage of flooded soil (puddling), followed by rice transplantation, and growing the crop in a submerged environment from crop establishment to close to harvest (Gong 1983; Sahrawat 2005).

Paddy is a staple food in many countries worldwide, and many populations depend on it for survival and growth. For better production of paddy, many activities have been identified and could potentially contribute to the heavy metals pollution in paddy field soils. Fertilizers and pesticides could be one of the major threats to paddy and aquatic organisms (Sow et al. 2012a). Several organisms have been identified that could cause major problems to the paddy, such as Walker (*Scirpophaga incertulas*), rice field mice (*Rattus argentiventer*), and apple snails (*Pomacea* sp.). These organisms are predominantly dangerous, when they have large populations. By applying the pesticides and fertilizers regularly, paddy field soils are likely to accumulate the pollutant, since heavy metals in the soil are bound to the various phase components of the soil like Fe–Mn oxyhydrates, carbonates, clay minerals, sulfides, and organic or biological substances (Ismail 1994; Khairiah et al. 2009a; Sow et al. 2012b). The contaminated paddy soil could exert long-term risks to ecosystems and human health through the increased uptake and accumulation of heavy metals in paddy

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(Cobb et al. 2000) and aquatic organisms, particularly the paddy eel. Resuspension of persistent pollutants from the soil is a potential threat to existing organisms (Zulkifli et al. 2010a), especially during the plowing stage of a paddy cycle.

In this study, sequential extraction procedure was applied to paddy soil in order to investigate the mobility and bioavailability of Pb, Cd, and Ni collected from paddy cultivation around Tumpat. Therefore, it can provide information on the speciation of heavy metals, mode of occurrence, bioavailability, mobilization, and the transport of heavy metals (Pérez-Cid et al. 1999).

Materials and methods

Background of study area The study areas were conducted around paddy cultivation located at Tumpat, Kelantan, Peninsular Malaysia (Fig. 1). For this study, 2 months of paddy soil samples were collected in March and April, 2011. For each month, ten stations were identified and focused on as station points (S1–S10). Paddy cultivation of Tumpat was far away from the industrial area. Therefore, the possibility of pollutants might come from fertilizers and pesticides applied in paddy fields.

Sampling and storage The paddy soil was collected at each station. For each station, three bundles of paddy field soils were collected and mixed together thereafter, which are representative for each station. A depth of ≥20 cm of paddy soils was collected by using clean and acid-washed polyethylene scoop. According to Kögel-Knabe et al. (2010), anthraquic horizon (≥20 cm) is human-induced topsoil formed by wet cultivation and contains the puddle layer and the plow pan. After that, the paddy soil was placed in the polyethylene plastic bags. The samples were transported back to the laboratory and stored in the freezer at −20 °C until further analysis.

Sample preparation The paddy field soils were dried based on procedures established by Tanner et al. (2000). The collected paddy field soils were dried in the air circulating oven for at least 5–7 days at 60 °C until a constant dry weight obtained. Afterwards, the samples were ground by using mortar and pestle, until the soils became fine and small in size. Then, the samples were sieved through 63 μm size stainless steel sieve and shaken vigorously to produce homogeneity (Ismail 1993; Yap et al. 2002).

Sample digestion The procedure of acid digestion was applied in order to determine the total concentration of Pb, Cd, and Ni in paddy field soils. Firstly, 1 g of each dried paddy soil was weighed and placed in the digestion tube and labeled. Then, 10 mL of a combination of concentrated nitric acid (AnalaR Grade, BDH 69 %) and perchoric acid with ratio 4:1 was added into each digestion tube filled with dried paddy soil. After the addition, the digestion tubes were placed on the digestion block at 40 °C for 1 h and then increased up to 140 °C for at least 3 h (Ismail 1993; Ismail and Ramli 1997; Yap et al. 2002), until the chemical reaction fully stopped. Next, the samples were left to cool down for a short period at room temperature after the digestion process. The distilled water was added up into each digestion tube, until a certain volume (40 mL) was achieved. The diluted samples were filtered through Whatman no.1 filter paper, and the filtrate was stored in the cool room to wait for the metal determination.

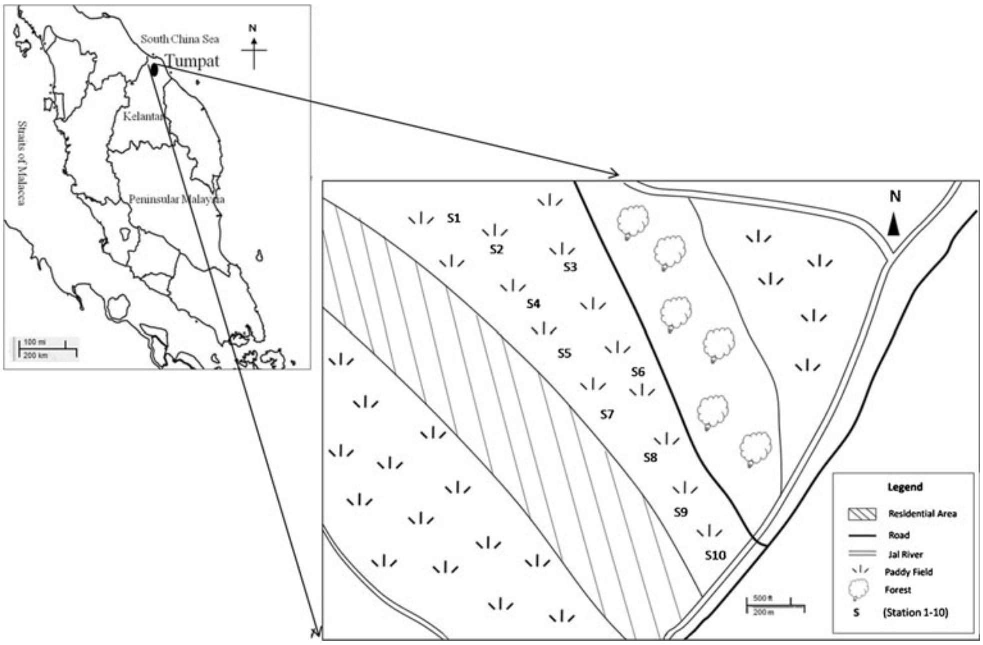

Fig. 1 March and April's sampling conducted at paddy field located at Tumpat, Kelantan

Geochemical fraction of Pb, Cd, Ni, Zn, and Cu in paddy soil Geochemical fraction of Pb, Cd, Ni, Zn, and Cu in paddy soil was extracted based on the modified sequential extraction technique, as described by Badri and Aston (1983) and Tessier and Campbell (1987). The four fractions can be abbreviated as EFLE (easily or freely leachable and exchangeable), acid-reducible, oxidizable-organic, and resistant fraction. The action taken for each fraction can be seen below:

1. EFLE: Ten grams of dried paddy soil was weighed. Fifty milligrams of 1.0 M ammonium acetate (NH_4CH_3COO), pH 7.0, was added into the sample and shaken for 3 h.
2. Acid reducible: The residue from (1) was weighed and recorded. Then, 50 mL of 0.25 M hydroxylammonium chloride ($NH_2OH{\bullet}HCl$) acidified to pH 2 with HCl was added into the residue (1). After that, the sample was shaken for 3 h.
3. Oxidizable organic: The residue from (2) was weighed and oxidized with 30 % H_2O_2 in a water bath at 90–95 °C. Next, 50 mL of 1.0 M ammonium acetate (NH_4CH_3COO) acidified to pH 2.0 with HCl was added into the sample and shaken for 3 h.
4. Resistant: The residue from (3) was digested with the combination of 10 mL concentrated nitric acid (AnalaR Grade, BDH 69 %) and perchloric acid (AnalaR Grade, BDH 60 %) in the ratio 4:1 as performed as acid digestion method.

The residue for each fraction was weighed before the next fraction was performed. After the filtration process of each fraction, the residue was washed with 20 mL of deionized distilled water and filtered through Whatman no.1 filter paper. The filtrate was stored in the polyethylene pill box and kept in the cool room until metal determination. A blank was employed for each fraction to ensure that the chemicals and samples used and processed in analysis from start to end point was free from any contamination.

Metals determination In this study, elements of Pb, Cd, Ni, Zn, and Cu were determined using an air–acetylene flame atomic absorption spectrophotometer, PerkinElmer Model AAnalyst 800. The value obtained was presented in $\mu g{\bullet}g^{-1}$ dw. Before metals determination was conducted, a standard solution of each element was freshly prepared from 1,000 $mg{\bullet}L^{-1}$ stock solution of each metal (Merck Titrisol).

Quality control Before any experiments or analysis were performed, all glassware and equipments were acid-washed in order to avoid possible contamination. For accuracy, the quality of the analysis applied to sample was checked with a certified reference material for soil (PACS-2) as shown in Table 1. In general, all selected metals showed a good and acceptable recovery percentage. The total concentration of each metal (Pb, Cd, Ni, Zn, and Cu) obtained through sum up of each fraction (EFLE, acid-reducible, oxidizable-organic, and resistant) was calculated and compared with the total concentration of Pb, Cd, Ni, Zn, and Cu from direct aqua regia method.

Enrichment factor (EF) and geoaccumulation index (I_{geo}) A number of calculation methods have been applied in this study to estimate the degree of metal enrichment in the soils (Ridgway and Shimmield 2002). In general, a common approach has been adapted to evaluate how much the anthropogenic impact on the soil is by calculating the enrichment factor for metal concentrations above uncontaminated background levels (Huu et al. 2010). The *EF* method normalizes the measured heavy metal concentration with respect to samples references, such as Fe, Al, or Zn (Mediolla et al. 2008). Therefore, in this study, the *EF* of heavy metals examined was calculated by the following equation (Huu et al. 2010):

$$EF = \frac{\left[\frac{C_{\text{metal}}}{C_{\text{normalizer}}}\right] soil}{\left[\frac{C_{\text{metal}}}{C_{\text{normalizer}}}\right] control}$$

in which ($C_{\text{metal}}/C_{\text{normalizer}}$)soil is the soil sample concentrations ratio of the heavy metal, while ($C_{\text{metal}}/C_{\text{normalizer}}$) control is the concentrations of background or baseline reference material such as average shale value (Salomas and Förstner 1984). Sutherland (2000) reported that when the value of *EF* increases, the distribution of anthropogenic inputs also rises. Therefore, five classifications of *EF* are created based on the degree of metal in soil, as seen in the following (Sutherland 2000):

EF <2	is deficiency to minimal enrichment
EF 2–5	is moderate enrichment
EF 5–20	is significant enrichment
EF 20–40	is very high enrichment
EF >40	is extremely high enrichment

Apart from this method, the geoaccumulation index (I_{geo}) has been widely used in many studies recently to estimate the degree of metal contamination or pollution (Tijani and Onodera 2009). The I_{geo} of metals studied in soil can be calculated according to this formula (Müller 1969):

$$I_{\text{geo}} = \log_2\left(\frac{C_{\text{metal}}}{1.5C_{\text{control}}}\right)$$

where C_{metal} represents the concentration of heavy metals in certain soil, and the C_{control} is the concentration of the

Table 1 A comparison of certified value (PACS-2) of Pb, Cd, Ni, Zn, and Cu with measured value ($\mu g \cdot g^{-1}$ dw)

Element	Certified value (a)	Measured value (b)	Percentage of recovery (b/a)
Pb	183.00±8.00	181.30±4.61	98.91
Cd	2.11±0.15	2.88±0.08	136.49
Ni	39.50±2.30	35.12±0.83	88.91
Zn	364±23	274.92±0.12	75.53
Cu	310±0.15	262.17±0.11	84.57

metal in control. A 1.5 factor is added into this formula to minimize the effect of possible variations in the background or to control values which belong to lithogenic variations in the soil (Stoffers et al. 1986). Muller's classification of the geoaccumulation index, which are practiced is used as reference, is divided into seven classes, as seen below:

$I_{geo} <0 =$	as unpolluted
$0< = I_{geo} <1$	as unpolluted to moderately polluted
$1< = I_{geo} <2$	as moderately polluted
$2< = I_{geo} <3$	as moderately to strongly polluted
$3< = I_{geo} <4$	as strongly polluted
$4< = I_{geo} <5$	as strongly to very strongly polluted
$I_{geo} > = 5$	as very strongly polluted

By applying these two calculation methods in this current study, the degree of metal contamination can be estimated in paddy field soils for both months (March and April).

Statistical analysis Statistical analysis of data was carried out by using SPSS statistical package program version 19. The *t* test was used in this study to compare the concentration of heavy metals between the 2 months examined. Therefore, the result obtained indicates that there is a nonstatistical ($p>0.05$) difference between the level of heavy metals (Cd, Pb, Ni, Zn, and Cu) for 2 months during observations. Furthermore, a nonstatistical difference ($p>0.05$) was obtained when a regression test was applied for a 2 months heavy metal comparison.

Results and discussion

Table 2 shows the mean concentrations of Pb, Cd, Ni, Zn, and Cu in paddy field soils collected in March. Resistant fraction was recorded as the highest accumulation of Pb (77.48±3.97 $\mu g \cdot g^{-1}$), followed by Zn (46.40±1.46 $\mu g \cdot g^{-1}$), and the lowest was found in Cd (0.70±0.08 $\mu g \cdot g^{-1}$). The concentrations of Ni and Cu in resistant fraction were 14.34±1.16 and 19.00±1.21 $\mu g \cdot g^{-1}$, respectively, compared to other fractions studied. A relatively low content of Pb (3.90±0.38 $\mu g \cdot g^{-1}$), Ni (0.24±0.13 $\mu g \cdot g^{-1}$), and Cu (0.07±0.01 $\mu g \cdot g^{-1}$) was detected in the acid-reducible fraction. However, Cd (0.06±0.02 $\mu g \cdot g^{-1}$) and Zn (4.12±0.75 $\mu g \cdot g^{-1}$) were found to be low in oxidizable-organic and EFLE fraction, respectively. Based on Fig. 2, the resistant fractions were much higher than nonresistant, except for Cu, where the resistant fraction was slightly lower than nonresistant. This situation might be due to the repeated application of agrochemical fertilizers by farmers to ensure their paddies growth in good condition, since Cu is one of the ingredients in agrochemical fertilizers used. The concentrations of Pb Cd, Ni, Zn, and Cu in paddy field soils collected on April were shown in Table 3. All measured mean metal concentrations were found within the range of previously reported mean concentrations in soils and sediments worldwide (Table 4). In general, the resistant fraction in this study gave relatively higher concentration of Pb (41.01±3.48 $\mu g \cdot g^{-1}$), Cd (0.83±0.19 $\mu g \cdot g^{-1}$), Ni (11.96±0.75 $\mu g \cdot g^{-1}$), Zn (52.33±3.97 $\mu g \cdot g^{-1}$), and Cu (15.76±2.02 $\mu g \cdot g^{-1}$) in paddy field soils than the bioavailable form (EFLE, acid-reducible, and oxidizable-organic fractions). Among the metals studied from various fractions of sequential extraction in the paddy field soils, the lowest concentrations of Pb (3.86±0.91 $\mu g \cdot g^{-1}$), Cd (0.04±0.01 $\mu g \cdot g^{-1}$), Ni (0.20±0.10 $\mu g \cdot g^{-1}$), and Cu (0.13±0.06 $\mu g \cdot g^{-1}$) were detected in the acid-reducible fraction, but not for Zn metal, in which EFLE fraction was marked as lowest concentration, which is 1.55±0.49 $\mu g \cdot g^{-1}$. Therefore, the resistant fraction of heavy metals was markedly higher than the nonresistant fraction, as shown on Fig. 3.

EF and geoaccumulation factor (I_{geo}) were calculated and shown in Table 5. For sampling conducted in March, the enrichment factor for Pb, Cd, Ni, Zn, and Cu are 2.85, 16.04, 0.30, 0.67, and 0.70, respectively. As can be seen, the enrichment factor of Pb, Cd, Ni, Zn, and Cu for April are 2.74, 5.63, 0.15, 0.54, and 0.60, respectively. The geoaccumulation index (I_{geo}) for March and April of each metal was shown in Table 5 as: Pb (2.96), Cd (−0.76), Ni (2.97), Zn (3.61), and Cu (2.97) for March and Pb (2.95), Cd (−1.20), Ni (2.67), Zn (3.51), and Cu (2.91) for April.

Pb in paddy soil Pb content in the paddy field soils collected from March and April were shown in Tables 2 and 3. Among the soil fractions studied for March and April paddy soil, the highest amount of Pb was only detected in the resistant fraction, and the lowest Pb concentration was

Table 2 Pb, Cd, Ni, Zn, and Cu concentration (μg•g^{-1} dw ± SD) in geochemical fractions of paddy field soils collected on March

Element	EFLE	Acid-reducible	Oxidizable-organic	Resistant	∑Nonresistant	Total (resistant+ nonresistant)
Pb	4.35±0.64	3.90±0.38	12.32±1.38	77.48±3.97	20.56±1.99	98.04±4.89
	(3.22–5.08)	(3.42–4.57)	(10.07–14.25)	(70.22–84.21)	(17.21–22.91)	(87.43–104.91)
Cd	0.26±0.04	0.15±0.05	0.06±0.02	0.70±0.08	0.46±0.06	1.17±0.07
	(0.21–0.33)	(0.06–0.22)	(0.02–0.08)	(0.57–0.83)	(0.37–0.54)	(1.02–1.23)
Ni	0.36±0.18	0.24±0.13	2.32±0.25	14.34±1.16	2.92±0.35	17.26±1.35
	(0.09–0.54)	(0.09–0.53)	(1.84–2.65)	(12.36–16.30)	(2.20–3.34)	(14.56–19.00)
Zn	4.12±0.75	7.72±1.13	7.23±0.81	46.4±1.46	19.07±2.46	65.47±3.51
	(3.16–5.69)	(6.31–10.38	(6.13–8.84)	(44.47–48.64)	(17.50–24.92)	(61.73–72.71)
Cu	0.51±0.04	0.07±0.01	19.77±1.89	19.00±1.21	20.34±1.89	39.34±2.79
	(0.45–0.57)	(0.04–0.08)	(16.88–22.56)	(17.32–20.74)	(17.46–23.13)	(34.78–42.69)

Parentheses indicates the range of values found in this study

found in the acid-reducible fraction. Moreover, the results indicate that the resistant percentage of Pb is much higher than percentages of nonresistant on both months. A study conducted by Khairiah et al. (2009a) on heavy metals in agricultural areas showed that the highest Pb content was found in FELDA Chini soil. This finding could probably depend mainly on the basic existing rock material in the sampling site. Generally, a predominantly high Pb concentration in resistant fraction could explain that there is a low distribution of anthropogenic sources; therefore the application of fertilizers and pesticides into paddy field soils is not likely to increase the Pb concentration.

In comparison, the Pb concentration in acid-reducible and EFLE was generally low. In paddy cultivation located at Kedah and Perlis, the Pb concentration was very low in both fractions (acid-reducible and EFLE) studied (Khairiah et al. 2009b). According to Adriano (1986), organic matter and clay are the important constituents contributing to Pb adsorption. It also has a tendency to be bound with oxides of Fe and Mn in soils. Therefore, the clayey nature of soils, the presence of high amounts of organic matter, and Fe and Mn are associated and responsible for the fixation of Pb in the unavailable form. Yet, the potential source of Pb could be from the transportation of vehicles and motorcycles in this study. A comparison of Pb concentration in surface soil collected from Peninsular Malaysia shown is much higher than in the present study, as shown in Table 4. However, the findings of Pb concentrations in paddy soil were low in bioavailable form compared to unbioavailable form.

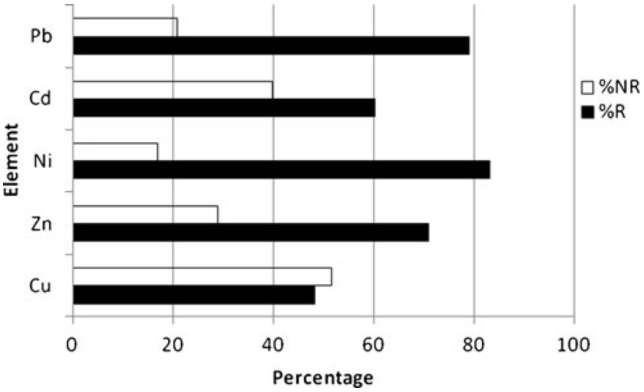

Fig. 2 Percentage of heavy metals in resistant (*R*) and nonresistant (*NR*) form of paddy soil collected on March

Cd in paddy soil Most of the Cd present in the paddy field soils collected on March and April was found to be highest in resistant fraction. The resistant percentage accounted for 60.21 and 81.05 % of total Cd for both months. For March, the total Cd concentration in paddy soil ranged from 1.02–1.25 μg•g^{-1}, while for April, it was 0.66–1.36 μg•g^{-1}. This value is slightly less than those reported for the paddy field soils of Kedah and Perlis, which ranged from 0.07–2.35 mg•kg^{-1}. But, as the studies conducted at Kedah and Penang of paddy field soils indicated, the total Cd concentration in the soil ranged from 3.54 to 20.86 mg•kg^{-1} (Habibah et al. 2011). These values were much higher than in the current study. In this study, the high amount of Cd in paddy field soils indicates that soils are naturally high in Cd.

Secondarily highest after resistant was EFLE (0.26 μg•g^{-1}) and the oxidizable-organic (0.11 μg•g^{-1}) fraction for March and April, respectively. These fractions indicating certain amounts of Cd were present in the bioavailable form. When comparing the Cd level deposited in Yan (0.12 mg•kg^{-1}) and Kerpan (0.21 mg•kg^{-1}) with the current study, we see that the utilization and application of agrochemical fertilizers and pesticides (Habibah et al. 2011) on paddy fields for several years could have increased the bioavailability of Cd in the study areas. Since the paddy field soils were collected during

Table 3 Pb, Cd, Ni, Zn, and Cu concentration ($\mu g \bullet g^{-1}$ dw ± SD) in geochemical fractions of paddy field soils collected on April

Element	EFLE	Acid-reducible	Oxidizable-organic	Resistant	∑Nonresistant	Total (resistant+ nonresistant)
Pb	4.82±1.31	3.86±0.91	12.82±1.48	41.01±3.48	21.49±2.26	62.50±5.34
	(2.51–6.94)	(2.50–4.88)	(9.80–15.02)	(35.07–46.53)	(17.99–25.670	(53.06–72.20)
Cd	0.05±0.01	0.04±0.01	0.11±0.05	0.83±0.19	0.20±0.06	1.03±0.24
	(0.03–0.06)	(0.02–0.06)	(0.05–0.18)	(0.52–1.09)	(0.10–0.28)	(0.66–1.35)
Ni	0.24±0.09	0.20±0.10	1.00±0.24	11.96±0.75	1.44±0.28	13.39±0.76
	(0.1–0.37)	(0.08–0.24)	(0.54–1.35)	(10.84–12.90)	(0.95–1.93)	(12.15–14.21)
Zn	1.55±0.49	4.25±1.18	3.91±0.61	52.33±3.97	9.72±2.00	62.05±4.64
	(0.59–2.41)	(2.98–6.46)	(3.25–5.20)	(47.56–59.53)	(7.74–14.07)	(56.03–68.68)
Cu	0.48±0.09	0.13±0.06	12.43±2.07	15.76±2.02	13.05±2.09	28.80±3.90
	(0.36–0.60)	(0.06–0.20)	(8.88–16.59)	(12.83–19.17)	(9.54–17.27)	(23.36–36.44)

Parentheses indicates the range of values found in this study

the plowing season of paddy cycle, the rapid plowing activity by farmers might possibly resuspend the Cd in paddy field soils and consequently increase the distribution of Cd concentration on paddy top soil. Cd is a normal element of phosphate fertilizers and contains different concentrations of Cd, depending on the source of phosphate rock (He et al. 2005). The phosphate fertilizers were applied on paddy fields to ensure the better growth and production of paddy. Unfortunately, the repeated and regularly application of fertilizers and pesticides could increase the Cd content in soils and become bioavailable to rice through the plant uptake. The manufactured fertilizers usually are not totally purified, and the presence of several impurities, particularly heavy metals, could be one of the behind reasons of high Cd amounts (Gimeno-Garcia et al. 1996). Apart of these factors, the soil type and redox condition might possibly be attributed to high amount of Cd in paddy soil. In flooded paddy soil, Cd in the strongly grayed soils tends to occur as sulfide mineral greenockite (CdS), hence the low solubility of Cd in the paddy field soils. After the paddy field soils return to the oxidizing state condition, the Cd^{2+} and $SO^{2-}{}_{4}$ are formed, and so increase in the mobility and bioavailability of Cd (Alloway 1995). The resulting of flooding and draining in the paddy field is likely to promote the formation of both the insoluble CdS (resistant fraction) and the soluble Cd^{2+} ion (ELFE fraction) (Habibah et al. 2011). The bioavailability of Cd concentrations in oxidizable-organic fraction indicates that paddy field soils were enriched with organic matter. The decomposition of organic matter is likely to be faster under tropical conditions (Khairiah et al. 2009b), and this might have resulted in the high Cd concentrations in paddy field soils collected from Tumpat. Kirkham (2006) has stated that organic matter tends to accumulate in the surface and subsurface layers of the soil profiles, either bioavailable as bulk materials or coating on particular matter. In addition, organic matter is well-known as an important reactive component in soils, with potential to retain the metal cations.

Ni in paddy soil Something similar was observed as Pb and Cd; the Ni concentrations were detected as highest in

Table 4 Comparison of Pb, Cd, Ni, Zn, and Cu concentration with other studies worldwide

Location	Element					Reference
	Pb	Cd	Ni	Zn	Cu	
Surface sediment, Peninsular Malaysia*	93.11	1.06	36.29	609.2	150.81	Zulkifli et al. (2010b)
North Sulawesi, Indonesia*	12	–	39.72	932.2	62.9	Edinger et al. (2008)
Spanish agricultural soil, European Mediterranean**	23	–	21	53	23	Micó et al. (2006)
Mangrove area, Singapore*	37.14	0.44	16.14	134.13	46.32	Cuong et al. (2005)
Agricultural soil, Cameron Highland**	7.49	0.51	–	78.39	77.39	Khairiah et al. (2009a)
Pearl River Delta, China**	49.9	0.9	32.7	107	43.9	Wong et al. (2002)
Dinapur, India**	35	8.3	34.45	121.85	52.6	Sharma et al. (2007)
Paddy field, Kelantan*	80.29	1.1	34.07	63.76	46.32	In this study

* unit in $\mu g \bullet g^{-1}$ dw; ** $mg \bullet kg^{-1}$ dw

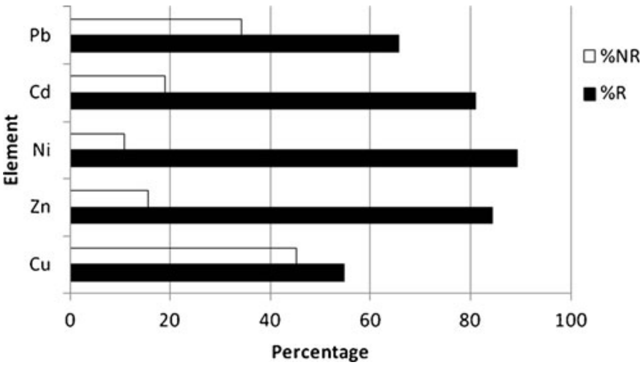

Fig. 3 Percentage of heavy metals in resistant (*R*) and nonresistant (*NR*) form of paddy soil collected on April

resistant fraction and the lowest in acid-reducible fraction for both March and April, as shown in Tables 2 and 3. This resulted in higher resistant percentages than that of nonresistant percentages. Therefore, resistant fraction accounted for 83.10 % in March and 89.28 % in April, for total Ni concentration. This indicates that the Ni element was likely to occur as basic rock material. The elevation of the bioavailable Ni in resistant fraction reflected that most of Ni of Tumpat paddy soil originated from natural soil deposits. Therefore, this study suggests the paddy field soils of Tumpat were unlikely to receive anthropogenic inputs of Ni due to the high Ni amounts in resistant fraction.

Oxidizable organic fraction was reported as being the second highest after resistant fraction for both months. Similarly, the Pb and Cd concentrations were also detected as secondarily highest in oxidizable-organic fraction, indicating there is bioavailability of Ni in paddy field soils. When paddy soil is polluted by those heavy metals from application of pesticides and agrochemical fertilizers, microorganisms and fishes species in the soil are the first living organisms subjected to their impacts, which contributes to the high organic matter in the paddy soil (He et al. 2005). Normally, the decomposition of organic matter is fast under tropical conditions (Khairiah et al. 2009b).

In the current study, low Ni concentration was recorded in acid-reducible and EFLE fractions for both months, thus demonstrating low Ni concentration in bioavailable form. A study conducted by Khairiah et al. (2009c) on heavy metals in guava plants and soils suggested that Ni was undetected in the EFLE, acid-reducible, and oxidizable-organic fraction. Therefore, Ni is required in small amounts due to its limited bioavailable in paddy field soils.

Zn in paddy soil Tables 2 and 3 show the concentration of Zn in paddy soil for March and April, respectively. The highest accumulation of Zn in paddy soil during March was recorded in resistant fraction (46.40±1.46 μg•g^{-1}) and 52.33±3.97 μg•g^{-1}for April. This result indicates that the accumulation of Zn was likely to occur in resistant fraction among other fractions examined in this study, indicating the Zn was not derived from anthropogenic inputs but mainly came from an unavailable form. From the study conducted by Khairiah et al. (2009b) on paddy field soils from Kedah and Perlis, the finding showed that resistant fraction was recorded as the highest fraction compared to the other fractions. From a study done by Habibah et al. (2011) on heavy metals in paddy soil from Kedah and Penang, Zn amounts were found to be a likely occurrence in resistant fraction. EFLE was the lowest accumulated Zn fraction in paddy soil, which is 4.12±0.75 for March and 1.55±0.49 μg•g^{-1}for April, consequently providing the percentage of resistant much higher than percentage of nonresistant, indicating low Zn bioavailability in paddy soil of Tumpat as illustrated in Figs. 2 and 3. In addition, the present of low amount of Zn in paddy field soils suggest that the study areas were deficient of Zn. This situation could be found in Spanish agricultural soil, which was 53 mg•kg^{-1} dw, as tabulated in Table 4. Manceau et al. (2005) stated that in clayey paddy soil, Zn was predominantly bound to hydroxyl-Al interlayers sandwiched between 2:1 vermiculite layers in the fine soil matrix. The study also stated that Zn is able to associate with the ferromanganiferous mottles in paddy field soils (Manceau et al. 2005). Paddy field soils in Kedah plain showed the abundance of phyllosilicate minerals in the clay fractions, such as kaolinite, smectite, and mica, consequently becoming a good binder for Zn accumulation (Paramananthan 1989). In this study, the usage of fertilizers

Table 5 Enrichment factor (*EF*) and geoaccumulation factor (I_{geo}) of Pb, Ni, Cd, Zn, and Cu (μg/g dw) in paddy field soils of March and April

Element	Mean concentration (March)	Mean concentration (April)	Shales (Wedepohl, 2004)	EF^a	$I_{geo}{}^a$	EF^b	$I_{geo}{}^b$
Pb	62.77	60.18	22	2.85	2.96	2.74	2.95
Ni	20.52	10.32	68	0.3	2.97	0.15	2.67
Cd	2.09	0.73	0.13	16.04	−0.76	5.63	−1.2
Zn	63.99	51.5	95	0.67	3.61	0.54	3.51
Cu	31.61	26.98	45	0.7	2.97	0.6	2.91

[a] Value for March

[b] Value for April

and pesticides might not elevate Zn metal in paddy field soils, even though the fertilizers used by farmers (particularly NPK) were containing Zn metal. According to the analysis report of NPK fertilizers under Food Security Policy 2009 of Agricultural Department of Malaysia, the percentages of Zn that contained in NPK fertilizers are 0.18 over 0.5 %. However, the low Zn levels in EFLE fractions showed that the usage and application of fertilizers would not increase the Zn concentrations in paddy field soils.

Cu in paddy soil The concentration of Cu was found to be highest in oxidizable-organic fraction, which is 19.77±1.89 μg•g^{-1}, and the lowest was found in acid-reducible fraction, with amount of 0.07±0.01 μg•g^{-1} for March as shown in Table 2. The elevation of high accumulation and bioavailability of Cu metal in oxidizable-organic fraction in paddy soil of March attributed to the high percentage of nonresistant (51.65 %) compared to the resistant (48.35 %), in this study. The high Cu in paddy soil also could be found in a study conducted by Khairiah et al. (2009b) at Kedah and Perlis. The finding indicates that the oxidizable-organic was the highest fraction accumulated of Cu in paddy soil. Certainly, there is a strong association between soil Cu and the organic fraction, since Cu forms the most stable complexes with organic matter (Adriano 1986; Wong et al. 2002). The low Cu in acid-reducible fraction in paddy soil of Tumpat suggests that the affinities for Cu in this fraction of the paddy field soils were not high. Calmano and Forstner (1983) suggested that the reducing conditions are mainly caused by decomposition of organic matter that is mediated by microorganisms.

For sampling conducted in April, the highest level of Cu was identified in resistant (15.76±2.02 μg•g^{-1}), and acid-reducible (0.13±0.06 μg•g^{-1}) fraction was remarked as the lowest of accumulating Cu metal as shown in Table 3. However, in April, resistant fraction was recorded as being much higher than nonresistant, which is 54.80>45.20 %, as shown in Fig. 3. These results showed that the inputs Cu sources were mainly derived from the breakdown of silicate rocks on the continents during weathering and soil formation (Chen and Yen 1972). The present of Cu metal in resistant fraction could explain that paddy soil of Tumpat paddy cultivation areas is naturally rich with Cu metal. Badri and Aston (1983) reported that the resistant fraction was probably trapped within silicate minerals. Therefore, this natural fraction contained Cu strongly incorporated into the crystalline lattices of the minerals. A lot of research has been done on heavy metals in paddy soil, and one that stands out in particular is Habibah et al. (2011) in Kedah and Penang. The study suggests that the Cu found in soil sample potentially came from the natural marine alluvial deposits. Consequently, low Cu levels in marine alluvial soils were also mentioned in Khairiah's et al. (2009b) study. However, Cu concentration in Spanish agricultural soil also indicates the low level, which was 23 mg•kg^{-1} dw, as seen in Table 4.

Based on the analysis report of NPK fertilizers under the Food Security Policy 2009 by the Agricultural Department of Malaysia, there was a distinct presence of Zn and Cu in the fertilizers that were supplied to the farmers. The permissible limit of Cu in the NPK fertilizers is 0.46 over 0.5 %. This indicated that the presence of Cu in the paddy soil of Kelantan may have mainly come from the fertilizers supplied to paddy fields over several years to ensure the crops production in better yields.

Enrichment factor and Geoaccumulation index The results of the calculation of the enrichment factor and geoaccumulation index of heavy metals in the paddy field soils are shown in Table 5. The *EF* in the paddy field soils of March reflected that Pb (2.85), Cd (16.04), Ni (0.30), Zn (0.67), and Cu (0.70) and Pb (2.74), Cd (5.63), Ni (0.15), Zn (0.54), and Cu (0.60) for April. *EF* of Pb indicates moderate enrichment in the paddy soil for both months. Although Pb was rich in the paddy field soils of Tumpat, the results indicated that the majority of Pb concentrations were in abundance in resistant fraction and accounting to 65.64 and 79.04 % of total Pb for March and April, respectively. This is different from what was observed in Cd, which was categorized as very high enrichment for March and a significant enrichment for April. The current study found that most of the high Cd contents in paddy soil are deposited in resistant fraction. *EF* of Ni was classified as deficiency to minimal enrichment for both months. In general, Ni content was of low bioavailability in paddy field soils. Therefore, in this present study, Ni was bioavailable with abundant presence in resistant fraction, therefore, contributing to low Ni in paddy field soils. Both Zn and Cu showed low *EF* values, which are 0.67 (March), 0.54 (April), 0.70 (March), and 0.60 (April), respectively, which is defined as a deficiency of Zn and Cu enrichment in paddy soil. Based on the Cu and Zn fractionation earlier, Zn was found to be abundant in resistant fraction and low in bioavailable forms, which are EFLE, oxidizable-organic, and acid-reducible. Similarly was observed in Cu metal, in which classified as deficiency of Cu metal enrichment in paddy soil of Tumpat. The low enrichment of Zn and Cu metals in paddy soil might be efficiently uptake by the paddy plant for growing. Although, the *EF* values of metals studied ranged from 0.15 to 16.04 and are categorized as significant enrichment, this most possibly reflect the background of anthropogenic inputs. However, most of the heavy metals are solely bioavailable in resistant fraction. In comparison, the EFLE fraction of each heavy metal was detected as the lowest, reflecting that the paddy soil of Tumpat was unlikely to originate from anthropogenic activities. Therefore, the agrochemical fertilizers and pesticides used might not increase the levels of heavy metals in examined paddy soil.

As a continuation of the foregoing study, the data in Table 5 shows the I_{geo} of Pb, Cd Ni, Zn, and Cu for both months. The elements studied, which are Pb, Ni, and Cu in March and April, showed class 3, which is moderately polluted. In March, the *EF* of Cd was −0.76 and −1.20 in April, indicating that the paddy field soils of Tumpat was unpolluted by Cd. A study done by Emmanuel and Edward (2010) on heavy metal pollution in soil based on dry and rainy season found −2.35 and −1.49 values for Cd, respectively. However, the accumulation of Zn in paddy soil for both months was heavily polluted. The application and repeated use of agrochemical fertilizers and pesticides in paddy soil could be attributed to the Zn pollution in paddy field of Tumpat, Kelantan. As mentioned above, most of the metals studied in this current study were likely found to accumulate and abundant in resistant fractions. The I_{geo} index is not readily comparable with the enrichment factor because of the natural I_{geo} calculation, which involves a logarithm function and multiplication factor of 1.5 (Emmanuel and Edward 2010). Reimann and de Caritat (2005) stated that the average concentrations of shales have been estimated for the 90 naturally occurring elements in the Earth's crust; they are not reflecting local lithogenic or soil properties. However, this was aggressively extended to the study of soil, lake sediment, and other environmental materials (Ragaini et al. 1977; Loska et al. 1997).

Conclusion

The present study indicated that the selected heavy metals studied (Pb, Cd, Ni, Zn, and Cu) in paddy soil of Tumpat was in the resistant and oxidizable-organic fraction for both months. Based on the enrichment factor calculation, Cd was significantly enriched. However, both Zn and Cu showed a deficiency pattern in paddy soil. These findings suggest that heavy metals examined (Pb, Cd, Ni, Zn, and Cu) might be slightly derived from anthropogenic activities such as the application of agrochemical fertilizers, pesticides, and plowing activities, which could resuspend the heavy metals that has been accumulating for a long period of time in paddy soil. However, regular monitoring of heavy metal content should be made in the future to avoid any heavy metal toxicity to human beings and plants.

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