Estımatıon Of The Transfer Of Mıcronutrıent (Cu, Mn, Zn) And Toxıc Elements (Cd, Cr, Ni, Pb) From Soıls To Rıce (*Oryza Sativa*) In Evros-Ergene Rıver Basın

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ABSTRACT

In this study, concentrations of nonessential toxic heavy metals (Cd, Cr, Ni and Pb) and the micronutrients (Cu, Zn, Mn) in twenty brown rice (*O.sativa*) and relevant paddy soil samples were investigated. Soil and brown rice samples from Evros-Ergene river basin were extracted for their total heavy metals content by dry digestion method and then determined using ICP OES. Results of this study revealed that the concentrations of Cd, Cr and Ni in the soils of studied sites were above than the maximum permissible levels. The average concentration of all metals in brown rice samples was below than the maximum permissible levels of WHO guideline. The ranking order of bioaccumulation factor (BAF) for heavy metals was Zn > Cu > Mn > Ni> Cr = Cd = Pb indicating that the accumulation of micronutrients was more than that of nonessential toxic heavy metals. It can be concluded that these rice samples cannot be regarded as a complete source to determine all metals posing threat to human health.

Keywords: heavy metals; rice, paddy soil pollution; Bioaccumulation Factor (BAF); Ergene-Evros Basin

INTRODUCTION

Trakya region, with its soil and water resources, is one of the most important agricultural regions in Turkey. Ergene Basin is located in East Trakya, and is surrounded by North Marmara Basin, Evros (Maritza) Basin, and Bulgaria. The increasing industrialisation and agricultural activities in Trakya region cause threat to water resources. The use of this water, even indirectly, for irrigation of paddy fields causes salinization, contamination in terms of heavy metals, and also desertification (Kocaman *et al*. 2015). Heavy metals such as cadmium, lead, chrome, cobalt, nickel, and copper, which are widely used in industry, are dumped into the ecological environment without any treatment (Adiloglu 2016). Due to domestic and industrial waste, pollution in water used for irrigation of rice is at fearful rates. Taking into consideration that in in wetlands of paddy farming, when water used in irrigation with high concentration of heavy metals, it remains on the soil surface, accumulates, and causes accumulation in paddy plant itself, and therefore, it is inevitable that the toxic effect causes great health problems on people (Kabata-Pendias and Mukherje 2007). Also factors such as local climate, atmospheric dry depositions, physicochemical properties of soil including pH, cation exchange capacity (CEC), soil texture, organic matter content, degree of maturity of plants at the time of harvest and metal species exhibit noticeable effect on bio-available fraction of heavy metals from soil to crops (Chen *et al*. 2016).

The main objective of this study is to conduct a risk assessment of seven heavy metals (Cd, Cr, Cu, Ni, Pb, Mn and Zn). Concentrations of these heavy metals were determined in soil and brown rice grain from Evros-Ergene river basin to assess pollution levels and potential health risks based on the national/international standard limits.

MATERIALS AND METHODS

This study was carried out in Evros-Ergene River Basin. Soil and rice samples were collected from farms in wetlands located closer to Ergene River within the area from 40^o 54´ to 41^o 17´ north latitude and from 26^o 21´ to 26^o 41´ east longitude. All sample sites were recorded using a handheld Global Position System (GPS). For convenience, the stations were grouped into three main sites, namely Ipsala (IP), Meriç (MR) and Uzunköprü (UK).

A total of 20 rice samples and 20 soil samples were collected directly in the study area during the 2015 harvest season. Twenty top soil samples from root zone at 0-30 cm depth were taken from this paddy field using Ponar Grab sampler. Rice grain was grown in the corresponding soil sampling site and bought from local peasants. Each individual rice sample was composed of at least five sub-samples which were taken from the same rice paddy. Each topsoil sample was obtained by mixing at least five adjacent sub-samples from one paddy field. At least 2 kg of soil was collected for each soil sample and 1 kg rice grain for each rice sample. The paddy plants and soil samples were inserted into labelled plastic bags and placed on ice while transported to the laboratory, then kept in the refrigerator at 4 ^{0}C for preservation before analysis.

Soil samples were air-dried in the laboratory for several days at ambient temperature. They were sieved through a 0.149 mm (100 mesh) nylon screen for digestion. Rice grain samples were oven-dried at 105 ^{o}C for 1 h, and later at 70 ^{o}C to constant weight. Husks were removed. Then, brown rice grain samples were ground in agate mortar until it could pass through a 63-micron mesh sieve and stored in closed polyethylene bags for digestion.

Well homogenized soil and brown rice samples were weighed (0.5 g) into separate teflon acid digestion tubes, then 8 ml % 65 HNO_3 and 2 ml 30% H_2O_2 were added in each digestion tube and were tightly closed with screw caps. The samples were placed in a CEM microwave system Model Mars 907511 (CEM Cooperation, Mathews, North Carolina, USA) and digested at 200 ^oC for 30 min. with a microwave power of 1000 W (Thompson and Walsh 2003). The digested samples were diluted to 100 ml with deionised water. Samples were kept in 4^0C refrigerator until heavy metal analysis was done.

Heavy metal concentrations were determined using ICP-OES Spectrometer machine Agillent 700 series for lead, cadmium, chromium, copper, manganese, nickel and zinc elements. Internal standard solutions were used to calibrate the instrument. Data obtained from the experiment were subjected to statistical analysis ANOVA and correlation using EXCEL. All samples were measured in duplicate. The recoveries of reference elements were within 15% of the actual values. Limits of Detection (LODs) were defined as 3 times the standard deviation of 10 runs of blank measurements. LODs of Cd, Cr, Cu, Mn, Ni, Zn and Pb were 0.07, 0.65, 0.66, 0.09, 0.91, 0.50 and 0.75 $mgkg^{-1}$ respectively. Concentrations of heavy metals were expressed in terms of $mgkg^{-1}$ on dry weight basis. In this study, the concentrations of Cd, Cr and Pb in the extracted solution of rice were below the limit of detection. Therefore, the concentration of Cd, Cr and Pb in rice was not detected.

Assessment Bioaccumulation Effect of Rice on the Uptake of Heavy Metals from the Soils

The BAF (bioaccumulation factor, the ratio of the concentration of the element in the grain to that in the corresponding soil) was calculated for each rice sample to quantify the bioaccumulation effect of rice on the uptake of heavy metals from the soils (1). The BAF was computed as

$$BAF = C_r/C_s, \quad (1)$$

where heavy metal concentration in the edible parts of the plant and soil are represented by C_{rice} and C_{soil}, respectively. When the BCF is <1 or the BAF=1, it indicates that the plant only absorbs but does not accumulate heavy metals; when the BCF is >1, it is an indication that the plant accumulates metals (Liu *et al*. 2005).

RESULTS AND DISCUSSION

Heavy Metal Concentration in Brown Rice Grains

In brown rice grains, among all metals, Mn and Zn were in more elevated concentrations than Cu and Ni. Concentration of Mn and Zn ranged between 7.89 mg kg^{-1} and 30.06 mg kg^{-1}, 7.47 mg kg^{-1} and 27.71 mg kg^{-1} respectively among the sixty sites, which did not exceed the maximum permissible limit within FAO/WHO (2002) for human consumption.

In addition, the concentrations of Cu and Ni ranged between 1.09 mg kg^{-1} and 5.18 mg kg^{-1}, 1.25 mg kg^{-1} and 3.86 mg kg^{-1} respectively among the sixty sites, which did not exceed the values defined by the FAO/WHO (2002), WHO (1996), TFC (2011) and the critical concentration in plants based on Kabata-Pendias and Pendias (1992) (Table 1). Cd, Pb and Cr concentrations were not found in any stations.

Table 1. Concentration of heavy metals (mgkg-1 dry matter) brown rice grain across the sampling sites

Brown rice samples	Cu	Zn	Cd	Cr	Pb	Mn	Ni
R-IP1	2.4±0.45	22.7±0.41	BDL	BDL	BDL	17.8±0.61	BDL
R-IP2	3.3±0.5	20.0±0.33	BDL	BDL	BDL	15.9±0.3	BDL
R-IP3	3.6±0.62	21.3±0.42	BDL	BDL	BDL	24.7±0.31	BDL
R-IP4	2.2±0.52	23.5±0.24	BDL	BDL	BDL	15.3±0.6	BDL
R-MR1	3.1±0.25	17.1±0.48	BDL	BDL	BDL	19.7±0.34	BDL
R-MR2	3.0±0.41	17.7±0.42	BDL	BDL	BDL	20.5±0.52	BDL
R-MR3	4.1±0.43	23.0±0.74	BDL	BDL	BDL	15.1±0.33	3.6±0.03
R-MR4	3.86±0.21	24.8±0.34	BDL	BDL	BDL	28.0±0.24	1.7±0.05
R-MR5	3.8±0.26	20.2±0.34	BDL	BDL	BDL	21.6±0.53	1.5±0.1
R-MR6	2.2±.0.42	13.4±0.75	BDL	BDL	BDL	18.1±0.1	BDL
R-MR7	1.7±0.45	12.5±0.23	BDL	BDL	BDL	21.9±0.42	1.0±0.0.1
R-UK1	2.2±0.51	13.1±0.34	BDL	BDL	BDL	21.0±0.51	BDL
R-UK2	3.4±0.42	17.9±0.36	BDL	BDL	BDL	24.9±0.45	BDL
R-UK3	3.6±0.9	17.4±0.42	BDL	BDL	BDL	16.1±0.24	BDL
R-UK4	3.3±0.42	19.6±0.24	BDL	BDL	BDL	16.7±0.42	BDL
R-UK5	2.4±0.52	19.4±0.52	BDL	BDL	BDL	16.0±0.45	BDL
R-UK6	2.5±0.62	16.2±0.34	BDL	BDL	BDL	16.0±0.42	BDL
R-UK7	2.9±0.78	15.8±0.53	BDL	BDL	BDL	16.0±0.34	BDL
R-UK8	3.8±0.25	19.5±0.21	BDL	BDL	BDL	23.6±0.43	BDL
R-UK9	3.7±0.46	17.6±0.34	BDL	BDL	BDL	21.2±0.45	BDL
FAO/WHO (2002)	20	50	0.2	-	0.2	-	-
WHO (1996)	-	-	-	-	-	-	10
TFC (2011)	-	-	0.2	-	0.2	-	-
Kabata-Pendias (1992)							
Normal Range	5-20	1-400	0.1-2.4	0.03-14	-	-	0.02-5
Toxic Range	20-100	100-400	5-30	5-30	-	-	10-100

BDL:Below detection limit

Table 2. Concentration of heavy metals (mgkg-1 dry matter) soil samples across the sampling sites

Soil Samples	Cu	Zn	Cd	Cr	Pb	Mn	Ni
S-IP1	7.2±0.66	45.9±0.5	BDL	BDL	23.5±0.75	27.6±0.09	7.03±0.91
S-IP2	17.9±0.51	137.8±0.24	1.0±0.07	29.0±0.65	12.2±0.55	391.8±0.02	35.8±0.47
S-IP3	18.4±0.21	64.9±0.12	1.3±0.11	30.6±0.2	27.4±0.62	440.5±0.03	30.2±0.54
S-1P4	11.5±0.33	35.5±0.3	0.9±0.02	32.5±0.52	10.2±0.53	250.0±0.07	37.4±0.65
S-MR1	9.7±0.31	29.1±0.42	0.7±0.09	31.8±0.41	9.1±0.24	252.0±0.03	30.6±0.85
S-MR2	10.9±0.52	36.8±0.5	0.6±0.12	16.1±0.31	10.3±0.72	418.7±0.09	27.9±0.41
S-MR3	11.6±0.26	42.8±0.1	1.14±0.21	45.9±0.33	8.7±0.62	328.7±0.08	32.3±0.51
S-MR4	16.0±0.41	53.1±0.21	1.0±0.18	33.8±0.55	9.9±0.45	418.0±0.01	71.2±0.62
S-MR5	8.3±0.47	34.1±0.6	BDL	BDL	7.7±0.2	89.7±0.12	25.5±0.84
S-MR6	16.9±0.63	55.1±0.3	1.9±0.15	81.76±0.24	16.5±0.33	615.0±0.09	68.3±0.9
S-MR7	20.0±0.33	67.1±0.54	2.1±0.11	94.4±0.49	18.1±0.59	882.1±0.01	83.0±0.54
S-UK1	20.1±0.24	169.1±0.21	1.6±0.02	65.8±0.24	17.6±0.6	436.2±0.01	63.3±0.62
S-UK2	18.5±0.21	29.3±0.1	1.1±0.03	34.1±0.32	10.1±0.41	589.6±0.05	39.3±0.85
S-UK3	27.9±0.15	64.9±0.43	2.5±0.04	68.3±0.41	10.5±0.53	564.9±0.04	51.3±0.45
S-UK4	29.8±0.24	67.7±0.25	2.6±0.01	74.7±0.24	11.2±0.22	430.1±0.12	56.1±0.53
S-UK5	20.1±0.41	52.3±0.33	1.9±0.01	82.2±0.34	8.7±0.5	522.2±0.2	106.1±0.81
S-UK6	19.5±0.54	52.6±0.35	2.1±0.06	95.6±0.41	9.3±0.22	642.3±0.03	119.6±0.23
S-UK7	18.0±0.32	50.6±0.22	1.8±0.05	76.7±0.62	9.4±0.63	662.6±0.01	106.4±0.61
S-UK8	28.9±0.45	74.4±0.34	2.4±0.07	96.4±0.14	10.7±0.54	499.5±0.01	117.5±0.48
S-UK9	27.1±0.54	69.9±0.44	2.4±0.06	105.3±0.52	10.9±0.21	523.2±0.1	134.8±0.62
RSCP 2001	50	150	1	100	50	-	30
Kabata-Pendias 1992	2-100 (normal range)	-	-	-	-	850 (toxic level)	-

BDL:Below detection limit

When brown rice samples are compared according to their region, there was difference only in terms of **Ni** among regions, which is statistically significant ($p<0.05$). Among these three regions, mean of Ni concentration was found out to be higher in MR region. There was no Ni concentration detected in IP region. The permissible limit of Nickel in plants recommended by WHO (1996) is 10 $mgkg^{-1}$. Ni concentration was detected in MR and IP regions; however these measurements were below the limit level. Kocaman *et al*. (2015), have reported that Cd and Co accumulated only in the roots. Fe and Mn were the most accumulated elements in all parts of the plants. These were followed by Zn and Cu. Ni accumulated in all parts of the plants from Ergene area while it accumulated only in roots, stems and grain with husk in the other locations.

The relation between "Zn-Cu", "Mn–Cu" ve "Mn–Zn" concentrations in brown rice samples in IP region was found out to be significant ($p<0.01$). Accordingly, there is a positively linear relation between "Zn-Cu" concentrations. The relations among concentration of "Zn-Cu", "Mn-Cu", Ni-Cu", "Mn-Zn" and "Ni-Zn" variables of brown rice samples in MR region were found out to be significant ($p<0.05$ and $p<0.01$). Accordingly, there is a positively linear relation among these variables. The highest relation was determined to be between "Zn-Cu" (85.6%). Also it is reported by Mohammed et.al. (2015) that the strongest correlation was found between Cu and Zn indicating a common source in Zanzibar, while a moderate negative relationship between these two elements was found in rice (−0.36) in North-western Italy (Brizio *et al*. 2016). In brown rice samples of UK region, the relation of concentration of "Zn-Cu" and "Ni-Mn" variables were determined as significant. Accordingly, there is a positively linear relation among these variables. Most of the previous studies considered total contents of trace elements a reliable indication for their toxicity; however, their available indices are more important than their total contents in determining their uptake and distribution in different plant parts (Abbas and Abdelhafez 2013). In many studies carried out on parts of paddy plants (root, shoot, husks and grains) showed that most of the metals accumulate in the root of the plant. When the results of this study are compared to previous studies, it can be said that heavy metals may be accumulated in the roots or other parts of the plants (Kocaman *et al*. 2015; Abdelhafez *et al*. 2015).

Heavy Metal Concentration In Soil

It was found that, in the essential heavy metals in the paddy soil, Mn concentration ranged from 27.69 to 882.14 mg kg^{-1}, Zn concentration ranged from 29.1 to 169.15 mg kg^{-1}, Cu concentration ranged from 7.25 to 29.81 mg kg^{-1} in the paddy field soils, and in the concentrations of nonessential toxic metals, Pb ranged from 7.25 to 27.44 mg kg^{-1}, Cr ranged from 16.15 to 105.31 mg kg^{-1}, Ni ranged from 7.03 to 134.86 mg kg^{-1} and Cd from 0.68 to 2.65 mg kg^{-1}. The paddy soil was acidic (pH 5.01) possibly attributed to continuous irrigation of paddy soils with contaminated river water. The ranking order of occurrence of the heavy metals in the paddy field soils was Mn > Zn > Ni > Cr > Cu > Pb > Cd indicating that Mn followed by Zn was at maximum concentration and Cd was at minimum concentration. Most of these metals arise from agrochemicals like fertilizers and pesticides, which have been used over a long period in the farm. It is known that phosphate fertilizers are manufactured from phosphate ore, which are contaminated with metals like Pb, Mn, and Cr (USEPA 1999; Wu *et al.* 2016). When soil sample concentrations were compared, there were differences determined in terms of Cu, Cd, Cr, Pb, Ni, and these differences were found out to be significant ($p<0.05$). It is reported by Wang et.al. (2016) that, except that no correlations were found between Cd, Cu, Ni, and Hg, all the heavy metals (As, Cr, Pb, Zn) were significantly correlated with each other at $p < 0.01$ level. Liu *et al.* (2011) have reported strong correlations among Cu, Ni, and Cr in soil around an electroplating plant and has implied that the metals have the same pollution source. Considering the mean of Cu variable, the group revealing difference was UK region. Cu concentration detected in soil samples of UK region was found high according to permissible limits stated in Regulation of Soil Pollution Control (RSCP). There was no significant difference of Cu values observed between IP and MR regions. The difference between the regions in terms of Ni variable is also statistically at significant level ($p<0.05$). Accordingly, the group revealing difference is UK region. There was no significant difference observed between the other regions (IP and MR). In all regions, Ni concentration was found above the limit value stated by RSCP. Domingo and Kyuma (1983) was found that Cheju rice farms has lower mean levels of Cu, Zn, V, Co, and Cr and higher mean level of Ni than paddy soils of 12 countries. Determined concentrations of Cr, which is another variable, revealed a significant difference among regions, and the region that caused this difference was again UK. In soil samples of UK, MR, and IP, Cr concentration was found out to be high according to permissible limits stated in RSCP, and UK region was found out to have the highest level among the groups. Similarly, when Cd concentrations were taken into consideration, the difference detected among the 3 groups was determined to be significant ($p=0.03$). UK region with the highest mean of Cd concentration in soil samples deviates from the other regions by causing the difference. Cd concentration detected in soil samples in UK region was found high according to permissible limits stated in RSCP. However, there was no difference detected in IP and MR regions. Wang *et al.* (2015) have reported that continuous application of wastewater has led to accumulation of heavy metals in the soil, and Cd, Zn, and Hg were the main pollutants. Zn and Cd were more mobile than other metals. In the area, the use of contaminated irrigation water is an important source which resulted in the increase of metal levels observed in soil. Especially in UK region, paddy fields being irrigated with water from Ergene River, causes concentrations of toxic heavy metals being at high level. Even though heavy metal concentrations did not exceed permissible limits of Turkish standards, their concentrations found in soil is at fearful rates (RSCP 2001), and the critical concentration in soil based on Kabata-Pendias and Pendias (1992). Determined concentration of Pb variable was at highest level in IP region and the difference was found out to be statistically significant ($p<0.05$). However there was no significant difference detected between Pb concentrations in MR and UK regions. Pb concentration in soil samples were found out to be below the permissible limits of Turkish standards. There was statistically no significant difference among regions in terms of other variables (Zn and Mn) ($p>0.05$). Samples of highest Zn concentration were detected in UK region; whereas Mn concentration was determined to be at toxic level in MR region in only one sample. In this study, the concentration of Mn and Zn were lower than the toxic level. Moradi *et al.* (2013) reported a positive correlation ($p < 0.01$) between the levels of Zn in agricultural soil and in rice collected in industrial sites in Iran.

In MR region, concentrations of all bilateral variables in soil samples were determined to be significantly in relation ($p<0.05$ and $p<0.01$). Accordingly, there was a positively linear relation among these variables. The highest relation was found out to be between “Zn-Cu” (97.5%). The degree of relation (Pearson correlation coefficient) was detected to be at 1% and 5% levels and was determined as significant. There was no variable detected without mutual relation. There was a positive correlation coefficient (r) between both micronutrients, Cu and Zn. A significant correlation was found between pH and soil available Cu and Zn from the samples that were taken from the surface depth. Soil acidity influences micronutrient content, with slightly acidic soil series containing more Cu and Zn compared to strongly acidic soils (Babar 2016). The spatial distribution of Cu and Zn correlated to the soil organic matter distribution.

Bioaccumulation Factor

BAF is a key process for human exposure to toxic heavy metals through the food chain (Zhuang *et al.* 2009). The bioaccumulation factors (BAFs) that were calculated for the transmission of heavy metals from soils to the brown rice plants. The BAF values of the heavy metals such as Zn, Mn, Cu, and Ni were found out to be in a range from 0.07 to 0.6, 0.02 to 0.6, 0.08 to 0.4, and from 0.01 to 0.5 respectively. The trend in the BAF for heavy metals in the study sites was in the ranking order of Zn > Cu > Mn > Ni. BAF is an index for evaluating the transfer potential of a metal from soil to plant (Zheng *et al.* 2007). The results suggest that Zn and Cu had relatively higher mobility from soil to rice, followed by Mn and Ni consistent in this study. Abdul Aziz *et al.* (2015) almost got similar findings. Yadav *et al.* indicated that essential elements, i.e. Zn, Co and Cu, had higher bio-concentration in rice grains as compared to other toxic metals. Also studies by Saphaty *et al.* (2014) indicated that the trend in the BAF for heavy metals in the study sites was in the ranking order of Zn > Mn > Cu. Among the heavy metals, BAF values were found to be higher for Zn and Mn whereas relatively lower BAF values were found in Cu. BAF values of less than 1 were obtained for Cu, Zn, Mn, and Ni in brown rice grains. Although Cu, Zn, Mn, and Ni concentrations were detected to be above Turkish and international limits in soil, BCF results showed that the bioavailability of metals was low in the study area. Abdul Aziz *et al.*(2015) almost got similar findings, where they found that heavy metals do not accumulate in the rice grain even though the concentrations of heavy metals were high in the soils.

CONCLUSIONS

In this study, the distribution of concentrations of nonessential toxic heavy metals (Cd, Cr, Ni and Pb) and the micronutrients (Cu, Zn, Mn,) in paddy soil and brown rice grain from Evros-Ergene River Basin were examined, and availability of these metals to the paddy plants were found out to be quite low. While studies carried out in the last decade showed that heavy metal pollution in this region was at a fearful rate, and that the migration of heavy metals from soil to plant is a key process for human exposure to heavy metals through the food chain, it was determined in this study that heavy metal concentrations were high in paddy soils probably occurring from using chemical fertilizers and pesticides, yet these concentrations were transmitted to brown rice grains at a level not causing any acute health risk.

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