



## **Source and Health Risk Assessment of Heavy Metals in Non-Certified Organic Rice Farming at Nakhon Nayok Province, Thailand**

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### **Abstract**

Heavy metals contamination is a problem in some non-certified organic rice farms that do not have buffer zones. Soil monitoring is therefore required to estimate the potential risk of such organic products. The objectives of the present study are to determine the extent of heavy metal contamination, sources of contamination and assessment of non-carcinogenic health risks to local consumers. Concentrations of toxic heavy metals were determined in soil and rice grain to assess the bioaccumulation factor. The health risk assessment was analyzed following Target Hazard Quotients (THQ) and the Hazard Index (HI). Sources of heavy metal contamination were determined by a correlation study of heavy metal contents, THQ and HI with some physical properties of these non-certificated organic rice fields. The occurrence of heavy metals in agricultural soils and rice grain were ranked in the following order: Pb > Mn > Zn > Cu > Ni and Zn > Mn > Cu > Ni. However, Pb and Zn contamination exceeded maximum permissible levels in rice grain. Non-certified organic rice from these locations might therefore present a health risk for consumers; the high HI values of rice consumption for adult males (5.10-35.09) and 6.12-42.08) indicated a serious adverse health risk for consumers. Individual correlation analysis and principal component analysis indicated that the THQ of Zn was positively correlated with its content in soil and in the grain. Main roads and community activities were found to be the main source of contamination for Zn and Mn, while Pb and Cu contamination mainly derived from paddy field activities such as fertilizer application. This finding will contribute to raising public awareness of the health risks of non-certified organic rice farming.

**Keywords:** Correlation; Health index; Lithogeny; Heavy metal; Organic rice

## Introduction

Heavy metal contamination in rice grain is a serious problem in many Asian countries. Levels of Pb and Cd were reported as exceeding the maximum allowable concentration (MAC) in Taizhou city in Zhejiang province, China [1], while high levels of Cd were found in rice grain in Mae Sot district in Tak Province, Thailand [2]. The main sources of contaminants in agricultural fields include inorganic fertilizers, pesticides and manure are well known [3-4]. Organic farming emerged as an attempt to produce safer, healthier food through reduced contamination. However, certification of organic farms is a rigorous process and much non-certified organic produce is sold directly from the farm or via local fresh markets [5]. In Thailand, one of the world's top five rice producers [6] most smallholder organic farmers are non-certified. Some non-certified organic farms are located near to main roads, communities or chemical farming, where the absence of a buffer zone poses risks of heavy metal contamination from traffic and other human activities. Although some reports have indicated that heavy metals accumulate mainly in the roots of rice plants, grains and paddy soils [2, 7], few studies have been conducted to assess the levels and distribution of contamination in non-certified organic rice fields.

In Nhongsang District, Nakhon Nayok Province, Thailand, non-certified organic rice farming has been practiced for more than 10 years. Buffer zones are not used to shield the crop against chemical contamination. Studies in many Asian countries have reported that Zn, Cu, Ni and Pb were found to accumulate mainly in the roots of the rice plant, grain, paddy soils and livestock manure, with high levels also found in drainage water from irrigated top soils [8]. At the same time, Mn, Zn and Cu were strongly absorbed by rice

plants [6]. The current study focused on Pb, Mn, Zn, Cu and Ni in the study area.

The objectives of the present study were to determine levels of heavy metal contaminants, the sources of contamination and non-carcinogenic health risks for local consumers. Concentrations of heavy metals were determined in soil and rice grain to assess bioaccumulation. A health risk assessment was conducted following Target Hazard Quotients (THQ) and the Hazard Index (HI). Sources of heavy metal contamination were determined by a correlation study of heavy metal content, THQ and HI with some physical properties of these non-certified organic rice fields.

## Methodology

### 1) Study sites

The study areas were located in Nakhon Nayok Province, Thailand, where organic rice farming has been practiced for over a decade. The sites were located as follows: Jareun paddy field (JR); latitude 14.2204 and longitude 101.3127, Songsri paddy field (SS); latitude 14.22474 and longitude 101.311, Lamai paddy field (LM); latitude 14.23298 and longitude 101.3143, Jarunsri paddy field (JS); latitude 14.21998 and longitude 101.3094, Chem paddy field (CM); latitude 14.2204 and longitude 101.3127 (Figure 1). Physical properties of the study sites were determined including atmospheric CO<sub>2</sub> (ppm), atmospheric relative humidity (%RH). Soil moisture and soil pH were measured using a digital meter. Data collection was performed after crop harvest (September to November 2018).

### 2) Sampling of soil and rice grain

Soil samples were collected from five sites (JR, SS, LM, JS, and CM). The three 5x5 m representative crop sites were separated by at least 10 m. Soil samples of 500 mg were collected from the 0-15 cm layer, with 4 samples collected from the corners and 1 from the

middle of the sample sites. The soil samples were then mixed to make a single composite sample. Rice grains were collected from the corresponding plants located above the soil sample sites in order to compute correlations between heavy metal concentrations in soil and rice grains. All samples were stored in clean polyethylene bags and brought to the laboratory for analysis. The hulls were removed from the rice grains, which were then washed thoroughly with deionized water. All subsamples were oven-dried at 60°C for 72 h, and the dried samples were weighed and ground with an agate mortar to fine powder. The soil samples were then air-dried at room temperature for several days, then pulverized and sieved through a 0.25 mm stainless steel mesh. All samples were stored in clean plastic bags for further analysis.

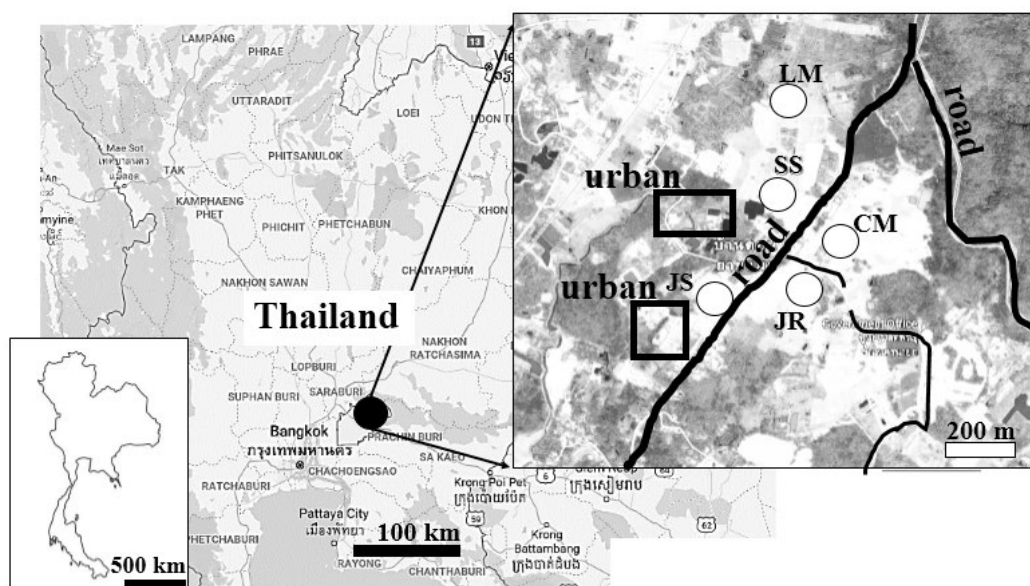
### 3) Heavy metal content and bioaccumulation factor (BAF) analysis

The digestion method followed Neeratanaphan [9]. For heavy metal analysis, one milligram of rice fine powder and sieved soil samples were digested after adding 15 mL of HNO<sub>3</sub> with three replicates at 80°C until digestion was complete and the solution was transparent.

After cooling, the digested sample was diluted to 50 ml with distilled water. The samples were then filtered to remove small particles in solution, then the volume was adjusted to 50 mL in a volumetric flask. The filtered samples were then analyzed using atomic absorption spectroscopy (AAS) and the heavy metal concentrations determined by flame oxidizing with AAS (Agilent—Model 280AA). The sample digestion was prepared with three replicates and the method of absolute calibration with known concentration of stock solution was used for heavy metal content determination. The BAF, the ratio of the concentration of the element in the rice grain to that in the corresponding soil, was calculated following Neeratanaphan [9].

### 4) Non-carcinogenic human health risk assessment

The daily heavy metal intake dose depended on the heavy metal concentration and the amount of any respective food consumed (in this case, rice). The estimated daily intake [EDI, mg d<sup>-1</sup> kg<sup>-1</sup>] is expressed as a daily dose per unit body weight (Eq. 1), and is usually calculated using the following formula of Phimol et al. [10].



**Figure 1** Map of organic rice paddy fields, JR JS LM SS CM in Nhongsang District, Nakhon Nayok Province, Thailand.

$$EDI = [CxCons]/BW \quad (\text{Eq. 1})$$

where C was the mean heavy metal content ( $\text{mg kg}^{-1}$ ), each element's concentration measured during this study was used to calculate the daily average consumption of rice ( $\text{kg d}^{-1}$ ) of Thai people: 0.43126 for male and 0.31928 for female [11]. The BW (reference body mass) was the average body weight for Thai people (68.83 kg for male and 57.40 kg for female) [12].

To analyze human non-carcinogenic risk (THQs), the reference dose (RfD), which was the United States Environmental Protection Agency's (USEPA's) maximum acceptable oral dose for a toxic substance, was used in the calculation. THQs are usually calculated using the following Zeng et al. [13] (Eq. 2).

$$THQ = [EDI]/RfD \quad (\text{Eq. 2})$$

RfD was the oral reference dose ( $\text{mg kg}^{-1} \text{d}^{-1}$ ) for heavy metals in this research for Pb, 0.0035 [12]; Zn, 0.300 [12]; Cu, 0.040 [12]; Mn, 0.014 [14]; Ni, 0.020 [15]. The HI, arising from the sum of THQs, was calculated (Eq. 3):

$$HI = \sum_{n=1}^i THQ \quad (\text{Eq. 3})$$

## 5) Statistical analysis

Correlation analysis was done to establish the relationship between heavy metal concentrations in field soil, rice and grain samples. A one-sample Kolmogorov-Smirnov test was conducted to test the normal distribution for each parameter. When the data were normally distributed, a Pearson correlation analysis was conducted; otherwise, a Spearman rank-order correlation analysis was performed. Relationship analysis was undertaken to assess the relationship between Pb, Cu, Ni, Mn, Zn content and the area's physical properties, BAF or heavy metal content in soils and rice grain at  $P=0.05$  or  $P=0.01$  level according to statistical software. As the preferred tool for identifying

pollution sources, principal component analysis (PCA) was applied to the data from this study [16-17] analysis.

## Results and discussion

### 1) Some physical properties of organic rice fields

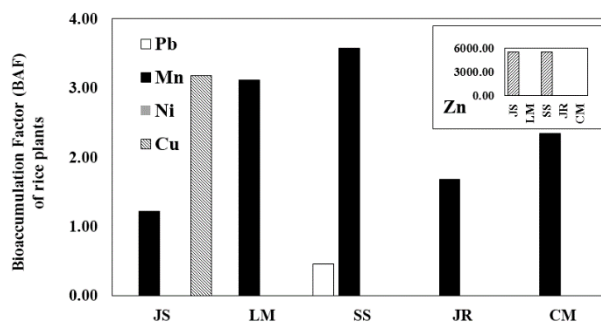
All sites (SS, LM, CM, JR and JS) were located far away from industries but close to urban areas; thus, some human activities might account for heavy metal contamination in the fields. The atmospheric  $\text{CO}_2$  (ppm) values ranged from 8.67-6.67ppm, with  $CM > JS$ ,  $LM > SS$ , JR. The relative humidity (%RH) values were 55-49.67 with  $SS > LM > CM > JR$ , JS. Soil moisture were 9-2.17 with  $JR > CM > SS > LM > JS$ . Soil pH values ranged from 6.3-5.7 with  $JR > CM > SS$ , LM and LS. For distance from forest showed that  $JS > JR$ ,  $CM > SS > LM$ , distance from urban showed that  $LM > JS > SS > JR$ , CM and distance from main road showed that  $LM > JS > CM$ ,  $JR > SS$ .

### 2) Heavy metal content analysis and soil to rice metal BAF

Analysis of soil samples found Mn levels ranging from 8.82-18.6  $\text{mg kg}^{-1}$ , Pb levels were 21.2-34, 27  $\text{mg kg}^{-1}$ , Zn were 0.14-0.5  $\text{mg kg}^{-1}$ , and Cu were 0.5-1.01  $\text{mg kg}^{-1}$ . Meanwhile, Ni levels were found at 0.43  $\text{mg kg}^{-1}$  only in LM. Mn was detected in rice grain samples at all sites, at levels in the range of 16.20-51.63  $\text{mg kg}^{-1}$ , Pb was detected at 9.64  $\text{mg kg}^{-1}$  only in SS, while Cu was detected at 2.54  $\text{mg kg}^{-1}$  only in JS. Zn was found only in SS and JS, at levels of 771.01 and 1995.5  $\text{mg kg}^{-1}$ , respectively. Ni was not detected in rice grain samples at any site (Table 1). These results showed lower levels of heavy metal contamination in organic rice farms compared with previous reports at other sites (16-20  $\text{mg kg}^{-1}$  of Cu and 55-66  $\text{mg kg}^{-1}$  of Zn in soil, 1.05-2.47  $\text{mg kg}^{-1}$  of Cu and 11.65-12.89  $\text{mg kg}^{-1}$  of Zn in rice grain) in paddy fields in Phraek Nam Daeng Sub-

district, Samut Songkhram Province, Thailand [7], and in paddy soils in the Khorat Basin, Northeast Thailand [18]. Nevertheless, some heavy metals are still finding their way into organically grown crops. One possible source of heavy metals is animal manure, as an important source of heavy metals in the environment [19]. In addition, with the proximity of roads to many non-certified organic rice farms in the study area, vehicles are a second likely source of contamination.

Soil to plant metal BAF was a significant indicator of the metal uptake capability of plants. Soil-to-plant transfer is a key route for human exposure to toxic heavy metals through the food chain. BAF factor values above 1 indicate that the plant accumulates heavy metals. The analysis found that rice had a higher Zn bioaccumulation capacity in SS and JS, while that of Mn was highest in SS (Figure 2). The highest BAF values for Pb and Cu were observed in SS and JS, respectively. These data suggest that Zn was relatively strongly bioaccumulated in rice, followed by Mn, Pb and Cu, consistent with previous reports [20]. Heavy metals including Fe, Mn, Zn, and Cu are micronutrients, and play an essential role in a range of enzymatic activities, photosynthesis and growth [21]. However, mean concentrations of Mn, Cu, Zn and Pb found in rice grain and soil were higher than their maximum permitted levels, implying additional contamination pathways for these metals from anthropogenic sources such as farm machinery and their engines.



**Figure 2** Bioaccumulation Factor (BAF) of rice plants. Mn, Ni, Pb, Cu (main box) and Zn (small box).

### 3) Risk Assessment of THQ and HI

Since risk factors such as THQ are used to assess potential health risks to humans, a risk assessment was conducted. The THQ of heavy metals from rice consumption showed that Zn levels from JS were higher than for other heavy metals, 25.893 and 32.791 for males and females, respectively (Table 2). The THQ values all exceeded one, therefore, Mn, Zn, Pb and Cu posed potential non-carcinogenic risks for consumers. The HI for rice consumption of the five elements was high (up to 40), indicating a high non-carcinogenic risk from ingestion of rice from these non-certified organic rice farms.

With high values of EDI due to high concentrations of heavy metals in rice grain, the THQs were also high. EDI was calculated from the daily average consumption of rice ( $\text{kg d}^{-1}$ ) of Thai people;  $0.43126 \text{ kg d}^{-1}$  for males and  $0.31928 \text{ kg d}^{-1}$  for females. The reference body weight (BW) was the average BW for Thais ( $68.83 \text{ kg}$  for males and  $57.40 \text{ kg}$  for females). However, these daily averages were higher than actual observed consumption in participants, at  $0.14958 \text{ kg d}^{-1}$  for males and  $0.11846 \text{ kg d}^{-1}$  for females [22].

**Table 1** Concentrations ( $\text{mg kg}^{-1}$ ) of Zn, Ni, Pb, Cu and Mn in soil and rice grain

	JR	CM	SS	JS	LM	Mean values <sup>a</sup>		Maximum permissible level in soil <sup>b</sup>
	Soil					Paddy soils	Surface soils	Soil
Mn	10.90±1.7	8.82±0.8	14.40±0.7	13.30±0.8	18.60±1.2	0.39	0.27–0.53	1.80
Zn	BDL	BDL	0.14±0.06	0.36±0.07	0.50±0.07	61.00	45–100	0.30
Ni	BDL	BDL	BDL	BDL	0.43±0.09	–	–	1.60
Pb	34.00±2.3	25.90±1.3	21.20±0.9	34.00±1.4	27.00±1.8	23.30	22–44	0.40
Cu	BDL	BDL	0.50±0.07	0.80±0.09	1.01±0.5	20.70	13–24	0.10
	Grain					Maximum permissible level in vegetable <sup>b</sup>		
Mn	18.35±1.5	20.65±2.3	51.63±2.4	16.20±2.3	58.00±3.6	0.50		
Zn	BDL	BDL	771.00±43.1	1995.38±50.2	BDL	0.10		
Ni	BDL	BDL	BDL	BDL	BDL	0.067		
Pb	BDL	BDL	9.64±1.7	BDL	BDL	0.003		
Cu	BDL	BDL	BDL	2.54±0.5	BDL	0.073		

**Remark:**

- Values of heavy metal content in the present study are given as mean, samples were analyzed in triplicates.
- BDL: Below Detectable limit. Zn 0.01 ppm, Ni 0.10 ppm, Pb 0.10 ppm, Cu 0.03 ppm and Mn 0.02 ppm following optimum working range for Agilent manufacturing recommendation.
- <sup>a</sup> Satpathy et al., 2014
- <sup>b</sup> Pollution Control Department, Thailand

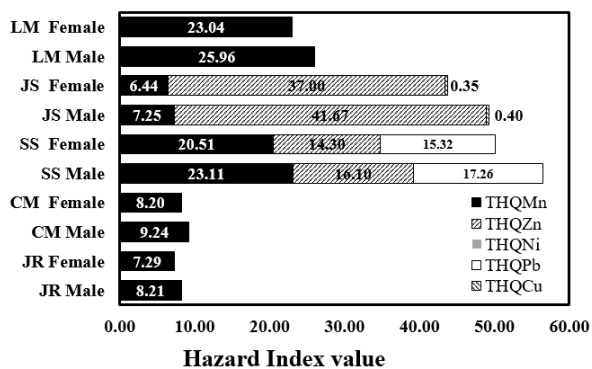
**Table 2** The estimated daily intake (EDI,  $\text{mg d}^{-1} \text{kg}^{-1}$ ) and the target hazard quotient (THQ)

Study sites	Estimated daily intake (EDI)							
	Mn		Zn		Pb		Cu	
	Male	Female	Male	Female	Male	Female	Male	Female
JR	0.11	0.11	ND	ND	ND	ND	ND	ND
CM	0.13	0.13	ND	ND	ND	ND	ND	ND
SS	0.32	0.31	4.83	4.69	0.06	0.06	ND	ND
JS	0.10	0.10	12.50	12.15	ND	ND	0.02	0.02
LM	0.36	0.35	ND	ND	ND	ND	ND	ND
	Target hazard quotient (THQ)							
JR	8.21	7.98	ND	ND	ND	ND	ND	ND
CM	9.24	8.98	ND	ND	ND	ND	ND	ND
SS	23.11	22.46	16.10	15.65	17.26	16.77	ND	ND
JS	7.25	7.05	41.68	40.50	ND	ND	0.40	0.39
LM	25.96	25.23	ND	ND	ND	ND	ND	ND

**Remark:**

- ND = Not determined
- \* Recommended Daily Intake (RDI) Mn=2.3 ( $\text{mg d}^{-1}$ ) for Male 1.8 ( $\text{mg d}^{-1}$ ) for female, Cu = 0.9 ( $\text{mg d}^{-1}$ ) for Male and female, Zn = 11 ( $\text{mg d}^{-1}$ ) for Male 8 ( $\text{mg d}^{-1}$ ) for female [24].

The estimated HI was mainly due to Mn, Zn and Pb with HI >1 (5.10-42.07); their THQs were calculated at 4.91-19.34, 10.01-31.05 and 10.72-12.86, respectively (Figure 3). These results indicated that Mn, Zn and Pb posed high non-carcinogenic risks to consumer health. Although daily intake of heavy metals via rice was an important exposure pathway, other studies reported that humans were also significantly exposed to heavy metals through ingestion of vegetables [23].



**Figure 3** The HI and THQ for rice consumers with the contamination of heavy metals for male and female adults (ages >15 years). The numbers indicate the values of THQ.

#### 4) Correlation study

Correlation analysis, Pearson correlation and PCA were used to distinguish the possible origins of heavy metals. Analysis of the results pointed to significant negative correlations between the distance of study site to the nearest main road, and distance from community to study site, with Zn concentration, with Pearson coefficient calculated as -0.944 and -0.960 ( $P < 0.05$ ), respectively (Table 3). Therefore, Zn contamination probably occurred through human activities such as emissions and improper disposal of wastes from the community or household garbage and vehicular transportation. A previous study also concluded that sources of Zn contamination had been reported as mainly anthropogenic in nature [24]. This is in contrast to soil Ni, Cu, which tend to originate from a combination of anthropogenic and lithogenic sources. In the PCA, two principal components with

eigenvalues above 1 were extracted; these two components explained 87.422 % of the variance in the data (Table 4). There were strong correlations between the three metals (soil Ni, Cu and Pb) and component 1, while strong correlations were also observed between the three metals (Zn, Mn) and component 2. However, the first component explained more of the variance than the second component (61.429% versus 25.993%).

Our empirical results are consistent with many other reports using PCA, confirming the linkage between Zn contamination and human activity. The Pearson correlation analysis and PCA indicated that agricultural activities, human activities and traffic emissions were the main sources of Zn and Cu, while Ni had a predominantly lithogenic origin, arising from soil parent materials [17, 26]. In addition, Ni BAF was found to be negatively correlated with soil Zn content; this could be explained by absorption kinetic studies, in which absorption of  $\text{Ni}^{2+}$  by the intact plant and its transfer from root to shoot were found to be inhibited by the presence of  $\text{Cu}_2\text{O}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{CO}_2^{+}$  [27]. Moreover, soil Zn is positively related to Zn BAF (Pearson 0.894 at  $P < 0.05$ ); high Zn content in soil increased transport to the rice plant and resulting bioaccumulation in plant tissues. This results in a high health risk index for rice (positive correlate with THQ and HI of Zn at  $P < 0.05$ , Table 3). These results were consistent with the significant correlations between grain Zn levels with DTPA-extractable soil Zn [28].

**Table 3** Correlations of heavy metals concentrations and bioaccumulation factor in soils and rice

Factors	Heavy metal content in soil				
	Pb	Ni	Cu	Mn	Zn
D Community	0.280	-0.602	-0.399	0.833	<b>-0.944*</b>
D Road	0.250	-0.595	-0.395	0.782	<b>-0.960**</b>
BAFMn	0.3489	0.391	0.138	0.493	-0.427
BAFZn	0.346	-0.060	-0.037	0.156	<b>0.894a*</b>

**Table 3** Correlations of heavy metals concentrations and bioaccumulation factor in soils and rice (continued)

Factors	Bioaccumulation factor for rice (BAF)				
	BAFPb	BAFNi	BAFCu	BAFMn	BAFZn
D Community	-0.284	0.994**	-0.285	0.657	-0.346
D Road	-0.354	0.993**	-0.234	0.310	-0.428
BAFMn	0.681	0.417	-0.405	1.00	0.007
BAFZn	0.609	-0.408	-0.408	0.007	1.00
	THQMn	THQZn	HI		
D Community	0.657	-0.255	-0.042		
D Road	0.609	-0.314	-0.134		
BAFMn	<b>0.900*</b>	-0.415	0.256		
BAFZn	0.040	<b>1.00**</b>	<b>0.942*</b>		

**Remark:** \* denotes a significant correlation in elemental concentration at  $p \leq 0.05$ , \*\* denotes a significant correlation in elemental concentration at  $p \leq 0.01$ , 'a' denotes the significance was tested by a Spearman rank-order correlation analysis; otherwise, it was tested by a Pearson correlation test.

**Table 4** Principle component analysis component score coefficient matrix

	Component		Communalities
	1	2	
soilMn	-0.123	<b>-0.903</b>	0.831
soilZn	0.181	<b>0.883</b>	0.812
soilNi	<b>0.838</b>	0.388	0.852
soilPb	<b>-0.979</b>	-0.054	0.962
soilCu	<b>0.946</b>	0.140	0.914
Eigen value	3.071	1.300	
% of Variance	61.429	25.993	
% of Cumulative	61.429	87.422	

**Remark:** - Extraction method: Principal Component Analysis.  
 - Rotation method: Varimax with Kaiser Normalization.  
 - Bold values represent the high loadings in this principle.

## Conclusions

Organic rice smallholders could not meet some criteria required for certification due to land tenure or proximity to roads or settlements. Therefore, monitoring and assessment of agricultural soils is required to evaluate the potential risk to consumers from soil and grain contamination by heavy metals from anthropogenic sources. High concentrations of heavy metals were detected in organic rice fields even through chemical fertilizers and pesticides were not used in farm operations. This implied that the contamination occurs via other pathways. Vehicles and animal manure

were identified as likely sources, with leaded gasoline, dust from storage areas or unmanaged waste dumps as key sources of heavy metals. Since Cu and Zn are added to pig's diets as nutritional supplements, and poultry health products also can contain As, manures produced from these animals can contain high concentrations of As, Cu, and Zn. These results suggest that farm location and the high use of animal manures contribute to heavy metal contamination in non-certified organic rice farms at the study sites.

The occurrence of heavy metals in paddy field soils was in a ranking order of  $Pb > Mn >$

Zn > Cu > Ni. However, concentrations of elements in the paddy soils were comparable to those of normal soils around the world. The ranking of heavy metals in rice was in the order: Zn > Mn > Pb > Cu. While Ni levels below detection limits, Pb and Zn exceeded maximum permissible levels in rice. The bioaccumulation factor showed that Zn and Pb were strongly accumulated in rice and many other crops. Correlation analysis showed that distance from main road and community were the main pathways for Zn contamination. The THQ and HI of the studied metals: Pb, Cu, Ni, Mn, and Zn, from consumption of rice remains serious in these study area. Therefore, organic rice farming should be located far away from road and traffic area, and heavy metal should be monitored in rice production.

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