Dhaka Univ. J. Biol. Sci. **24**(1): 1-16, 2015 (January)

ASSESSMENT OF THE PHYTOAVAILABILITY OF Cu AND Ni USING VARIOUS EXTRACTION PROCEDURES

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Key words: Heavy metal, Phytoavailability, Single and sequential extraction, Correlation

Abstract

The phytoavailability of copper (Cu) and nickel (Ni) in soils from Bangladesh was assessed. The uptake by *Ipomoea aquatica* and *Oryza sativa* L. was measured and a range of extractants tested on soils and plant tissue samples. Extractants tested were distilled water, 1 M NH_4Cl, 0.01 M $CaCl_2$, 0.005 M diethylenetriamine penta-acetic acid (DTPA), 0.1 M ethylenediamine tetraacetic acid (EDTA), 0.1 M HCl and 1 M HCl. The extractability of the metals varied depending on the metal species, the crop and the extractant used. The best extractant was 1 M HCl, which extracted the highest amount of the heavy metals and correlated most strongly with their plant uptake measures. The use of 1 M HCl is, therefore, recommended for first-level screening of soils contaminated with heavy metals if only one extractant is to be used. Sequential extraction showed that Cu was associated mostly with the 0.005 M DTPA and 0.1 M EDTA extractable fractions, while Ni was associated with the 0.1 M HCl and 1 M HCl fractions in most cases. The fractions of metals extracted using the sequential extraction procedure varied compared to single extractions for all soil types.

Introduction

Heavy metal contamination of the soils of Bangladesh has not yet reached a level for concern(1). However, heavy metal contamination of arable soils through industrial activities is likely to reach alarming levels in the near future. Industrial waste has been found to increase the heavy metal load in surrounding agricultural soils(2). These metals are taken up by plants and from there enter into the food chain where they may cause health hazards (3).

Chemical extraction procedures enable the prediction of changes in heavy metal mobility or bioavailability in soils(4). In order to understand the chemistry of heavy metals in their interactions with other soil components, such as clay minerals, organic matter and the soil solution, or to assess their mobility and retention as well as their availability to plants, the usual approach is to use selective chemical extraction(5). Single and sequential extraction methods have been used in the study of nutrient element

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deficiency in agricultural crops and animals as well as in environmental pollution analysis(1,4). The ultimate goal of such studies has been to see whether different pools of elements in soil are obtained using different extractants, both in single and sequential extraction. These properties could be linked to phytoavailability, phytoremediation and/or soil reclamation. However, data on plant uptake and the pool of elements extracted using different extractants are lacking. The present study aims at assessing the phytoavailability of heavy metals in soils varying in their degree of contamination and using various extraction procedures.

Materials and Methods

Three surface soils (0 - 150 mm), two from sites suspected to be contaminated and one from a site with no known source of industrial contamination were used. The suspected contaminated soils (steel mill and textile mill soils) were collected from the Sonargaon industrial area of Narayanganj (23°39.051' N, 90°34.919' E and 23°39.065' N, 90°34.903' E) and the apparently non-contaminated soil was collected from an agricultural land located in Dhamrai, Dhaka (23°54.749' N, 90°10.842' E). A sample of artificially spiked (as described below) Dhamrai soil was also included in the study. The soils were designated NC (apparently non-contaminated, Dhamrai series), SM (steel mill soil, Sonatala series), TM (textile mill soil, Shilmandi series) and Sp (spiked soil, Dhamrai series). The collected soil samples were processed following the procedures described by Imamul Huq and Alam(6).

In order to study the phytoavailability of heavy metals in soil and their extractability by different extractants, a pot culture experiment was set up with the soils of NC, SM, TM and Sp. Two crop species were used: An upland crop, *Ipomoea aquatica*, which is a leafy-vegetable plant commonly known as "Kalmi Sak"; and a lowland crop, rice (*Oryza sativa* L.; BRRI dhan-29). Upland and submerged conditions were maintained for growing Kalmi and rice, respectively. All experiments were performed in triplicate, as follows:

- 12 pots (2 × 2 × 3) for the two suspected contaminated soils (each soil with two plant species);
- 6 pots (2 × 3) for the non-contaminated soil (with two plant species), representing the control; and 6 pots (2 × 3) containing the spiked soils (with two plant species).

Earthen pots (5 kg and 7 kg) with no hole at the bottom were used. Air-dried 5 mm sieved soil samples amounting to 4 kg and 5 kg were placed in each of the earthen pots to grow *Ipomoea* and rice, respectively.

For spiking, solutions of Cu and Ni (as $CuSO_4.5H_2O$ and $NiSO_4$), at concentrations one and a half times that of the standard background levels of the corresponding metals in the Dhamrai soil were used (Table 1). The metal salts used were $CuSO_4.5H_2O$ and

$NiSO_4$. Solutions were made separately using 300 ml distilled water and mixed uniformly with the soils adding 100 ml of each to the pots. The spiked pots were allowed to stand for two weeks before plantation. The same spiking procedure was used for an additional pot that was used to measure the amount of the elements two weeks after spiking. This measure was the background value for the spiked soils.

Table 1. Spiking rates of the heavy metals.

Element	Standard background value* (mg/kg)	Spiking rate (mg/kg)
Copper (Cu)	26	39
Nickel (Ni)	20	30

*Source: Imamul Huq and Alam(6).

Ipomoea seeds were obtained from a local market and rice seedlings (BRRI dhan-29) were collected from a farmer's field. Ten *Ipomoea* seeds were sown in each pot. After 12 days the seeds began to germinate and 5 seedlings/pot were allowed to grow. For rice, soil was puddled and 9 rice seedlings/pot were randomly transplanted and allowed to grow. All pots were placed in a net-house in a randomized arrangement. Plants were watered with tap water as and when required. A blanket application of urea N was made after transplantation of seedlings. Positions of the pots were changed every alternate day to allow equal exposure of each pot to sunlight. Adequate plant protection measures were taken and the appearances of any symptoms were noted during the growing period.

At harvest, the plants and soils were collected individually for chemical analysis. *Ipomoea* plants were harvested manually by uprooting them carefully after 45 days of emergence and were prepared for analysis(6). The plant samples were separated into roots and edible parts (shoots and leaves). Rice plants were harvested 110 days after transplantation. Fresh and dry weights of roots and shoots of the plants together with the weights of 1000 grains were recorded.

The properties of the soil samples were analyzed in the laboratory(6). The background concentrations of total Cu and Ni (pre- and post-experiment) of the soils were determined by digestion of the soils using aqua regia. The total concentrations of the metals in the plants and soils were measured by flame-atomic absorption spectrometer (Varian-Spectra 220). The quality control/quality assurance (QC/QA) of the analyses was as described by Imamul Huq *et al.*(7).

The various extractants used are presented in the Table 2. Soils were both singly and sequentially extracted. Extraction of all four soil types with each of the extractants was performed in triplicate. Required amounts of the extractants were prepared and the specific ratios of soil to extractant were according those listed in Table 2. For the sequential extraction procedure (SEP), 10 g soil (2 mm sieved fraction) was placed in a 50 ml polycarbonate centrifuge tube and the extractions were performed sequentially in the order of strength of the extractants (distilled H_2O < 1 M NH_4Cl < 0.01 M $CaCl_2$ < 0.005 M DTPA (diethylenetriamine pentaacetic acid) < 0.1 M EDTA (ethylenediamine tetra-acetic acid) < 0.1 M HCl < 1M HCl) at a ratio of 1 : 2 and following the duration given in the Table 2. Extractions were performed in triplicate for each sample. All the extracts were centrifuged for 5 minutes at 2000 rpm and the supernatants were collected.

Table 2. Extraction methods used in the study.

Extractant	Ratio (w/v)/ time/temperature	Pool	Reference
Distilled H_2O	1 : 10, 1 h, room temp.	Water soluble	-
1 M NH_4Cl	1 : 6, 16 h "	Neutral salt soluble/exchangeable	Krishnamurti *et al.*(8)
0.01 M $CaCl_2$	1 : 5, 16 h "	Neutral salt soluble/ exchangeable	Ahnstrom and Parker(9)
0.005 M DTPA	1 : 2, 2 h "	Chelating extractable	Lindsay and Norvell(10)
0.1 M EDTA	1 : 2, 2 h "	Chelating extractable	Lindsay and Norvell(10)
0.1 M HCl	1 : 10, 0.5 h "	Weak acid extractable	CSTPA(11)
1 M HCl	1 : 33.3, 2 h "	Weak acid extractable	ANZECC and ARMCANZ(12)

The data were statistically evaluated using Pearson correlation coefficients and descriptive statistics were performed using Sigma Plot (v.12.0) and Minitab (v.16, Minitab, Inc.).

Results and Discussion

The selected soils were analyzed to ascertain the levels of Cu and Ni in them. The total Cu values were 63.93, 35.59, 26.24 and 139.23 mg/Kg in the NC, SM, TM and Sp soils, respectively while the total Ni values were 115.34, 98.68, 57.59 and 183.62 mg/Kg in NC, SM, TM and Sp soils respectively. Soil pH ranged from 4.4 to 5.8 in the contaminated soils. These lower pH values may be attributed to acid effluent coming from nearby industrial operations as well as high organic content.

Among the seven extractants used, 1 M HCl extracted the highest proportion of Cu (28.68 – 50.30%) and Ni (6.77 - 50.54%) from the soils (Tables 3a and 3b). Regardless of the soils and extractants, the relative metal extractability was observed to be in the order: Cu > Ni. Evidently, 1 M HCl was the most efficient agent for extracting Cu (av. for all soils 41%), followed by Ni (av. for all soils 28%) from the soils. There was a wide difference in metal extractability between 0.1 M HCl and 1 M HCl extractants. The amount of Ni extracted by H_2O in the suspected contaminated soils (SM and TM) varied from 3.05 to 5.4%, whereas the H_2O extractable fractions of Cu in the soils were below the detection

Table 3a. Levels (mg/kg) and per cent of total (in parentheses) Cu in the soils after individual extractions.

Soil	Extractants	Range (Min. to max.)	Sd	% of Total (Min. to max.)	Mean (%)
NC	Distilled H_2O	BDL	BDL	BDL	BDL
	1 M NH_4Cl	0.24 - 0.54	± 0.15	0.38 - 0.84	0.38 (0.59)
	0.01 M $CaCl_2$	0.085 - 0.09	± 0.003	0.13 - 0.14	0.088 (0.14)
	DTPA	2.97 - 3.27	± 0.17	4.65 - 5.12	3.17 (4.96)
	0.1 M EDTA	8.05 - 8.40	± 0.17	12.59 - 13.13	8.23 (12.87)
	0.1 M HCl	9.73 - 12.38	± 1.37	15.22 - 19.36	10.86 (16.99)
	1 M HCl	18.33 - 19.33	± 0.50	28.68 - 30.24	18.84 (29.48)
	Boiled aqua regia	61.73 - 66.06	± 2.17	-	63.93
SM	Distilled H_2O	BDL	BDL	BDL	BDL
	1M NH_4Cl	0.01 - 0.53	± 0.30	0.02 - 1.48	0.19 (0.52)
	0.01M $CaCl_2$	0.10 - 0.15	± 0.03	0.27 - 0.41	0.12 (0.32)
	DTPA	5.68 - 8.70	± 1.51	15.96 - 24.46	7.17 (20.14)
	0.1 M EDTA	10.03 - 10.10	± 0.04	28.20 - 28.38	10.06 (28.26)
	0.1 M HCl	10.61 - 18.80	± 4.67	29.81 - 52.83	13.41 (37.67)
	1 M HCl	16.93 - 17.90	± 0.52	47.58 - 50.30	17.52 (49.24)
	Boiled aqua regia	34.96 - 36.56	± 0.85	-	35.59
TM	Distilled H_2O	BDL	BDL	BDL	BDL
	1M NH_4Cl	0.01 - 0.02	± 0.01	0.02 - 0.07	0.01 (0.05)
	0.01 M $CaCl_2$	0.14 - 0.18	± 0.02	0.53 - 0.69	0.16 (0.60)
	DTPA	3.50 - 3.68	± 0.09	13.35 - 14.01	3.57 (13.61)
	0.1 M EDTA	6.62 - 7.16	± 0.27	25.22 - 27.29	6.87 (26.19)
	0.1 M HCl	6.93 - 9.20	± 1.29	26.41 - 35.07	7.71 (29.37)
	1M HCl	9.73 - 10.50	± 0.39	37.10 - 40.02	10.17 (38.75)
	Boiled aqua regia	25.90 - 26.84	± 0.52	-	26.24
Sp	Distilled H_2O	BDL	BDL	BDL	BDL
	1 M NH_4Cl	0.01 - 0.58	± 0.30	0.004 - 0.42	0.34 (0.25)
	0.01 M $CaCl_2$	0.12 - 0.16	± 0.02	0.09 - 0.11	0.14 (0.10)
	DTPA	13.54 - 14.37	± 0.42	9.72 - 10.32	13.96 (10.03)
	0.1 M EDTA	29.16 - 29.28	± 0.06	20.94 - 21.03	29.22 (20.99)
	0.1 M HCl	43.94 - 55.92	± 6.63	31.56 - 40.16	51.57 (37.04)
	1 M HCl	65.07 - 67.20	± 1.20	46.73 - 48.27	65.81 (47.27)
	Boiled aqua regia	126.51 - 160.33	± 18.40	-	139.23

Sd = Standard division, BDL = Below detection limit.

Table 3b. Levels (mg/kg) and per cent of total (in parentheses) Ni in the soils after individual extractions.

Soil	Extractants	Range (Min. to max.)	SD	% of total (Min. to max.)	Mean (%)
NC	Distilled H_2O	2.96 - 3.26	± 0.16	2.41 - 2.65	3.15 (2.56)
	1 M NH_4Cl	0.32 - 2.36	± 1.11	0.26 - 1.92	1.08 (0.88)
	0.01 M $CaCl_2$	1.79 - 1.99	± 0.11	1.45 - 1.62	1.86 (1.52)
	DTPA	0.56 - 0.60	± 0.02	0.46 - 0.49	0.58 (0.47)
	0.1 M EDTA	2.30 - 2.44	± 0.07	1.87 - 1.99	2.38 (1.93)
	0.1 M HCl	5.29 - 6.59	± 0.66	4.31 - 5.36	5.89 (4.79)
	1 M HCl	20.90 - 21.30	± 0.22	17.01 - 17.34	21.04 (17.13)
	Boiled aqua regia	119.54 - 125.47	± 3.02	-	122.85
SM	Distilled H_2O	3.37 - 3.70	± 0.17	2.92 - 3.21	3.52 (3.05)
	1 M NH_4Cl	5.89 - 6.76	± 0.45	5.11 - 5.86	6.39 (5.54)
	0.01 M $CaCl_2$	4.35 - 5.36	± 0.51	3.77 - 4.64	4.89 (4.24)
	DTPA	4.23 - 4.28	± 0.03	3.67 - 3.71	4.25 (3.68)
	0.1 M EDTA	4.92 - 5.11	± 0.10	4.26 - 4.43	5.03 (4.36)
	0.1 M HCl	5.90 - 11.64	± 0.94	5.12 - 10.09	8.32 (7.21)
	1 M HCl	56.80 - 58.30	± 0.75	49.24 - 50.54	57.54 (49.89)
	Boiled aqua regia	112.57 - 117.02	± 2.42	-	115.34
TM	Distilled H_2O	2.86 - 3.26	± 0.22	4.97 - 5.66	3.11 (5.40)
	1 M NH_4Cl	2.72 - 3.15	± 0.22	4.73 - 5.47	2.92 (5.07)
	0.01 M $CaCl_2$	2.00 - 2.15	± 0.07	3.47 - 3.72	2.07 (3.59)
	DTPA	1.36 - 1.52	± 0.09	2.37 - 2.65	1.42 (2.46)
	0.1 M EDTA	2.52 - 2.72	± 0.11	4.38 - 4.72	2.60 (4.51)
	0.1 M HCl	4.53 - 6.20	± 0.94	7.87 - 10.77	5.12 (8.89)
	1 M HCl	21.77 - 24.03	± 1.14	37.79 - 41.73	22.82 (39.63)
	Boiled aqua regia	54.70 - 61.63	± 3.60	-	57.59
Sp	Distilled H_2O	3.23 - 3.76	± 0.30	1.76 - 2.05	3.42(1.86)
	1 M NH_4Cl	4.66 - 6.92	± 1.15	2.54 - 3.77	5.65 (3.08)
	0.01 M $CaCl_2$	1.99 - 2.10	± 0.06	1.08 - 1.14	2.03 (1.11)
	DTPA	2.25 - 2.57	± 0.16	1.22 - 1.40	2.41 (1.31)
	0.1 M EDTA	13.87 - 15.75	± 1.00	7.55 - 8.58	14.61 (7.96)
	0.1 M HCl	5.90 - 11.64	± 2.98	3.21 - 6.34	8.32 (4.53)
	1 M HCl	12.43 - 13.87	± 0.78	6.77 - 7.55	12.97 (7.06)
	Boiled aqua regia	176.78 - 195.87	± 10.63	-	183.62

Sd = Standard division.

limit. $CaCl_2$ extracted a relatively smaller amounts (0.1 - 0.7% Cu, 1.1 - 4.6%) of total Cu and Ni than NH_4Cl (0.004 - 1.5% Cu, 0.3 - 6% Ni) from the soils. The chloride salts (NH_4Cl and $CaCl_2$) were found to be weak extractants for Cu (up to 1.5%). EDTA was a better extractant than DTPA for the metals. DTPA extracts metals that are thought to represent the plant-available fractions(4,10,13). DTPA and EDTA form soluble complexes with metals, reducing their activity in the soil solution; therefore, ions desorbed from the surface enter into the extraction solution(1). It is clear that HCl is a better extractant for the metals though the 1 M concentration performed better than the 0.1 M HCl. The extractability, in general, of the metals with different extractants was in the order: 1 M HCl > 0.1 M HCl > 0.1 M EDTA > DTPA > 1 M NH_4Cl > 0.01 M $CaCl_2$ > H_2O. It is, therefore, apparent from these results that 1 M HCl is the best choice for Cu and Ni extraction from soil.

The extent of contamination due to heavy metal deposition in the NC, SM, TM and Sp soils was identified by using information of background levels of the metal elements for Bangladesh soils(14) and the tolerable and in excess of tolerable level was calculated on the information of Kloke(15). Background concentrations for Cu and Ni of Bangladesh soils were 27 and 22 mg/kg, respectively, whereas the maximum tolerable concentrations were defined as 100 and 50 mg/kg for Cu and Ni, respectively. The total heavy metal contents of the soil samples (Table 4) showed a wide variation in values ranging from levels similar to background to a level reflective of severe contamination. The levels of Cu in all the soils were within the tolerable limit (> 27 – ≤ 100 mg/kg), whereas Ni concentration in the soils were found to be in excess of tolerable level (≥50 mg/kg) with the exception of the TM soil, which had a total Ni concentration of 57.6 mg/kg, which is within the tolerable level.

The apparently non-contaminated soil (NC) contained relatively higher amounts of Cu and Ni than the suspected contaminated soils (SM or TM soils). The higher values for the elements in the NC soil were perhaps due to the sampling site of the Dhamrai soil being adjacent to the Dhaka-Manikganj highway. Levels of some heavy metals have previously been reported to be high in soils along the highways due to automobile exhaust(16). However, of the two industrial sites in this study, higher contents of the metals were found in the soils of the steel mill area. The heavy metals found in soils make the plants and soil biota living there vulnerable to metal toxicity. The extent of contamination was observed to be higher in the steel mill area than in the textile mill area and this might be due to an excessive amount of heavy metals in the discharged effluents.

Evidently, 1 M HCl was the most efficient agent for extracting Cu (41.2%) and Ni (28.4%) from the soils. This indicates that 1 M HCl extracted most of the non-residual fraction of the metal elements. The sequential extraction procedure (SEP) showed that Cu and Ni were associated mostly with the DTPA and 1 M HCl extractable fractions, respectively. Copper was also associated with the EDTA, 0.1 M HCl and 1 M HCl fractions (Figs 1 and 2). Water, NH_4Cl and $CaCl_2$ were very poor extractants, as observed in the SEP data. It is apparent that the fractions of the metals extracted from the soils using the SEP varied widely compared to the single extractions. Chowdhury *et al.*(1) observed similar variations for cadmium, lead and zinc in the same soils.

Dry matter produced for *Ipomoea* and rice was better in the SM and TM soils than in the NC and Sp soils. The mean dry weights of different parts of *Ipomoea* and rice along with 1000 grain weight of rice grown in the soils are presented in the Table 4.

Table 4. Dry weights of plant tissue of *Ipomoea aquatica* and rice.

Soil	*Ipomoea aquatica*			Rice				
	Wt. of roots (g/pot)	Wt. of shoots and leaves (g/pot)	Total wt. of plants (g/pot)	Wt.of roots (g/pot)	Wt.of straw (g/pot)	Wt. of unhusked grains (g/pot)	Total weight of plants (g/pot)	Wt.of unhusked grains (1000 grain wt, g)
NC	1.51	1.31	2.82	4.10	18.27	15.53	37.90	20.30
SM	1.58	2.06	3.64	7.35	34.40	26.73	68.49	20.06
TM	3.45	3.20	6.65	5.65	28.14	32.61	66.40	19.84
Sp	1.29	1.08	2.37	3.46	15.82	14.07	33.35	20.84

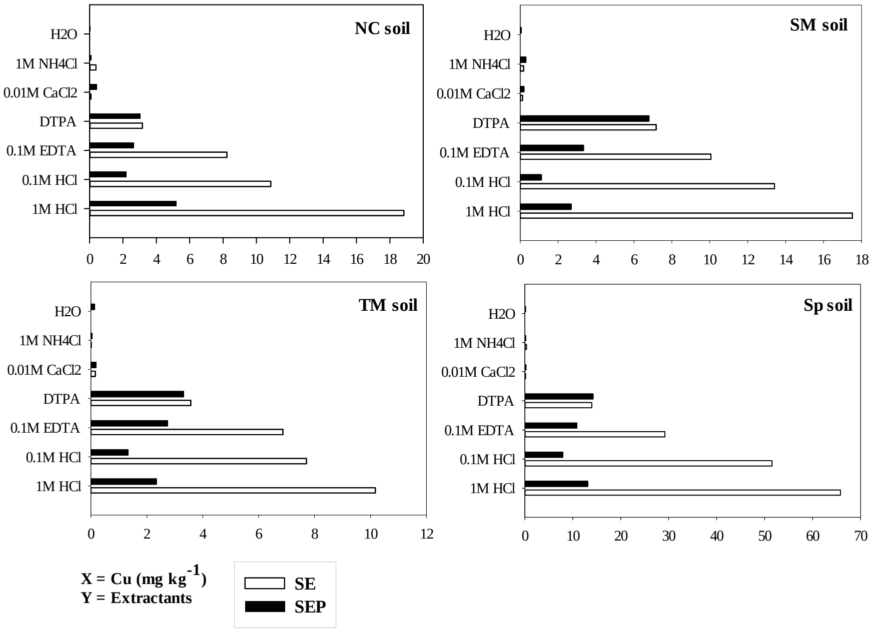

Fig. 1. Mean proportion of Cu in each of the single extractants (SE) compared with the corresponding fraction in the sequential extraction procedure (SEP) for NC, SM, TM and Sp soils.

The maximum concentration of Cu and Ni (51.4 and 33.2 mg/kg) were observed in the edible parts of *Ipomoea* (shoots + leaves) grown in the SM soil, while in the rice grains, the metal concentrations (11.9 and 13 mg/kg, respectively, for Cu and Ni) were found to be the highest in the Sp soil (Table 5). Copper and Ni contents were found to be much higher in the edible parts of *Ipomoea* than in the rice grains (Fig. 3).

According to Kabata-Pendias(17), the normal levels of Cu and Ni in mature leaf tissue vary from 5 - 30 and 0.1 - 5 mg/kg, respectively and the toxic levels of the same metals range from 20 - 100 and 10 - 100 mg/kg, respectively. The tolerable concentrations of Cu and Ni in agronomic crops range from 5 - 20 and 1 - 10 mg/kg, respectively. In the present study, the total contents of Cu in *Ipomoea* (74.7 - 120.8 mg/kg) and rice plants (107 - 256.5 mg/kg) grown in the soils were much higher than the toxic level. The concentration of Cu in rice grains (6.3 - 11.9 mg/kg) was found within the tolerable limit, whereas in the edible parts of *Ipomoea* the concentration (30.7 - 51.4 mg/kg) exceeded the toxic limit. The total concentration of Ni in *Ipomoea* (55 - 73.2 mg/kg) was within the toxic limit exceeding the normal and tolerable limits, while in the rice plants the concentration (116.1 - 151 mg/kg) was found to be much higher than the toxic level. In all the soils, the concentrations of Ni in the edible parts of both the plant species (10.9 - 13 mg/kg in rice and 20.9 - 33.2 mg/kg in *Ipomoea*) were found to be within the toxic range.

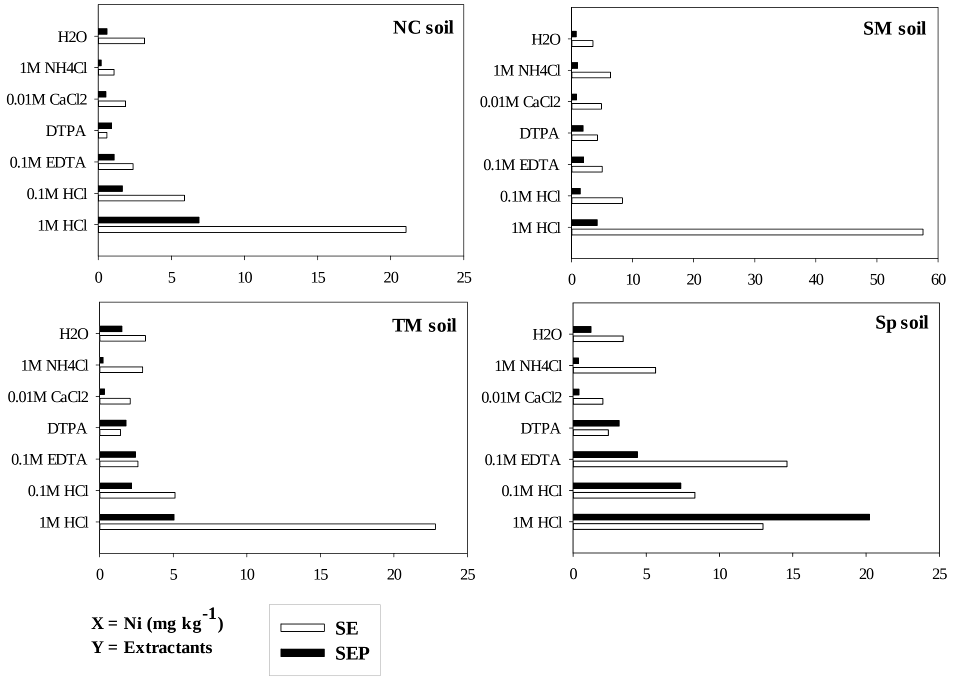

Fig. 2. Mean proportion of Ni in each of the single extractants (SE) compared with the corresponding fraction in the sequential extraction procedure (SEP) for NC, SM, TM and Sp soils.

However, the concentrations of the metal elements in the plants were found much higher in the steel mill soil than the soil in textile mill area. This may be due to the fact that the metals were in a more labile state than those in the TM soil. The TM soil would have been contaminated primarily with cellulosic organic waste, with the metals bound to these organic materials rendering them less mobile for plant uptake. Elevated levels of Cu and Ni in plants near metal smelters have previously been demonstrated in other investigations (18-21).

Table 5. Levels (mg/kg) of Cu and Ni in *Ipomoea* and rice.

		Ipomoea			Rice					
Metal	Soil	Root	Shoot + leaf	Total	Root	Straw	Grain	Husk	Grain + husk	Total
Cu	NC	43.4	31.3	74.7	55.6	32.3	9.0	10.1	19.1	107.0
	SM	69.4	51.4	120.8	87.4	39.2	9.1	12.5	21.6	148.1
	TM	78.6	30.7	109.3	63.8	30.9	6.3	7.9	14.2	108.9
	Sp	81.6	34.2	115.8	189.1	42.2	11.9	13.3	25.2	256.5
Ni	NC	33.7	29.6	63.3	58.0	17.9	12.8	27.4	40.2	116.1
	SM	40.0	33.2	73.2	70.8	25.9	11.5	30.7	42.2	138.9
	TM	34.2	20.9	55.0	62.6	23.1	10.9	22.6	33.5	119.2
	Sp	40.7	28.2	68.9	87.2	27.4	13.0	23.4	36.4	151.0

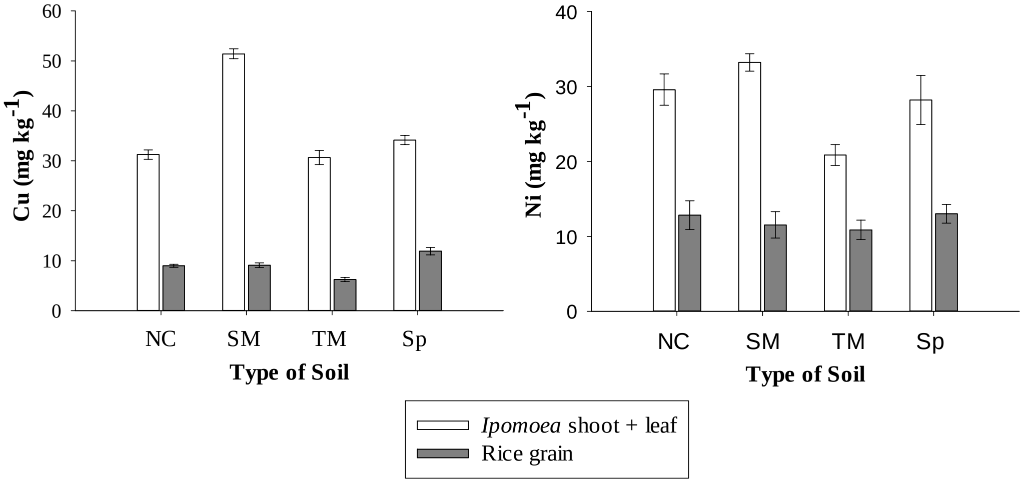

Fig. 3. Comparison of Cu and Ni in the edible parts of *Ipomoea* and rice.

The maximum uptake of Cu in *Ipomoea* was found for the TM soil (0.37 mg/pot), with 73% in the roots and 27% in the edible parts. The highest Cu levels in rice plants were found in the SM soil (2.6 mg/pot), with the major portion taken up by rice straw (up to 53% of total uptake) in all soil types (Fig. 4). In the edible parts of rice (husk + grains), the maximum uptake of Cu was observed for the SM soil (0.58 mg/pot), followed by the TM, Sp and NC soils.

The maximum uptake of Ni by *Ipomoea* was observed in the TM soil (0.18 mg/pot), followed by the SM (0.13 mg/pot), NC (0.09 mg/pot) and Sp (0.08 mg/pot) soils. The maximum uptake of Ni by the roots of *Ipomoea* was found in the TM soil (0.12 mg/pot) and by the edible parts in both the SM and TM soils (0.07 mg/pot) (Fig. 4). These values represented 65.5% (by roots) and 37.1% (by shoots and leaves) of the total Cu accumulation in *Ipomoea*. The level of Ni uptake by rice plants was in the order: SM soil > TM soil > Sp soil > NC soil (Fig. 4). The uptake of Ni in the edible parts of rice (husk + grains) was found to be the highest in the SM soil (1.13 mg/pot; 44.4% of the total uptake) and the quantity in the TM soil was 1.1 mg/pot (52.1% of the total uptake).

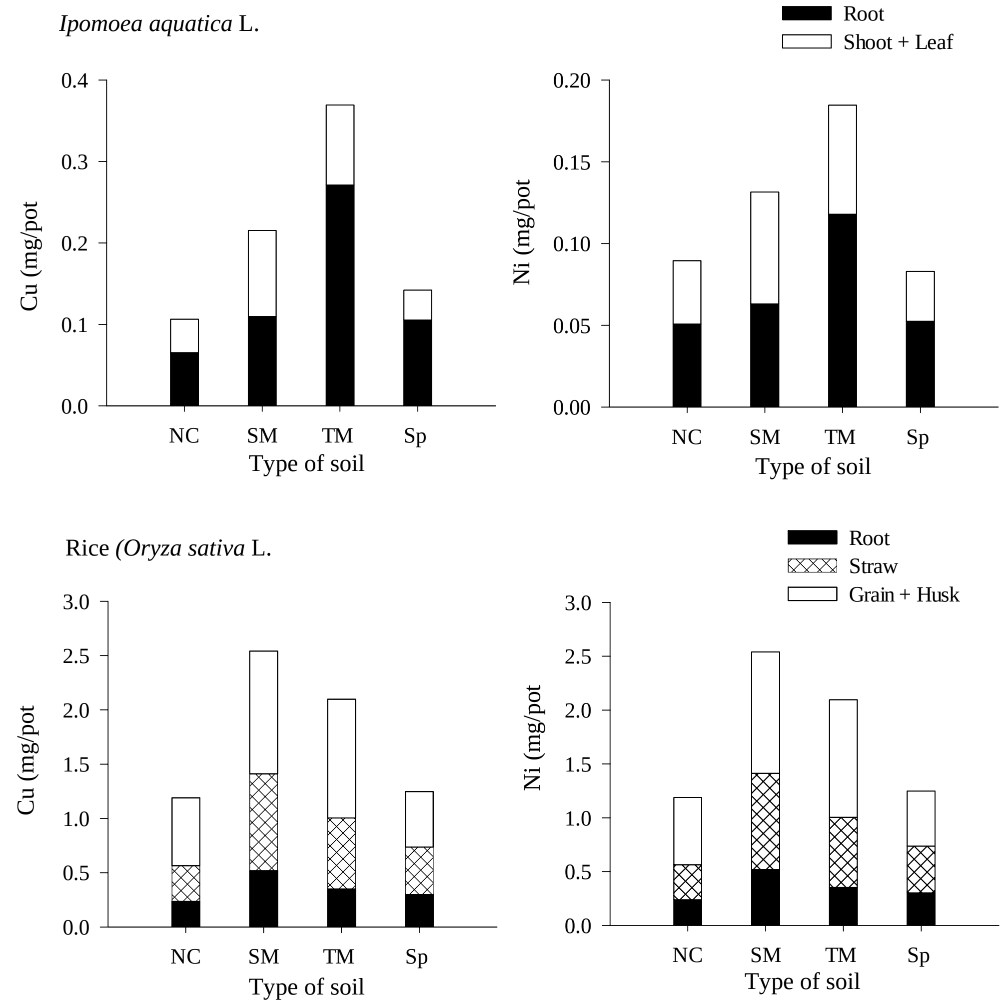

Fig 4. Uptake of Cu and Ni by *Ipomoea* and rice.

Table 6. Pearson correlation coefficients between the fractions of the metals and pH in the soils.

	T Cu	T Ni	SE$_1$ Cu	SE$_1$ Ni	SE$_2$ Cu	SE$_2$ Ni	SE$_3$ Cu	SE$_3$ Ni	SE$_4$ Cu	SE$_4$ Ni	SEP$_1$ Cu	SEP$_1$ Ni	SEP$_2$ Cu	SEP$_2$ Ni	SEP$_3$ Cu	SEP$_3$ Ni	SEP$_4$ Cu	SEP$_4$ Ni	pH
T Cu		0.92^{a}	0.97^{a}	−0.58^{b}	0.90^{a}	NS	0.83^{a}	NS	0.94^{a}	0.90^{a}	0.99^{a}	0.97^{a}	0.95^{a}	0.89^{a}	0.86^{a}	0.68^{b}	0.89^{a}	0.71^{a}	0.73^{a}
T Ni	0.92^{a}		0.90^{a}	NS	0.84^{a}	0.43^{a}	0.83^{a}	NS	0.87^{a}	0.85^{a}	0.89^{a}	0.84^{a}	0.84^{a}	0.75^{a}	0.86^{a}	0.58^{b}	0.81^{a}	NS	0.57^{b}
SE$_1$ Cu	0.97^{a}	0.90^{a}		NS	0.97^{a}	NS	0.93^{a}	NS	0.99^{a}	0.97^{a}	0.98^{a}	0.99^{a}	0.98^{a}	0.96^{a}	0.94^{a}	0.81^{a}	0.97^{a}	0.84^{a}	0.59^{b}
SE$_1$ Ni	-0.58^{b}	NS	NS		NS	NS	NS	0.77^{a}	NS	NS	−0.60^{b}	−0.62^{b}	-0.60^{b}	−0.58^{b}	NS	NS	NS	NS	-0.86^{a}
SE$_2$ Cu	0.90^{a}	0.84^{a}	0.97^{a}	NS		NS	0.93^{a}	NS	0.98^{a}	0.96^{a}	0.94^{a}	0.95^{a}	0.97^{a}	0.96^{a}	0.92^{a}	0.82^{a}	0.98^{a}	0.88^{a}	NS
SE$_2$ Ni	NS	0.43^{a}	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SE$_3$ Cu	0.83^{a}	0.83^{a}	0.93^{a}	NS	0.93^{a}	NS		NS	0.95^{a}	0.98^{a}	0.85^{a}	0.87^{a}	0.87^{a}	0.88^{a}	0.98^{a}	0.91^{a}	0.92^{a}	0.86^{a}	NS
SE$_3$ Ni	NS	NS	NS	0.77^{a}	NS	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.68^{a}
SE$_4$ Cu	0.94^{a}	0.87^{a}	0.99^{a}	NS	0.98^{a}	NS	0.95^{a}	NS		0.99^{a}	0.96^{a}	0.97^{a}	0.97^{a}	0.97^{a}	0.96^{a}	0.85^{a}	0.98^{a}	0.88^{a}	NS
SE$_4$ Ni	0.90^{a}	0.85^{a}	0.97^{a}	NS	0.96^{a}	NS	0.98^{a}	NS	0.99^{a}		0.92^{a}	0.94^{a}	0.93^{a}	0.94^{a}	0.98^{a}	0.90^{a}	0.96^{a}	0.90^{a}	NS
SEP$_1$ Cu	0.99^{a}	0.89^{a}	0.98^{a}	−0.60^{b}	0.94^{a}	NS	0.85^{a}	NS	0.96^{a}	0.92^{a}		0.99^{a}	0.98^{a}	0.94^{a}	0.87^{a}	0.72^{a}	0.93^{a}	0.77^{a}	0.72^{a}
SEP$_1$ Ni	0.97^{a}	0.84^{a}	0.99^{a}	−0.62^{b}	0.95^{a}	NS	0.87^{a}	NS	0.97^{a}	0.94^{a}	0.99^{a}		0.99^{a}	0.97^{a}	0.89^{a}	0.78^{a}	0.95^{a}	0.84^{a}	0.68^{a}
SEP$_2$ Cu	0.95^{a}	0.84^{a}	0.98^{a}	−0.60^{b}	0.97^{a}	NS	0.87^{a}	NS	0.97^{a}	0.93^{a}	0.98^{a}	0.99^{a}		0.98^{a}	0.87^{a}	0.75^{a}	0.97^{a}	0.85^{a}	0.67^{a}
SEP$_2$ Ni	0.89^{a}	0.75^{a}	0.96^{a}	−0.58^{b}	0.96^{a}	NS	0.88^{a}	NS	0.97^{a}	0.94^{a}	0.94^{a}	0.97^{a}	0.98^{a}		0.88^{a}	0.83^{a}	0.98^{a}	0.93^{a}	NS
SEP$_3$ Cu	0.86^{a}	0.84^{a}	0.94^{a}	NS	0.92^{a}	NS	0.98^{a}	NS	0.96^{a}	0.98^{a}	0.87^{a}	0.89^{a}	0.87^{a}	0.88^{a}		0.93^{a}	0.90^{a}	0.85^{a}	NS
SEP$_3$ Ni	0.68^{b}	0.58^{b}	0.81^{a}	NS	0.82^{a}	NS	0.91^{a}	NS	0.85^{a}	0.90^{a}	0.72^{a}	0.78^{a}	0.75^{a}	0.83^{a}	0.93^{a}		0.81^{a}	0.90^{a}	NS
SEP$_4$ Cu	0.89^{a}	0.81^{a}	0.97^{a}	NS	0.98^{a}	NS	0.92^{a}	NS	0.98^{a}	0.96^{a}	0.93^{a}	0.95^{a}	0.97^{a}	0.98^{a}	0.90^{a}	0.81^{a}		0.92^{a}	NS
SEP$_4$ Ni	0.71^{a}	NS	0.84^{a}	NS	0.88^{a}	NS	0.86^{a}	NS	0.88^{a}	0.90^{a}	0.77^{a}	0.84^{a}	0.85^{a}	0.93^{a}	0.85^{a}	0.90^{a}	0.92^{a}		NS
pH	0.73^{a}	0.57^{b}	0.59^{b}	−0.86^{a}	NS	NS	NS	−0.68^{a}	NS	NS	0.72^{a}	0.68^{b}	0.67^{b}	NS	NS	NS	NS	NS	

a - denotes significance at $p \leq 0.01$, b - denotes significance at $p \leq 0.05$, NS - not significant, T - Total, SE1 - Single extraction with 1 M HCl, SE2 - Single extraction with 0.1 M HCl; SE3 -Single extraction with DTPA, SE4 - Single extraction with 0.1 M EDTA, SEP1 - Sequential extraction procedure with 1 M HCl, SEP2 - Sequential extraction procedure with 0.1 M HCl, SEP3 - Sequential extraction procedure with DTPA, SEP4 - Sequential extraction procedure with 0.1 M EDTA.

The total concentrations of Cu and Ni in the soils were found to be significantly correlated (*r* = 0.92, $p \leq 0.01$). Highly significant positive correlation (*r* = 0.83 - 0.99, $p \leq 0.01$) between total and extractable (1 M HCl, 0.1 M HCl, 0.1 M EDTA and DTPA) Cu was observed (Table 6). Highly significant correlation (*r* = 0.81 - 0.90, $p \leq 0.01$) was also observed between the total Ni and the extractable Cu fractions. Soil pH showed significant relationship with the 1 M HCl extractable and total metal concentrations, extractable and total metal concentrations. Soil organic matter did not show any significant relationship and hence these results are not reported in the text. Water, NH_4Cl and $CaCl_2$ extractable fractions of Cu and Ni showed poor and non-significant relationships. Correlations among the other extractable metal fractions were found to be positively significant in most cases.

Table 7. Correlation coefficients between extracted metal elements and their corresponding plant contents irrespective of soil types.

Metal element	Extractants	Single extraction				Sequential extraction			
		Ipomoea (Shoot)		Rice (Grain)		*Ipomoea* (Shoot)		Rice (Grain)	
		r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Cu	Distilled H_2O	−0.14	0.66	0.46	0.14	-0.43	0.16	0.19	0.57
	1 M NH_4Cl	−0.05	0.89	0.63	0.03	0.68	0.02	0.02	0.95
	0.01 M $CaCl_2$	−0.13	0.69	-0.08	0.80	0.27	0.39	0.38	0.23
	0.005 M DTPA	0.17	0.60	0.78	0.003	0.26	0.42	0.18	0.58
	0.1 M EDTA	−0.07	0.83	0.82	0.001	0.03	0.93	0.19	0.55
	0.1 M HCl	−0.09	0.78	0.83	0.001	0.02	0.96	0.26	0.42
	1 M HCl	−0.12	0.71	0.83	0.001	0.11	0.74	0.29	0.37
	Boiled aqua regia	−0.24	0.45	0.78	0.003				
Ni	Distilled H_2O	0.47	0.13	0.53	0.08	−0.71	0.01	−0.07	0.82
	1 M NH_4Cl	0.41	0.19	0.04	0.91	0.38	0.22	0.03	0.93
	0.01 M $CaCl_2$	0.55	0.06	−0.16	0.63	0.78	0.003	0.10	0.77
	0.005 M DTPA	0.51	0.09	−0.08	0.81	0.04	0.91	0.11	0.75
	0.1 M EDTA	0.16	0.62	0.17	0.59	−0.18	0.58	0.04	0.91
	0.1 M HCl	0.35	0.26	−0.14	0.67	−0.08	0.81	0.20	0.53
	1 M HCl	0.51	0.09	−0.17	0.59	0.02	0.95	0.24	0.46
	Boiled aqua regia	0.50	0.10	0.26	0.42				

r = Correlation coefficient, *p* = p value, n=12.

Copper content in rice grains showed highly significant ($p < 0.01$) positive correlations with the total and most of the single extractable fractions in the soils, while for *Ipomoea* the relationships were mostly negative and not significant (Table 7). In general, poor correlations were observed between the concentrations of Cu and Ni in both the plant species and those in the soils and the relationships were mostly not significant. The positive relationships among the soil and plant metal contents could be related to heavy metal toxicities in both plants and animals through their entry into the food chain. Poor and negative correlations observed among the soil and plant metal contents might be due to differences in soil types, the nature of the plants concerned and the extent and type of industrial effluents.

The concentrations of Cu in the soils after harvest of both of the crops were found to be increased by 190, 635, 609 and 45 (for *Ipomoea*) and 135, 144, 118 and 8% (for rice) in the NC, SM, TM and Sp soils, respectively (Fig. 5), while the concentration of Ni in the residual soils showed irregular pattern. The decreases in metal content in the soils after plant culture indicate accumulation of the elements into the plants. On the other hand, the increase in the concentration of Cu and Ni in soils was perhaps due to the release of endogenous metals from the plants, caused by the release of organic acid exudates from the plant roots.

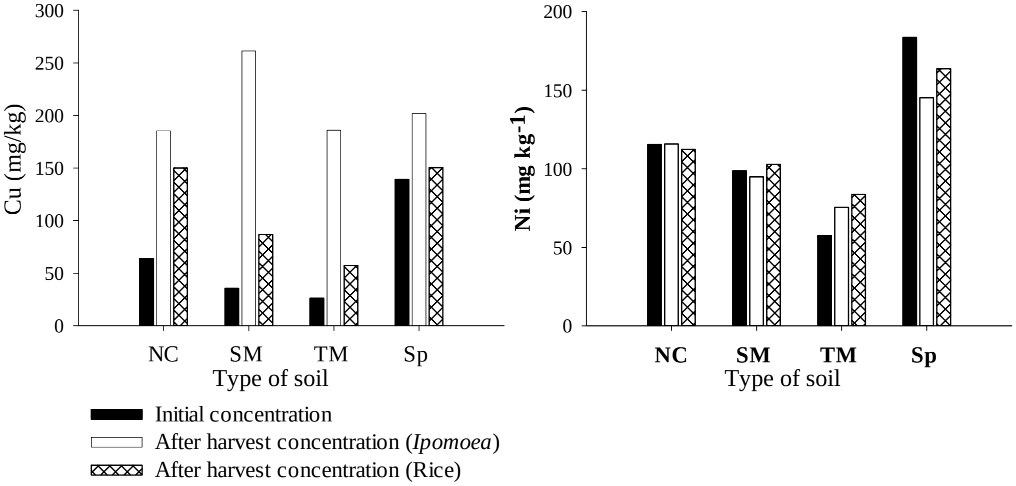

Fig. 5. Concentrations of Cu and Ni in the soils before and after harvest of the crops.

The results of the present study suggest that agricultural soils of Bangladesh in the vicinity of different industrial areas are at risk of heavy metal contamination/pollution. The concentration of Cu and Ni in the soils and the plant tissues and a strong correlation among the extractable metal fractions and metals content in the plants indicate the phytoavailability of the heavy metals. However, the assessment of the phytoavailability of the elements considered in this study was found to be dependent on the method of extraction, the crop and metal species, as well as the soil type. The present study indicates that a mild extractant such as 1 M HCl can be used to assess the phytoavailability of the heavy metals Cu and Ni.

Acknowledgment

The work was conducted as a part of research grant of Bangladesh University Grants Commission. The authors acknowledge it.

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(Manuscript received on ....; revised on ....)