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THRESHOLD VALUES OF ARSENIC, CADMIUM AND LEAD IN SOIL FOR RICE (*ORYZA SATIVA* L.) AND KALMI (*IPOMOEA AQUATICA*)

Swarnali Mahmood[1], M. Shahjahan Choudhury[2] and *S.M Imamul Huq[1]
[1]Department of Soil, Water and Environment, University of Dhaka, Dhaka 1000, Bangladesh
[2]Bangladesh-Australia Centre for Environmental Research (BACER-DU)
University of Dhaka, Dhaka 1000, Bangladesh

ABSTRACT

A pot experiment was conducted to assess the threshold values of arsenic (As), cadmium (Cd) and lead (Pb) in soil for rice (*Oryza sativa* L.) and kalmi (*Ipomoea aquatica*). Rice and kalmi were grown on a silt loam soil treated separately with different levels of As (0, 0.5, 1, 2 and 5 mg As/L of irrigation water), Cd (0, 5, 10, 15 and 20 mg Cd/kg soil) and Pb (0, 25, 50, 100 and 200 mg Pb/kg soil). The contents of As, Cd and Pb in soil for 10% reduction in dry matter yield of rice and kalmi were taken as the threshold values which were 14.10 mg, 4.34 mg and 46.17 mg per kg soil for As, Cd and Pb, respectively, while the same for kalmi were 4 mg, 6.57 mg and 34.84 mg per kg soil, respectively, for the three elements.

Keywords: Dry matter yield, threshold value, arsenic.

INTRODUCTION

Among the chemical contaminants, trace elements are considered to have specific ecological, biological, and health significance (Kabata-Pendias, 2011). All elements, even those that are metabolically essential (*e.g*., Cu, Mn, etc.) are toxic when present in excess (Achard-Joris *et al*., 2007). Although metal contamination in the soils of Bangladesh has not reached a level of concern yet, the industrial wastes have been found to increase the metal loads in the surrounding agricultural soils (Joardar *et al*., 2005). The uptake of metals from contaminated soils by plants comprises a prominent path for such metals to enter the food chain where they may cause health hazards (Imamul Huq *et al*., 2000).

Arsenic (As), cadmium (Cd) and lead (Pb) are typical toxic trace elements in soils. Excess of these elements produces a harmful effect on biological systems. Irrigation with As contaminated groundwater leaves a risk of accumulation of this toxic trace element in soil and the eventual exposure of the food chain through plant uptake and animal consumption (Imamul Huq and Naidu, 2005). In plants, As interferes with the metabolic processes (Martin *et al*., 1992), and inhibits plant growth and biomass accumulation (Stepanok, 1998; Stoeva *et al*., 2003). Cd inhibits seed germination (Koeppe, 1977; Yu, 1991) and interferes with several physiological processes resulting in low productivity (Obata and Umebayashi, 1997). High doses of Pb exposure can restrict plant biomass (Gopal and Rizvi, 2008; Gichner *et al*., 2008; Islam *et al*., 2008; Piotrowska *et al*., 2009; Singh *et al*., 2010). Pb strongly inhibits seed germination, seedling development, root elongation, plant growth, transpiration, chlorophyll production, and water and protein content of plant (Pourrut *et al*., 2011), thereby, poses a serious problem for agriculture (Johnson and Eaton, 1980).

From plant growth, animal, and human health standpoint, soils are not considered polluted unless a threshold concentration exists that affects its biological processes (Kabata-Pendias, 2011). The threshold dose-response model is considered as the most dominant model in toxicology (Calabrese and Baldwin, 2003). Threshold values are not fixed physiological facts or physical constants but are statistical points representing the best estimate values from a group of responses (Mohapatra, 2006). Plant growth is commonly used as a general parameter to study the influence of excess trace elements, with growth rate inhibition often being the most obvious plant reaction (Fodor, 2002; Hagemeyer, 2004). Plant populations become stressed when a biotic or abiotic factor affects plant growth and development (Jackson, 1986). Once plant stress is detected, identified and quantified, thresholds for plants can be developed to indicate when control measures are needed (Nutter, 1990).

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*Corresponding author e-mail: imamhuq@hotmail.com

For agricultural soils, the maximum acceptable concentrations of metals have been established by different countries (McLaughlin *et al*., 2000). The maximum acceptable concentration of As for agricultural soil recommended by the European Union is 20 mg/kg (Rahaman *et al*., 2013). The maximum acceptable concentrations of Pb and Cd in soils fall in the vast ranges of 50-300 and 1-20 mg/kg, respectively (Council Directive 86/278/EEC, 1986; McLaughlin *et al*., 2000). Although much debate is there on the contamination of our food crops by non-essential trace elements like As, Cd and Pb, there is, however, no information about the limit of these elements to cause yield reduction of crops. With these views in mind, the present work aimed to establish the threshold values of arsenic, cadmium and lead in soil for 10% yield reduction of two most commonly grown crops: rice and kalmi.

MATERIALS AND METHODS

Soil collection and preparation

The soil samples were collected from the experimental field of Bangladesh Jute Research Institute, Atigram Union, Manikganj Sadar upazilla, Manikganj district, Bangladesh, the geo-location being 23°52´60´´ N and 90°02´12´´ E. The sampling site was a medium high land and used as a vegetable land. The soil samples were collected from the surface to 15 cm of depth using a spade. After being transported into the laboratory, the whole sample was mixed thoroughly to make it a homogeneous sample.Then the collected soil samples were processed for pot experiment as well as for analysing the background properties following the procedures described in Imamul Huq and Alam (2005).

Plant culture

The seeds of rice (*Oryza sativa* L. var. BRRI dhan 41) and kalmi (*Ipomoea aquatica* var. BARI Gimakalmi-1) were collected from the local market. A total of 78 plastic pots of 2 L size each with sealed bottom were used in order to prevent draining of irrigation water. One kg processed soil was taken in each pot labelled with treatment symbols. Recommended fertilizers (BARC, 2012) were mixed with the soil to ensure optimum growth of the plants. Rice and kalmi were grown in semi-controlled net-house condition for 50 days and 42 days, respectively, and treated separately with different levels of As (0, 0.5, 1, 2 and 5 mg As/L of irrigation water), Cd (0, 5, 10, 15 and 20 mg Cd/kg soil) and Pb (0, 25, 50, 100 and 200 mg Pb/kg soil). The sources of As were 80% arsenite as sodium meta arsenite ($NaAsO_2$) and 20% arsenate as sodium arsenate (Na_2HAsO_4. H_2O) in solutions. The As treatments were applied to the selected pots with irrigation water and records were maintained about total amount supplied in each pot to keep track of the total As contents in soil for different treatments. A week before sowing the seeds, the soils of the selected pots were spiked with different Cd and Pb treatments separately as the solution of $CdCl_2.H_2O$ and $Pb(NO_3)_2$, respectively. All treatments were applied in triplicates. The pots were arranged in the net-house in a completely randomized design. The positions of the pots were changed every alternative day to allow equal exposure to sunlight.

For kalmi, 10 seeds were sown in each pot. For rice, soils were puddled and 10 seedlings at 7 days after germination were transplanted in each pot. The selected plants were supplied with arsenic mixed irrigation water every day as arsenic treatment. For other pots, irrigation was applied with tap water every day. Each pot received 50 ml of irrigation water every day, except rainy days. The records were kept for total water supplied in each pot as irrigation. The pH of the tap water used for irrigation was in the range of 6.3 to 6.7. The records of soil pH change were kept, but there was no significant change. The tap water was also analysed for trace element contents, but the concentrations of As, Cd and Pb were found to be below detection limit (2 µg/kg).The weeds were mainly grasses which were removed manually by uprooting just after germination not to allow adequate time for them to take up any metal or nutrients. The visual symptoms and growth parameters were noted during the plant growth.

Plant sample collection and preparation

The sampling of the plants was done by uprooting them carefully from the pots. The plants were put into the plastic bag with proper labelling and brought into the laboratory. After washing, the fresh weights of the collected plants were taken with an electric balance. Then the samples were first air-dried and then oven-dried at 70° ± 5°C for 48 hours. The oven dry weights of the plants were also taken. The oven-dried plant samples were then ground. The ground plant samples were mixed thoroughly to make it composite sample and kept in plastic containers with proper labelling and stored in dry place for chemical analyses.

Laboratory analyses

The soil sample was analysed in the laboratory for chemical and physicochemical properties before plant growth following the procedures described in Imamul Huq and Alam (2005). The concentrations of As, Cd and Pb were determined using atomic absorption spectrophotometer on aqua-regia digest of the soil samples and on nitric acid digest of the plant samples. The quality control/quality assurance (QC/QA) of the analyses was as described by Imamul Huq *et al*. (2008).

Estimation of threshold values

The threshold values of As, Cd and Pb were estimated through linear regressive dose-response curves in which the percent relative dry matter yields of plants were plotted against the total trace element contents present in soil. The percent relative dry matter yield of plant was calculated as the percentage of dry matter production of a given treatment to that at control (zero treatment). The content of trace element (mg/kg) present in soil for 10% reduction in dry matter yield of the plants under study was taken as the threshold.

Data analyses

The experimental data of the experiment were statistically evaluated using Microsoft Excel and Minitab (version 17).

RESULTS AND DISCUSSION

Soil properties

The USDA textural class of the homogeneous experimental soil sample was silt loam(13.9% sand, 74.1% silt and 12% clay fractions) with slightly acidic pH (6.52), low organic matter (1.77%) content, total As and Pb contents of 1.37 and 1.13 mg/kg soil, respectively, and a total Cd content below detection limit (2 µg/kg).

% Relative yield as affected by arsenic

The effects of soil As on relative yields of rice (Fig. 1a) show that the % relative dry matter yield of rice initially increased at the lowest application rate of As and then significantly decreased with increasing As treatments ranging from 2% (at 6.37 mg As/kg soil) to 25% (at 26.37 mg As/kg soil). Increase in yield for small additions of As has also been observed for corn, potatoes, rye and wheat (Gulz *et al*., 2005). Similar observation that at low concentration of an antagonistic or otherwise non-essential element favours growth, were made by Imamul Huq and Larher (1983) with Na for a different crop. It could be due to the fact that at low concentration of As, the P availability or Fe availability was favoured which caused this yield increase. At low levels, however, As increases P uptake by plants, possibly resulting from As-induced physiological plant P deficiency (Carbonell *et al*., 1998; Burlo´ *et al*., 1999). On the other hand, Fe is the only micronutrient element which has been found to be synergistic with As in several plant culture experiments (Barrachina *et al*., 1994). The As concentration at which rice yield shows a decrease of 10% is judged to be the maximum allowable limit or critical content of arsenic in soil (Yan-Chu, 1994). For the present experiment, 10 % decline in rice yield was observed at 14.10 mg As/kg soil (Fig. 1a) which may be considered as the critical content or threshold value of As in soil for rice plants under the present study. Xiong *et al*. (1987) reported 10% rice yield decrease in sierozen soil at 25 ppm of As. Yan-Chu (1994) reported 10% yield loss of rice in purple soil for 10 ppm total As, in yellow-brown soil for 51 ppm total As.

The dry matter yield of kalmi plants also significantly decreased with increasing As treatments (Fig. 1b). Decline in relative yield of kalmi ranged from 4% (at 2.87 mg As/kg soil) to 44% (at 16.37 mg As/kg soil). In the present experiment, 10% yield reduction of kalmi plants was observed at 4 mg As/kg soil which may be considered as the critical level or threshold value of As in soil for kalmi plants.

% Relative yield as affected by cadmium

The relative dry matter yields of rice under elevated soil Cd treatments significantly decreased from 8% to 51% at 5 and 20 mg Cd/kg soil, respectively (Fig. 2a). In the present experiment, the threshold value for 10% decrease in rice yield was observed at 4.34 mg Cd/kg soil and moreover, 50% decline at 16.82 mg Cd/kg soil.

The relative dry matter yields of kalmi significantly declined from 5% (at 10 mg Cd/kg soil) to 54% (at 20 mg Cd/kg soil) as compared to the control (Fig. 2b). In the present experiment, the threshold value of Cd for 10% decline in the relative yield of kalmi was observed at 6.57 mg Cd/kg soil, and 50% yield decline at 20.12 mg Cd/kg soil.

% Relative yield as affected by lead

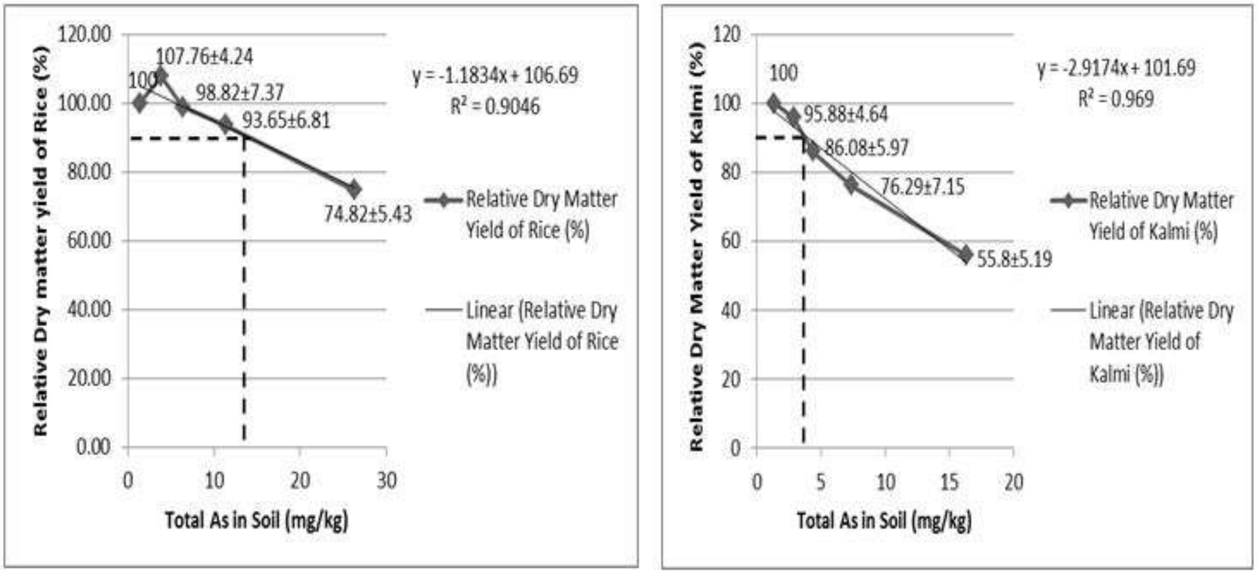
The relative dry matter yield decline of rice under elevated soil Pb concentrations (Fig. 3a) ranged from 5% (at 25 mg Pb/kg soil) to 34% (at 200 mg Pb/kg soil). In the present experiment, the threshold value of Pb for 10% yield reduction of Rice was observed at 46.17 mg Pb/kg soil.

The yield of kalmi significantly declined ranging from 10% (at 25 mg Pb/kg soil) to 52% (at 200 mg Pb/kg soil) as compared to the control (Fig. 3b). In the present experiment, the threshold value of Pb for 10% yield reduction of kalmi was observed at 34.84 mg Pb/kg soil, and for 50% yield reduction was observed at 194.26 mg Pb/kg soil.

Relationship between trace elements in soils and in plants

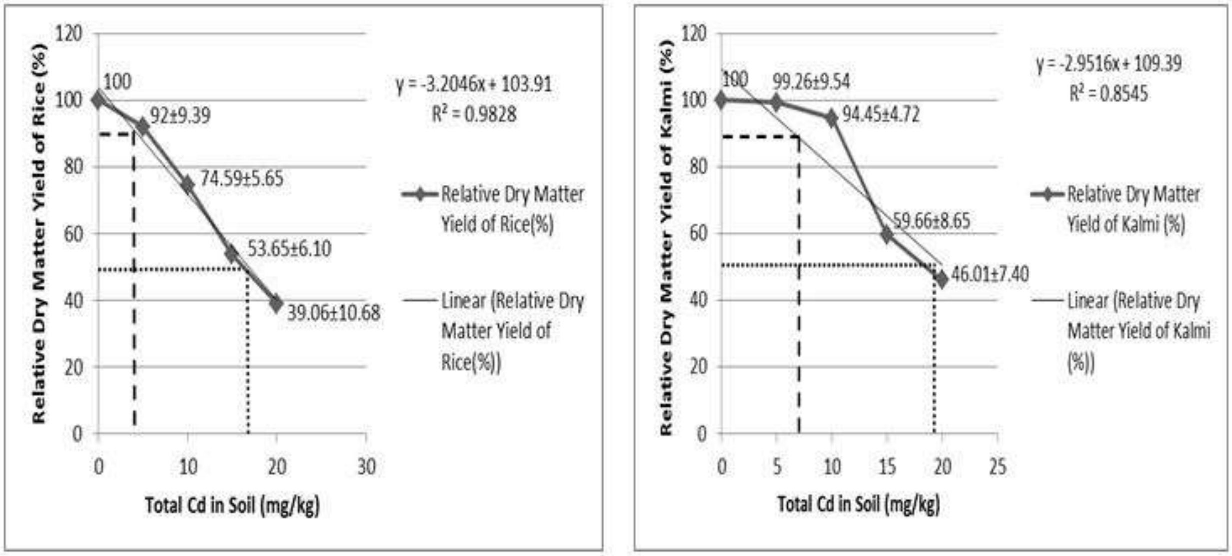
The As concentrations in rice plants ranged between 3.62 to 37.57 mg As/kg dry weight of plants (Fig. 4a) and that in kalmi plants ranged between 3.02 to 26.92 mg/kg dry weight of plants (Fig. 4b). The maximum concentrations of As in both rice and kalmi were observed at the highest application rate of As (5 mg As/L of irrigation water). The correlation analysis between the total As in soils and the corresponding As concentrations in plants for both rice and kalmi plants showed significant positive relationship ($r = 0.870864$, $p<0.1$ for rice; and $r = 0.963095$, $p<0.01$ for kalmi).

According to Kashem and Singh (1999), the normal and toxic Cd levels in plants vary from 0.2-2 mg/kg and 15-20 mg/kg, respectively. In the present experiment, the Cd contents in rice exceeded the toxic range at the treatments



a **b**

Fig. 1. Relative yields of (a) rice and (b) kalmi vs. soil total As concentrations. The vertical dash-lines indicate the threshold values of As for 10% yield reduction.



a **b**

Fig. 2. Relative yields of (a) rice and (b) kalmi *vs*. soil total Cd concentrations. The vertical dash-lines and dot-lines indicate the threshold values of Cd for 10% and 50% yield reduction, respectively.

of 10, 15 and 20 mg Pb/kg soil (Fig. 4c). For kalmi, the Cd contents exceeded the toxic range at 15 and 20 mg Cd/kg soil (Fig. 4d). The correlation analysis between the total Cd in soils and the corresponding Cd concentrations in plants for both rice and kalmi showed significant positive relationship (r = 0.948534, $p<0.02$ for rice; and r = 0.984829, $p<0.01$ for kalmi). Lund *et al*. (1981) also found significant positive correlations between Cd in the soil and Cd concentrations in the leaves of several crop species.

The normal and toxic Pb levels in plants vary from 0.1-2 mg/kg and 10-20 mg/kg, respectively (Kashem and Singh, 1999). In the present experiment, the Pb contents in rice

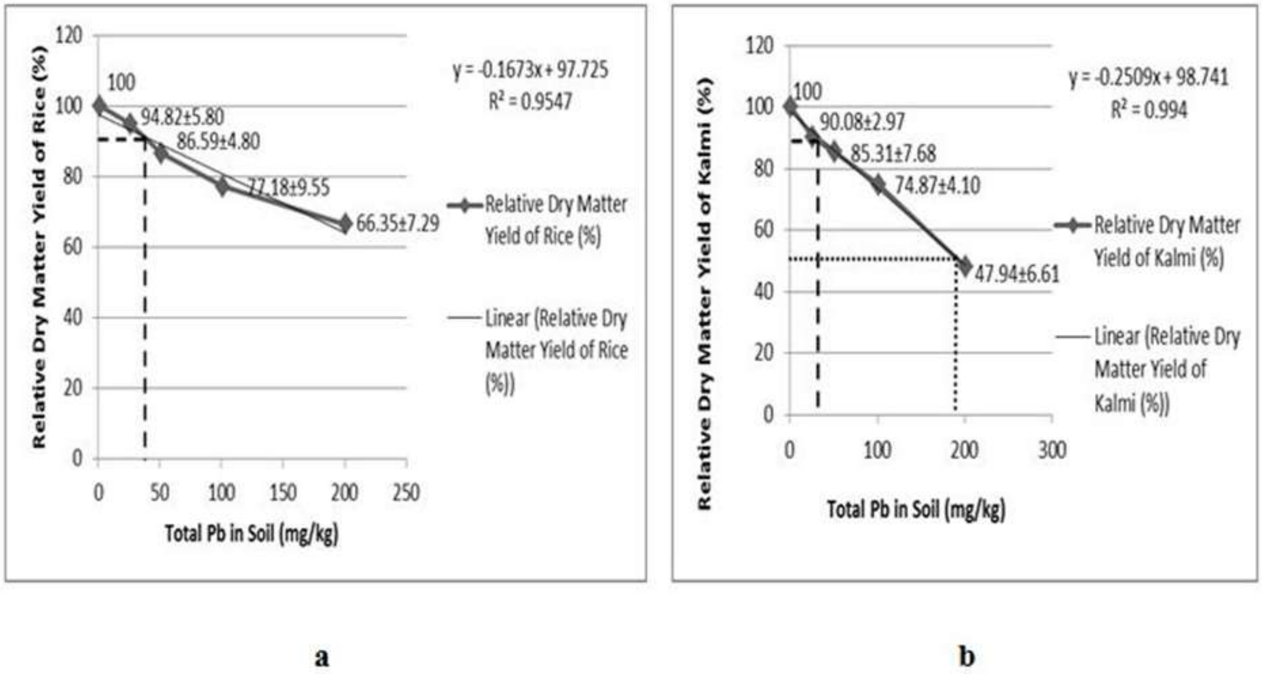


Fig. 3. Relative yields of (a) rice and (b) kalmi *vs*. soil total Pb concentrations. The vertical dash-lines and dot-line indicate the threshold values of Pb for 10% and 50% yield reduction, respectively.

plants were observed to exceed the toxic range, except at control (Fig. 4e). Similarly, Pb contents in kalmi plants exceeded the toxic level at 100 and 200 mg Pb/kg soil (Fig. 4f). The correlation analysis between the total Pb in soils and the corresponding Pb concentrations in plants for both rice and kalmi showed significant positive relationship (r = 0.972214, $p<0.01$ for rice; and r = 0.979699, $p<0.01$ for kalmi). Korcak and Fanning (1985) also found a positive relationship between the concentration of Pb in the soil and that in the plant.

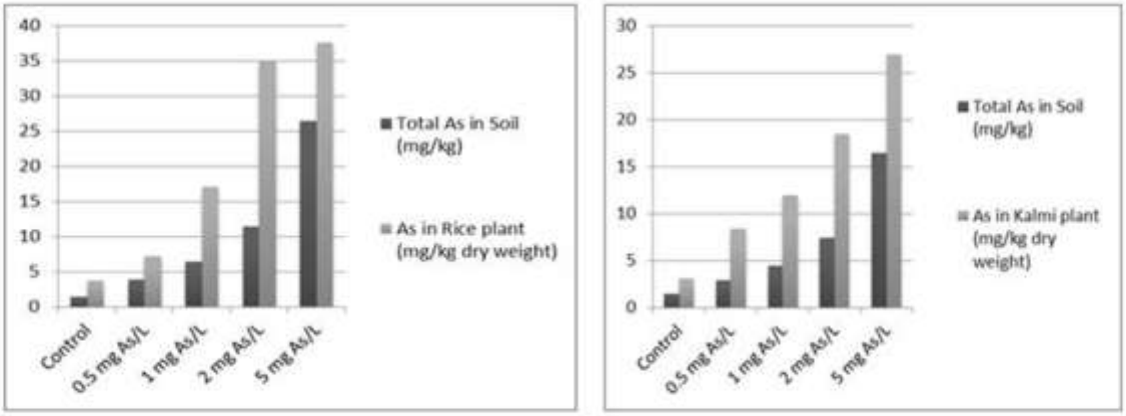
Accumulation of trace elements in plants

The accumulation of As, Cd and Pb in rice and kalmi plants was calculated by multiplying the concentration of trace elements in dry matter of plants (µg/g dry weight) with the corresponding total dry matter production (g/100 plants) and expressed as mg/100 plants. In most cases in this experiment, it was observed that the accumulation of trace elements in plants gradually increased and finally decreased at the highest application rate (Table 1). This might be due to the reduction of water movement from root to the shoots, lower germination of the seeds as well as reduction in fresh and dry weights of the plants under study. Another probable reason could be that some older leaves died at higher levels of treatments which were not taken to measure trace elements.

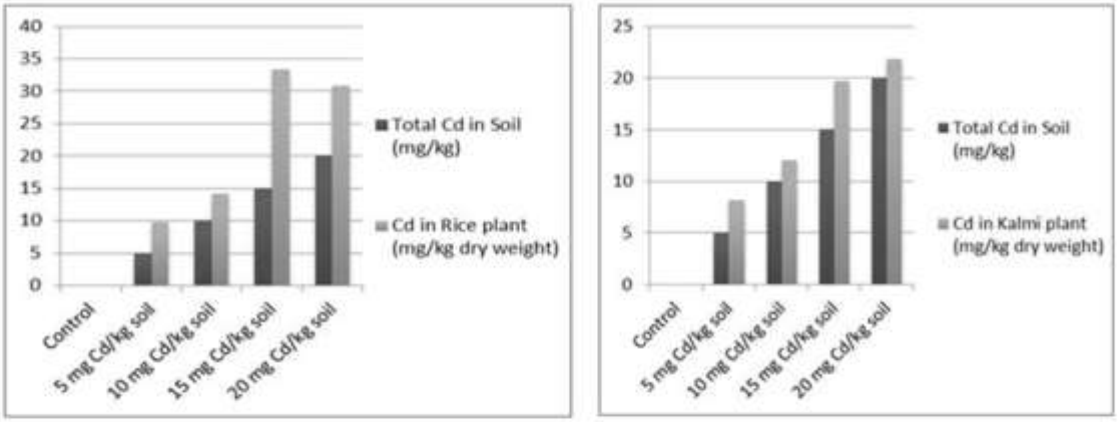
The total accumulation of As, Cd and Pb in both rice and kalmi was significantly affected by elevated treatments (Table 1). The highest As accumulation in rice (0.135 mg/100 plants) was observed at the rate of 2 mg As/L of irrigation water, and in kalmi (0.117 mg/100 plants) at the rate of 5 mg As/L of irrigation water. The maximum accumulation of Cd in rice (0.076 mg/100 plants) and in kalmi (0.091 mg/100 plants) was observed at the treatment of 15 mg Cd/kg soil. Pb accumulation in rice was observed to be the maximum (0.289 mg/100 plants) at the rate of 50 mg Pb/kg soil. The maximum Pb accumulation in kalmi (0.169 mg/100 plants) was observed at 100 mg Pb/kg soil.

Transfer of trace elements from soil to plants

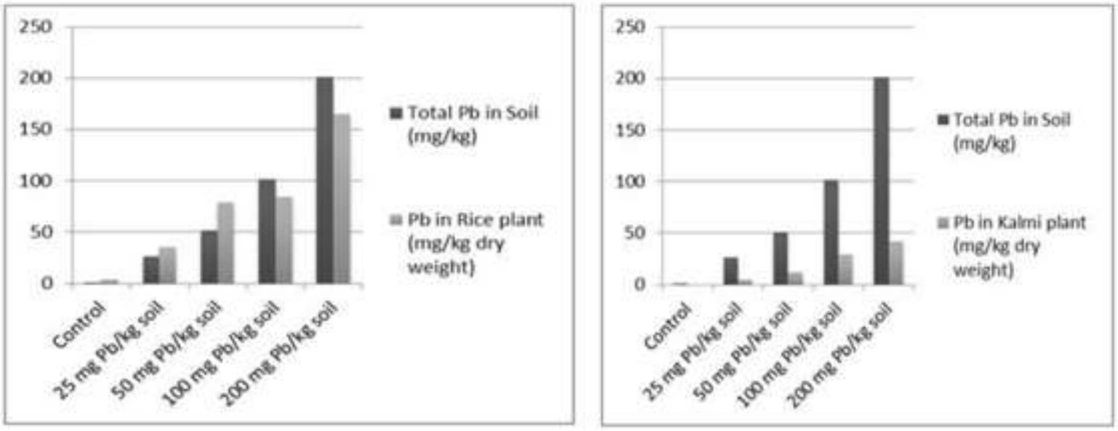
To estimate the potential transfer of As, Cd and Pb to the food chain, the soil/plant transfer factor (Table 2) was calculated as the ratio of the total trace element in plant (mg/kg dry weight) to the corresponding total trace element in soil (mg/kg). In case of soil contamination with As, the maximum transfer to rice (3.06) was observed at 11.37 mg As/kg soil and that to kalmi (2.91) at 2.87 mg As/kg soil. In case of soil contamination with Cd, the maximum transfer to rice (2.23) was observed at 15 mg Cd/kg soil and that to kalmi (1.63) at 5 mg Cd/kg soil. In case of soil contamination with Pb, the maximum transfer to rice (3.22) was observed at 1.13 mg Pb/kg soil and that to kalmi (0.29) at 101.13 mg Pb/kg soil. Transfer factor values more than 0.1 indicate the affinity of the element towards the plant species (Farago and Mehra, 1992). It appears from the present observations that all the three elements showed their affinity at different concentrations in soil. Moreover, this affinity also differed with the growing conditions of the plant species as well as their genetic make-up.



a) As content in soil and rice **b)** As content in soil and kalmi



c) Cd content in soil and rice **d)** Cd content in soil and kalmi



e) Pb content in soil and rice **f)** Pb content in soil and kalmi

Fig. 4. As, Cd and Pb contents in soil and plants.

CONCLUSION

It is apparent from the present study that the threshold values for trace toxic elements vary with the nature of soil and the plant species. The threshold value for As in soil is higher than the average As contents in Bangladesh soil except some cases where As accumulation in soil has been reported due to irrigation with As contaminated ground water (Imamul Huq, 2008). Likewise, the threshold values for Cd and Pb are also above the average

Table 1. Accumulation of As, Cd and Pb in rice and kalmi plants at different levels of soil contamination

Mean accumulation of As (mg/100 plants)		
Treatments of As (mg/L of irrigation water)	Rice	Kalmi
0	0.015^{de}	0.023^{e}
0.5	0.033^{d}	0.062^{d}
1	0.071^{c}	0.079^{c}
2	0.135^{a}	0.109^{ab}
5	0.120^{ab}	0.117^{a}
Mean accumulation of Cd (mg/100 plants)		
Treatments of Cd (mg/kg soil)	Rice	Kalmi
0	-	-
5	0.038^{d}	0.063^{d}
10	0.045^{c}	0.089^{ab}
15	0.076^{a}	0.091^{a}
20	0.051^{b}	0.078^{c}
Mean accumulation of Pb (mg/100 plants)		
Treatments of Pb (mg/kg soil)	Rice	Kalmi
0	0.015^{d}	-
25	0.141^{c}	0.035^{d}
50	0.289^{a}	0.077^{c}
100	0.276^{ab}	0.169^{a}
200	0.265^{ab}	0.156^{ab}

Any two means having a common letter(s) in a column are not significantly different at 5% level of significance.

Table 2. Transfer factors of soil As, Cd and Pb for rice and kalmi plants at different levels of soil contamination

Treatments* Crops	1	2	3	4	5
As					
Rice	2.64	1.86	2.66	3.06	1.42
Kalmi	2.20	2.91	2.71	2.50	1.65
Cd					
Rice	-	1.96	1.41	2.23	1.54
Kalmi	-	1.63	1.20	1.31	1.04
Pb					
Rice	3.22	1.34	1.53	0.83	0.82
Kalmi	-	0.19	0.23	0.29	0.21

*As: 1= zero, 2= 0.5 mgL^{-1}, 3= 1 mgL^{-1}, 4= 2 mgL^{-1}, 5= 5 mgL^{-1}
*Cd: 1= zero, 2= 5 $mgkg^{-1}$, 3= 10 $mgkg^{-1}$, 4= 15 $mgkg^{-1}$, 5= 20 $mgkg^{-1}$
*Pb: 1= zero, 2= 25 $mgkg^{-1}$, 3= 50 $mgkg^{-1}$, 4= 100 $mgkg^{-1}$, 5= 200 $mgkg^{-1}$

levels of these elements in Bangladesh soils. The present piece of information could be used to make risk analysis for soil contamination. However, further research with more plant species and different soils are emphasized.

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