The Chittagong Univ. J. B. Sci.,Vol. 5(1 &2):135-147, 2010.

INFLUENCE OF SOIL APPLIED CADMIUM ON THE GROWTH AND CADMIUM CONTENT IN INDIAN SPINACH (*BASELLA RUBRA* L.)

M. G. KIBRIA[*] , K. T. OSMAN, M. ISLAM AND M. J. AHAMMAD
Department of Soil Science, University of Chittagong, Chittagong 4331, Bangladesh.

ABSTRACT

Growth and accumulation of cadmium (Cd) in Indian spinach (*Basella rubra* L.) grown in three different textured soils treated with various levels of Cd was studied by a pot experiment. In general**,** Cd application caused a suppression of dry weights of leaves, stems and roots. The increasing application of Cd from 1 to 9 mg Cd kg^{-1} soil significantly increased Cd concentration in leaf, stem and root from that of the control. The highest concentrations of Cd were found in roots and the lowest concentrations were found in the stems with moderate values in leaves in clay loam soil while in other two soils, the Cd concentrations in leaves and stems were almost similar being lower than those in roots. Positive correlations were found between 0.1 M HCl extractable Cd in soils after harvest and concentrations of Cd in plant parts of Indian spinach.

Key words: Cadmium, Indian spinach, Leaf, Stem, Root.

INTRODUCTION

Human exposure to toxic heavy metals via dietary intake is of increasing concern. Cadmium is a potentially hazardous heavy metal that is being introduced in agricultural soil through anthropogenic activities such as sewage sludge application (Sposito *et al*. 1982), application of commercial fertilizer (He and Singh 1993) and through weathering of soil minerals. Long term use of Cd-containing phosphorus fertilizer at high rates has caused significant increases in Cd concentration in soil (Mendes *et al*. 2006).

Most edible crops are indiscriminate in their extraction of nutrients from the soil and thus will extract the non-desirable heavy metals along with the required essential nutrients. This may predispose the populace to heavy metals through the consumption of such agricultural products thereby serving as a point of entry of heavy metals into the food chain. Cadmium is not known essential elements for crop plants.

[*] Corresponding author

However, there are evidences indicating that various plant species have the ability to absorb Cd by roots and translocate Cd from the roots to the shoots (Shute and Macfie 2006, Murakami *et al.* 2007). Cadmium poses a potential threat to human health as it enters the food chain (Obata and Umebayashi 1997). People will suffer from renal tubular disease if they consume rice with relatively high Cd content (Watanabe *et al*. 1998). The Codex Alimentarius Commission an international foods standards organization, has set a limit of 0.2 mg Cd kg^{-1} in cereals and legumes for human consumption (Codex Alimentarius Commission, 2001). Therefore, minimizing the intake of Cd from food is an important health issue. For ecological safety and human health, Cd uptake by food crops needs to be understood in order to limit its accumulation in the food chain. Physical and chemical properties of soils such as texture, pH and organic matter play a very important role in the solubility of Cd and its availability to plants (Eriksson *et al.*, 1996; Adams *et al.* 2004). Plant species and cultivars differ widely in their ability to absorb, accumulate and tolerate Cd (Dunbar *et al.* 2003, He *et al.* 2006).

Contaminations of agricultural soils of Bangladesh by heavy metals especially Pb, Cd and Cu have been reported by Khan (2001) and Kibria (2008). With these views in mind, the present study has been designed to investigate the effects of Cd on growth of and accumulation in Indian spinach (*Basella rubra* L.), a very popular leafy vegetable crop in Bangladesh.

MATERIALS AND METHODS

Pot experiment

Soils of three different textures namely clay loam, sandy clay loam and sandy loam were collected from (1) agricultural field located near new Science Faculty Building of Chittagong University, (2) agricultural field near Shahid Minar of Chittagong University, and (3) Syed Para at Hathazari Upazilla under Chittagong district from a depth of 0-15 cm on the basis of composite sampling method. Soil samples were air dried and larger and massive aggregates were broken down by gentle crushing with wooden pluck. Dry roots, grasses and other particulate materials were discarded from the soils and processed for pot experiment. A portion of the soils passed through 2 mm sieve was preserved for laboratory analyses. Characteristics of the soils are presented in Table 1. Particle size distribution was determined by hydrometer method of Day (1965) and organic carbon (wet –oxidation method) and cation exchange capacity of the soils by the method of Jackson (1973). Soil pH was measured by glass electrode pH meter using soil: water ratio of 1:2.5. Total nitrogen was determined by Kjeldahl method as described by Jackson (1973). Soil samples were digested with a mixture of HNO_3 and HCl at the ratio of 1:3 (Jackson 1973) for determination of total P, K, Fe, Mn, Zn, Cd and Pb. Phosphorus was determined by vanadomolybdophosphoric yellow color method in nitric acid system according to Jackson (1973). Potassium was determined by flame photometer and Fe, Mn, Zn, Cd and Pb were determined by atomic absorption spectrophotometer (Varian spectra AA-220).

TABLE 1 : PROPERTIES OF SOILS USED IN POT EXPERIMENT.

Properties	Clay Loam Soil	Sandy Clay Loam Soil	Sandy Loam Soil
Sand (%)	30	57	63
Silt (%)	38	22	20
Clay (%)	32	23	17
pH (1:2.5 H_2O)	5.4	5.5	5.1
Organic carbon (%)	0.97	0.83	0.61
CEC (cmolkg^{-1}soil)	7.86	6.93	6.43
Total N (%)	0.15	0.09	0.04
Total P (%)	0.023	0.029	0.020
Total K (%)	0.36	0.24	0.26
Total (Fe %)	1.574	0.736	1.472
Total Cd (mg kg^{-1})	0.07	bdl*	bdl*
Total Pb (mg kg^{-1})	11.2	7.8	9.6
Total Zn (mg kg^{-1})	108.7	58.96	55.02
Total Mn (mg kg^{-1})	185.06	190.20	86.90

*bdl= below detection limit (<0.002 mg kg^{-1})

A total number of 54 pots having diameter and depth 28 and 25 cm, respectively were used. Eight kilograms soils were placed in each earthen pot. The fertilizer doses of N, P, K and S (99 kg N, 18 kg P, 60 kg K and 18 kg S ha^{-1}) were applied from Urea, TSP, MP and zinc sulfate respectively. According to Bangladesh Agricultural Research Council (2005) recommendation, half N and K, and whole of P and S were applied during soil preparation. Five healthy and uniform seeds of Indian spinach were sown at equal distance in each pot. After two weeks of seedling emergence, one seedling was kept in each pot and different levels of Cd (0, 1, 3, 5, 7 and 9 mg Cd kg^{-1} soil) were applied in solution form as cadmium sulfate ($3CdSO_{4}.8H_2O$). Care was taken to keep uniform seedlings in the pots. Each treatment was replicated thrice and the pots were arranged in randomized block design. Remaining N and K were applied in two equal installments after 2 and 4 weeks of seedling emergence. Water was applied regularly in the pots to maintain the field capacity of the soils. Plants were harvested after five weeks of Cd application. Leaf, stem and roots were separately collected. Oven dry (65^0 C to constant weight) weights of leaf stem and roots were recorded.

Plant analysis

Oven dried (65^0 C to constant weight) and ground plant samples were digested with ternary acid (HNO_3, H_2SO_4 and $HClO_4$ mixture at a ratio of 5:1:2) (Jackson 1973). The concentrations of Cd in the digested solution were measured by atomic absorption spectrophotometer (Varian Spectra AA 220).

Data analysis

The significance of differences between the means of the treatments was evaluated by one way analysis of variance followed by Duncan's Multiple Range Test at the significance level of 5%. Pearson's correlation coefficient was estimated to test the relationships between metal concentrations in plant tissues and metal contents in soils.

RESULTS AND DISCUSSION

Effects of Cd on growth of Indian spinach

The variation in growth response of Indian spinach after five weeks of metal exposure in three different textured soils treated with Cd is presented in Figure 1. Dry weight of leaf, stem and root ranged from 4.38 to 10.07, 1.80 to 8.80 and 0.58 to 1.75 g pot^{-1}, respectively. In general**,** Cd application caused a suppression of dry weights of leaves, stems and roots. Cadmium application even at the lowest level (1 mg Cd kg^{-1} soil) in sandy loam soil significantly reduced leaf dry weight of Indian spinach whereas in clay loam and sandy clay loam soil, Cd application at 3 and 5 mg Cd kg^{-1} soil, respectively caused reduction in leaf dry weight. In sandy loam soil, both stem and root dry weights began to significantly decrease at 5 mg Cd kg^{-1} soil treatment compared to the control. Reduction in stem dry weight began from 7 and 5 mg kg^{-1} Cd application in clay loam and sandy clay loam soil. On the other hand, root dry weight was reduced only at 9 mg Cd kg^{-1} soil in both the soils. Saber *et al.* (1999) reported that exposure of sunflower seedlings to Cd^{2+} at concentrations of 0.05 mM or 0.2 mM severely reduced the fresh and dry masses of shoots and roots, particularly at higher concentrations of Cd^{2+}. The results found by Zhang *et al*. (2002) with two wheat cultivars grown on soils under 5 Cd levels ranging from 0 to 1.0 mg kg Cd showed that the growth and dry matter accumulation were stimulated in low Cd concentration (0.3 mg kg^{-1}) for both cultivars, while inhibited significantly under high concentration (>0.3 mg kg^{-1}).

Cadmium application at the highest rate (9 mg Cd kg^{-1} soil) in the present study produced the lowest dry weight biomass in all the three soils. Dry weight biomass of leaf, stem and root were reduced from 32 to 57, 33 to 55 and 33 to 49 % of control at the highest dose of Cd (9 mg Cd kg^{-1} soil) in sandy loam, sandy clay loam and clay loam soil, respectively. Shute and Macfie (2006) reported that application of Cd at 100 mg kg^{-1} reduced plant height and dry weight of soybean (*Glycine max* L.) down to 40% and 34% compared to control, respectively.

The reduction of dry weight may be related to the inhibition of photosynthesis (Greger and Örgen 1991). Decrease in fresh and dry weights of plant organs is one of the frequently used parameters describing the toxicity of metals (Costa and Spitz 1997). These changes can be induced by direct Cd action on plants, e.g. by decreased elasticity of cell walls inhibiting cell growth (Barceló and Poschenrieder 1990), or indirect action resulting from disturbances of water and mineral relations, respiration and photosynthesis (Costa and Morel 1994; Prasad 1995).

Concentration of Cd in Indian Spinach

Figure 2 shows the variation in Cd concentration in leaf, stem and root in relation to the various doses of Cd in three different textured soils. Cadmium concentrations in leaf, stem and root varied as a function of different doses of the metal in all the three soils. The increasing application of Cd from 1 to 9 mg Cd kg^{-1} soil significantly increased Cd concentrations in leaf, stem and root over control. This is in agreement with Jiang *et al.* (2001) who found that the Cd content in roots of garlic increased with increasing solution concentrations of Cd^{2+}. Cadmium supplying at 9 mg Cd kg^{-1} soil increased leaf and root Cd concentrations about 4 fold than that in 1 mg Cd kg^{-1} soil supplement in clay loam soil in the present study. Cadmium concentration in stem was zero in this soil with 1 mg Cd kg^{-1} soil treatment. In sandy loam and sandy clay loam soils, the leaf Cd concentration increased by 6 and 4 fold, stem Cd concentration by 3 and 7

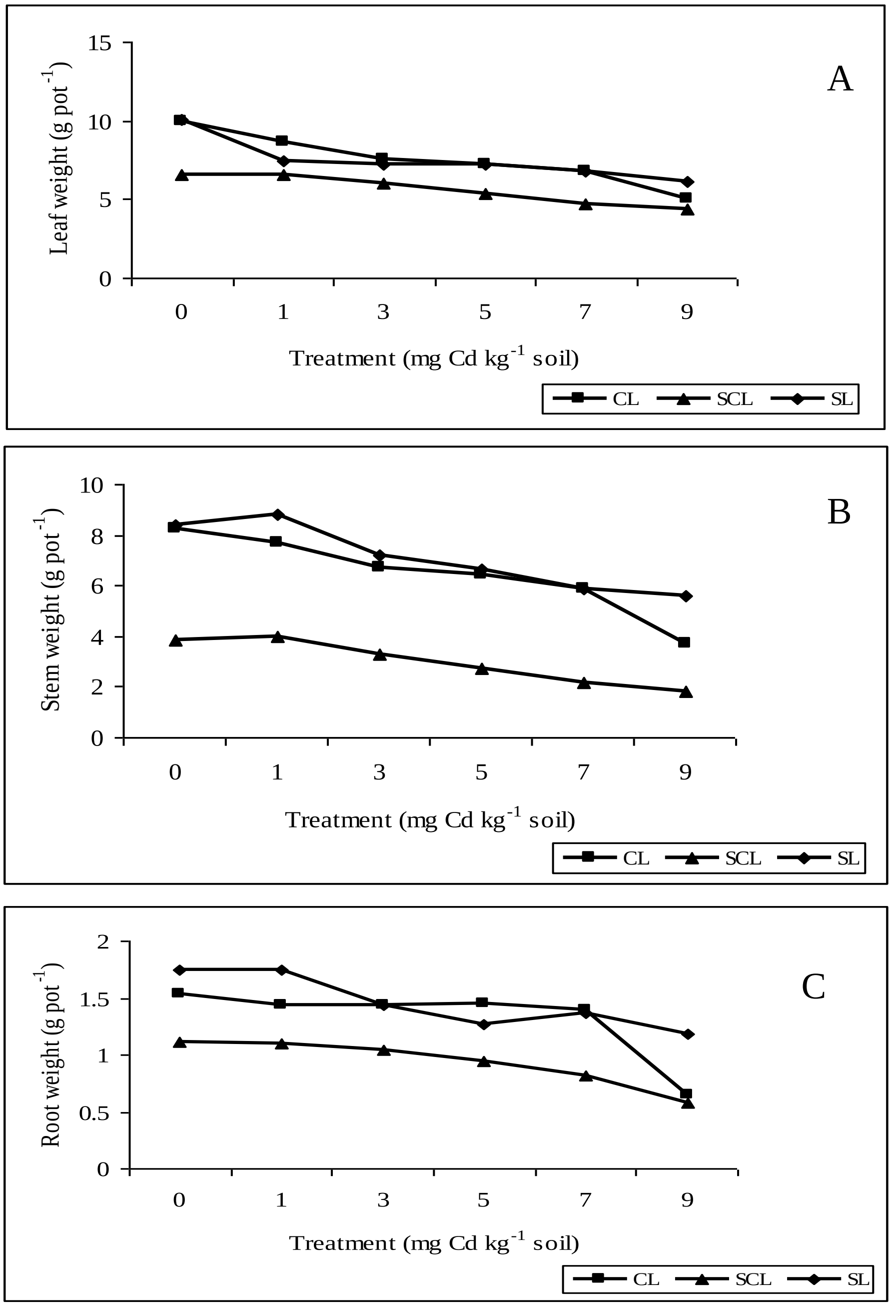

FIGURE 1 : EFFECTS OF CADMIUM ON LEAF (A), STEM (B) AND ROOT (C) WEIGHT OF INDIAN SPINACH (CL-CLAY LOAM SOIL, SCL- SANDY CLAY LOAM SOIL AND SL-SANDY LOAM SOIL).

fold and root Cd concentration by 3 and 7 folds, respectively, with Cd application at 9 mg kg^{-1} soil than that of 1 mg kg^{-1} soil. The roots of garlic plants treated with 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M Cd was reported to accumulate Cd approximately 1826, 114, 59, 24 and 4 times to that of the control (Jiang *et al.* 2001). The highest leaf, stem and root Cd concentrations in the present study obtained at 9 mg Cd kg^{-1} soil treatment were in the ranges 21.18-27.86, 17.68-30.21 and 26.29-76.31 mg kg^{-1}, respectively.

The highest concentrations of Cd were found in roots and the lowest concentrations were found in the stems with moderate values in leaves in clay loam soil while in other two soils, the Cd concentrations in leaves and stems were almost similar being lower than those in roots. Shute and Macfie (2006) reported the Cd the highest concentration of Cd in roots, moderate in stems and leaves, and the lowest in the pods and seeds of soybean. Arao *et al.* (2003) also reported similar patterns of distribution of Cd in soybean.

Bioaccumulation coefficients (BC) of Cd in leaf, stem and root of Indian spinach were in the ranges of 00 - 7.03, 0.00 - 6.36 and 2.92 - 35.5, respectively (Table 2). These ranges were very much lower than the reported values of bioaccumulation coefficients of Cd (Salt *et al.* 1995). Bioaccumulation coefficient of Cd in leaf and root of Indian spinach decreased with the increase of Cd application except for root in sandy clay loam soil. Pinto *et al.* (2004) reported that the increase of soil contamination level led to an increase of root BC but a decrease was observed in shoot BC in sorghum. The decrease in root BC of Cd in the present study might be influenced by the larger retention of Cd in soil. The increase of Cd application led to an increase of stem BC in clay loam soil. The opposite trend was observed in sandy loam soil while there was no definite trend of variation of stem BC in sandy clay loam soil. Bioaccumulation coefficient of Cd in root of Indian spinach was higher than leaf and stem indicating lower translocation of Cd from root to stem and leaf.

Extractable Cd in soils after harvest of Indian spinach

A small amount of background Cd (0.07 mg kg^{-1}) was in clay loam soil used for this experiment as mentioned in the Materials and Methods section. In control soils, 14 % of the total Cd in clay loam soil was estimated to be 0.1M HCl extractable. In soils, to which Cd was added, 38-61, 43-57 and 43-56 % of the Cd was found to be 0.1M HCl extractable in clay loam, sandy clay loam and sandy loam soil respectively (Table 3). Metals extracted with 0.1 M HCl are thought to represent the mobile fraction of metals and may reflect the bioavailability of metals (Kashem *et al.* 2007).

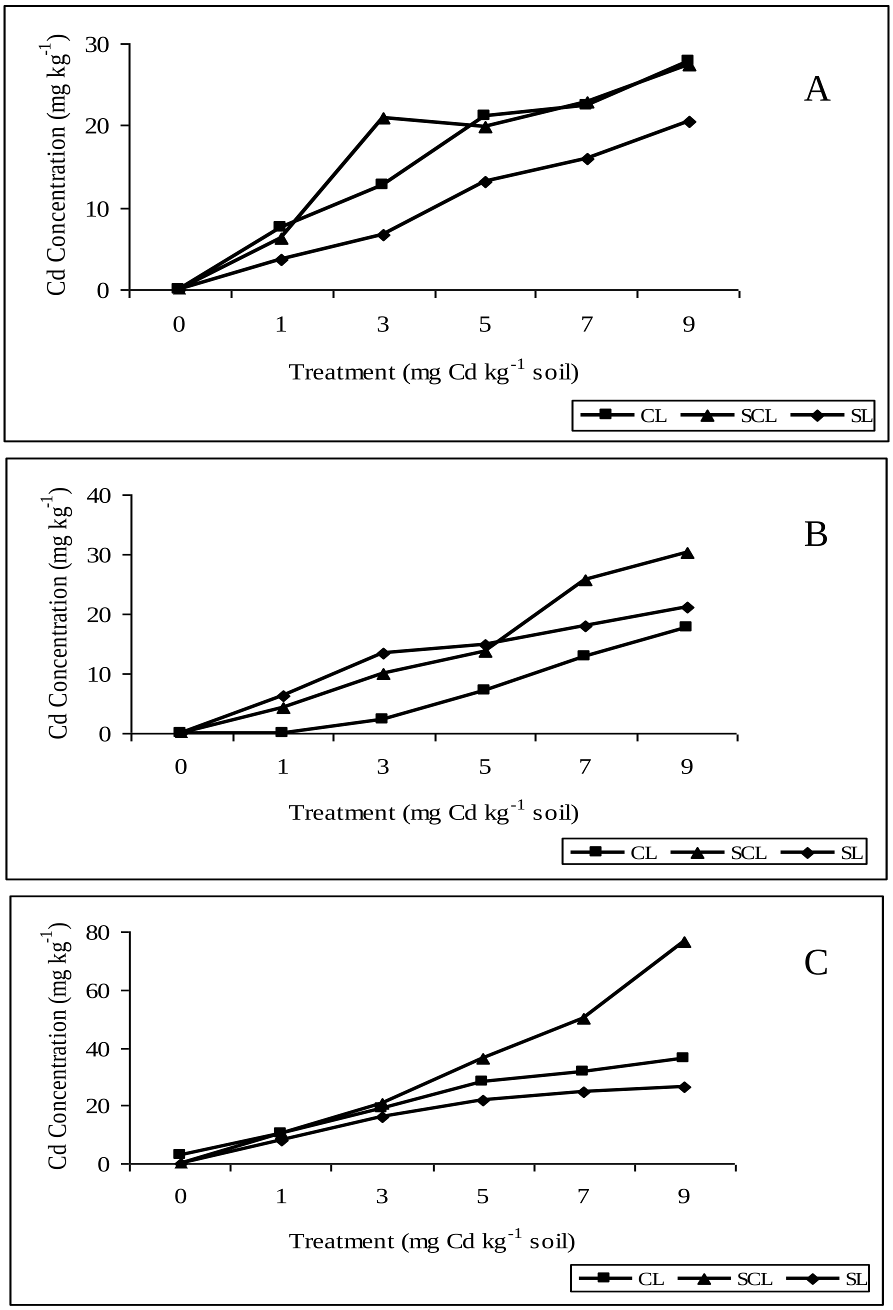

FIGURE 2 : EFFECTS OF CADMIUM ON CONCENTRATION OF Cd IN LEAF (A), STEM (B) AND ROOT (C) OF INDIAN SPINACH (CL-CLAY LOAM SOIL, SCL- SANDY CLAY LOAM SOIL AND SL- SANDY LOAM SOIL).

TABLE 2 : BIOACCUMULATION COEFFICIENT OF Cd IN DIFFERENT PLANT PARTS OF INDIAN SPINACH GROWN IN cd SPIKED SOILS.

Treatment (mg Cd kg^{-1} soil)	Clay Loam Soil			Sandy Clay Loam Soil			Sandy Loam Soil		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
0	0.00	0.00 d	37.53 a	0.00 c	0.00 d	0.00 c	0.00 c	0.00 c	0.00 e
1	7.02 a	0.00 d	9.53 b	6.23 a	4.39 a	10.5 a	3.64 a	6.36 a	7.80 a
3	4.12 b	0.77 c	6.14 c	6.99 a	3.36 bc	6.89 b	2.20 b	4.46 b	5.41 b
5	4.18 b	1.40 b	5.59 c	3.99 b	2.72 c	7.23 b	2.63 b	2.99 c	4.34 c
7	3.16 c	1.83 ab	4.47 c	3.28 b	3.66 ab	7.14 b	2.29 b	2.56 c	3.56 cd
9	3.07 c	1.95 a	4.03 c	3.04 b	3.36 bc	8.48 ab	2.28 b	2.35 c	2.92 d
Sig. of F Value (P)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Mean values in the column followed by the same letter (s) are not significantly different according to DMRT ($P \leq .05$).

In Japan, a single extraction with o.1M HCl has been widely used to determine the level of extractable Cd concentration in soils (Murakami *et al.* 2007). A highly positive correlation between 0.1 M HCl extractable Cd and concentrations of Cd in plant parts of Indian spinach was found in all the three soils (Table 4). Further, Cd concentrations in plant parts of Indian spinach were also highly correlated with total soil Cd added. This is in conformity with Nehnevajova (2005) who reported that sunflower seedlings showed a highly positive correlation between external Cd concentration in nutrient solution and root Cd concentration in a hydroponics study.

Cadmium application, in general, caused a suppression of dry weights of leaves, stem and root of Indian spinach. Cadmium concentration in leaf, stem and root of Indian spinach significantly increased from that of the control with increasing application of Cd in soil.

TABLE 3 : 0.1 M HCL EXTRACTABLE Cd IN SOILS AFTER HARVEST OF INDIAN SPINACH.

Treatment (mg Cd kg^{-1} soil)	Extractable Cd (mg kg^{-1}of soil)		
	Clay Loam Soil	Sandy Clay Loam Soil	Sandy Loam Soil
0	0.01± 0.01 (14*)	0.04 ± 0.01 (00)	0.00 (00)
1	0.66 ± 0.11 (61)	0.49 ± 0.29 (49)	0.56 ± 0.01 (56)
3	1.78 ± 0.12 (58)	1.32 ± 0.19 (44)	1.60 ± 0.02 (53)
5	2.44 ± 0.08 (48)	2.57 ± 0.37 (51)	2.64 ± 0.27 (53)
7	2.67 ± 0.04 (38)	3.99 ± 0.61 (57)	2.98 ± 0.54 (43)
9	4.80 ± 0.14 (53)	3.88 ± 0.62 (43)	4.68 ± 1.37 (52)

* Percentage of total Cd extracted by 0.1 M HCl is given in parenthesis.

TABLE 4 : CORRELATION COEFFICIENTS BETWEEN TOTAL Cd IN SOILS AND Cd CONCENTRATIONS IN PLANT PARTS AND BETWEEN 0.1 M HCL EXTRACTABLE Cd IN SOILS AND Cd CONCENTRATIONS IN PLANT PARTS OF INDIAN SPINACH.

Cadmium	Soil	Leaf	Stem	Root
Total	Clay Loam	0.953 **	0.961 **	0.964 **
	Sandy Clay Loam	0.901 **	0.972 **	0.958 **
	Sandy Loam	0. 970 **	0.935 **	0.945 **
0.1 M HCl Extractable	Clay Loam	0.953 **	0.944 **	0.947 **
	Sandy Clay Loam	0.885 *	0.975 **	0.944 **
	Sandy Loam	0.987 **	0.950 **	0.936 **

* and ** represent that correlation is significant at the 0.05 and 0.01 level, respectively.

The translocation of accumulated Cd in leaves was more pronounced in clay loam and sandy clay loam soil while in sandy loam soil it was mostly translocated in the stem at lower Cd application. A highly positive correlation between 0.1 M HCl extractable Cd and concentrations of Cd in plant parts of Indian spinach was found in all the three soils

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Manuscript received on 26.7.11; Accepted on 29.9.11
The Chittagong University Journal of Biological Sciences, Vol. 5 (1 & 2). Page No:135-147