Research article

Heavy Metal Uptake of Leafy Vegetable Irrigated with Different Source of Industrial Effluents

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Abstract

Keywords

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leafy vegetable

The accumulation of heavy metals in vegetable and food crops irrigated by different industrial effluents is considered a consequential environmental problem in several countries such as Bangladesh, where wastewater is routinely used as a water source for irrigation of crop fields. The present investigation was conducted to assess the prevalence of different heavy metals like iron, manganese, zinc, copper, nickel, cadmium, chromium, and lead in Indian spinach irrigated with three different (pharmaceutical, beverage and dyeing) sources of industrial effluents. The results showed a considerable amount of heavy metals present in the effluent water and deposited in the soil after irrigation. The plant (root, stem and leaves) also accumulated the heavy metals from the contaminated soil. The range of different metals in effluent treated plant leaves was 177-294, 14.14-23.13, 19.15-33.99,7.53-12.49, 7.38-12.55, 0.98-1.17, 5.26-7.06, and 4.12-7.35 mg/kg for iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr) and lead (Pb), respectively. The transfer factor from soil to other plant parts was highest in the root for all the three types of effluents and the transfer factor from root to other parts was more than 1 in case of copper (Cu) and nickel (Ni).

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1. Introduction

Recently, in Bangladesh, consumer demand for safe and high quality vegetables has increased. However, the good appearance of a vegetable cannot give an assurance of the safety vegetables. Plants can contain different toxic metals in their edible parts because of the source of irrigation. At present, the expansion of industries, urbanization and different anthropological actions leads to the spread of different heavy metals in the environment [1]. Industrial wastewater with heavy metals has been released over cultivable land and irrigation canals, and farmers continue to use such water source in the dry season for vegetable cultivation. As a result, crops, and especially leafy vegetables accumulate different metals and become contaminated. Moreover, both the edible and non-edible parts of vegetables accumulate the metals. Helencha (*Enhydra fluctuans*) is a common leafy vegetable, becomes highly contaminated with Cd when grown on heavy metal contaminated soil and the contaminated plant with Cd can cause cancer [2]. The consumption of vegetables that contain high quantities of heavy metals in their tissues cause clinical problems as well as physiological disorders to human beings because there are no good remediations for the elimination of metals from the human body [3, 4].

Gazipur is an industrial area of Bangladesh, and the use of industrial wastewater that contains a considerable amount of toxic and heavy metals is a common irrigation practice in this area [5-7]. Wastewater discharge from industries is responsible for the contamination of soil and surface water with Zn, Cr, Cu, Pb, and Cd in Dhaka and Gazipur District [8, 9]. Vegetables from the Dhaka Export Processing Zone (DEPZ) area contaminated with elevated levels of Cr, Zn, Cu, Fe, Pb, Ni, and Cd [10] are considered as a source of vegetables for the capital city, Dhaka. However, industrial and municipal sewage wastewater are the main sources of soil and water pollution. On agricultural land, the continual use of wastewater significantly contributes to the accumulation of these heavy metals in soils and plants; an accumulation that has nowadays become a serious issue [7, 11]. Leafy vegetables are particularly susceptible to pollution in wastewater because they accumulate more heavy metals than fruits or grain crops [7]. The major concern is that people consume the leafy portion, which also contains different metals. Indian spinach was selected for this study because it is one of the important leafy vegetables during the dry season in Bangladesh, and the edible parts are leaves and stems. The present study was conducted to identify the heavy metals and their concentrations in different industrial wastewaters (washing, dyeing and pharmaceuticals), and to quantify the levels of heavy metals in different parts of the leafy vegetables that had been irrigated with the wastewaters.

2. Materials and Methods

2.1 Industrial effluents and study area

The irrigation water for the pot experiments was collected from the discharge points of pharmaceutical, beverage and dyeing industries of Rajendrapur, Monipur Bazar and Hotapara regions of Gazipur district using big (200 litres) plastic containers (Figure 1). The control water was supplied from a deep tube well in Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The experiment was conducted at Horticulture Research field under the net house of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh, which is situated at the centre of Madhupur Tract.

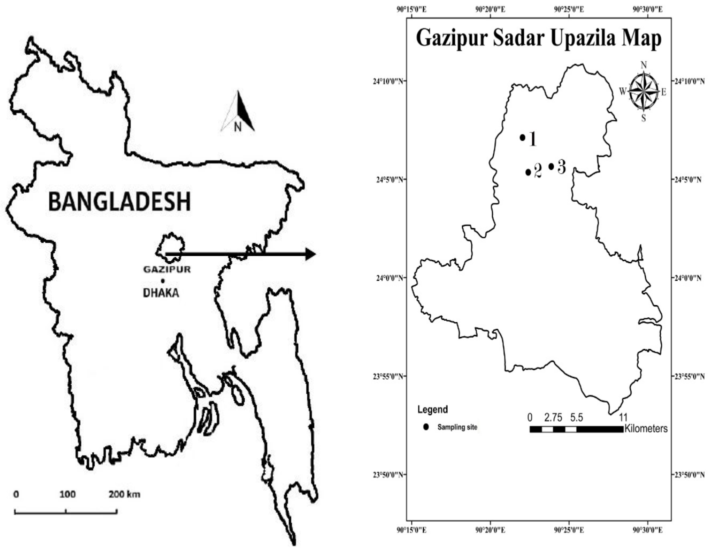

Figure 1. Different industrial effluents collection points in the industrial area of Gazipur sadar upazila. 1. Monipur; 2. Rajendrapur; 3. Hotapara

2.2 Pot preparation

The soil for the experiment was silty clay with a pH of 6.4. Immediately after collection, soil samples were air-dried, crushed, mixed thoroughly and sieved through a 2-mm nylon sieve. Finally, each pot was filled up with a total of 10 kg of sieved soil. Recommended doses of manures and fertilizers were applied in each pot according to FRG [12].

2.3 Experimental design

The experiment was laid out in a completely randomized design with four replications. The seeds of Indian Spinach (*Basella alba* L.) were sown in the filled pots and irrigated regularly with three different industrial effluents of pharmaceutical, beverage and dyeing industries, separately. Soil irrigated with underground water was considered as control. Necessary intercultural operations were done and when required. The crops were uprooted at 45 days after sowing, at which time they were edible, and then prepared for heavy metal analysis.

2.4 Effluents, soil and plant sample preparation

After collection of effluents from their respective discharge points, 500 ml of each effluent was separated for analysis. For preservation until analysis, a drop of 65% HNO_3 was added to each filtered sample to maintain the pH, and the samples were stored in a refrigerator at 4ºC. After uprooting, all the leafy vegetables were washed with double distilled water and separated into three-parts (root, stem and leaf). The separated roots, stems and leaves were oven-dried at 70ºC for 72 h to eliminate all moisture. The dried samples were ground using a plant grinder and sieved through a 0.05 mm sieve, and stored in polythene zip-bags. After the uprooting of vegetables, about 500 g soil samples were collected from each pot. The soil samples were air-dried, grounded with a pestle and mortar, and passed through a 2-mm sieve. Then, further analysis was carried out.

2.5 Analytical methods

The heavy metals Fe, Mn, Zn, Cu, Ni, Cd, Cr and Pb in the plant and soil samples were analyzed. One gram of sieved soil samples and 1 g of sieved plant samples were carefully digested with a mixture of concentrated HNO_3 and $HClO_4$ at 5:1 (v/v) ratio, separately [13], until a clear solution had appeared. Then, the clear solution from the digestion was used for the analysis of heavy metals for both plant and soil sample using Atomic Absorption Spectrophotometer (AAS) (GBC, Avanta) according to their respective wavelengths.

2.6 Statistical analysis

The collected data from the experiment were analyzed using the statistical package program MSTAT-C to illustrate the statistical significance of the experimental results. The means differences were compared by Least Significant Difference (LSD) test at 5% or 1% level of significance.

2.7 Transfer factor

The transfer of different heavy metals from soil to different plant parts [14] was calculated to estimate the relative uptake of heavy metals by the plants with respect to soil.

$$\text{Transfer factor with respect to soil (TFs)} = \frac{\text{The concentration of metal in the plant part}}{\text{The concentration of metals in soil}}$$

The transfer factor with respect to roots was calculated [15] to determine the relative movement of heavy metals from roots to different plant parts.

$$\text{Transfer factor with respect to the root (TFR)} = \frac{\text{The concentration of metal in the plant part}}{\text{The concentration of metals in root}}$$

3. Results and Discussion

3.1 Chemical properties of different industrial effluents

To assess the contamination load of industrial effluents that were used for irrigation, the samples were analyzed for different physio-chemical properties and the results were compared with the prescribed limits set by the Food and Agricultural Organization [16] and are presented in Table 1. The pH of the different effluents ranged from 6.25 to 9.23, which was under the prescribed limit. Here, Fe had the highest concentration and Cd had the lowest concentration in pharmaceutical effluent followed by dyeing wastewater. The enrichment of the heavy metals was in the order of Fe>Pb>Zn>Mn>Cu>Ni>Cr>Cd in pharmaceutical and Fe>Pb>Zn>Mn>Cu>Ni>Cd>Cr in dyeing, and Fe>Pb>Mn>Zn>Cu>Ni>Cr>Cd in beverage wastewater. This high concentration of metals in effluent water indicates the availability and distribution of metals in the vegetables when grown with effluent water as a source of irrigation. The dyeing effluent contained a high concentration of metal that reflected the high use of the chemicals in the textile factory. Beverage effluents were

Table 1. Chemical properties of different industrial effluents and control water

Parameters	**Types of Effluents**				
	Control	**Pharmaceutical**	**Beverage**	**Dyeing**	**Permissible level***
pH	7.15	9.23	6.25	8.85	6-9
Iron (mg/l)	1.78	5.15	2.97	4.28	2.0
Manganese (mg/l)	0.12	1.32	0.48	1.60	0.20
Copper (mg/l)	0.09	0.87	0.24	1.04	0.20
Zinc (mg/l)	0.24	1.78	0.27	1.97	2.0
Nickel (mg/l)	0.03	0.37	0.12	0.44	0.20
Cadmium (mg/l)	0.001	0.13	0.09	0.28	0.01
Chromium (mg/l)	0.003	0.16	0.11	0.24	0.10
Lead (mg/l)	0.10	4.05	1.02	3.42	5.0

* Food and Agricultural Organization (FAO) standard [16]

comparatively lower in metal concentrations than the other effluents. The permissible limits for Fe and Zn were 2.0 mg/l, where Fe concentration was above the permissible limit in all types of effluent but Zn concentration was in an acceptable limit. The concentrations of Mn, Cu and Ni were also higher than the permissible limit (0.20), except for the concentration of Ni in beverage effluent which was under the permissible limit. The concentration of Pb was lower than the critical limit (5.0) in all three effluents.

3.2 Spatial variability of heavy metals in soil

The concentrations of different heavy metals in the soil after harvesting the crop are abridged and presented in Table 2. In effluent irrigated soil, Fe (561.6 mg/kg) had the highest and Cd (4.18mg/kg) had the lowest concentration in pharmaceutical effluent followed by the dyeing effluent (Fe= 446.5 mg/kg, Cd=3.66 mg/kg). The sequence of metal concentrations was Fe>Zn>Mn>Cr>Ni>Cu>Pb>Cd in pharmaceutical and beverage irrigated soils, and Fe>Zn>Mn>Cr>Ni>Pb>Cu>Cd in dying effluent irrigated soil. The high deposition of iron in the soil may be due to the increased amount of iron in the effluent water. The metal concentration of the soil increased because of wastewater irrigation during the crop growing season. However, the bioavailability and mobility of heavy metals is influenced by the physical and chemical properties of soil. In addition, the metal concentration does not only depend on the properties of soil but also depends on environmental factors, precipitation reactions, and adsorption-desorption characteristics of soils [17, 18]. The amount of metals found in the current experimental soil did not exceed the allowable limits. Similar types of experimental results were also reported by various authors [19-22]. This could be because other experiments were conducted on soil samples gathered from industrial areas, but in this study, the soil samples were irrigated with industrial effluent for a short time. Long term use of effluents increases metal deposition, contaminates the soil and makes the soil less fertile for crop cultivation.

Table 2. Mean heavy metal (mg/kg) content in wastewater irrigated soil

Metals	Present Study Soil				Uncontaminated Soil			Safe Limit[d]
	Control	Pharmaceutical	Beverage	Dyeing	Soil[a]	Soil[b]	Soil[c]	
Fe	127.1	561.6	256.9	446.5	-	-	1000	-
Mn	20.63	46.68	36.69	59.48	-	-	-	-
Zn	42.44	61.36	50.88	61.75	150	50	100	300-600
Cu	4.92	15.06	18.86	14.82	25	20	30	135-270
Ni	5.36	24.02	24.86	16.95	1	40	30	75-150
Cd	0.5	4.18	3.66	3.83	1	0.06	1	3-6
Cr	20.31	31.47	28.5	38.87	30	100	100	11
Pb	5.17	12.61	8.44	16.48	50	100	50	250-500

[a]Source: [19]; [b]Source: [20]; [c]Source: [21]; [d]Source: [22]

3.3 Heavy metal concentration in root, stem and leaf of Indian spinach

The mean concentration of different heavy metals in different parts (roots, stems and leaves) of Indian spinach are presented in Figure 2. It can be seen that the concentration of all the heavy metals uptake was higher in wastewater-irrigated plants than in the freshwater (control) irrigated plants. In pharmaceutical effluent, Fe showed the highest concentration (395.5 mg/kg) followed by dyeing effluent (306.3 mg/kg), and Cd had the lowest concentration in beverage (1.11 mg/kg) followed by dyeing (1.49 mg/kg) wastewater irrigated plants in the roots. The pattern of heavy metal concentration was found similar in case of roots and leaves for all the effluent treatments. The sequence of heavy metal concentration was Fe>Zn>Mn>Cr>Ni>Cu>Pb>Cd in pharmaceutical and Beverage, and Fe>Zn>Mn>Cr>Pb>Ni>Cu>Cd in dyeing wastewater irrigated plants for both roots and leaves.

Furthermore, in the case of stem, the order of heavy metal concentration was Fe>Zn>Mn>Ni>Cu>Cr>Pb>Cd in pharmaceutical, Fe>Zn>Mn>Cu>Cr>Ni>Pb>Cd in beverage and Fe>Zn>Mn>Cr>Cu>Ni>Pb>Cd in dyeing effluent treatments. Nevertheless, in parallel with roots and leaves, stems also showed the highest Fe content in pharmaceutical (168 mg/kg) followed by dyeing (115.9 mg/kg) and the lowest Cd content in beverage (0.68 mg/kg) wastewater irrigated plants.

The range and mean concentrations of heavy metals (mg/kg) in Indian spinach are compared with the safe limits in Table 3. The results of the present study showed that the concentrations of Fe, Zn, Cu were within the safe limits for all three sources of effluents. However, the concentrations of Cd, Cr and Pb exceeded the permissible limits of all references [22-24]. According to Bhatnagar and Awashthi [22], Ni concentration was above the safe limit in the vegetable samples for all three types of effluent.

Table 3. Heavy metal concentration (mg/kg) in vegetables grown in different industrial effluents

Metals	Present study vegetable			Safe limit[a]	Safe limit[b]	Safe limit[c]
	Pharmaceutical	Beverage	Dyeing			
Fe	285.86(168.1-395.5)	149.66(95.88-202.5)	199.73(115.9-306.3)	450	-	-
Mn	17.75 (11.98-23.22)	13.51 (10.14-16.25)	23.13 (17.2-29.27)	-	-	-
Zn	29.72 (20.55-39.27)	22.41 (15.81-32.28)	33.39(24.32-41.85)	60	50	100
Cu	10.08 (8.12-12.49)	8.38 (7.3-9.37)	6.19 (5.32-7.53)	40	30	20
Ni	10.90 (9.44-12.55)	8.14 (6.7-9.7)	6.25 (5.58-7.38)	20	1.5	10
Cd	1.28 (0.95-1.79)	0.92 (0.68-1.11)	1.23 (1.04-1.49)	0.3	1.5	0.2
Cr	10.90 (6.55-17.51)	8.65 (5.26-12.95)	13.35 (7.06-21.05)	5	20	0.5
Pb	5.76 (4.01-8.01)	4.06 (3.09-4.97)	7.74 (5.88-10)	5	2.5	9

[a]Source: [23]; [b]Source: [22]; [c]Source: [24]

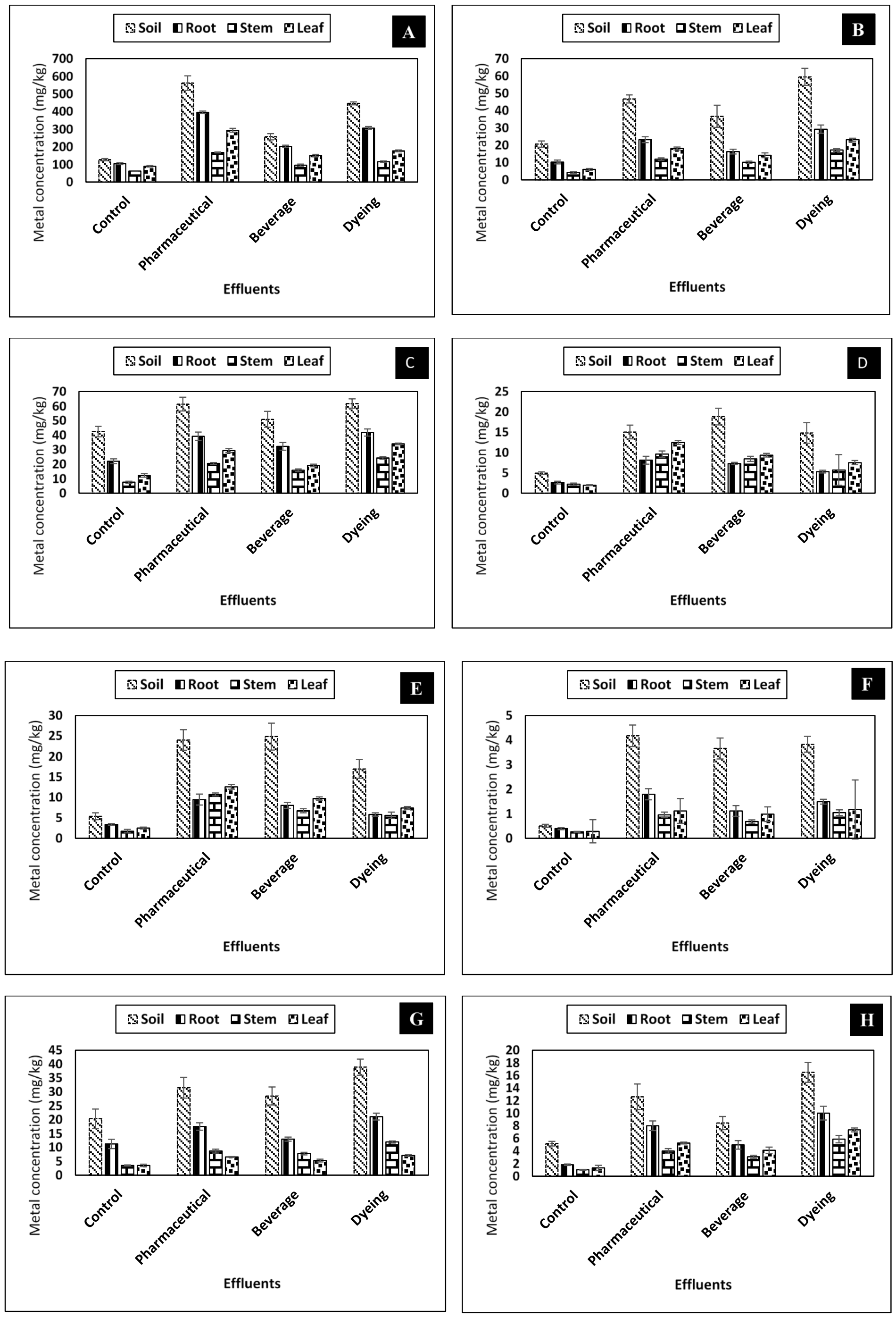

Figure 2. Heavy metal concentration in the soil and different parts of Indian spinach grown with different industrial effluents. The error bars indicate the standard error of the mean. A) Iron, B) Manganese, C) Zinc, D) Copper, E) Nickel, F) Cadmium G) Chromium, and H) Lead

Generally, trace metals are uptaken by the plant biomass and stored in the plant tissues. Absorption and gathering of metals in the plant tissues was influenced by numerous factors, which included pH, moisture, temperature, organic matter and available nutrient [11]. In this study, it is clear from the result that effluent treated vegetable uptook more metals than the control water treated vegetable, which is in agreement with many researchers [11, 25-27]. One of our concerns was the metal accumulation in the leaf portions of Indian spinach, and we have found a considerable amount of all the heavy metal in the leaves from all sources of effluents [26, 28]. There was a variation in the metal concentration for availability in the plant, and this might be due to differences in the ion uptake mechanism and absorption competition among the heavy metals [26, 29].

3.4 Transfer of heavy metal from soil to plant parts

The movement of heavy metals from soil to plant parts (TFS) and root to plant parts (TFR) was estimated to determine the uptake of metals from soil and root to plant parts (Table 4). The results show that the root uptook more metals than the other parts of the vegetable from the soil, except for Cu and Ni. Cu and Ni are more readily absorbed by the leaves than the soil and roots. This is mainly due to the fact that the cations of these heavy metals are less mobile in plant than many nutrients, and after uptake are mainly accumulated in root tissues. Roots are the first tissues that have contact with heavy metal ions and the time of exposure is a key factor regarding the final concentration. The transfer ratio of Ni from root to stem and leaf was more than 1 for all three types of industrial effluents. The proportion of heavy metals between soil and plant parts is a major index used to assess the contamination of soil with heavy metals. The proportion “>1” indicates the higher enrichment of heavy metals in plant parts than in soil [30]. The TFR value of Ni is more than 1, meaning that it is highly mobile from the root to other parts. Lower transfer value indicates low accumulation and less mobility of the metals to plants.

4. Conclusions

Wastewater irrigation is a regular practice in the industrial area of Gazipur, Bangladesh. This study shows that there is a high accumulation of metals in the growing soil and translocation into the edible parts of the vegetable. It is a great concern in the case of leafy vegetables because the leaves are the edible portions of the plant, which contain significant amounts of heavy metals. Long term deposition of heavy metals in the soil may increase the concentrations of heavy metals and transfer them into the food chain which is a major health and safety issues.

5. Acknowledgements

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Table 4. Transfer factor (TF) of heavy metals in different parts of Indian spinach

	Fe		**Mn**		**Zn**		**Cu**		**Ni**		**Cd**		**Cr**		**Pb**	
Pharmaceutical	TF_S	TF_R	TF_S	TF_R	TF_S	TF_R	TF_S	TF_R	TF_S	TF_R	TF_S	TF_R	TF_S	TF_R	TF_S	TF_R
Root	0.64	-	0.50	-	0.64	-	0.53	-	0.39	-	0.43	-	0.56	-	0.64	-
Stem	0.32	0.50	0.26	0.52	0.33	0.52	0.45	0.85	0.45	1.13	0.23	0.53	0.27	0.49	0.32	0.50
Leaves	0.42	0.66	0.39	0.78	0.48	0.75	0.40	0.76	0.52	1.33	0.27	0.62	0.21	0.37	0.42	0.66
Beverage																
Root	0.59	-	0.44	-	0.63	-	0.54	-	0.32	-	0.30	-	0.45	-	0.59	-
Stem	0.37	0.62	0.28	0.62	0.31	0.49	0.64	1.18	0.27	0.84	0.19	0.61	0.27	0.60	0.37	0.62
Leaves	0.49	0.83	0.39	0.87	0.38	0.59	0.83	1.54	0.39	1.21	0.27	0.88	0.18	0.41	0.49	0.83
Dyeing																
Root	0.61	-	0.49	-	0.68	-	0.39	-	0.34	-	0.39	-	0.54	-	0.61	-
Stem	0.36	0.59	0.29	0.59	0.39	0.58	0.45	1.16	0.33	0.96	0.27	0.70	0.31	0.57	0.36	0.59
Leaves	0.45	0.74	0.39	0.79	0.55	0.81	0.50	1.28	0.44	1.27	0.31	0.79	0.18	0.34	0.45	0.74
Control																
Root	0.35	-	0.50	-	0.52	-	0.36	-	0.63	-	0.78	-	0.55	-	0.35	-
Stem	0.20	0.57	0.20	0.41	0.18	0.34	0.39	1.08	0.32	0.51	0.52	0.67	0.17	0.31	0.20	0.57
Leaves	0.25	0.72	0.29	0.59	0.29	0.56	0.51	1.42	0.46	0.73	0.56	0.72	0.17	0.31	0.25	0.72

TFS = transfer factor with respect to soil, TFR = transfer factor with respect to root

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