Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Toxicity assessment of effluent from flash light manufacturing industry by bioassay tests in *Trigonella foenumgracum*

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Publication Info

Paper received:
23 July 2013

Revised received:
12 December 2013

Accepted:
08 January 2014

Abstract

A rapid bioassay test was conducted to study heavy metal accumulation and biochemical changes in *Trigonella foenumgracum* (methi) irrigated with 25, 50, 75 and 100% of effluent from flash light manufacturing industry at 60 days after sowing. Total metal concentration in effluent samples was: Cr=0.12<Cd=0.18<Pb=0.24<Cu=2.68 mg l^{-1} whereas, metals were not detected in control. An increase in photosynthetic pigments of exposed plant was noticed up to 50% concentrations of the effluent followed by a decrease at higher concentration as compared to their respective control. An enhanced lipid peroxidation in the treated plants was observed, which was evident by increased level of antioxidants: proline, cysteine, malondialdehyde and ascorbic acid content. The treated plants accumulated metals in the following order: Cu>Pb>Cr>Cd in the roots and shoots.

Key words

Antioxidant, Bioassay, Heavy metal, Industrial effluent, *Trigonella foenumgraecum*

Introduction

Irrigation of agricultural fields using industrial wastewater in developing countries is well documented (Bhargava *et al*., 2008; Sahu *et al*., 2008; Chandra *et al*., 2009). The quantity and type of pollutants present in the effluent depends on the manufacturing processes involved and raw materials used in the industry. Several authors have reported that the industrial effluent contains several nutrients which can increase the growth and crop productivity (Bhargava *et al*., 2008). However, due to the presence of various hazardous heavy metals it has shown toxic symptoms like stunted growth, necrosis, decrease in germination, decrease in photosynthetic pigment and biomass content in various plant species (Nagajyothi *et al*., 2009; Kumar *et al*., 2009) besides transportation of these metals into food chain (Barman *et al*., 2000). Further, the quality of treated effluent may not violate the discharge limits, but their use for irrigation purposes may be toxic to the humans and the environment (Slabbert, 1996). Plants irrigated with industrial wastewater, when consumed by people result in a number of diseases like diarrhea, mental retardation, liver and kidney damage etc. (Matsuno *et al*., 2004; Uzair *et al*., 2009). It is therefore, important to evaluate the characteristics of industrial effluents prior to their application in agriculture (Sahu *et al*., 2008; Singh *et al*., 2010; Kumar *et al*., 2013a,b). Besides, evaluating the quality of effluent following chemical methods, bioassays should also be performed to determine the total effect of effluent quality on biological systems (Sahu *et al*., 2008).

The present study was aimed to determine the impact of effluent from flash light manufacturing industry following bioassays by using *T. foenumgracum* as test material in terms of photosynthetic pigments and antioxidants. Further, efforts have been made to study the accumulation and translocation of Cu, Cd, Cr and Pb in this species.

Materials and Methods

Sample collection : Effluent samples were collected in 50 l gallons from a waste water treatment unit of a flash light manufacturing industry located at Lucknow, Uttar Pradesh, India. Physico-chemical characteristics of effluent along with the control (tap water) were analysed following the standard methods of APHA (2005).

Experimental setup : Four irrigational treatments *viz.*, 25, 50, 75 and 100% of effluent were prepared using tap water, whereas tap water served as control. Healthy seeds of *Trigonella foenumgracum* cv. Pusa Early were procured from a local seed shop at Lucknow. Seeds were sterilized by soaking them in 0.1% $HgCl_2$ solution for 5 min to avoid fungal/bacterial contamination and then washed thoroughly with running tap water followed by deionized water. Ten sterilized seeds were sown in earthen pots filled with 7 kg of garden soil; later plants were thinned to three plants per pot and irrigated with different irrigational treatments at 3 days interval. The experiment was performed in six replicates.

Biochemical parameter : Biochemical responses in *T. foenumgracum* was observed at 60 days after sowing (DAS) by analyzing photosynthetic pigment contents (chlorophyll a, b and total) following Lichtenthaler and Wellburn (1983), carotenoids by Duxbury and Yentsch (1956), ascorbic acid by Keller and Schwager (1977), cysteine by Gaitonde (1997), proline by Bates *et al*. (1973) and malondialdehyde (MDA) by Heath and Packer (1968).

Heavy metal analysis : The plants were uprooted on 60 DAS, washed thoroughly using tap water followed by deionized water to remove soil. The plants were segregated into roots and shoot, dried, grounded and stored into PEP bottles for further analysis. Powdered plant samples were analysed for Cu, Cr, Cd and Pb on atomic absorption spectrophotometer (AA 240 FS, Varian) after digesting the samples in nitric acid and perchloric acid mixture (3:1 v/v).

Translocation factor (TF) and Concentration Index (CI) : Translocation factor and concentration index ratio were computed following Mattina *et al*. (2003) and Kiekens and Camerlynck (1982) respectively.

Statistical analysis : The data obtained were statistically analyzed following two tailed *t* test and one way analysis of variance (SPSS, Statistical package and MS Excel) using Duncan multiple range tests (DMRT) to determine the significance of differences among treatments at probability (p) 0.05 and 0.01.

Results and Discussion

The effluent was found to be slightly alkaline having pH 7.22. Total dissolved solid (TDS) and total suspended solid (TSS) were found to be 1230.90 and 791.22 mg l^{-1}, respectively. Total alkalinity and hardness of the effluent samples were recorded as 441.69 and 386.36 mg l^{-1}. Sulphates and chloride contents were 7.30 and 96.37 mg l^{-1}. The dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD) were 3.86, 163.61 and 480.27 mg l^{-1} respectively. The inorganic phosphate concentration was 3.13 mg l^{-1}. Potassium, sodium and calcium concentration were 10.78, 68.42 and 38.24 mg l^{-1} respectively. All the parameters analysed were found to be significantly (p< 0.01) higher, whereas turbidity, pH and Ca were found to be higher at significance level of p< 0.05. Metal concentration in the effluent samples was found in the following order : Cr=0.12<Cd=0.18<Pb=0.24<Cu=2.68 mg l^{-1} whereas, metals were not found in control (Table 1).

An increase in Chl a, Chl b, Total Chl and carotenoid contents was observed in leaves of *T. foenumgracum* plants treated up to 50% effluent, however, these pigments decreased significantly (p<0.05) beyond 50% effluent treatment (Fig. 1). At 50% effluent treatment, Chl a increased by 20%, however, at 100 % treatment it deccreased by 10% over their respective control in the leaves of *T. foenumgracum*. Chl b content in these plants significantly (p<0.01) reduced at all the treatments, except a non-significant increase was noted at 50% treatment. Maximum reduction in Chl b was noticed at 100% treatment in the leaves of this plant (Fig. 1B). Carotenoid content in *T. foenumgracum* decreased significantly (p<0.01) at higher effluent concentration.

An increase in effluent concentration in irrigation water resulted in enhanced proline production in the plant (Fig. 2A). It ranged from 27.80 to 138.72 µg g^{-1} f.wt. in leaves of the plants.

Table 1 : Physico-chemical characteristics of effluent and tap water

Parameter	Tap water	Effluent
pH	6.95 ± 0.03	7.22 ± 0.09*
Odor	odorless	pungent
Color	colorless	muddish
Turbidity	2.06 ± 0.23	1138 ± 50.0*
TDS	80.24±1.35	1230.90 ± 108.61**
TSS	21.23±1.06	791.22 ± 55.23**
TS	101.47±4.77	2022.11 ± 62.46**
Total Alkalinity	42.82 ± 0.87	441.69 ± 39.60**
Total hardness	15.65 ± 1.17	386.36 ± 41.01**
Sulfate	3.22 ± 0.23	7.30 ± 0.20**
Chloride	5.22 ± 1.59	96.37 ± 10.31**
DO	6.72 ± 0.19	3.86 ± 0.30**
BOD	0.62 ± 0.02	163.61 ± 14.34**
COD	1.49 ± 0.07	480.27 ± 40.03**
Phosphate	1.18 ± 0.02	3.13 ± 0.37**
K	18.65 ± 2.52	10.78 ± 1.36**
Na	26.08 ± 1.79	68.42 ± 5.44**
Ca	24.24 ± 2.39	38.24 ± 5.30**
Pb	ND	0.24±0.004
Cd	ND	0.18±0.0038
Cu	ND	2.68±0.35
Cr	ND	0.12±0.02

All the values are mean of six replicates ± S.E. Two tailed t-test was performed to compare the values of effluent and control where, ns=not significant at p<0.05, *=significant at p<0.05, **=significant at p<0.01. All the parameters have same unit mg l^{-1} except colour (visual) and turbidity (NTU)

Cysteine and ascorbic acid content in the leaves of the species was enhanced at initial treatment levels (up to 50% effluent concentration), however, cysteine content decreased significantly ($p<0.05$) beyond 50 % effluent treatment (Fig. 2B-C). It was found maximum in leaves of the plants irrigated with 25% effluent concentration. The observed values were approximately 676 and 576 % higher than their respective control. After 60 days of effluent treatment, cysteine content ranged between 65.61 (100%) to 120.95 µg g^{-1} f.wt (25%) (Fig. 2B).

Ascorbic acid content in treated plants ranged between 397.06 (100%) to 622.51µg g^{-1} f.wt. (50%) (Fig. 2C). Maximum ascorbic acid content was found at 50% effluent treatment, which was approximately 63% higher than control (Fig. 2C). MDA content, in leaves of the plant, increased with increasing concentrations of industrial effluent in irrigation water (Fig. 2D). It ranged between 2.76 (control) and 6.98 n mol g^{-1} (100 % effluent) at 60 DAS in the leaves of *T. foenumgracum*. At 100% effluent treatment, MDA content in *T. foenumgracum* increased by 253% over their respective control.

Application of wastewater led to change in the physico-chemical characteristics of soil and consequently enhanced the heavy metal uptake by plants. Cu concentration in shoots of *T. foenumgracum* irrigated with various amendments of effluent ranged from 23.81 to 30.81 µg g^{-1} d. wt. Similarly, Pb ranged from 4.50 to 21.73 Cr from 2.98 to 7.83 and Cd from 1.83 to 6.56 µg g^{-1} d.wt. respectively.

The concentration of Cu in roots of *T. foenumgracum* ranged from 28.25 to 39.75 µg g^{-1} d.wt. Similarly, Pb ranged from 8.57 to 39.01, Cr from 4.73 to 25.06 and Cd from 1.76 to 10.46 µg g^{-1} d. wt. respectively.

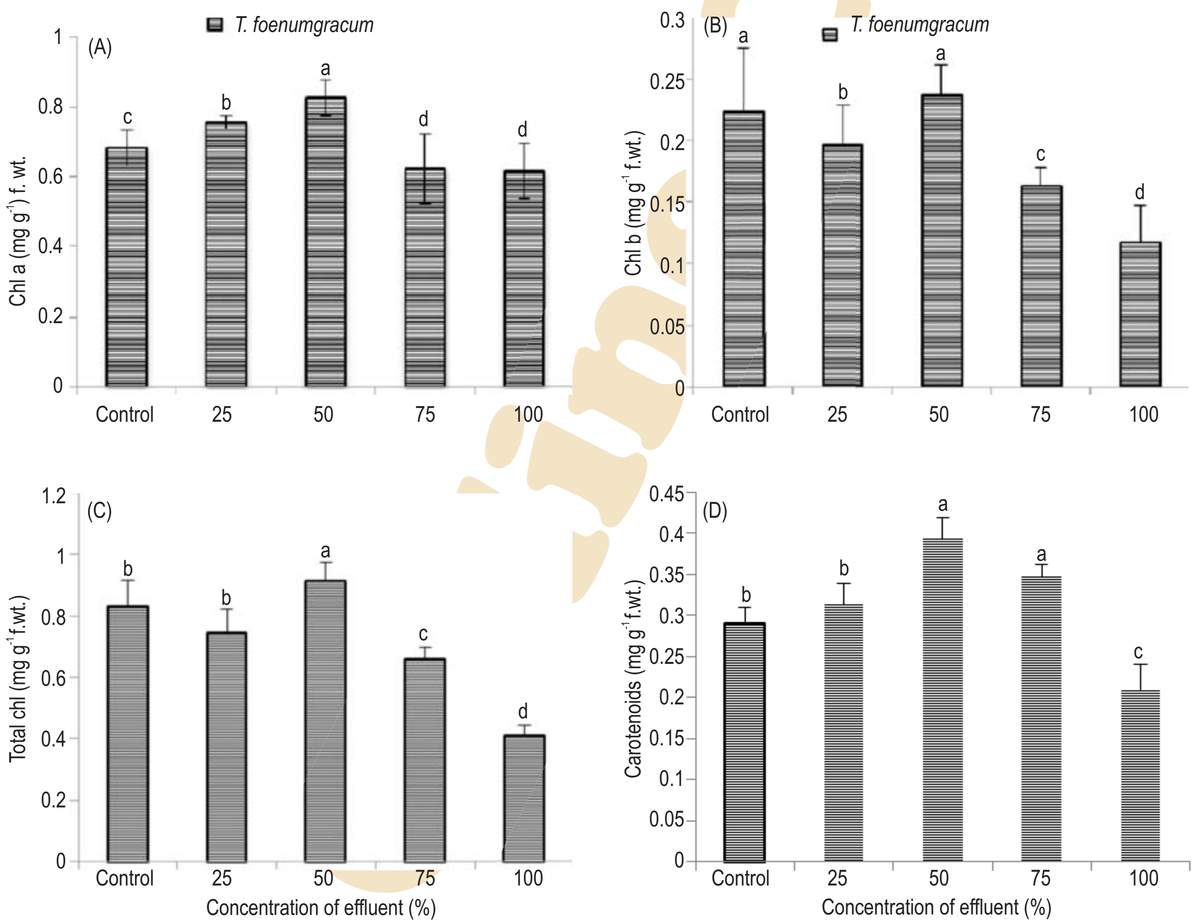

Fig. 1 : Effect of different treatments of Flash light manufacturing industry effluent on the chl a [A], chl b [B], total chl [C] and carotenoids [D] contents ± SE (n=6). One way ANOVA (DMRT) was performed to compare the means. Different letters on the bars show significant differences between the treatments at $p< 0.05$

Translocation factor (TF) was found to be more than one for all metals except for Cr at 25 % treatment (Table 2). TF ranged from 0.78 to 0.83, 0.45 to 0.85, 0.26 to 1.69 and 0.38 to 1.13 for Cu, Pb, Cr and Cd, respectively.

Concentration index (CI) of all metals increased with increase in effluent concentration in the irrigation water. Results revealed that CI for roots and shoots ranged between 2.20 to 3.10, 2.26 to 2.77 (Cu), 2.32 to 10.57, 1.43 to 6.94 (Pb), 1.08 to16.04, 1.76 to 3.88 (Cr), 4.22 to 9.34, 1.22 to 5.22 (Cd), respectively (Table 2).

Results of physico-chemical analysis of effluent (Table 1) revealed higher concentration of inorganic contaminants and toxic metals, which might be due to the gravitational settling of pollutants along the treatment system. pH is generally acknowledged to be the principle factor governing concentration of soluble and plant available metals (Malaviya and Rathore, 2007). High turbidity may be due to dissolved solids and salt (Hinchman *et al*., 1998). Total hardness of the effluent was found to be 386.36 mg l^{-1}. The anions responsible for the hardness are mainly carbonate, bicarbonate, sulphate, floride, nitrate and silicate (Sahu *et al*., 2008). These ions may become part of the effluent because of their interaction with soil system and/or due to use of various inorganic chemicals in the industrial processes. BOD and COD of industrial effluent were found to be 163.61 and 480.27 mg l^{-1} respectively, which were much higher than the prescribed standard i.e. 30 and 250 mg l^{-1} for the final discharge of effluent (Peng *et al*., 2004; Malaviya and Rathore, 2007).

An increase in photosynthetic pigments in leaves of *T. foenumgracum* was observed at lower concentration of the

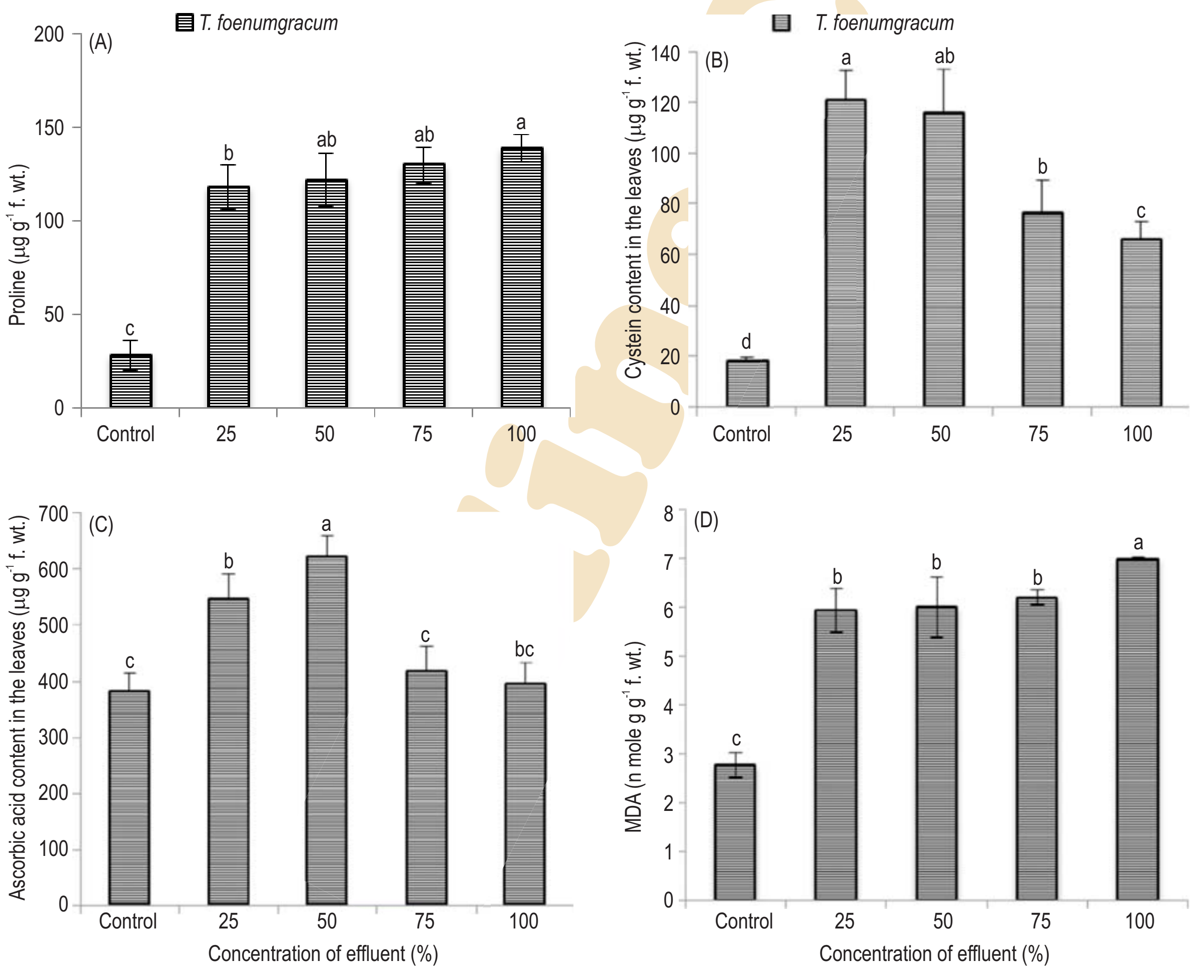

Fig. 2 : Effect of different treatments of Flash light manufacturing industry effluent on the proline [A], cysteine [B], ascorbic acid [C], and MDA [D] content ± SE (n=6). One way ANOVA (DMRT) was performed to compare the means. Different letters on the bars show significant differences between the treatments at $p < 0.05$

Table 2 : Translocation factor and concentration index of metals with respect to roots and shoots of *T. foenumgracum*

Effluent conc. (%)	Cu	Pb	Cr	Cd
		Translocation Factor (TF)		
0	0.825±0.01	0.850±0.02	1.04±0.02	1.13±0.02
25	0.804±0.02	0.525±0.02	1.69±0.01	0.380.01±
50	0.76±0.03	0.519±0.02	0.55±0.0	0.56±0.02
75	0.778±0.01	0.447±0.01	0.27±0.01	0.611±0.05
100	0.775±0.01	0.74±0.02	0.26±0.02	0.74±0.02
		Concentration Index for roots (CI_R)		
25	2.20±0.02	2.32±0.01	1.08±0.02	4.22±0.15
50	2.705±0.12	3.95±0.02	5.50±0.03	6.55±0.26
75	2.903±0.35	9.93±0.21	16.04±0.02	8.26±0.58
100	3.100±0.01	10.57±1.08	15.46±0.01	9.34±0.12
		Concentration Index for shoots (CI_S)		
25	2.26±0.21	1.43±0.02	1.76±0.02	1.22±0.12
50	2.30±0.03	2.41±0.22	2.93±0.12	2.74±0.02
75	2.50±0.01	5.23±0.12	3.64±0.12	3.77±0.01
100	2.77±0.21	6.94±0.25	3.88±0.02	5.22±0.25

All the values are mean of six replicates ± S.E.

effluent which might be due to availability high nutrients in the effluent. This may be probably facilitated by the optimum availability of Fe and Mg (Kisku *et al*., 2000; Arora *et al*., 2008). An increase in carotenoid content is suggested as a defense strategy in many plants to combat metal stress (Sinha *et al*., 2007; Kannan *et al*., 2012). Many authors have reported a reduction in chlorophyll content in the plants irrigated with wastewater (Sinha *et al*., 2007; Bhargava *et al*., 2008).

Proline is a metabolite, which has been shown to accumulate in plants under abiotic stresses, especially heavy metal stress (Zhao, 2011; Bauddh and Singh, 2012a, 2012b). In *T. foenumgracum*, proline content was found to be increased with increase in effluent concentration in the irrigation water (Fig. 2B). Similar results have been reported by Sinha and Singh (2005) and Bhargava *et al*. (2008). The decreased cysteine content in plants treated with higher effluent concentration was probably due to decreased activities of sulfate reduction enzymes, ATP sulfurylase and adenosine-5-phosphosulfate sulfo-transferase under metal stress (Nussbaum *et al*., 1988). MDA is a major indicator of lipid peroxidation in plants, and it was found to be enhanced with increase in effluent concentration in irrigation water. The increased MDA level was probably due to the presence of heavy metals in industrial effluent (Nwaogu *et al*., 2011; Muneer *et al*., 2011; Zhao, 2011). Under heavy metal stress an increased MDA level has been reported in *Vigna radiata* (Muneer *et al*., 2011), *Zea mays* and *Triticum aestivum* (Zhao *et al*., 2011).

T. foenumgracum accumulated Cu, Pb, Cr and Cd in their roots and shoots. The maximum permissible limit of Cu, Pb, Cr and Cd, in vegetables is 20, 9, 0.5 and 0.1-0.2 mg kg^{-1} respectively, on dry weight basis SEPA (2005). Chromium is a highly toxic heavy metal accumulated by roots of the plants and then subsequently transported towards the shoot via vascular system (Leghouchi *et al*., 2009). Cr content in the aerial part of *T. foenumgracum* ranged from 2.99 to 6.57 μg g^{-1} d. wt. Cu is an essential micronutrient for normal plant metabolism but it has been reported to be toxic at higher concentrations (Li and Xiong, 2004). Cu contamination brings changes in nitrogen metabolism with a reduction in total nitrogen and it increases free amino acid (Llorens *et al*., 2000). In the present study, Cu has been accumulated in higher amount by the studied plant species. Pb concentration in plants grown on uncontaminated areas was reported as 0.05 to 3.0 μg g^{-1} (Kabata-Pendias and Pendias, 2001) and 5 to 10 μg g^{-1} (Carranza-A´lvarez *et al*., 2008). Present findings showed that Pb concentration in the roots and shoots of *T. foenumgracum,* ranged between 3.69 to 39.01 μg g^{-1} d.wt. and 3.13 to 21.73 μg g^{-1} d.wt. respectively. Cd is not an essential element for metabolic processes and is a cumulative poison. Kabata-Pendias and Pendias (2001) reported that both root and leaf can uptake Cd effectively. The results of the present study revealed that the concentration of Cd in the roots and shoots of *T. foenumgracum* ranged between 1.12 to 10.46 and 1.50 to 7.83 μg g^{-1} d.wt., respectively.

Translocation factor is an important parameter and can be used to monitor the movement of metals from root to shoot. *T. foenumgracum* was found to have TF > 1. TF > 1.0 indicates a proficient capability of plants to transport metal from root to shoots, most likely due to efficient metal transporter system (Zhao *et al*., 2003) and possible sequestration of metals in the leaf vacuoles and apoplast (Lasat *et al*., 2000). Concentration index was found to be > 1.0 for both roots and shoots of the plants, which confirm that the studied plant, effectively up-to metals present in the growing medium. It may be concluded that, continuous application of effluent in the agricultural fields may not only adversely affect the plant growth but also result in metal accumulation in the crops and pose a threat of metal toxicity. Hence, the agricultural use of industrial effluent requires comprehensive investigation for its suitability for irrigation purposes.

Acknowledgement

Authors are thankful to The Head, Department of Environmental Science, BBAU, Lucknow, India for providing infrastructure support.

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