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ORIGINAL ARTICLE

Evaluation of phytoremediation efficacy of *Trigonellafoenum-graecum*(Fenugreek)on copper contaminated soil

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ABSTRACT
Heavy metals are considered one of the major environmental contaminants due to their toxicity and harm they could cause with their presence in various environmental matrices. Phytoremediation is an eco-friendly approach, that employs plants as a tool to remove the pollution from the environment. The following study investigated the efficiency of Trigonell afoenum-graecum (Fenugreek), for metal remediation on copper contaminated soil medium. Fenugreek plants were grown across five test treatments of copper contamination– T1 (Control), T2 (50 ppm), T3 (100 ppm), T4 (150 ppm), T5 (200 ppm), for 30 days. At the end of exposure, the plants were analyzed for the presence of copper using Atomic Absorption Spectroscopy. The AAS results showed that T2, T3, T4, and T5 have shown a comparatively higher copper accumulation in plants against the control treatment. Metal accumulation was observed to have occurred mostly within the root region of Fenugreek plants, followed by its translocation to the leaves. Therefore, the results suggest that Trigonella foenum-graecum can be used for phytoremediation of copper from contaminated soils.
Keywords: *Phytoremediation, Trigonella foenum-graecum, Copper, Bioaccumulation, Translocation, Atomic Absorption Spectroscopy (AAS).*

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INTRODUCTION
Heavy metals are considered significant environmental pollutants due to reasons like toxicity effects, persistence in the environment, and bio-accumulative nature, potential to affect living organisms and environment [1]. Metals' concentration in the environment has accelerated drastically to alarming levels with the rapidly developing industrial sectors. Exposure to heavy metals through any routes can induce severe toxicity and adverse health conditions in terrestrial and aquatic beings, and to the ecosystem[1]. The soil matrix serves as a major reservoir or a transporting media for the metals because the association properties between the soil and the metals is rich and possess diverse binding characteristics [5]. Most metals do not undergo microbial or chemical degradation, thus, they persist in the environment for a long time [9]. Conventional technologies for cleaning up metal contaminated sites often do not serve as an ideal method due to limitations like cost, labor requirement, and may also involve non-eco-friendly approaches. Phytoremediation employs plants capable of accumulating metals within various plant's parts or stabilize the distribution or mobility of metals, which helps in the restoration of the site. Plants do not disturb the natural balance of the medium, where they are made use of. Phytoremediation is useful for remediation for a wide range of pollutants and is said to be 10-fold cheaper than conventional techniques [10].
Copper is an abundant trace element present on the Earth, and an essential microelement involved in physiological functions of both plants and animals. However, copper, when present in concentrations higher than optimum can pose serious risks to animal health, and to the ecological system. Copper toxicity can cause failure of vital organs like liver and kidney in humans [12]. Repeated application of copper-based fungicides and pesticides cause soil toxicity affecting the soil microbial population, and soil fertility [13]. Copper is considered one of the most toxic metals to aquatic organisms and ecosystems [12].

Trigonella foenum-graecum (Fenugreek) is an annual legume, and a plant of high nutritional values and medicinal properties. Kaur. L., 2016 showed that Fenugreek accumulated Lead and translocated the metal to the harvestable parts of the plant. Metal accumulation increased with increasing metal concentrations. The maximum lead was observed in the root than the shoot. The dry matter yield of Fenugreek and chlorophyll content decreased with increasing metal concentrations. In another study to compare the accumulation potential of Indian Mustard, *Brassica juncea,* and Fenugreek, *Trigonella foenum-graecum* in Lead and Nickel contaminated soils, Kaur, L., 2018 found that both plant species were able to accumulate the metals in root and shoot parts. The study incorporated EDTA and Salicylic Acid in the treatments. In all the treatments, the roots had accumulated more metal than the aerial shoot parts. The concentration of metal accumulated increased in treatments amended with EDTA. Fenugreek showed better translocation of Lead, while Mustard showed better results with Nickel translocation. Perveen. S *et al.,* 2012 used *Trigonellafoenum-graecum* (Fenugreek) and *Vigna mungo* (Black gram), two important pulse crops that are grown in India to identify the phytoremediation potential of the plants in induced Cd toxicity. Cd caused significant damage to the plants. It was found out that this toxicity was significantly reduced when the plants earlier treated with Cd was treated with different neem plant parts. Highest improvement was noted in plants treated with neem fruits alone while the minimum was noted in plants treated with neem leaf. The effect in Fenugreek plants was higher when compared to Black gram plants.
The undertaken study aims to evaluate the phytoremediation efficiency of *Trigonellafoenum-graecum* in Copper contaminated soil. The objectives include 1. Experimental contamination of soil medium with Copper, 2. Pot experiment – Growing Fenugreek in contaminated soils for 30 days, 3. Atomic Absorption Spectroscopic analysis for the presence of Copper in plants, 4. Quantification of Copper extracted from the soil – Bioaccumulation Factor (BAF) and Translocation factor (TF).

MATERIAL AND METHODS

Sample collection

The soil sample required for the study was taken from the garden of the residence located at Tambaram, Chennai. Fenugreek seeds of good quality were collected from the market.

Soil analysis

Soil analysis was performed to derive basic physicochemical parameters of the soil used for the study. Soil analysis was done at National Agro Foundation, Taramani, Chennai. The testing methods adopted for the study were from '*Methods of analysis of soil, plants, water, fertilizers, and organic manure*' by HLS Tandon (FDCO) (2009) and '*Guide to laboratory establishment for plant nutrient analysis*' by FAO UN (2008).

Pot Experiment

To understand the phytoremediation technique, a pot experiment was performed. Copper sulphate salt was used as the source of copper. Five test treatments were: T1- Control, T2- 50 ppm, T3- 100 ppm, T4- 150 ppm, T5- 200 ppm. 50 mg, 100 mg, 150 mg, 200 mg of $CuSO_4$ were weighed to make up the respective test concentrations [1 ppm = 1 mg/kg of the contaminant in soil] [4]. Duplicates were maintained for each treatment. Pots were filled with 1 kg of garden soil each. The soil was spiked with corresponding concentrations of $CuSO_4$, and mixed thoroughly for equal distribution of copper. The pots with experimentally contaminated soils were sown with the fenugreek seeds (50 seeds/pot). Within 4-5 days, the seeds began to germinate. The plants were grown in the contaminated soils for30 days. They were watered on alternative days to maintain the moisture in the soil matrix to facilitate plant growth, and was given enough exposure to sunlight. Excessive watering was avoided to prevent leaching of metals.
At the end of the exposure period, the plants were harvested from their respective pots separately.. The duplicates of each treatment were pooled together to get enough samples for each treatment. The harvested plants were washed under tap water to remove soil, and dirt from the plants, and then rinsed in distilled water.

Processing of plant samples for metal analysis

The plants were dried in the hot air oven for 48 hours at 70ºC. The dried plants were separated into their plant parts: roots, stem, leaves, and ground using a mortar and pestle. The ground plant parts were stored in individually labeled Ziploc bags for further analysis. Phytoextraction property of accumulating plants causes the plants to extract the metals and translocate them to various plant parts, and store/degrade them within the plant tissues. Hence, categorizing the plants into individual parts allows the estimation of metal accumulated in the respective plant part, and conduct a comparative study.

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Acid digestion for extraction of metals

Estimation of metal concentration in the plant samples was done by adopting the Wet acid digestion method, done using Aqua Regia, a mixture of Conc. HCl and Conc. HNO_3(3:1). The ground plant samples were transferred into 50 ml beakers and digested using 12 ml of Aqua Regia. The mixture was heated on a hot plate until the fumes reduced, and the digest became clear. The digestion was performed inside the fume hood as the acid mixture can release toxic fumes. Following digestion, the mixture was allowed to cool. The digested mixture was filtered through Whatman filter paper of Grade 1 into a 25 ml Standard Measuring Flask. The final volume was made up to 25 ml using double distilled water. Metal concentrations in the digested samples were determined by Atomic Absorption Spectroscopy (AAS) analysis.

Quantification of copper

Estimation of translocation factor

Translocation factor (TF) is a ratio of the ability of the plants to translocate the metal from its root to the aerial parts. Represented in mg/kg. The following formula is used to calculate TF,

$$Translocation\ Factor = \frac{Metal\ concentration\ in\ aerial\ parts}{Metal\ concentration\ in\ roots}$$

Estimation of bioaccumulation factor

Bioaccumulation factor (BAF) is calculated to quantify the metal accumulation efficiency in plants by comparing the concentration in plants and soil. Represented in mg/kg. The following formula is used to calculate BAF,

$$Bioaccumulation\ Factor = \frac{Metal\ concentration\ in\ aerial\ parts}{Metal\ concentration\ in\ soil}$$

RESULTS AND DISCUSSION

Physicochemical parameters of soil

The physicochemical properties of the garden soil used for the phytoremediation study were established through soil analysis (Table 1). Parameters like pH, Electrical conductivity, Organic matter, CEC, and level of presence of macro (N, P. K, Ca, Mg, S) and micronutrients (Na, Zn, Mn, Fe, Cu, B) were established from the analysis. The non-contaminated soil had pre-existing copper of concentration of 1.33 mg/kg.

Metal distribution in plant parts

The Atomic Absorption Spectroscopy results to determine the distribution of metals in individual plant parts are presented in Figure 1. The results denote that the plants across treatments have extracted copper showing concentrations slightly higher than control. However, only trace amounts of metal had been extracted by the plants, showing a significant difference between the copper concentration introduced into the soil and copper concentrations that were detected in the plant parts.

Copper was found to have accumulated more within the roots of the plants throughout treatments, followed by the leaves. The presence of metal in the leaves could be a result of the translocation of the metal from the roots. Highest metal concentration in roots was observed at treatments 100 ppm and 150 ppm, and the lowest at 200 ppm. Copper concentration was noted to increase in the leaves as concentrations increased. The highest was noted at 200 ppm treatment with a total metal concentration of 1.02 ppm. Despite higher concentrations of metal in the vicinity of Fenugreek, the plants were able to thrive in the environment. Plants exhibit tolerance to copper toxicity by adopting mechanisms on a cellular level to prevent the accumulation of copper to toxic concentrations within the sensitive sites of the cell thereby preventing the damaging effects of the metal rather than developing proteins to resist the heavy metal effects [15]. Organic acids excreted by the plants can facilitate metal uptake but these molecules can also inhibit the uptake of metals through the formation of a complex with it outside the root.

Quantification of copper in plant parts

The Bioaccumulation factor (BAF), and the Translocation Factor (TF) were calculated substituting the concentration of copper analyzed within the plant parts.

Bioaccumulation factor

A Bioaccumulation factor with a value greater than 1 categorizes the plant as an effective accumulator [1], but the results exhibit bioaccumulation lesser than 1. Increased Bioaccumulation factor was noted at 50 ppm concentration and 100 ppm with values 0.0245 mg/kg and 0.0247 mg/kg with least variation between the treatments. Metal accumulation was not much evident with the increasing concentrations (Figure 2.). More of notable bioaccumulation happened within the root system of the plants, which could be considered as phytostabilization, and this could suggest that metal accumulation happens immediately within the roots, and then the other plant regions. In a similar study performed [5], it was studied that the

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metal was primarily sequestered within the root system. The least accumulation of 0.0084 mg/kg noticed at 200 ppm could be due to the reduced biomass observed at that respective treatment.

Translocation factor

Translocation factor with value lesser than 1 denotes no effective translocation has occurred. The data is represented in Figure 3. The highest translocation factor of 1.02 was noticed in leaves at 200 ppm. Despite the scarce bioaccumulation that had occurred in the plants, metal accumulation was noticed in the leaves of the plants suggesting that the metal had been translocated to the aerial plant parts. This accumulation could have happened by employing the xylem and phloem system, transporting the metal from the roots to the leaves. Compared to the metal concentration observed in root and leaves, the stem had reduced concentration present in them, which also denotes reduced accumulation of metal had taken place within the stem.

Table 1. Physicochemical parameters of soil

S. No.	Parameters	Unit	Result
1	pH	-	7.0
2	Electrical Conductivity	mS/cm	0.498
3	Organic Matter	%	0.50
4	Nitrogen	mg/kg	38.5
5	Phosphorous	mg/kg	11.93
6	Potassium	mg/kg	203
7	Calcium	mg/kg	979
8	Magnesium	mg/kg	262
9	Sulphur	mg/kg	62.7
10	Sodium	mg/kg	404
11	Zinc	mg/kg	0.95
12	Manganese	mg/kg	11.74
13	Iron	mg/kg	5.14
14	Copper	mg/kg	1.33
15	Boron	mg/kg	0.8
16	CEC	meq/100g	9.36

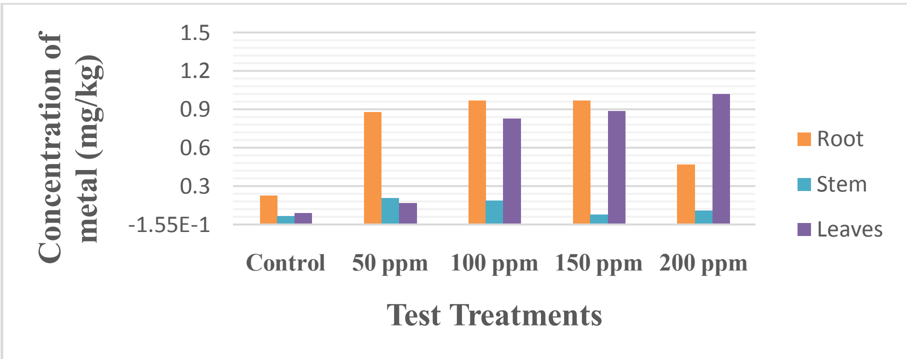

Figure. 1. Distribution of copper in plant parts

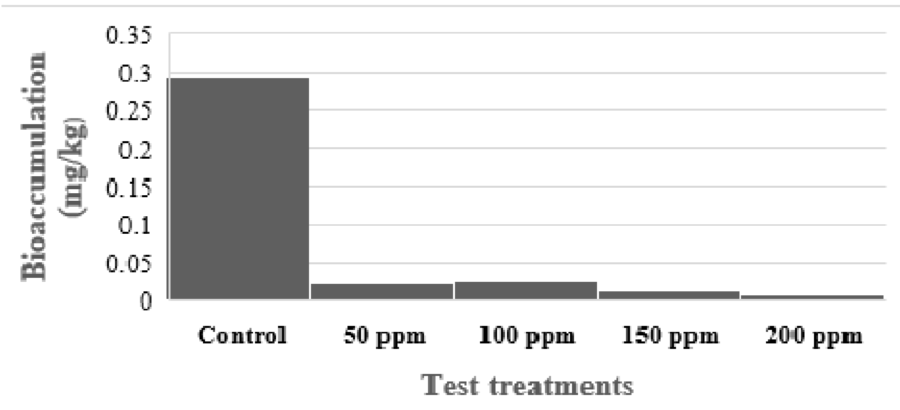

Figure. 2. Bioaccumulation of copper in Fenugreek with respect to the concentration added to the soil

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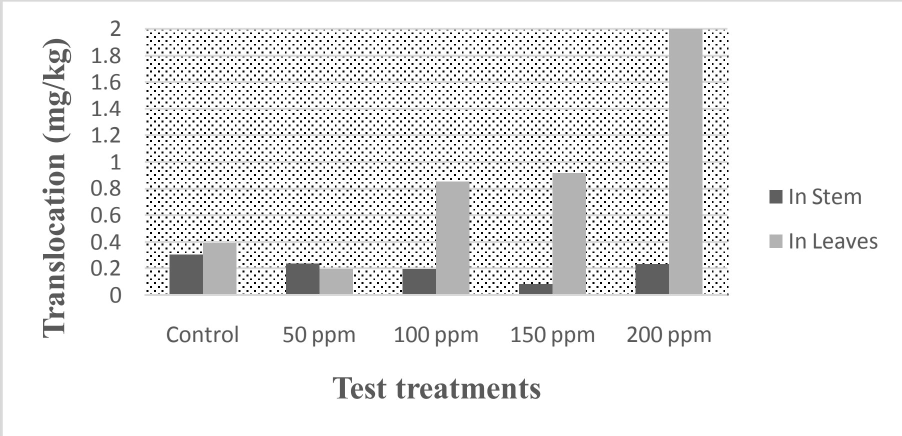

Figure. 3. Translocation of copper in Fenugreek – Concentration of metal translocated from the roots to shoot parts

The results obtained indicated reduced bioaccumulation and translocation to have taken place in the plants. Comparing the results with control treatment that did not have an externally amended copper in the medium, the plants that were grown in soils with amended copper indeed accumulated copper from the soil system the plants were grown in. Various factors could serve as a reason for the reduced remediation of copper that had taken place in the plant, *Trigonella foenum-graecum.* Bio-availability of a metal in the corresponding medium determines the quantity of metal available for the plants to take in. The organic matter in the soil exhibit strong binding to copper, therefore restricting metal mobility, and the total copper available for plant uptake can be low [7]. The chemical form of copper in the soil must have also influenced the metal's bioavailability. The pH of the soil is also a key factor for metal bio-availability. Ariyakanon and Winaipanich, 2006 suggest that the pH of the soil can determine the solubility and mobility of metal in the soil. At a pH lower than 7.0, copper exists in cuprous form and not cupric form. Therefore, in its cuprous form, the solubility and mobility of copper are higher when compared to its cupric form. This suggests that the availability of copper for plants increases with a decreasing pH. The soil pH used for the study was of neutral nature (pH 7); this could have also been a factor for the reduced intake of copper. The biomass of the plant and the intensive root system that the plant could develop play a great role in effective phytoremediation. Fenugreek produced low biomass even at control treatment, which may also suggest if at all fenugreek could be used for phytoremediation, it can only be used for remediation on a small scale level pertaining to its biomass.

The effectiveness of remediation could also be improved by adding various organic amendments into the contaminated soil system that shall further help to enhance the chelation of metals to the roots and therefore the metal uptake, and also reduce metal induced toxicity, thus helping the plants to thrive.

CONCLUSION

Phytoremediation gained much attention with its eco-friendly approach to remove toxic environmental pollutants with over hundreds of species having been identified with the potential to extract/immobilize metals from/in the medium where the plants are employed. Few countries have approached the method as a means to restore the ecological health of a polluted site. Despite the certain limitations it has, the process still can be approached given all necessary associated factors are taken care of, for effective results and this effectiveness can vary between laboratory conditions and field conditions.

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