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RESEARCH PAPER

Phytoremediation of zinc contaminated water by marigold (*Tagetes Minuta* L)

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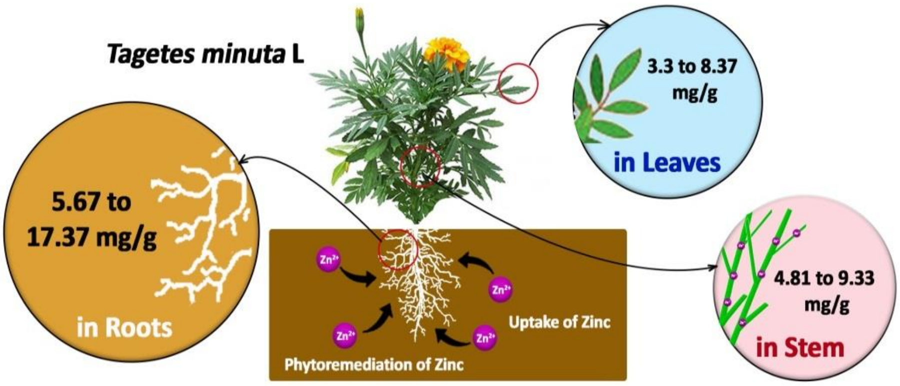
Highlights

- Heavy metals affect adversely the aquatic and terrestrial environments.
- Phytoremediation is an approach used to detoxify contaminated areas.
- Plant can be a useful material for remediation of heavy metals from contaminated sites.

Graphical Abstract



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Abstract

The heavy metals like zinc affect adversely the aquatic and terrestrial environments. Phytoremediation technique is most effective, low cost and environmental friendly method used to remove pollutants such as heavy metals from both water and soil. The present study aims to evaluate marigold effect in phytoremediation of zinc from waste water and investigate the growth parameters under zinc metal stress. To this aim, marigold plant was used to remove zinc from synthetic waste water at different concentrations (0, 100, 200,300, and 400 μM). The experiment was conducted for 15 days in different steps including plant collection, growth in controlled environment, pre-analysis, post-analysis, drying, separation of parts, grinding digestion, filtration and metal detection by using atomic absorption spectrophotometer. Zinc accumulation was checked by detection of zinc metal in roots, shots, and leaves of plants. According to results, in roots, stem and in leaves zinc found in range of (5.67 to 17.37); (4.81 to 9.33) and (3.3 to 8.37), respectively. It could be concluded that this plant have zinc accumulation capacity and it is useful to treat zinc contaminated site.

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1. Introduction

Defilement by overwhelming reasons for soil, water, air contamination, and human wellbeing (Yoon et al., 2006; Aleagha and Ebadi, 2011). In ongoing decades, the yearly worldwide arrival of substantial metals accomplished 22,000 t (metric/ton) for cadmium, 939,000 t for copper, 783,000 t for lead, and 1,350,000 t for zinc (Singh et al., 2003). The harmfulness of overwhelming metals is a fundamental issue since they have a long ingenuity on the earth. These toxic components stayed in the soil up to over 20 years and extended around the world. Fifty-three components are named substantial metals. Their densities surpass 5 g/cm^3, and they are known as all-inclusive toxins in mechanical territories (Garbisu and Alkorta, 2001). Also, plants' substantial metals ingestion may create harmfulness in human nourishment and subsequently lead to intense and ceaseless sicknesses. For instance, Zn and Cd can cause intense gastrointestinal and respiratory, heart, cerebrum, and kidney harms. Likewise, high centralizations of substantial metals in soil can unfavorably influence crop development on account of physiological and biochemical procedures, a hindrance to so that in any event, prompting the demise of plants (Garbisu and Alkorta, 2001; Schmidt, 2003; Schwartz et al., 2003). Intense poisonous impacts of breathed in Zinc have been accounted for in mechanical laborers presented to zinc exhaust. The side effects incorporate pneumonic trouble, fever, chills, and gastroenteritis. In a little scope concentrate on zinc-treatment facility laborers, no proof was found of expanded mortality from a malignancy. Current traditional techniques to remediate overwhelming metal defiled soil and water, for example, ex-situ unearthing, landfill of the top tainted soils detoxification(Ghosh and Singh, 2005), and Physico-chemical remediation are expensive, time-consuming, labor exhaustive by destroying soil structure and increasing contaminants mobilization (Ghosh and Singh, 2005).

Phytoremediation is a methodology used to detoxify polluted zones by utilizing the plants (Garbisu and Alkorta, 2001). Many plant-based techniques can be used to remediate soil depending upon the site conditions, the level of cleanup required, and the types of plants and phytoremediation technology. Phytoremediation is a minimal effort, long haul, ecologically and stylishly well-disposed strategy for immobilizing/balancing out, corrupting, moving, evacuating (Susarla et al., 2002; Jadia and Fulekar, 2008; Zhang et al., 2010). As a green innovation, it is relevant for various types of natural and inorganic toxins and gives stylish advantages to the earth by planting trees and making green spaces, which are through and through socially and mentally advantageous (Ghosh and Singh, 2005). Heavy metals such as Zinc and arsenic are more toxicants, and they cause major destruction in the environment. In different Pakistan areas, water resources (surface water and groundwater) have been contaminated with heavy metals due to various human activities. Phytoremediation is a low cost, easily understandable, and natural process compared to other conventional methods. There is a need to find the accumulation capacity of different plants for phytoremediation treatment at a low cost. *Tagetes minuta* has not been used to treat wastewater through phytoremediation. This research evaluates how marigold helps in zinc phytoremediation from wastewater and study the growth parameters under zinc metal stress.

2. Materials and Methods

2.1. Plant material

The plant material used for the present study was the Common name **(**marigold**)** local name **(**satbarg**)** belongs to *the* Asteraceae family.

2.2. Plant collection

Plants collection was done from Harnoi Abbottabad. The same height, healthy green plants were collected randomly from the non-contaminated site for heavy metal analysis in this research.

2.3. Growth medium

1.5 kg air-dried nutrient-free Sand (washed with 1 NHCL and washed three times with distilled water). 100 g Plants were measured by using measuring balance were grown in each pot containing 1.5 kg of nutrient-free sand.

2.4. Nutrient medium

Plants were placed in the lab by providing proper sunlight, air, and Hoagland solution. Hoagland's solution was used as a growth medium before experiments and supplied to plants when stored at nursery. It had the following composition (Mirza et al., 2010).

2.5. Pre-analysis

In pre-analysis, different parameters such as chlorophyll analysis, plant height, no. of leaves, root length, etc. was considered.

2.6. Chlorophyll analysis

Chlorophyll was done before and after zinc treatment—new leaves of both of when medicines were gathered for the assurance of Chlorophyll a and b substance. Powder of Fresh leaves (1 g) of various examples was taken in test cylinders, and 10 ml of 80% Acetone arrangement was additionally included. These test tubes were put for the time being for finished extraction. Photosynthetic shades were resolved spectrophotometric ally utilizing the obvious frequencies of 646 and 663 nm for Chlorophyll a and b, separately.

Table 1. Standard estimations of different morphological parameters (root, stem length, and no. of leaves) before zinc treatment.

Treatments	Shoot length (cm)	Root length (cm)	No. leaves
# 1	74±12.1	7±0.6	191±12
# 2	75±7.8	8±0.6	221±8
# 3	70±10	10±1.5	302±5
# 4	90±1.5	10±2.1	339±12
# 5	113±9.5	14±3.6	313±14

2.7. Treatment

Zinc treatments were done at different concentrations. Every concentration has three replicates. Every pot was properly labeled according to given treatments, as shown in the above table. The experiment was placed for 15 days after treatment by providing sunlight and Hoagland's solution daily.

Table 2. Zinc treatment at different concentration in each replicate.

Treatment	(plants / pot) (g)	Zinc (μM/L)	Replicates
# 1	100	0	X,Y,Z
# 2	100	100	X,Y,Z
# 3	100	200	X,Y,Z
# 4	100	300	X,Y,Z
# 5	100	400	X,Y,Z

2.7.1. Parameters

Different parameters of *Tagetes minuta* were concentrated under zinc pressure, including chlorophyll investigation, plant stature (cm), number of leaves per plant, and normal root length, poisonousness manifestations when the zinc medicines.

2.8. Post-analysis

After 15 days of zinc treatment, plants were removed carefully from the sand, washed thoroughly with tap water, and divided into roots and shoots. Chlorophyll analysis and lengths of both roots and shoot were used as an indicator for studying the effect of Zinc on plant growth. The outcomes demonstrate that the root length, root surface region, and root volume of NHE diminished fundamentally with expanding Zn^{2+} fixation in development media, while the root development of HE was not unfavorably influenced, and even advanced, by 500 μmol/L Zn^{2+} (Jadia and Fulekar, 2009).

2.9. Chlorophyll analysis after treatment

1 gram of fresh leaves was taken in a test tube, and 10 ml of 80% acetone placed for 24 h; after this time solution was filtered and checked at 663, 646, and 710 nm.

2.10. Analytical procedures

After a week, the dried plants were weighted to get the dry weight and were separated into roots, leave and stem for grinding. Dry samples were ground into a fine powder using mortar. Then using the $HNO_3/HClO_4$ Plant samples were digested, and 0.5 g of 100 mL conical flask was separated as a sample. At that point, each tapered jar 10 mL of Per-chloric and Nitric corrosive blend (1:2 proportions) was included and left for the time being. The following day, glass pipes were put at the mouth of every flagon, so that channel stem remained, at any rate, one inch over the outside of fluid. The cups were then positioned on a hot plate, and the temperature was continuously expanded to consider successful assimilation. HNO3 volatilized as nitrous oxide exhaust, and afterward, the white vapor of Per-chloric corrosive came out from the cup inside 15 to 20 minutes. The outcome was a white arrangement in the carafe. After absorption, the cups were expelled from the hot plate permitted to cool and barely any refined water milliliters included (Yang et al., 2006). Digestion products were diluted up to 50 ml with distilled water and filtered using the man 42 filter paper. A similar method was used for all 45 samples of roots, shoots one by one. Heavy metals were measured by using Atomic absorption Spectroscopy (Perkin Elmer Model 920).The filtered samples were kept in the sample bottles after maintaining a volume of 50 ml.

3. Results and Discussion

Results show that the accumulation of Zinc was increased in roots, stem, and leaves of *T. minuta* with an increase in Zinc concentration. The concentration of zinc metal in different parts of the plant decreased in order to roots> stem> leaves. The information shows that the grouping of Zn in leaves and stems expanded with expanding Zn gracefully levels. The conveyances of the metals were accounted for as stem> leaf> root for Zn. These outcomes demonstrate that Sedum alfredii has an exceptional capacity to endure Zn poison levels (Xiong et al., 2004). The toxic limit of Zn in the majority of the plant species is 500 mg/kg (Schwartz et al., 2003). However, plants with Zn< 20 mg/kg are considered to be Zn deficient (Schmidt, 2003). Hence, Zn content is considered to be deficient in the majority of plant species. Zinc is an essential element in a specific amount; however, Zinc's higher concentration causes toxic effects in a living organism (Jadia and Fulekar, 2009).

Table 3. Zinc accumulation in each part of the plant according to the given treatment.

Treatment (μM)	Root (mg)	Stem (mg)	Leaves (mg)
0	5.67	4.81	3.3
100	7.93	4.91	4.8
200	9.87	7.3	5.77
300	13	8.2	6.4
400	17.37	9.33	8.37

3.1. Effect of zinc concentration on roots growth

The Zinc Concentration in the *Tagetes minuta* plant has been studying at the lab scale. Fig. 1 shows the accumulation of Zn in roots of *Tagetes minuta* and its effects on plant growth (Farooq et al., 2010). The minimum Concentration of Zinc found in plant roots was about 5.67 mg/g in control, while its concentration increases by increasing given treatments. As zinc treatment increased in plant roots, the maximum zinc concentration was found on 400 µM was about 17.3 mg/g. zinc accumulation in roots is showing in the Fig. 1.

The root morphology and Zn^{2+} take-up energy of HE and NHE of S. alfredii were explored utilizing hydroponic strategies and the radiotracer transition technique (Jadia and Fulekar, 2009). The outcomes show that the root length, root surface region, and root volume of NHE diminished altogether with expanding Zn^{2+} fixation in development media, while the root development of HE was not unfavorably influenced, and even advanced, by 500 µmol/L Zn^{2+}. The groupings of Zn^{2+} in both ecotypes of S. alfredii. Shown a positive relation with root volumes, root length, and root surface area. However, no positive correlation was reported between concentrations of Zn^{2+} and root diameter. A fast direct stage portrayed the take-up energy for 65Zn2+ in the underlying foundations of both ecotypes of S. alfredii during the initial 6 h and a more slow straight stage during the resulting time of examination. The Michaelis-Menten condition could describe the fixation subordinate for S. alfredii ecotypes. The V max (most extreme take-up speed) for 65Zn2+ flood is 3-crease more noteworthy in the HE than in the NHE. To be sure, this demonstrates expanded assimilation of the root was one of the instruments of Zn hyper gathering. An altogether bigger V max esteem recommended a higher thickness of Zn transporters per unit film zone in HE roots (Jadia and Fulekar, 2009).

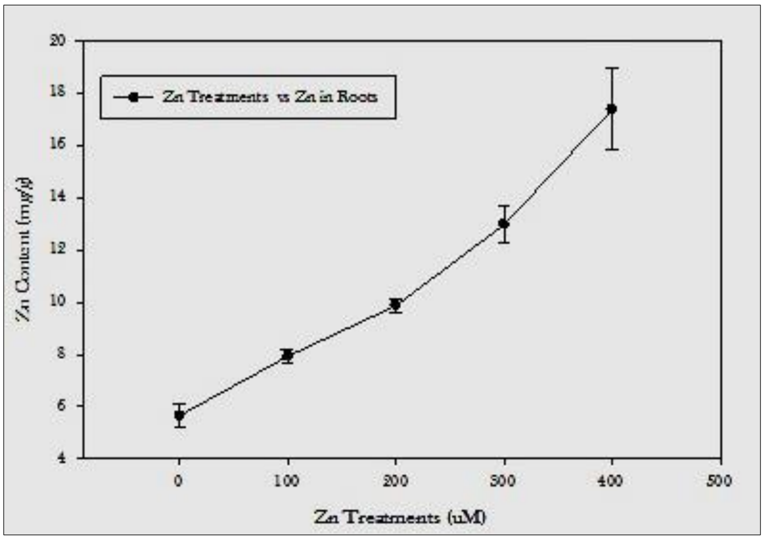

Fig. 1. Zn Accumulation in Roots of Treated Plants.

3.2. Zinc accumulations in plant stem

Zinc accumulation in plant stems at every treatment founded to be very clear, as shown in Fig. 2. In controlled treatment, zinc concentration was found 4.81, and 4.91 mg/g found at 100 µM. It shows that 4.81 mg/g zinc was present in plant stem naturally because it is essential for plant growth, almost 0.1 mg/g zinc accumulated by plant stem at 100 µM. As zinc concentration in treatment increased, Zinc in plant stem also increased at 400 µM maximum zinc observed in plant stem i-e 9.33 mg/g, if 4.81 was present naturally, then accumulated. Zinc was about 4.52mg/g in the plant stem. Zinc compartmentation contemplates including hyper accumulating, and non-hyper accumulating S. alfredii plants exhibited that examined plant can gather Zn in shoots over 2% of dry weight utilizing radioactive tracer transition procedure. Zn concentrations in Leaf and stem in hyper accumulating ecotype (HE) were 24, and 28 fold higher than NHE, respectively. Also, 1.4 fold more Zn was accumulated in the roots of the NHE. In non-hyper accumulating, in the root vacuoles, Zn accumulation was 2.7 fold more than HE.

3.3. Zinc accumulation in plant leaves

Zinc found in plant leaves naturally 3.3 mg/g, at 100 µM of Zinc, was found in plant leaves 4.80. This result showed that almost 1.5 mg/g zinc accumulated by plant leaves. Similarly, at 400 µM, Zinc found in plant stem was 8.37 mg/g. If 3.3 mg/g naturally existed in the plant stem, then 5.07 mg/g zinc up taken by plant leaves. As indicated by results, Zn fixations in the leaves and HE's stems were 34 and multiple times higher, though Pb focuses were 1.9 and 2.4 occasions higher, separately than those of the NHE (Jadia and Fulekar, 2009).

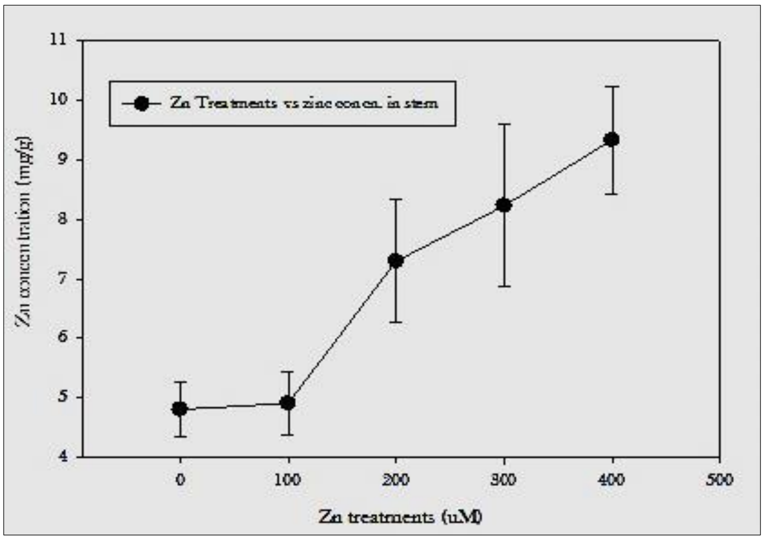

Fig. 2. Zinc Accumulation in the Stem of Treated Plants.

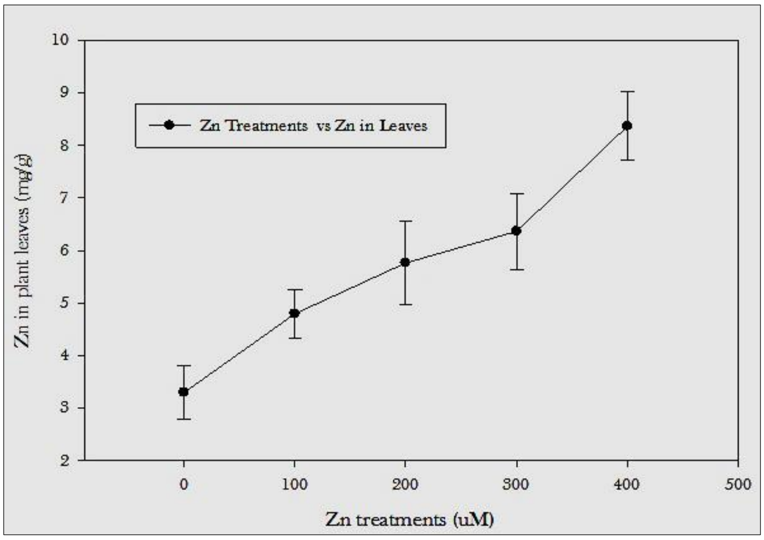

Fig. 3. Zn Accumulation in Levels of Treated Plants.

3.4. Effect of zinc on photosynthetic pigments

The effects of Zinc on the photosynthetic pigments, i.e., total Chlorophyll before treatment and total Chlorophyll after treatment, were presented in Fig. 4. Before zinc treatment, the total chlorophyll content observed between 21 to 25 mg/g was higher than the total chlorophyll content after zinc treatment range about 13 to 16 mg/g. chlorophyll efficiency was decreased after zinc accumulation by the plant. After zinc treatment, chlorophyll efficiency decreased as zinc concentration was increased. Fig. 3 shows that Zn's high collection influenced the plant chlorophyll. Plants assimilate harmful metals; they impervious to the pollution, translocate and amass it into their underlying foundations and leaves, and tidy up soils and water (Jadia and Fulekar, 2009). Phytoremediation is given as of late specific consideration in ecological investigations because of its positive effects, including financially savvy, natural cordial purposes, and the probability of reaping the plants for the extraction of assimilated contaminants, for example, metals that can't be effortlessly biodegraded for reusing among others (Mirza et al., 2010).

The phytoextraction capacity of *T. minuta* was evaluated, utilizing both the translocation factor (TF) and the bioaccumulation factor (BF) as follows. In light of point by point, concentrates substantial metals known to meddle with chlorophyll combination either through the direct hindrance of an enzymatic advance or initiate lack of a fundamental supplement (Lone et al., 2008).

The chlorophyll content is an essential factor in determining the photosynthesis intensity in plant leaves. Zinc is one of the most poisonous metals, and because of its versatility, it handily consumes by roots and can cause substantial harm to plants (Brekken and Steinnes, 2004).

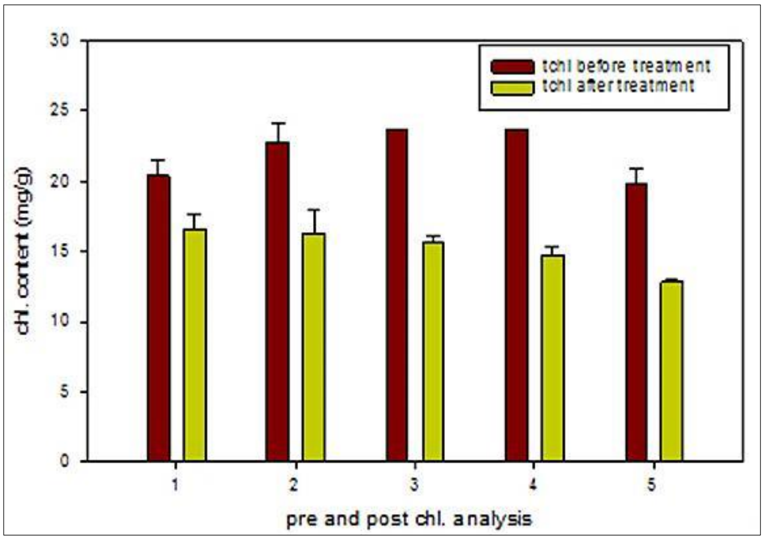

Fig. 4. Chlorophyll Content before and after Zinc Treatment.

3.5. Bioaccumulation factor

Bioaccumulation refers to the total conc. of metal that has been stored or trapped within different parts of the plants in the bioaccumulation factor (concentration in plant/habitat> 1) (Mirza et al., 2010; Wei and Chen, 2006).

$$BF = Zn\ conc. in\ shoots\ /Zn\ conc. in\ solution \quad (1)$$

BF value was in the range of 0 to 4.5 mg/g for various Zn treatments. As treatments increased, the bioaccumulation factor also increased. The highest value of BF 4.5 at 400 µM zinc treatment. Heavy metals Pb, Zn, Mn show greater affinity towards bioaccumulation in their study. Bio magnifications occur due to a higher concentration of heavy metals in plants. *Eichhorniacrassipes* is the unique property to accumulate heavy metals Cd, Cu, Pb, and Zn from the plant's root tissues.

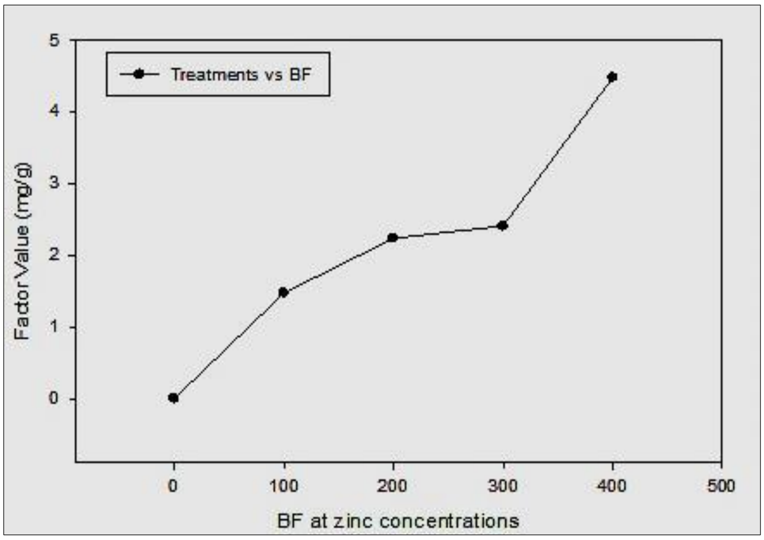

Fig. 5. Bioaccumulation Factor of the Whole Plant.

3.6. Translocation factor

Any substance or metal movement from root to stem and to leaves through plant transportation mechanism is called translocation. The translocation factor calculated by applying the following formula:

Translocation Factor TF = Cr conc. in shoots/ Cr conc. in roots (2)

In translocation factor (concentration in shoots/roots >1), metal concentrations in the shoots of a plant should be higher than those in the roots (Mirza et al., 2010). TF values were in the range of 0 to 1.2 for various Zn treatments. The highest amount of TC was found at 200 µM, which was about 0.2 mg/g. Data shows that the higher zinc translocation was done at 200 µM after that; plant growth affected by zinc and zinc translocation results reduced with increasing zinc concentration. Also, some toxicity symptoms appeared in the plants receiving that Zn treatment.

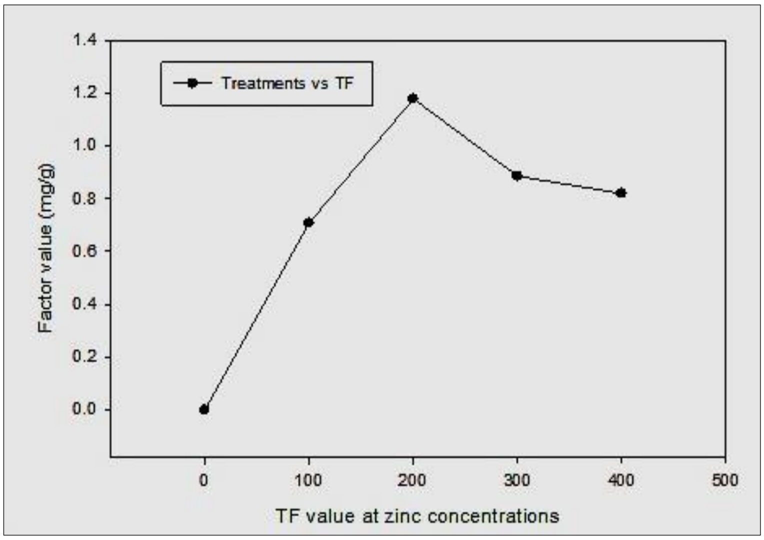

Fig. 6. Translocation Factor at Different Concentrations of Zinc in the Whole Plant.

4. Conclusions

Phytoremediation is one new cleanup idea that includes plants' utilization to clean defiled conditions, for example, soil and water. After this study, it concluded that *T. minuta* has an accumulation capacity of Zinc from wastewater. Maximum Zinc (17.37 mg/g) accumulated in plant roots at 400 µM concentration. In-plant stem zinc was observed at about (9.33 mg/g) and leaves (8.37 mg/g). Both bioaccumulation factor and translocation factor esteem for hydroponics were more prominent than those who recommended that the plant be a valuable material for remediation of Zn polluted conditions. The range of BF value observed (1.5 to 4.5) shows the *T.minuta* is useful for remediation of Zinc contaminate sites. While TF value was observed in the range of (0.7 to 1.2), zinc metal was easily transferred to other parts of the plant. Zinc metal is toxic for the plant at higher concentrations because, at the end of the experiment, leaves dried, and the translocation factor also decreased at 300 and 400 µM of zinc metal.

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