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# Sunflower *(Helianthus annuus)* for Phytoremediation of Zinc in Hydroponic System

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## ABSTRACT

*It has been noted that zinc contamination is hazardous, which induces researchers to seek new means to overcome it. One of the methods is to employ the sunflower plant to eliminate zinc in soil. However, there is insufficient information about zinc phytoremediation by sunflowers in the hydroponic system. Hence in this study, a 15-day experiment was conducted using zinc concentrations of 0, 10, 20, and 30 mg/L in a hydroponic system to investigate the effect of zinc towards the sunflower. The effects of zinc concentration on the plant growth performance (length of root, length of the stem, and the number of leaf), zinc uptake, and zinc translocation were evaluated. The findings showed that the plant growth was stunted but tolerated to a zinc stress condition, where the zinc concentrations had affected the growth of the sunflower root, length, and stem, and the zinc uptake significantly ($p<0.05$). It was also found that there was a significant variation of root length and zinc uptake in leaf within certain phases statistically ($p<0.05$). Then the translocation factor was found significantly*

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*different for the time parameter but not the zinc concentrations (p<0.05). Therefore, this experiment concluded that the sunflower plant was highly tolerant of zinc and able to remove the zinc from contaminated environments. Lastly, this study showed that sunflowers are the potential to phytoremediate zinc in a hydroponic system.*

**Keywords**: *Helianthus annuus, phytoremediation, hydroponic, zinc*

## INTRODUCTION

Zinc is an essential element to plants as it involves actively in the plants' protein synthesis and metabolism. The zinc activates the RNA polymerase in protein synthesis and acts as a co-factor and activator for enzymes [1]. The toxicology of heavy metal depends on the concentration in the environment and its mobility characteristic. Unlike organic compounds, heavy metal is non-biodegradable and persistent in the environment. Thus, the removal of heavy metal from contaminated sources is particularly challenging. Current practices for heavy metal remediation such as chemical precipitation, activated carbon and resin are costly, and generate secondary by-products. As a result, researchers and authorities have focussed on phytoremediation, a plant-based technology, to remediate heavy metal from contaminated sources.

Phytoremediation relies on the plant capacity to absorb heavy metal from the environment. The translocation factor is used as a tool to assess the potential of plants to remediate metals from contaminated sources. Particularly, it evaluates the capacity of plants to transfer metals from their roots to the upper parts. Having values of translocation higher than 1 shows that the plant is a reliable candidate for phytoremediation [2].

Weeds, sunflower, cabbage, canola, mustard, alfalfa, and ricinus are potential phytoremediation plants. It has also been found that the sunflower *(Helianthus annuus)* is an excellent candidate for phytoremediation. The sunflower plant remediates heavy metal, and the harvested sunflower for biofuel production is a sustainable approach [3-4]. The phytoremediation mechanisms mainly involve phytoextraction, phytotransformation, and translocation. Besides, rhizobacteria of sunflowers also improve phytoremediation performance and enhance the accumulation of metals [5].

Sunflower has been widely reported as a phytoremediation plant for soil and water contaminated with zinc. Studies [6-7] reported that sunflower capable of removing zinc from the soil-water environment, hence suitable to be used as a phytoremediation plant. Garousi *et al*. (2016) [8] found that sunflower is the right candidate in the phytoextraction and phytoaccumulation of selenium in the hydroponic system. However, there is limited study on sunflower remediating zinc in the hydroponic system. This study aimed to investigate the effect of zinc on the growth of the sunflower *(Helianthus annuus)* as well as its uptake and translocation of zinc in the tested plant.

## METHODOLOGY

### Plant Preparation and Hydroponic System Set-Up

First, sunflower seeds were obtained from the market. They were washed and rinsed with distilled water a few times. The seeds were then each placed in a hydroponic nutrient solution pot to grow. The seedlings were left to grow for three weeks under 14/10 hours of light/dark cycle. Meanwhile, the nutrient solution was aerated continuously by an air pump and renewed once a week. The deep culture hydroponic system was used. The container consists of 30 pots, and one seed was put in each pot.

### Zinc Solution Preparation

A litre of 1000 mg/L zinc solution was prepared from zinc sulphate heptahydrate salt to serve as a zinc stock solution. This stock was diluted to 10, 20, and 30 mg/L zinc concentrations.

## Phytoremediation Study

In this phase, the seeding plants were transferred to containers that contained zinc concentrations of 0 (control), 10, 20, and 30 mg/L zinc, respectively. Three replicates of the samples were executed for each treatment. During the treatment, observation on the length of root, stem, and the number of the leaf under the stress of zinc was recorded at a five-day interval for 15 days. The length of root and stem were measured using vernier caliper; meanwhile, the number of leaf was count visually. The average was calculated. The percentage of the inhibition of the plant growth was compared to the control. The calculated standard deviation of less than 10 was neglected.

During the treatment of zinc, three plants from each container were removed from the treatments every five-day interval for 15 days. Their roots, stems, and leaf were digested according to the EPA method 3050B [9-10]. The zinc concentrations were then determined by Atomic Absorption Spectrometry (AAS). The uptake of zinc was recorded. The phytoextraction ability of sunflower *Helianthus annuus* was calculated using the translocation factor (TF) as follows:

$$TF = \frac{concentration\ of\ zinc\ in\ aerial\ part\ of\ plant\ (stem\ and\ leave)}{concentration\ of\ zinc\ in\ root}$$

## Statistical Analysis

The experimental data were expressed as means and standard deviations. They were further analysed by one-way analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

The effects of zinc on the sunflower *Helianthus annuus* were evaluated by observing the plant growth performance, zinc uptake, and zinc translocation factor. It then was observed that the length of roots, stems, and the number of leaves in the sample experienced a significant reduction under zinc contamination compared to the control. Following, the zinc translocation factor was calculated to determine if this plant was potential for zinc bioaccumulation.

### The Growth Performance of the Sunflower Plants

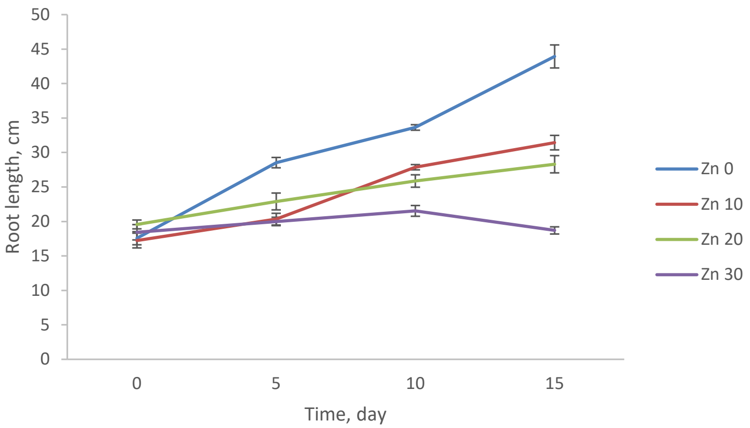
Figure 1(a) shows the root growth performance under zinc stress at different concentrations for 15 days. Results showed that at 10 mg/L, the root lengths were 17.23 ± 1.07 cm, 20.33 ± 0.85 cm, 27.87 ± 0.38 cm and 31.43 ± 1.05 cm on days 0, 5, 15, and 15, respectively. These indicate the low toxicity of zinc in this range of concentrations. However, at the treatment of 30 mg/L of zinc concentration, it initially promoted the root growth with the root length of 18.43 ± 1.11 cm, 20.00 ± 0.61 cm, and 21.53 ± 0.78 cm for day 0, 5 and 10, respectively. However, there was a reduction of root growth on day 15 (18.70 ± 0.52 cm), showing the toxic effects of zinc.

Figure 1(b) shows the percentage inhibition of the root length of the sunflower plant compared to the control. On day 15, the figure showed the highest root length inhibition, which is 99 % for the 30 mg/L zinc concentration. It is followed by 67% for 20 mg/L zinc concentration and 46% for 10 mg/L of zinc concentration. The concentration of zinc shows a significant effect on root length inhibition statistically ($p<0.05$).

The root elongation is an essential factor in metals removal in the hydroponic system [11]. Roots help plants to absorb water and nutrient from the substrate, transport through the stem to leaf for photosynthesis. Rhizobacteria of sunflower promote the growth of root under metals stress conditions and induce stress tolerance in plants [5]. Hence, sunflower root and root hair elongation occurred; the absorption surface area and absorption of water and nutrient, including zinc, increased.

It can be reported that a reduction of the number of root hair and growth root length was observed at 30 mg/L zinc concentration (Figure 1(b)). This indicates the zinc concentration had caused root toxicity symptoms. This result concurred with that reported by [12], which had a similar observation on the sunflower under zinc stress conditions in the soil.

(a)

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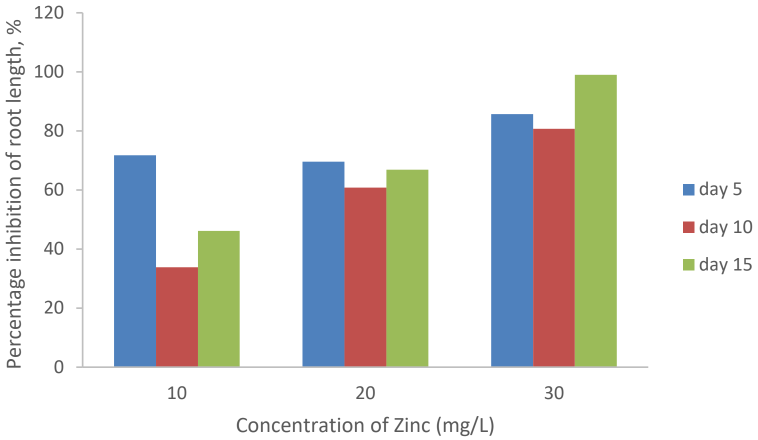


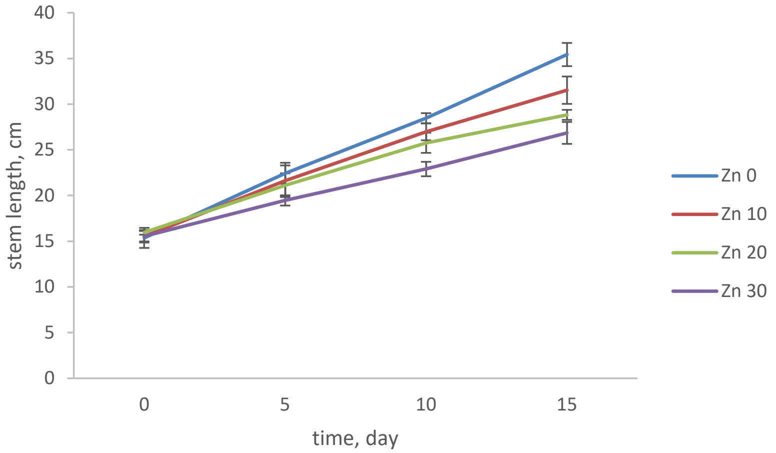

**Figure 1: Effect of Zinc Concentration on (a) Root Length and (b) Inhibition of Root Length in 15 days**

Figure 2(a) shows the stem length at different zinc concentrations. The stem length is statistical significantly under the influence of zinc concentrations for the entire 15 days of treatment. Overall, the stem length at 10 mg/L zinc concentration recorded the highest length where 15.53 ± 0.67 cm, 21.63 ± 1.66 cm, 26.97 ± 0.92 cm and 31.53 ± 1.50 cm on days 0, 5, 10 and 15, respectively. However, all three treated plants in three concentrations (10, 20, and 30 mg/L) have not exceeded the growth of the stems in the control group (0 mg/L). At concentrations of 10, 20 and 30 mg/L on day 15, the stem lengths were 31.53 ± 1.50 cm, 28.83 ± 0.55 cm and 26.85 ± 1.20 cm, respectively, compared to the control (35.43 ± 1.27 cm).

The inhibition of the stem length under the influence of zinc concentration is illustrated in Figure 2(b). Figure 2(b) shows the stem length is most inhibited, which is 44 %, at 30 mg/L of zinc concentration on day 15. Secondly, the 20 mg/L and 10 mg/L zinc concentration inhibits 36 % and 20 % stem length, respectively. There is a significant difference among the concentrations of zinc on the stem length ($p<0.05$).

It can be discussed that a low concentration of zinc serves as a micronutrient which promotes the growth of plants [13]. However, large doses cause of metal hormesis will stunt the growth of the shoot. Sunflower is efficiently transferring metals from roots to other parts of the plant [14]. Furthermore, metal stress conditions reduced chlorophyll and protein contents [15]. Hence, the length of the stem was inhibited.

(a)




(b)

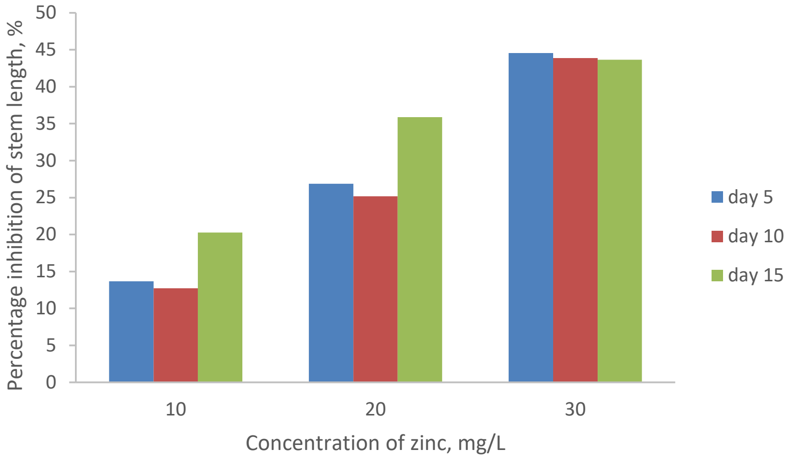


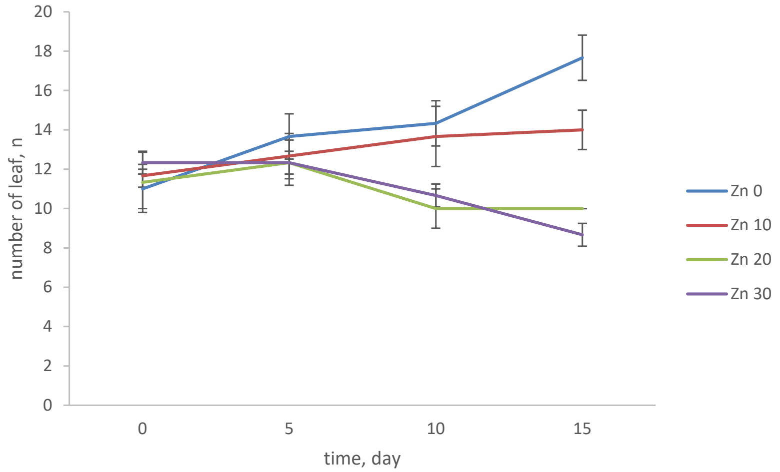

**Figure 2: The Effect of Zinc Concentration on (a) Stem Length and (b) the Percentage Inhibition of Stem Length in 15 days**

Figure 3(a) illustrates the number of leaf under the effect of zinc concentrations in 15 days. At the 0 mg/L and 10 mg/L zinc concentration, the number of leaf increased during treatment. But at 20 mg/L, the number of leaf decreased and recorded 12 ± 1, 10 ± 1, and 10 ± 0 on days 5, 10, and 15, respectively. At concentrations 30 mg/L, the number of the leaf also decreased, which was 12 ± 1, 11 ± 1, and 9 ± 1 on days 5, 10, and 15, respectively.

The effect of zinc concentrations on the leaf number compared to the control is shown in Figure 3. Generally, an increase of zinc concentration decreases the leaf number of the plants. The highest leaf number inhibition was found to be 155 % recorded by 30 mg/L zinc concentration on day 15. The concentration of zinc shows a significant difference in the percentage of inhibition of the leaf number ($p<0.05$).

It was observed too that the leaf chlorosis and browning occurred especially at a high concentration of zinc. This was due to the zinc ions exhibiting high mobility characteristics, which leads to a strong damaging effect on the leaf cell membranes. Besides, various metals binding proteins such as metallotheiones, phytochelations, and antioxidant enzymes enhanced the accumulation of metals through absorption and transportation mechanisms [15]. Al-Jobori and Kadhim (2019) [16] described that the sunflower *Helianthus annuus* showed stunted growth and a reduced leaf expansion compared to stem and root. This observation was supported by [17], where lead, cadmium, and nickel ions showing the sequence of plant growth as root, shoot, and finally leaf for reed.

(a)




(b)

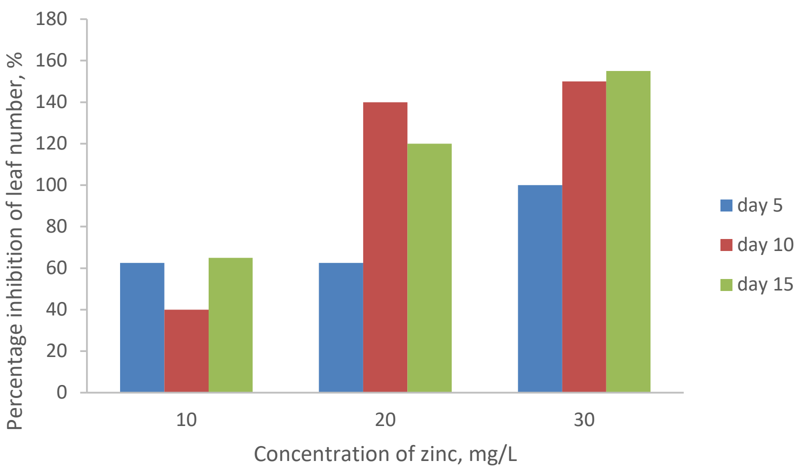



**Figure 3: The Effect of Zinc Concentration on (a) the Leaf Number and (b) Percentage Inhibition of Leaf Number in 15 Days**

Figures 1(a), 2(a) and 3(a) display the effect of time of the zinc concentration on the root growth, stem length, and the number of leaf. In general, the plants in the sample grew over time but did slower than control. It can be explained that the plants reduce the concentration of metal entering the cell through their extracellular precipitation, biosorption to cell walls, thus reducing the uptake or increase the efflux [18]. These mechanisms successfully reduce the effect of toxicity sensitive sites within the cells of the plants and thus preventing the damaging effects and maintaining the homeostasis of the plant [19]. There are no accurate reports on metals accumulation and distribution in sunflower plants as they involve several endogenous and exogenous factors.

In the experiment of the present study, the length of the root and the number of leaf were reduced at 30 mg/L zinc on day 15. It was also observed that the chlorosis of the leaf occurred. The statistical analysis shows a significant difference for the length of root ($p$<0.05), but not significant for the stem and number of the leaf ($p$>0.05). The plant root length is a well-known, established measure to determine the metal-tolerant ability of plants. It is known that the root is a specialised absorptive organ that has a direct response and bio-accumulating ability on toxic metals. Therefore, the effect of zinc over time becomes significant only on the root, but not on the stem and leaf. Hwang *et al* (2016) [11] also demonstrated a consistent result in their study using fern *Pteris vittata* and arsenic in a hydroponic system. Besides, [20] reported similar findings where zinc accumulation in the shoot of sunflower *Helianthus annuus* in the hydroponic system was observed.

## Zinc Uptake

Figure 4 illustrates the zinc uptake for root, stem, and leaf under the effect of zinc concentration. It was found that increased zinc concentration increased in zinc uptake in root, stem, and leaf. For roots and leaf, the zinc uptake kept increasing for the concentration of 0 mg/L and 10 mg/L throughout the treatment period. At 10 mg/L, the zinc uptake in roots were 50.473 ± 1.16 mg/L, 50.720 ± 1.17 mg/L, and 55.807 ± 0.54 mg/L for day 5, 10 and 15, respectively. The zinc uptake in leaf were 31.030 ± 0.38 mg/L, 31.747 ± 0.61, 35.153 ± 0.62 mg/L on day 5, 10 and 15, respectively. However, at a concentration of 20 mg/L and 30 mg/L, the uptake decreased between day 10 to day 15. At 30 mg/L, the zinc uptake in roots were 63.103 ± 1.76 mg/L, 75.070 ± 1.22 mg/L and 62.127 ± 1.41 mg/L on day 5, 10 and 15, respectively. The zinc uptake in leaf were 35.563 ± 0.45 mg/L, 42.130 ± 0.37 mg/L and 36.550 ± 0.91 mg/L for day 0, 10 and 15, respectively. For stems, the uptake of zinc increased in all concentration across the treatment period were at 20 mg/L, the zinc uptake in stem increased as much as 55.603 ± 1.12 mg/L, 70.807 ± 0.83 mg/L, and 82.337 ± 1.52 mg/L on day 5, 10 and 15, respectively.

Statistically, there is a significant difference in the effects of zinc concentration on root zinc uptake ($p$<0.05). However, uptake of zinc in stem and leaf exhibits no significant difference among zinc concentration treatment ($p$>0.05). One of the explanations for root to be more responsive to toxic metals in the environment might be that root was the specialised absorptive organ. It contacted directly with metals and subjected to the accumulation of more metals than any of the other organs. Also, a high concentration of zinc led to high concentration gradient and osmotic pressure; thus, the uptake of zinc and accumulation of zinc increased and caused root cell wall membrane damage [21]. For the hydroponic system, the plant with high metals uptake in the root is considered as a strong excluder for stabilising metals [22]. [23] also reported that the highest accumulation of metals in the hydroponic system is the root section. When an increase of zinc concentration, similar consistent findings for increased metals uptake of wetland plants was reported by [24-25] supported this study by stating the concentration of copper in sunflower root was five times higher compared to shoots and leaves. These are the main reasons that root length was usually used as a measure for determining the metal-tolerant ability of plants.

For time factor, Figure 5 also demonstrates uptake of zinc in root, stem, and leaf under the influence of different concentrations of zinc over time. Generally, an increasing trend in uptake of zinc in the plant was observed over time except at day 15 for 30 mg/L root zinc uptake as well as 20 and 30 mg/L leaf zinc uptake. Statistical result based on time factor shows significant zinc uptake in leaf ($p$<0.05) but not significant for root and stem ($p$<0.05). Bioaccumulation of zinc occurs over time and finally effects the final receiver, which is the leaf. Chlorosis and drop of leaf were observed revealed that leaf bioaccumulation of zinc ability decreased. This phenomenon was supported by Figure 4(c) and [26].

Besides concentration and time, the uptake and bioaccumulation of zinc by plants also depends on the plant species, type of metals, and the metals tolerance level by the plant parts.  This is due to these processes, which are affected by several physiological plant factors such as zinc uptake, xylem translocation from root to leaf, and the sequestration of metals. Niu *et al*. (2007) [27] reported the different responses or tolerance of plants towards metal types and concentrations.

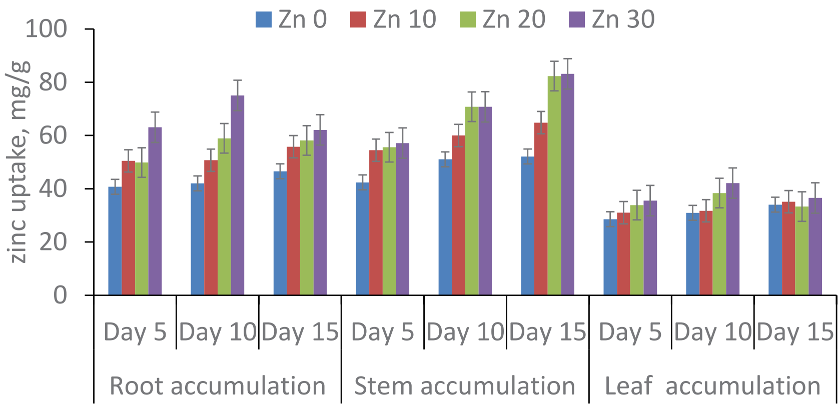



**Figure 4: The Effect of Zinc Concentration on the Uptake of Zinc in Root, Stem, and Leaf in 15 Days**

## Zinc Translocation Factor

Table 1 shows the zinc translocation factor in sunflower H*elianthus annuus*. The maximum value of the transfer factor is 1.9897 ± 0.0530 at 20 mg/L zinc concentration on day 15, and the minimum value is 1.4674 ± 0.0489 at 30 mg/L zinc concentration on day 5. Overall, the results show translocation of the factor higher than 1 in all samples. [28] and [2] also reported a similar result as in the present study. [24] recorded the same highest transfer factor at a concentration of 20 mg/L zinc for wetland plant *Typha angustifolia* in experiment zinc concentration ranging from 0 – 100 mg/L.

Statistical analysis shows a significant difference for the time factor ($p<0.05$) but not for the zinc concentration ($p>0.05$). This indicates that the sunflower *Helianthus annuus* has effectively translocated the zinc from the root to the leaf. The transportation across root the cellular membrane is a crucial means which initiates the metal uptake into plant tissues. The movement of the zinc from root to leaf and shoot occurs through the xylem driven by a high transpiration rate [19]. As zinc has high mobility and is an essential metal to plant photosynthesis, it is translocated easily over time. Besides, zinc may be translocated as it is essential to the plant metalloenzymes and photosynthesis [30].

The other factors contributing to the metals uptake, accumulation, and distribution in the different plant parts are the anatomical, biochemical and physical factors. Furthermore, a complex interaction between metals and nutrient elements exhibit synergistic or antagonistic effects on the sunflower plant [7]. Clemens (2006) [31] highlighted that the uptake, sequestration, and translocation of metal in the root of the plant and then storing them mainly in the vacuoles through metal binding peptides, which called phytochelatins (PCs). These processes occur as the levels of the PCs in *Helianthus annuus* increase as the metal level and time of exposure increase. The PCs are also involved in the homeostasis of zinc and Cu by providing transient storage for the ions. Considering the results of this study, the variation of the zinc uptakes by the different parts of the plants could be due to a series of defense mechanisms such as their PCs production, expressed by it to avoid toxicity. Hence, in conclusion, the sunflower *Helianthus annuus* is a potential candidate to be used in phytoremediation due to its high phytoextraction ability.

**Table 1: Zinc Translocation Factor in the Sunflower *Helianthus annuus***

| Zinc concentration, mg/L | Translocation factor (aerial/ root) | | |
|---|---|---|---|
| | Day 5 | Day 10 | Day 15 |
| 0 | 1.7428 ± 0.0285 | 1.9519 ± 0.0356 | 1.7826 ± 0.1808 |
| 10 | 1.6946 ± 0.0247 | 1.8094 ± 0.0308 | 1.7923 ± 0.0352 |
| 20 | 1.7969 ± 0.0849 | 1.8529 ± 0.0371 | 1.9897 ± 0.0530 |
| 30 | 1.4674 ± 0.0489 | 1.5044 ± 0.0451 | 1.9278 ± 0.0615 |

## CONCLUSION

It can be concluded that the sunflower *Helianthus annuus* can be an efficient phytoextractor plant. The zinc concentrations showed that inhibition of plant growth morphological parameters and root zinc uptake were significantly different. The length of the root and leaf zinc uptake was decreased significantly over time under being placed in several zinc stress conditions. The translocation factor, value higher than 1, indicated that the sunflower had performed well to phyto-extract the zinc ion. Therefore, the sunflower plant is a suitable plant to remediate zinc from contaminated sources. Further study on zinc-induced oxidative stress should be highlighted to elucidate the phytoremediation mechanisms pathway.

## ACKNOWLEDGEMENT

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