Proceedings Book of
9th ICEEE – 2018 International Conference on
"Climatic Change and Environmental (Bio) Engineering"
22nd - 24th of November, 2018
Budapest, Hungary
ISBN 978-963-449-105-7 **Publisher: ICEEE, RKK Óbuda University**

TOXIC EFFECTS OF HEAVY METALS (CU, ZN, PB, CD) ON EARLY GROWTH OF THREE TAGETES SPECIES

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Abstract

Objective: Heavy metals in soil pose potential threats to the environment, therefore remediation of heavy metal contaminated sites is an important issue. Tagetes species have been proposed as potential plants for phytoremediation of heavy metal contaminated soil. Although much research has been carried out previously to investigate the bioaccumulation ability of Tagetes species, little information is available on the toxicity of metals on these plants. Therefore, our objective was to investigate the toxic effects of heavy metals on these plants. Methods: In our study a seed germination test was conducted to measure the toxic effects of four heavy metals (Cu, Zn, Pb and Cd) on early growth of three different Tagetes species (Tagetes erecta, Tagetes patula and Tagetes tenuifolia). Results: Our results showed that all tested heavy metals had significant ($p<0.05$) toxic effects on seed germination and root/shoot elongation of the three plants. On the basis of IC_{50} values (concentration of a heavy metal which causes 50% inhibition) the following series of phytotoxicity was observed: Cd>Cu>Zn>Pb. Tagetes tenuifolia was the most sensitive plant to heavy metals, while Tagetes erecta and Tagetes patula were able to tolerate low concentration of metals (below 400 mg l^{-1} Cu, Zn, Pb, and below 16 mg l^{-1} Cd) without considerable decline in the measured growth parameters. However, our experiment was carried out under laboratory conditions, and the seeds were germinated in hydroponic solution, which means that these values could be much higher in natural soils. Conclusion: Our results indicate that Tagetes erecta and Tagetes patula could be suitable for remediating moderately heavy metal (Cu, Zn, Pb and Cd) contaminated soils. With the advantage that these plants can also beautify the environment, using them for phytoremediation has an important and practical significance.

Keywords: *heavy metals, phytotoxicity, Tagetes erecta, Tagetes patula, Tagetes tenuifolia, phytoremediation*

1. INTRODUCTION

Numerous studies have shown that heavy metals in soil pose potential threats to the environment [1-4]. Pollution sources of heavy metals mainly derive from anthropogenic sources such as agriculture, urbanization, industrialization, and mining [4-8]. Some heavy metals such as Zn and Cu are essential elements for many physiological progresses in low quantities, while others like Cd and Pb are without known biological function.

In excessive concentrations both essential and non-essential metals can be toxic to living organisms and endanger the health of humans and animals through the food chain [2, 3, 7, 9, 10]. In addition, heavy metals cannot be chemically or biologically degraded as organic pollutants, therefore they can be accumulated at relatively high concentrations in the topsoil [2, 11]. For these reasons, increasing attention has been paid in recent years to the remediation of heavy metal contaminated soils [10-12].

Phytoremediation is proposed as a cost-effective, environmental friendly and sustainable technique for restoration of these sites [13-15]. In recent years much research has been conducted to investigate the phytoremediation ability of ornamental plants [10, 12-14, 16, 17]. These plants have some advantages compared to other kinds of remediation plants: 1. They are apart from the food chain; 2. They can beautify the environment; 3. they usually have high biomass; and 4. ornamental plant industry improve new plant varieties with great stress tolerance and disease resistance [10, 12, 17, 18] Therefore, soil remediation with ornamental plants could be useful, especially in contaminated urban areas, where people have greater environmental requirements [10, 12].

Marigolds have been proposed as potential plants for phytoremediation of heavy metal polluted areas [19-21]. In most of the experiments common marigold (*Calendula officinalis*) were used as a test plant, but remediation potential of *Tagetes* species were also investigated.

Previous studies shown that African marigold (*Tagetes erecta*) can be utilized for the remediation of soils polluted by Cd, because it can accumulate high Cd content in its above-ground tissues [14, 21-24]. In Goswami & Das's (2017) study *T. erecta* accumulated Cd in the range 1719 to 3519 mg kg^{-1} dry weight, which is far above the average toxic (5–10 mg Cd kg^{-1}) ranges in other plants [21, 25]. According to other studies French marigold (*Tagetes patula*) can also hyperaccumulate Cd from combined contaminated soils [26, 27]. *T. erecta* and *T. patula* have also shown quite good capability to accumulate Pb [23, 28, 29]. According to Shah et al. (2017) Pb accumulation potential of *T. erecta* is higher at lower concentrations of Pb [29]. Afrousheh et al. (2015) classified *T. erecta* as a Cu tolerant species [30]. *T. erecta* can accumulate Cu within 2438 to 3767 mg kg^{-1} dry weight, which is far beyond the toxic (20–100 mg Cu kg^{-1}) ranges in other plants [21, 25]. According to Castillo et al. (2011) *T. erecta* colonized with *Glomus intraradices* can potentially phytostabilize Cu in contaminated soils [31]. *T. patula* also can accumulate Cu in its root tissues [26, 28]. Other studies have also revealed that *Tagetes* species bioaccumulate Zn, Cr, and Fe in its tissues [20, 24, 26].

High levels of heavy metals have the potential to become toxic to plants [7, 25, 32]. Much research has been conducted to investigate the bioaccumulation ability of *Tagetes* species, however, little information is available on the toxicity of heavy metals on these plants. According to Lal et al. (2008) and Goswami & Das (2017) both Cd and Cu stress reduce *T. erecta* biomass, which is unfavorable at phytoremediation [14, 21]. Wang & Zhou (2005) observed that 10 mg l^{-1} Cd in hydroponic solution had an obvious toxic effect on the root elongation of *T. erecta*, however it had little effects on seed germination and shoot elongation [16]. According to Shah et al. (2017) Pb accumulation *in T. erecta* had a very less negative effect on its growth parameters [29].

Seed germination tests are widely used to assess acute toxicity effects of different chemicals. Besides the germination rates of seeds, short-term shoot and root elongation are also often measured. The aim of this study was to compare the effects of selected metals on seed germination and shoot/root elongation of African marigold (*Tagetes erecta*), French marigold (*Tagetes patula*), and Signet marigold (*Tagetes tenuifolia*). In addition, we would like to determine which *Tagetes* species can tolerate the greatest amounts of heavy metals, and we would like to assess what is the highest amounts of metals which can be tolerated by tested plants without considerable decline in germination and shoot/root elongation. Results of this study provide new information for using *Tagetes* species to remediate heavy metal contaminated soil.

2. Materials and methods

Three different marigold species were used in the experiment: African marigold (*Tagetes erecta*), French marigold (*Tagetes patula*) and Signet marigold (*Tagetes tenuifolia*). Seeds were obtained from Rédei Kertimag Seed Trading Ltd. Heavy metal concentrations used in this experiment were based on previous studies [12, 14, 16, 21, 23, 29]. Pb, Zn, and Cu concentrations in the test solution were 0, 50, 100, 200, 400, 800, 1600, 3200, 6400 mg l^{-1}, while Cd concentrations were 0, 1, 2, 4, 8, 16, 32, 64, 128 mg l^{-1}. Heavy metals were added as $Pb(NO_3)_2$, $ZnSO_4*7H_2O$, $CuSO_4*5H_2O$, $Cd(CH_3CO_2)_2*H_2O$, which were obtained from Reanal Laboratory Chemicals LLC.

The experimental procedure was as follows: 3 g cotton-wool was placed in plastic pots (height: 40 mm, diameter: 120 mm) and moistened with approx. 50 ml test solution with a specific heavy metal concentration.

Twenty-five seeds were laid on cotton-wool pads and exposed to the solutions under controlled conditions. The pots were sealed with cellophane and set under a photoperiod of 12 h light and 12 h dark, and 25±1 °C temperature. After six days (144±1 h), the number of germinated seeds was recorded, and plant root and shoot elongation were measured. The experiment was conducted in a completely randomized design with four replications.

The data were recorded as means±standard deviations and analyzed by SPSS (version 25) and Graphpad Prism (version 6). Two-way analysis of variance (ANOVA) and Tukey multiple comparisons were carried out to test for any significant differences between the means. IC_{50} values (heavy metal concentration that cause 50 % inhibition effects on seed germination and root/shoot elongation) were determined after normalization with a log-logistic dose-response model. A 95 % significance level ($P < 0.05$) was used for all statistical analysis.

3. Results

Toxic effects of heavy metals on seed germination

Effects of heavy metals on seed germination of *Tagetes* species are shown in Figure 1. Germination rates were 89±3.83 %, 91±2.00 % and 55±3.83 % for *T. erecta*, *T. patula* and *T. tenuifolia* in control. Increasing concentration of heavy metals in the test solution significantly ($p<0.05$) decreased the germination rates of all tested species.

T. erecta and *T. patula* had significantly ($p<0.05$) higher germination rates than *T. tenuifolia* in all heavy metal treatments except for above 800 mg Cu l^{-1} concentration. There was a slight increase in germination rate of *T. tenuifolia* at 50 mg Cu l^{-1} concentration, however all three plants had less germination rates at 100 Cu mg l^{-1} compared with control. At highest Cu treatment dose (6400 mg Cu l^{-1}) germination was not noticeable. Zn also significantly reduced germination rates firstly at 100 mg Zn l^{-1} concentration compared with control, however the decline in germination rates of *T. erecta* and *T. patula* was less than Cu in all concentration. On the contrary, Zn was more toxic to *T. tenuifolia* than Cu. *T. tenuifolia* was not germinated above 800 mg Zn l^{-1}. Pb was the least toxic heavy metal to *T. erecta* and *T. patula*, because germination rates of these plants were the highest in all treatment concentration.

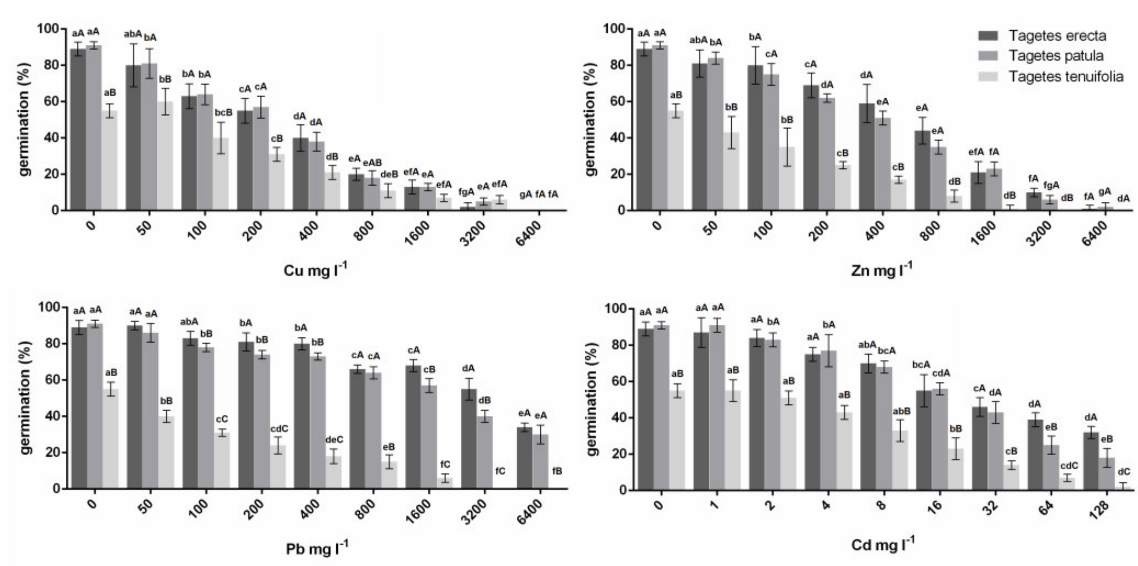

Figure 1. Seed germination (%) of *Tagetes* species exposed to different concentrations of heavy metals. The error bar represents standard deviation (n = 4). The same small letter above the column means there is no significant difference between the means of seed germination at different heavy metal concentration, and the same capital letter means there is no significant difference between the means of seed germination among plants by Tukey's multiple test (P <0.05).

From 100 to 3200 mg Pb l^{-1} concentration (except for 800 mg Pb l^{-1}) *T. erecta* had higher germination rates than *T. patula*. *T. tenuifolia* was more sensitive to Pb than the other plants, and it was above 1600 mg Pb l^{-1}. Cd toxicity is tested in much lower concentrations than other heavy metals. 4 mg Cd l^{-1} concentration significantly decreased the germination rates of all plants compared with control. At higher Cd treatment dose (64 and 128 mg Cd l^{-1}) germination rates of *T. erecta* were significantly higher than *T. patula*.

Toxic effects of heavy metals on root elongation

All tested heavy metals had a significant effect ($p<0.05$) on root lengths of the three plants. Root lenghts were 3.29±0.27 cm, 3.25±0.11 cm and 1.95±0.29 cm for *T. erecta*, *T. patula* and *T. tenuifolia* in control, and they were significantly reduced with increasing heavy metal concentration. Inhibition on root elongation was firstly observed at the lowest concentration (50 mg l^{-1}) of Cu, Zn and Pb, and at 2 mg Cd l^{-1}. The results are shown in Figure 2.

T. erecta and *T. patula* had significantly ($p<0.05$) higher root lengths than *T. tenuifolia* in all treatments except for those receiving more than 100 mg Cu l^{-1} and 1600 mg Zn l^{-1} concentration. Cu was the most toxic heavy metals to plant root formation. 100 mg Cu l^{-1} caused more than 50 %, while 800 mg Cu l^{-1} caused more than 90 % decline in root lengths of all plants. Zn had very similar effects to Cu. It decreased root lengths by 50 % firstly at 200 mg Zn l^{-1}, and by 90 % at 200 mg Zn l^{-1}. Pb also inhibited root elongation; however it was less toxic than Cu and Zn. More than 50 % inhibition on root elongation of *T. tenuifolia* was observed at 400 mg Pb l^{-1}, while it was occurred only at 800 mg Pb l^{-1} in the case of *T. erecta* and *T. patula*. Similarly to the results of germination rates, Cd was the most toxic heavy metals to plant root formation. 8 mg Cd l^{-1} decreased root lengths by more than 50 %, while 128 mg Cd l^{-1} decreased it by more than 90 %.

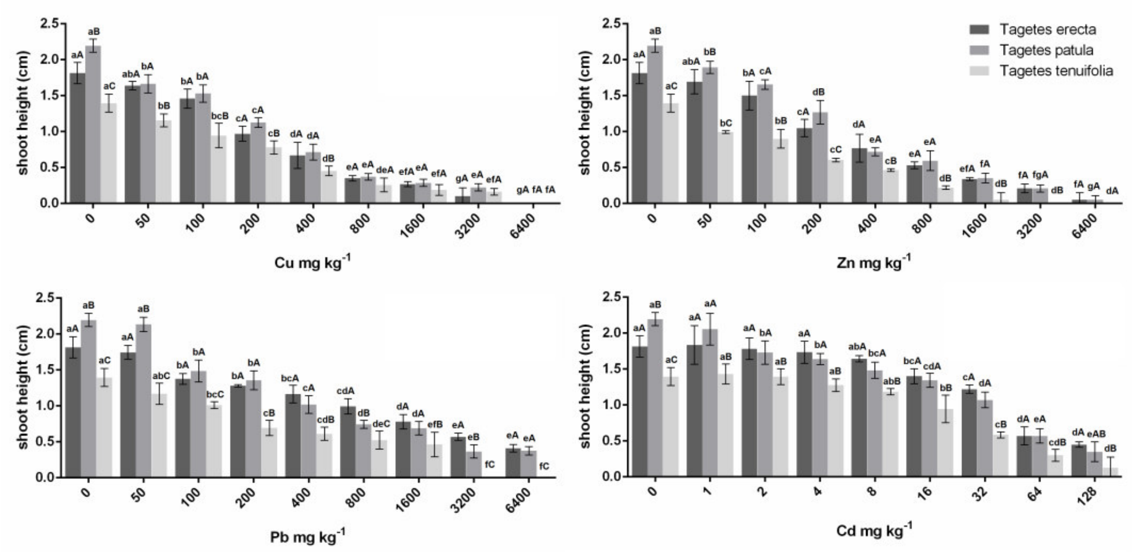

Figure 2. Root lengths (cm) of Tagetes species exposed to different concentrations of heavy metals. The error bar represents standard deviation (n = 4). The same small letter above the column means there is no significant difference between the means of root lengths at different heavy metal concentration, and the same capital letter means there is no significant difference between the means of root lengths among plants by Tukey's multiple test (P <0.05).

Toxic effects of heavy metals on shoot elongation.

Effects of heavy metals on shoot elongation are shown in Figure 3. Shoot heights were 1,82±0.15 cm, 2.20±0.10 cm and 1.40±0.13 cm for *T. erecta*, *T. patula* and *T. tenuifolia* in control. Shoot elongation was also significantly ($p<0,05$) inhibited by increasing concentration of heavy metals,
however less toxic effects were observable compared with the results of root elongation.

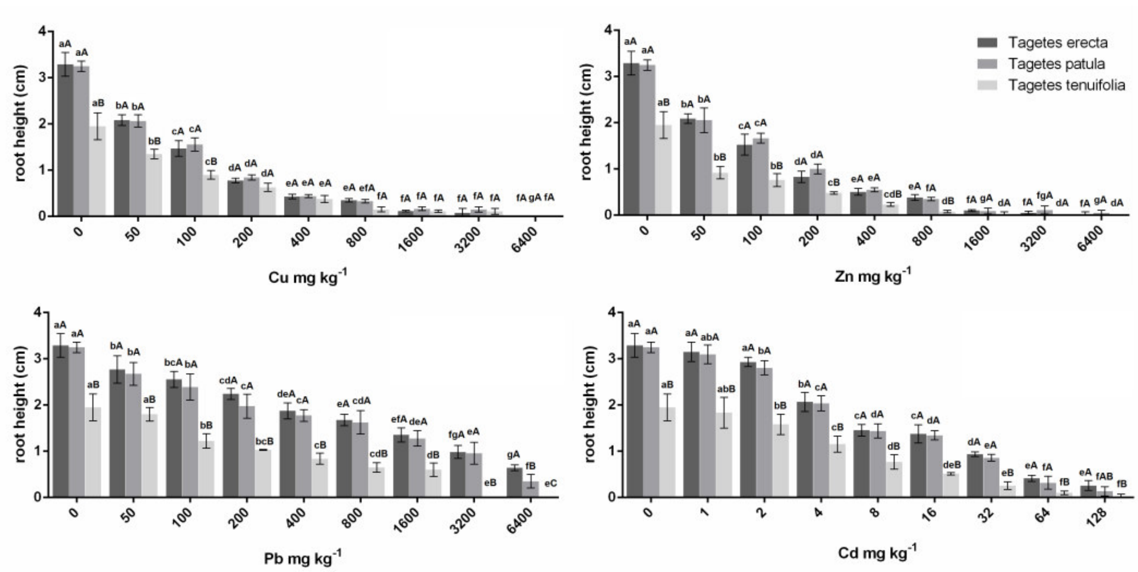

Figure 3. Shoot heights (cm) of Tagetes species exposed to different concentrations of heavy metals. The error bar represents standard deviation (n = 4). The same small letter above the column means there is no significant difference between the means of shoot heights at different heavy metal concentration, and the same capital letter means there is no significant difference between the means of shoot heights (cm) among plants by Tukey's multiple test (P <0.05).

T. erecta and *T. patula* had significantly ($p<0.05$) higher shoot heights than *T. tenuifolia* in all treatments except for above 400 mg Cu l^{-1} and at the highest concentration of Zn (6400 mg Cd l^{-1}) and Cd (128 mg Cd l^{-1}). Cu and Zn significantly reduced shoot heights of *T. patula* and *T. tenuifolia* firstly at 50 mg l^{-1} concentration compared with control. 400 mg Cu l^{-1} caused more than 50 %, and 3200 mg Cu l^{-1} caused more than 90 % decline on shoot heights of all three plants. Similarly to Cu, Zn also decreased plant shoot heights by 50 % at 400 mg l^{-1} concentration; however 90 % decline was observed only at the highest concentration (6400 mg Zn l^{-1}). Pb was toxic to plants firstly at 100 mg l^{-1} concentration. Shoot heights of *T. patula* and *T. tenuifolia* is decreased by 50 % firstly at 400 mg Pb l^{-1}, while only 1600 mg Pb l^{-1} reduced shoot heights of *T. erecta* at such a rate. At 50 mg Pb l^{-1} *T. patula* had significantly higher shoot heights than *T. erecta*, however at 800 and 3200 mg Pb l^{-1} *T. erecta* had higher shoot heights. Cd was the most toxic heavy metals to plant shoot heights. 2 mg Cd l^{-1} concentration significantly decreased the shoot heights of *T. patula* compared with control, while inhibition of shoot elongation was observed firstly at 16 mg Cd l^{-1} concentration for *T. erecta* and *T. tenuifolia*. 50 % decline on shoot heights were firstly at 32 mg Cd l^{-1} for *T. patula* and *T. tenuifolia*, and at 64 mg Cd l^{-1} for *T. erecta*.

Ecological toxicity based on IC_{50} values

IC_{50} values confirm previous results. All heavy metals used in this experiment were the most toxic to root elongation. Cu, Zn and Pb were least toxic to seed germination, while Cd was least toxic to shoot elongation (Table 1.).

Table 1. IC_{50} values of heavy metals (heavy metal concentration that cause 50 % inhibition effects) in *Tagetes* species based on seed germination and shoot/root elongation.

IC_{50} (mg l^{-1})		African marigold (*Tagetetes erecta*)	French marigold (*Tagetes patula*)	Signet marigold (*Tagetes tenuifolia*)
Cu	Seed germination	290.0	277.0	233.9
	Shoot elongation	261.4	199.5	220.5
	Root elongation	79.9	85.1	97.1
Zn	Seed germination	644.9	446.6	167.6
	Shoot elongation	310.4	249.8	158.6
	Root elongation	82.1	90.2	51.9
Pb	Seed germination	1462.0	812.7	154.5
	Shoot elongation	418.8	231.6	302.6
	Root elongation	349.2	380.8	286.3
Cd	Seed germination	12.2	15.5	11.2
	Shoot elongation	27.57	12.84	20.95
	Root elongation	7.3	7.7	5.9

IC_{50} values were determined after normalization with a log-logistic dose-response model.

There was no considerable difference in IC_{50} values among the three plants at Cu treatment. IC_{50} values were between 233.9 and 290.0, 199.5 and 261.5, 79.9 and 97.1 for seed germination, shoot elongation and root elongation. *T. erecta* was the least, and *T. tenuifolia* was the most sensitive plant to Zn treatments. The IC_{50} values based on seed germination were shown the greatest difference among plants. These values for *T. erecta*, *T. patula* and *T. tenuifolia* were 644.9, 446.6 and 167.6 mg Zn l^{-1}.

Large differences in IC_{50} values of the three plants were also observed at Pb treatments. The highest IC_{50} value of the experiment was based on seed germination of *T. erecta* (1462.0 mg Pb l^{-1}), but *T. patula* also had a high IC_{50} value (812.7 mg Pb l^{-1}).

On the contrary, for *T. tenuifolia* IC_{50} value based on seed germination was only 154.5 mg Pb l^{-1}, which is less than at Cu and Zn treatments. The IC_{50} values based on root elongation were 349.2, 380.8 and 286.3 mg Pb l^{-1}, based on shoot elongation were 418.8, 231.6 and 302.6 mg Pb l^{-1} for *T. erecta*, *T. patula* and *T. tenuifolia*. Among the three plants and based on shoot elongation as an indicator, *T. patula* was the most sensitive plant (IC_{50} = 12,84 mg Cd l^{-1}), however based on seed germination and root elongation *T. tenuifolia* was the most sensitive to Cd (IC_{50} = 11,2 and 5,9 mg Cd l^{-1}). *T. erecta* was the least sensitive plant to Cd in all indicator.

4. Discussion

The results showed that all tested heavy metals had significant ($p<0.05$) inhibitory effects on seed germination and root/shoot elongation of the three plants. It is expected, since plant seeds were in direct contact with the toxicity of heavy metals in the hydroponic solution, and inhibition effects of heavy metals on growth parameters of *T. erecta* has been also observed in previous studies [14, 16, 21, 29]. The reason for this is that excessive amounts of heavy metals may cause substantial inhibition of photosynthetic and enzymatic activity, or modification of mineral uptake and internal translocation [7,32].

According to our results heavy metals were the least toxic to seed germination. It is possible that plants absorbed nutrients internally from seed stored materials during germination or heavy metals could hardly penetrate seeds [16, 33]. Heavy metals had the greatest adverse effect on plant root lengths. Root elongation is known to be more sensitive than shoot elongation to heavy metal toxicity, because roots are responsible for absorption and accumulation of metals [7, 33]. In addition to this, roots of *Tagetes* species can accumulate higher amounts of heavy metals than its shoots [12, 29]. Heavy metals had less effect on shoot elongation. Probably, nutrition was provided by seeds after root elongation was inhibited [16].

On the basis of the IC_{50} values (average ranking of the three growth parameters) the following series of phytotoxicity was observed: Cd>Cu>Zn>Pb for *T. erecta* and *T. patula*, and Cd>Zn>Cu>Pb for *T. tenuifolia*. As the results show, Cd is the most toxic and Pb is the least toxic heavy metal to the tested species. Di Salvatore et al. (2008) assessed similar toxicity scales for various species (including lettuce, broccoli and tomato), however in Wong & Bradshaw (1982) studies Cu and Pb was more toxic to ryegrass than Cd [34, 35].

In our experiment seed germination and shoot heights of *T. erecta* were decreased by 50 % compared with control at 64 mg Cd l^{-1}, while root length at 8 mg Cd l^{-1}. Goswami & Das (2017) observed approximately 50 % reduction on root length and shoot/root dry biomass of *T. erecta* only at 300 mg Cd kg^{-1} dose on clay loam soil [21]. In Lal et al.

(2008) experiment shoot heights of *T. erecta* was reduced by 23 % at 32.6 mg Cd kg^{-1} dose on sandy loam soil [14]. Wang & Zhou (2005) determined a 16.1 mg Cd l^{-1} IC_{50} value for root elongation of *T. erecta* in hydroponic solution, which is higher than in our result (7.3 mg Cd l^{-1}) [16]. According to Wang & Zhou (2005), increasing Cd concentrations (0 to 15 mg Cd l^{-1}) increase shoot elongation of *T. erecta*, however there was a significant decline on shoot heights between these Cd concentrations in our experiment [16]. In Goswami & Das (2017) experiment 40 % decline was observed on root lengths and shoot/root dry biomass of *T. erecta* at 400 mg Cu kg^{-1} dose on clay loam soil [21]. Our results showed that 400 mg l^{-1} Cu in hydroponic solution reduce root lengths and shoot heights by 87 % and 63 %. According to Shah et al. (2017) 2500 mg Pb kg^{-1} in soil decrease root lengths and shoot heights of *T. erecta* less than 5 %, however in our experiment 1600 mg l^{-1} Pb concentration in the test solution decreased root lengths and shoot heights by 59 % and 57 % [29].

These comparisons show that in previous studies heavy metals were less toxic to growth parameters of *T. erecta*. The reason for this that hydroponic solution is quite different from natural soils. In soils heavy metal could be tied up in insoluble forms, and they are less available to plants [36]. In addition to this, many factors influence the uptake and the toxicity of metals in natural soils, such as temperature, soil pH, soil aeration, the type of plant and its size, the root system etc. [7, 37]. Although hydroponic experiments have very limited relevance to the natural environment, these researches can be useful in demonstrating the tolerance of a species to heavy metals [38].

Based on our results, *T. tenuifolia* is the most sensitive plant to the tested heavy metals among the three species. In addition, *T. tenuifolia* produce less biomass than *T. erecta* and *T. patula*, which decrease its phytoremediation potential [10, 39]. Between the effects of heavy metals on *T. erecta* and *T. patula*, no considerable differences were observed, however *T. erecta* was more tolerant to high levels of Pb (above 1600 mg Pb l^{-1}) and Cd (above 64 mg Cd l^{-1}). According to our results, these two *Tagetes* species can be used to remediate heavy metal (Cu, Zn, Pb and Cd) contaminated soils, because these plants have higher biomass production and can tolerate higher levels of heavy metals. Moreover, previous studies shown that *T. erecta* and *T. patula* have good capability to accumulate different heavy metals in their tissues [14, 21-23, 26-30].

High levels of heavy metals decrease above-ground biomass of plants, which result in less effective phytoremediation. In our experiment results of shoot heights can indicate the reduction of biomass. IC_{50} values based on shoot elongation (average of the three plants) were 227.1, 239.6, 317.7 and 20.5 at Cu, Zn, Pb and Cd treatment. It means that phytoremediation using *Tagetes* species could be effective below these heavy metal concentrations in soil.

However, our experiment was carried out under laboratory conditions, and the seeds were germinated in hydroponic solution, which means that these values could be much higher in natural soils [36].

It was concluded that all tested heavy metals were toxic to the plants, however *T. erecta* and *T. patula* were tolerant to low concentrations of heavy metals, which means that these plants could remedy moderately contaminated soil.

Therefore, with the advantage that these plants can beautify the environment, using them for phytoremediation in urban areas has an important and practical significance [10, 12]. Adding chelators, applying fertilizers or inoculating plant growth-promoting rhizobacteria (PGPR) to soil may increase the biomass and the phytoremediation ability of *Tagetes* species [10, 22, 40, 41].

Acknowledgment

This research has been supported by the ÚNKP-18-3-I-SZIE-38. New National Excellence Program of the Ministry of Human Capacities, Hungary

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