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Influence of Different Lead Concentrations on the Growth Parameters of French Marigold (*Tagetes patula* L.)

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Abstract: This study was carried out to investigate the effect of different lead (Pb) concentrations on some growth characteristics of French marigold (*Tagetes patula* L.). The research was carried out at Siirt University (Türkiye) Faculty of Agriculture, Agricultural Biotechnology Laboratory. In the study, French marigold (*T. patula* L.) seeds were used as plant material. Five different concentrations of lead (0, 200, 400, 600, and 800 ppm) were considered as research subjects. 7 pots were used for each concentration, and the laboratory experiment was set up in a randomized complete plots design with 7 replications. In the experiment, a 2:2:1 ratio peat:sand: soil mixture was used as the plant growth medium. Plant height (cm), stem thickness (mm), the number of branches per plant, the number of flowers per plant, single flower weight, and plant fresh and dry weights (g) were evaluated. According to the results, the difference between Pb concentrations was found to be significant in terms of all parameters except for the number of branches, and fresh and dry plant weights. In the study, according to Pb concentrations, plant height values were between 46.25-52.50 cm, stem thickness was between 4.13-5.77 mm, the number of branches was between 4.25-5.75 per plant, the number of flowers was between 3.25-6.25 per plant, single flower weight was between 0.84-1.49 g, plant fresh weight was between 11.66-14.32 g and plant dry weight varied between 1.10-1.29 g. In the study, promising results were obtained that the French marigold can be used for phytoremediation in Pb-contaminated areas.

Keywords: Lead, *Tagetes patula*, number of flowers, number of branches, plant weight

1. Introduction

Various industrial and chemical wastes arising as a result of industrialization, domestic wastes due to population growth, commercial fertilizers, and pesticides, have caused heavy metal accumulation in soil and underground and surface waters. Lead (Pb) is one of the heavy metals that is frequently encountered in nature due to its widespread use in industrial and agricultural activities and has no role in the plants' lives. Lead is found in the surface and near-surface layers of the soil, and its mobility is low compared to other heavy metals because it is generally absorbed by organic matter in the soil (Kınay and Erdem, 2019). Lead uptake of plants is mainly regulated by various physical and chemical parameters, such as pH, particle size, and cation exchange capacity of the soil (Nas and Ali, 2018; Giannakoula et al., 2021). Although Pb has no biological function, it can cause morphological, physiological, and biochemical dysfunctions in plants (Sarıyer, 2017). Studies have shown that when plants are exposed to Pb, seed germination (Wierzbicka and Obidzińska, 1998), root and stem growth (Malar et al., 2014; Zulfiqar et al., 2019), and chlorophyll content (Dao and Beardall, 2016) are reduced.

Recently, environmentally friendly plant breeding (eg; phytoremediation) methods have been used instead of costly traditional methods, which have high energy requirements, and are harmful to the environment in the removal of toxic metals such as Pb. The use of medicinal and aromatic plants for phytoremediation has gained attention since they are not directly connected to the food chain and are not directly consumed as human food. Labiatae (lavender, mint, bacillus, rosemary, sage), Compositae (chamomile) and Poaceae (vetiver, lemongrass, citronella, palmarosa) family plants gave positive results for phytoremediation of heavy metal contaminated areas (Pandey et al., 2019). In addition, it has been proven that the essential oil rate and content of the plants are not affected by heavy metal-contaminated areas (Zheljazkov et al., 2006; Khajanchi et al., 2013).

French marigold (*Tagetes patula* L.), a member of the Compositae family, has gained attention as a medicinal plant due to its active ingredients, as well as being an ornamental plant. Pigments obtained from French marigold are used in various areas such as vegetable oils, pasta, bread, and dairy products (Coelho et al., 2017), in the production of nutritional supplements to prevent vision loss due to aging in humans (Burlec et al., 2021), and for condensation in broiler meat and egg yolk (Martinez et al., 2004; Liu et al., 2011). It is reported that essential oils of *Tagetes* sp. are antibacterial (Singh et al., 2020; Walia et al., 2020), insecticidal (Singh and Thakur, 2019; Şahin, 2019), and nematicidal (Bhattacharyya, 2017; Massuh et al., 2017) activities. In addition, studies have shown that *T. patula* is a suitable plant for use in phytoremediation (Miao et al., 2022). It should be noted that studies on phytoremediation mostly focused on species such as corn, sunflower, *Ricinus*, rapeseed, and very few studies were conducted with ornamental, and medicinal, and aromatic plants. In addition, studies have focused on the metal-accumulating capacities of plants, and not much research has been done on their herbal properties. This study aimed to investigate the effect of Pb concentrations on some growth parameters of the French marigold (*T. patula* L.).

2. Materials and Methods

The research was carried out in the Agricultural Biotechnology Laboratory of the Faculty of Agriculture at Siirt University (Türkiye). In the study, French marigold (*T. patula*) seeds (Zengarden firm, Türkiye) were used as plant material. Five different concentrations of lead (0, 200, 400, 600, and 800 ppm) were considered as research subjects. The experiment was set up in a randomized complete plots design with seven replications. In the experiment, a 2:2:1 ratio of peat:sand:soil mixture was used as the plant growth medium. Some physico-chemical properties of the growth medium are given in Table 1. The soil of the plant growth medium was clayey-loamy textured, slightly alkaline, and salt-free. Lime content was "medium calcareous", organic matter content was "moderate", and available phosphorus (P) and exchangeable potassium (K) contents were "adequate". The available iron (Fe) content of the soil was "high", the copper (Cu) content was "adequate", and the zinc (Zn) and manganese (Mn) content were "low" (Table 1).

Table 1. Some physical and chemical properties of potting soil used in the research*

Soil properties	Value
Clay, %	36.63
Silt, %	43.10
Sand, %	20.27
pH	7.82
Electrical conductivity, mS cm^{-1}	0.818
Lime ($CaCO_3$), %	7.5
Organic matter, %	2.40
Available P, ppm	7.95
Exchangeable K, meq 100 g^{-1}	0.94
Available Fe, ppm	19.63
Available Cu, ppm	4.89
Available Zn, ppm	0.48
Available Mn, ppm	13.76

*: Analyzes were made in the Soil and Water Analysis Laboratory of the Black Sea Agricultural Research Institute.

The plants were grown under a 16/8 light/dark regime in a growth room with a relative humidity between 60-70% and temperature between 25-27 °C for seven weeks. Seeds were sown in viols in peat medium (Klasmann-Deilmann, potground H, Germany) on 14/02/2019. The seeds were germinated on 19/02/2019. A 2:2:1 ratio of peat:sand:soil was placed in the pots before planting, the pots were watered, the soil was brought to field capacity, and the weight of each pot at field capacity was equalized. When the plants had 2-3 leaves, they were planted in pots on 04/03/2019.

Lead nitrate [$Pb(NO_3)_2$] was used as the Pb source. Five different concentrations of lead (0, 200, 400, 600, and 800 ppm) were prepared and applied to plants in 3 equal parts with 3 days intervals (1st application: 06/03/2019, 2nd application: 09/03/2019, 3rd application: 12/03/2019). Tap water was used to control plants.

During the growth and development of the plants, foliar fertilizer was applied to each pot to ensure normal development. To keep the soil at field capacity, 50 ml of water was given daily using tap water. Plant height (cm), stem thickness (mm), number of branches per plant, number of flowers per plant, single flower weight (g), and plant fresh and dry weights (g) were evaluated.

The data obtained from the study were subjected to analysis of variance (ANOVA) according to the randomized complete plots experimental design. Differences between groups according to F test results were determined by Tukey's Honest Significant Difference multiple comparison test

(Açıkgöz and Açıkgöz, 2001). Error bars in all graphs show the mean ± standard error.

3. Results and Discussion

3.1. Plant height

The effects of Pb doses on the plant height of the French marigold were statistically significant at $p<0.05$ level. According to the results, the highest plant height was obtained in the control (52.50 cm). On the other hand, 200 to 600 ppm had slightly lower plant height values compared to the control, while 800 ppm had significantly lower plant height (Figure 1). Eid et al. (2018) reported that an 80 ppm dose of Pb application on *T. erecta* L. reduced plant height compared to the control, and this effect was less at lower Pb concentrations. Also, Shah et al. (2017) reported that plant height decreased continuously with increasing Pb concentrations in *T. erecta*, and this decrease was statistically significant. Mazher (2006) reported that increasing Pb application rates to *Leuceana leucocephala* reduced its vegetative growth characteristics. It was determined that plant height differed significantly between 110- and 220 mg Pb applications in the *Coriandrum sativum* L. in which Pb was applied for approximately 28 days (Garrett and Trott, 2019). Studies on sunflower (Kastori et al., 1998; Alaboudi et al., 2018), rice (Zhang et al., 2005), cotton (Bharwana et al., 2014), rapeseed (Rosca et al., 2021) and lavender (Pirsarandib et al., 2022) reported that plant height was negatively affected by Pb application while higher Pb doses decreased plant height. Lead shows significant growth inhibition by disrupting the functioning and mineral nutrition of plants (Mishra et al., 2006; Gopal and Rizvi, 2008; Islam et al., 2008; Dere and Doğan, 2020). High Pb concentrations delay cell division and differentiation and reduce their elongation. The anatomical and morphological changes caused by Pb negatively affect plant growth and development (Janjatovic et al., 1991; Kastori et al., 1998; Doğru, 2019; Kınay and Erdem, 2019).

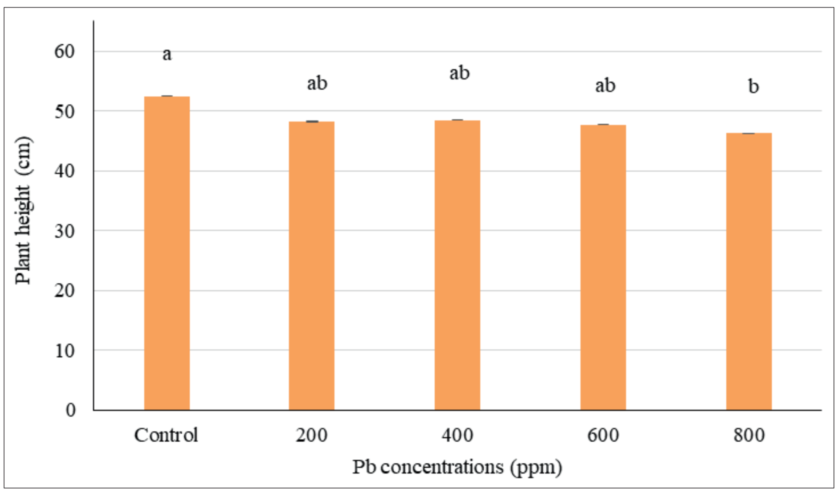

Figure 1. Plant height of French marigold exposed to different Pb concentrations

3.2. Stem thickness

The difference between Pb concentrations in terms of stem thickness of French marigold was statistically significant ($p<0.01$). The highest stem thickness value was obtained in the control (5.76 mm), and 200, 600, and 800 ppm had significantly lower stem thickness values, interestingly, the difference in 400 ppm was not as high as other Pb doses (Figure 2). Gülser and Çığ (2020) reported that stem thickness in *Hyacinthus orientalis* L. was increased at 40 mg kg^{-1} Pb concentration, while it was decreased at 80 mg kg^{-1} Pb dose. Similarly, Huang et al. (2017) reported that the stem thickness of *Robinia pseudoacacia* L. was affected positively in the presence of 0 and 90 mg kg^{-1} Pb in the soil, and negatively at 900 and 3000 mg kg^{-1} Pb. Akay (2022) reported that increasing Pb levels (0, 200, 400, 800, and 1600 mg kg^{-1}) in Cubana Kordes rose reduced the stem thickness significantly. Lead inhibits root and stem growth even at low concentrations (Islam et al., 2008), this becomes evident in the roots (Doğru, 2020). Verma and Dubey (2003) also reported that there was a 40% decrease in root growth and 25% in the stems. Lead accumulation in the roots is thought to be a defense mechanism to protect the aerial parts of the plant against Pb toxicity (Yerli et al., 2020).

3.3. Number of branches per plant

The effects of Pb concentrations on the number of branches in the plant were not significant. While the results were not significant, the number of

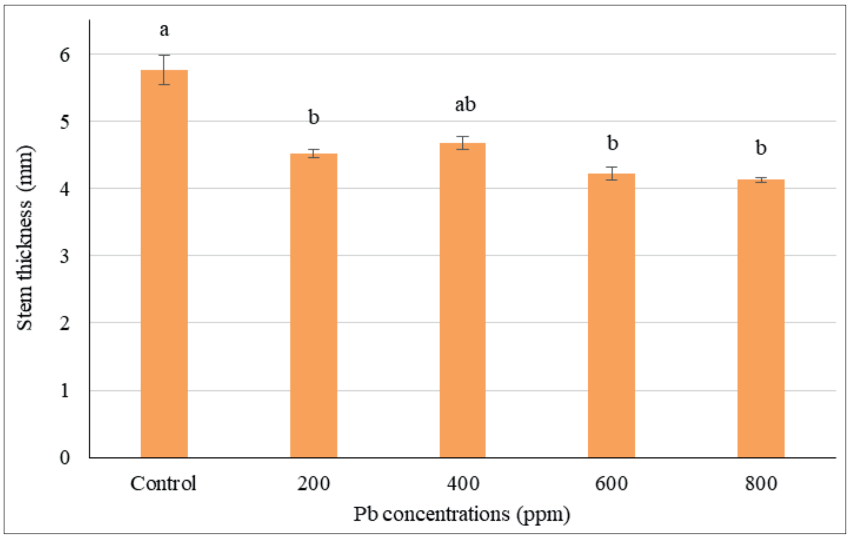

Figure 2. Stem thickness of French marigold exposed to different Pb concentrations

branches in the plant varied between 4.25 -5.75 number, and a higher number of branches per plant was obtained as the Pb dose increased (Figure 3). Eid et al. (2018) reported that Pb concentrations decreased the number of branches in *Tagetes erecta* L., and Matter (2016) reported that Pb application at increasing doses decreased the number of branches by 53.26% in roselle (*Hibiscus sabdariffa* L.) compared to the control. In another study, it was reported that Pb concentrations (250, 450, 650, and 850 mg kg^{-1}) applied to the *Salix mucronata* decreased the number of branches from the first dose, as well as plant height and stem thickness (El-Mahrouk et al., 2019). On the contrary, Sanaei et al. (2022) stated that the number of branches in guar (*Cyamopsis tetragonoloba* L.) decreased at a dose of 25 mg L^{-1} and increased at a dose of 50 mg L^{-1} compared to the control, which is similar to our results. Some soil factors (pH value of the soil, cation exchange capacity, mineralization, biological and microbial conditions, Pb amount, presence and amount of organic and inorganic compounds, competition between cations), plant species and varieties, alone or by interacting with each other, affect Pb behavior in the soil and uptake by plants (Bi et al., 2010; Gupta et al., 2010; Ak and Yücel, 2011; Doğru, 2020).

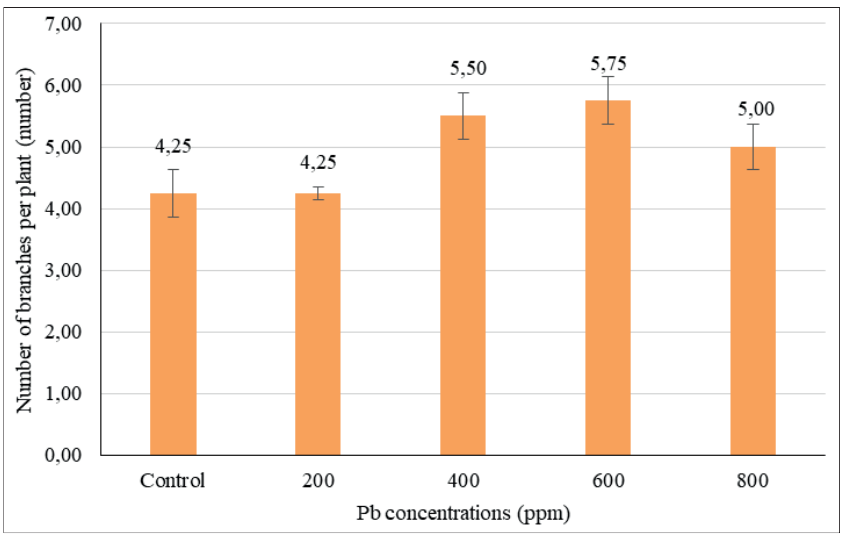

Figure 3. Number of branches per plant of French marigold exposed to different Pb concentrations

3.4. Number of flowers per plant

The effects of Pb concentrations on the number of flowers in the plant were found to be statistically significant ($p<0.05$). According to the results, there was a higher number of flowers per plant on all Pb-applied plants, compared to control. The highest number of flowers in the plant was determined at 800, 400 and 200 ppm doses (respectively, 6.25, 6.25 and 6.00 number plant^{-1}) and the lowest was determined in the control group with 3.25 per plant (Figure 4). On the contrary, Eid et al. (2018) reported that the number of flowers in the plant decreased as Pb concentrations increased in *Tagetes erecta*. According to Ma et al. (2022), different Pb concentrations (0, 100, 200, 400, 800, and 1600 mg kg^{-1}) significantly affected the number of flowers on three *Hydrangea* cultivars, and each cultivar reacted differently to heavy metal stress as Pb concentrations increased. As Pb concentrations increased, the number of flowers decreased in cv. 'Endless Summer', while it increased in 'Classic Rood' and 'Ayesha'. In the study conducted with *Salvia splendens* Sello cv. 'Torreador', flowering was not affected by Pb treatments, and the highest number of inflorescences was recorded at the highest Pb concentration (200 mg Pb dm^3) (Nowak, 2007). This difference between the studies is thought to be due to differences in species and varieties, the Pb content of the soil, and the application method. Indeed, Gleba et al. (1999) reported that the uptake of heavy metals by plants depends on both soil and plant factors. Nowak (2007) reported that the phytotoxicity mechanism of Pb involves different biochemical pathways in different plant species. In addition, in this study, it was observed that flowering was earlier in Pb-treated plants compared to the control group. Chauhan et al. (2020) also reported that flowering was earlier in plants grown in Pb-contaminated soils compared to the control in sunflower (*Helianthus annuus* L.).

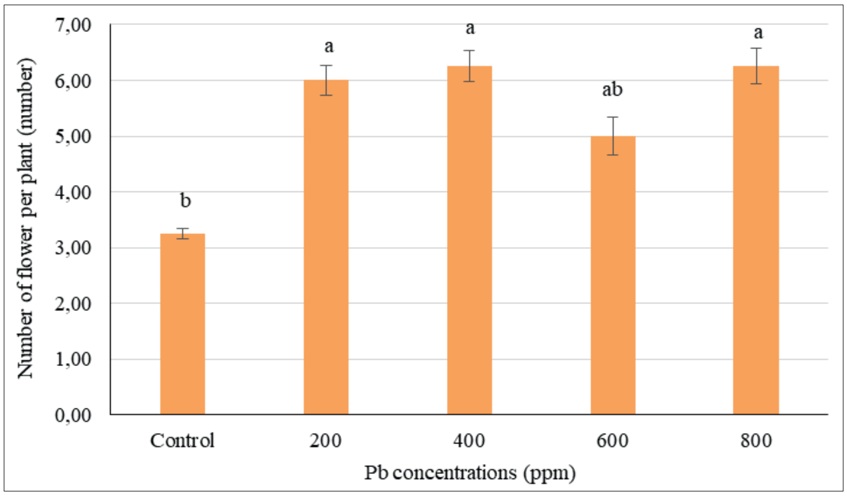

Figure 4. Number of flowers per plant of French marigold exposed to different Pb concentrations

3.5. Weight of single flower

According to the results of the research, the effects of Pb concentrations on single flower weight in *T. patula* were significant at $p<0.05$ level. In parallel with the increase in Pb doses, single flower weight decreased. The highest single flower weight was obtained in the control (1.49 g), the application of 200, 400, and 600 ppm doses reduced the weight of a single flower. A substantial reduction was obtained at 800 ppm Pb applied plants (Figure 5). According to Tabrizi et al. (2015), increasing Pb application to the soil reduces the fresh and dry weight of the pot marigold (*Calendula officinalis* L.) flower. Also, Eid et al. (2018) reported that increasing Pb concentrations (0, 20, 40, and 80 ppm) had significant effects on flower weight in *T. erecta*, and the lowest value was obtained from 80 ppm Pb application.

3.6. Plant fresh weight

In the study, the fresh weight of the plants varied between 11.66 and 14.32 g. Even though there was a continuous reduction in the plant fresh weight with increasing Pb dose, the difference was not significant (Figure 6). Similar to our observations, Pb applied to the switchgrass (*Panicum virgatum* L.) caused a slight decrease in the plant's fresh weight (Alacabey and Zorer Çelebi, 2020). The shoot fresh weight of the sunflower (*H. annuus* L.) was decreased with the increasing Pb concentration (Chauhan et al., 2020), and Pb chloride (0, 500, 1000 mM) was applied roselle plants had reduced above-ground fresh biomass (Matter, 2016). Lead applications also caused a decrease in the fresh weight at the first (24.55%) and second (32.72%) harvests in *Mentha piperita* (Amirmoradi et al., 2012). When these results were compared with our findings, it can be said that the effects of Pb concentrations differ depending on the plant species and soil factors. Begonia (1997) reported that Pb uptake from the soil and transport and accumulation mechanisms to the above-ground organs vary according to plant species.

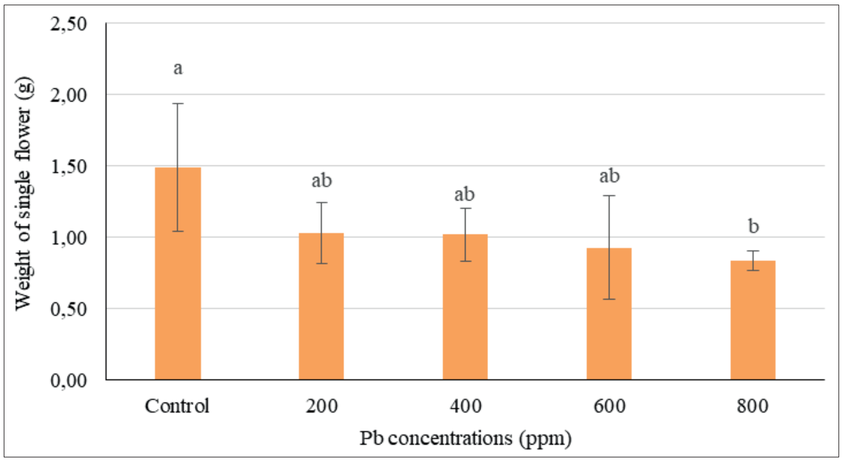

Figure 5. Weight of the single flower of French marigold exposed to different Pb concentrations

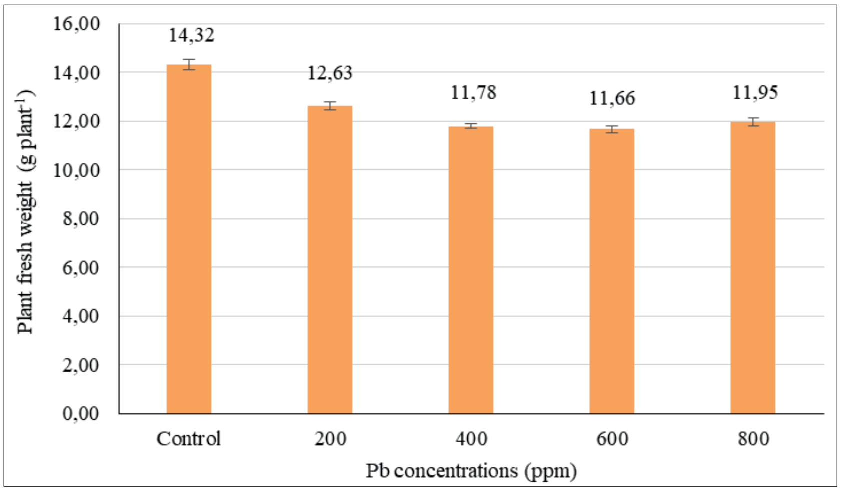

Figure 6. Plant fresh weight of French marigold exposed to different Pb concentrations

3.7. Plant dry weight

In the study, the plant above-ground dry weight of the French marigold was found to be statistically insignificant between different Pb applications. The plant above-ground dry weights varied between 1.10-1.29 g (Figure 7). Similar to our findings, shoot dry weight in dill (*Anethum graveolens* L., cv. Hercules), peppermint (Mentha x piperita L., cv. Mitchum), and basil (*Ocimum basilicum* L., cv. Broad Leaf Italian) did not change with increasing Pb concentration (Zheljazkov et al., 2006), while Pb concentrations applied to two different tobacco (*Nicotiana tabacum* L.) cultivars reduced the above-ground dry matter yield by 96.7-104.0% (Kinay and Erdem, 2019). Increasing Pb applications reduced the dry weight of greater plantain (Kosobrukhov et al., 2004), peppermint (Amirmoradi et al., 2012), sage (Amirmoradi et al., 2015), marigold (Tabrizi et al., 2015), basil (Youssef, 2021), and lavender (Pirsarandib et al., 2022). According to Lamhamdi et al. (2013), fresh and dry weights of wheat and spinach were decreased when exposed to Pb stress, and the effect of Pb was less in spinach than in wheat. In addition, the authors suggested that this decrease in plants exposed to Pb was mainly due to the inhibition of the uptake of potassium, phosphorus, calcium, and magnesium macroelements.

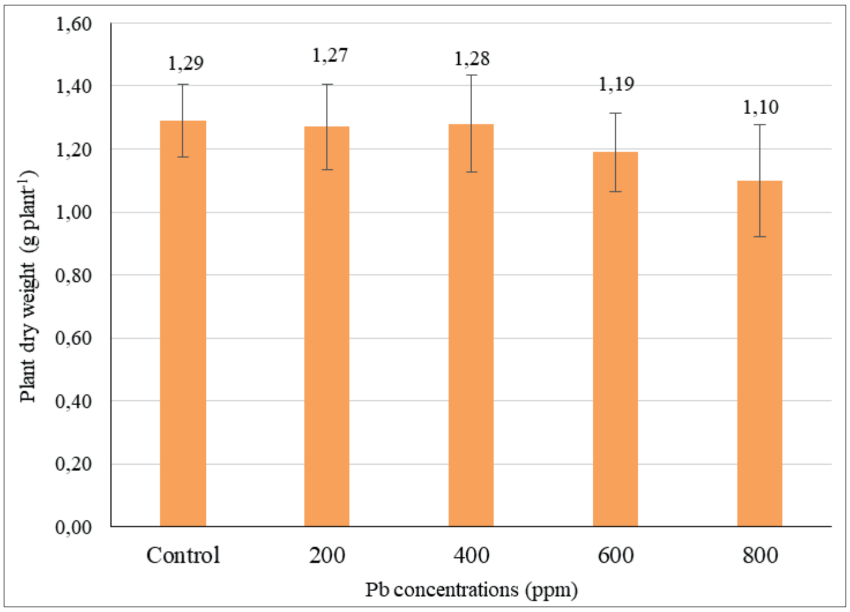

Figure 7. Plant dry weight of French marigold exposed to different Pb concentrations

4. Conclusions

Heavy metals in soil and water affect the growth and development of plant species and varieties differently. In this study, plant height, stem thickness, and single flower weight decreased with increasing Pb concentrations, while the number of flowers per plant increased compared to control. Plant fresh and dry weight decreased with increasing Pb application, but the plants remained viable until the end of vegetation without showing signs of toxicity. Marigold, which is an ornamental plant and is not directly consumed as human food, has medicinal importance due to its components. Our results showed Marigolds' promising potential in phytoremediation programs in Pb-contaminated areas, but the results of this study need to be validated under various field and soil conditions before they can be recommended.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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This research received no external funding.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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