

Impact of Phenolic Compounds on Heavy Metal Stress accumulation within *Tagetes erecta* L.

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Abstract:

Plant productivity can be negatively impacted by heavy metal stress due to a variety of harmful consequences that might hinder cellular functions at different metabolic levels. Plants use phenolic substances for important molecular and physiological responses to environmental stresses. In plants under heavy metal stress, increasing the production of phenolic substances helps shield the plants from oxidative stress. The effect of zinc (Zn) and lead (Pb) at 240 ppm concentrations on the accumulation of total phenol contents in *Tagetes erecta* L. was investigated using a pot experiment.

Key word

Heavy metal, Lead(Pb), Phytoremediation, Phenolic compound, *Tagetes erecta* L., zinc(Zn)

Introduction

Soil can undergo contamination by trace elements (TEs) originating from both natural phenomena, including seepage from geological formations, volcanic eruptions, wildfires, and atmospheric deposition, as well as anthropogenic activities such as mining, smelting, the application of sewage sludge, fertilizers rich in TEs, pesticides in agricultural settings, and the disposal of industrial and municipal waste. The contamination of soil by TEs resulting from anthropogenic activities currently represents a significant environmental challenge (Fan et al., 2017). TEs are identified as potentially hazardous elements. They exhibit non-biodegradable properties, are highly toxic even at minimal concentrations, and their mobility is influenced by various physio-chemical conditions, which depend on the speciation of TEs, their oxidation states, the presence of living organisms, and the physio-chemical characteristics of the soil (Hamelink et al., 1994). Consequently, the environmental remediation of soil contaminated with TEs has emerged as a prominent issue of concern. A variety of both ex-situ and in-situ remediation technologies have been developed for TEs-contaminated soil, including landfilling, soil washing, encapsulation, and vitrification; however, these methods often lack economic viability and environmental sustainability. Phytoremediation represents an innovative technology

that employs plants to extract TEs from the soil by bioaccumulating and concentrating these elements within their biomass, offering an economically viable and ecologically sound alternative (Ali and Chaudhury, 2016).

Zinc is recognized as an essential trace element that plays a vital role in the physiological and metabolic processes of numerous species. Nonetheless, elevated concentrations of zinc can be detrimental to both human health and plant vitality. This metal is integral to protein synthesis and exhibits a relatively low concentration in surface waters due to its limited mobility resulting from rock weathering and other natural sources (Noulas et al. 2018; Balafrej et al. 2020). Zinc is introduced into the environment through both natural and anthropogenic channels, with the latter contributing a greater volume than natural sources. Numerous studies have established that zinc is closely associated with the nitrogen metabolism of plants, leading to reductions in protein synthesis, as well as chlorosis and necrosis of foliage (Ebbs and Kochian 1997; Fageria and Stone 2004). The excessive application of zinc salts can provoke toxicity in humans, manifesting as fever, chills, nausea, vomiting, diarrhea, abdominal cramps, respiratory distress, gastroenteritis, and cardiovascular complications (Elinder 1986; Farooq et al. 2010). The presence of lead (Pb) in the bloodstream adversely impacts soft tissues and induces mineralization. In children, lead exposure can disrupt growth metabolism and, in severe instances, affect the central nervous system (Gonzalez et al., 2008).

Ornamental plants are utilized as subjects in phytoremediation studies owing to their substantial biomass, rapid growth rates, and enhanced capacity for heavy metal absorption through their root systems and subsequent translocation into their tissues. The integration of biomass cultivation with soil remediation constitutes a critical aspect of sustainable land management practices, wherein management strategies yield self-financing and additional environmental benefits alongside soil restoration. *Tagetes erecta* L., commonly found in India, is employed for decorative purposes and possesses significant market value. Accordingly, this plant was selected for phytoremediation technology in the present research endeavor. A multitude of studies have been conducted to evaluate the efficacy of ornamental plants in the realm of phytoremediation (Nakbanpote et al. 2016; Khan et al. 2021).

This research endeavour sought to elucidate the response of *Tagetes erecta* L. to abiotic stress induced by the presence of heavy metals (Zn and Pb) in the edaphic environment. It is posited that varying concentrations of heavy metals will elicit distinct physiological responses in the cultivars of the marigold plant. This investigation focused on the contributions of phenolic and flavonoid compounds in the context of oxidative stress precipitated by heavy metal exposure.

Material and method

Sampling and characterization of soil

Subsequent to the removal of the superficial layer, a soil sample was collected from the M. N. College campus in Visnagar, at a depth of 10–15 cm. The parameters of soil pH, electrical conductivity, and moisture content were quantified utilizing methodologies established in the botany laboratory M. N. College, Visnagar

Plant cultivation

Seeds of *Tagetes erecta* L. (Marigold) were sourced from Dantivada Krushi University, Gujarat, India. Prior to sowing, the seeds underwent soaking in distilled water. Sowing was conducted during the winter season of October 2023, utilizing 10 cm diameter plastic pots, each containing 1 kg of soil. Ten days post-sowing, a single seedling was selected per pot to ensure homogeneity in plant size and phenotypic characteristics. The pots were subsequently positioned within a greenhouse located on the college campus. Following a 60-day growth period, dry plant material was collected and grind to a powder for the subsequent assessment of phenolic content.

Estimation of phenolic content

The quantification of total phenolics in the extracts was conducted using the Folin-Ciocalteu reagent. Gallic acid was utilized as the standard for analysis. Concentrations of gallic acid at 0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml were prepared in methanol. Additionally, concentrations of 0.1 and 1 mg/ml of the plant extract were also formulated in methanol, with 1.5 ml of each sample being introduced into test tubes and combined with 1.5 ml of distilled water. Following this, 0.5 ml of Folin-Ciocalteu reagent was added, and the mixture was incubated for 4 minutes at room temperature. Subsequently, 2 ml of a 20% sodium carbonate solution was added. The test tubes were covered and allowed to incubation for 1 minute in a water bath prior to spectrophotometric measurement of absorbance at 765 nm (Harborne, J. B. 1998). The Folin-Ciocalteu reagent exhibits sensitivity to reducing agents, including polyphenols, resulting in the development of a blue chromophore upon reaction. This blue coloration is quantifiable through spectrophotometric analysis, thereby enabling the determination of total phenolic content. All measurements were conducted in triplicate for each of the three extracts.

Estimation of flavonoid content

The quantification of total flavonoid content was conducted utilizing an aluminum chloride colorimetric assay, as delineated by Zou et al. (2004).

Result and Discussion

Table 1: Showing total phenolic content in *Tagetes erecta* L.

Parameters	Control	Pb (240mg/l)	Zn (240mg/l)
Total phenol	0.762±0.04	0.625±0.03	0.611±0.03
Total flavonoid	2.045±0.125	3.165±0.146	4.465±0.131

Table 1 illustrates that the growth of the marigold (*Tagetes erecta* L.) plant exhibits variability in response to varying concentrations of heavy metals. The marigold plant demonstrated optimal growth at the initial concentrations of the respective heavy metals. Furthermore, the analysis of variance (ANOVA) methodology was employed to assess the significance of toxicity levels.

The objective of this research focuses on the major phenolic content in *Tagetes erecta* L. subjected to heavy metal accumulation. Under control conditions, the total phenolic content was quantified at (0.762±0.04), whereas the treatment with lead (Pb) and zinc (Zn) at 240 mg/l resulted in a diminished phenolic concentration. While total flavonoid content in control condition (2.045±0.125) and after treatment with lead and zinc at 240 mg/l resulted in an increasing flavonoid content. This is attributable to the accumulation of heavy metals (Zn and Pb) within *Tagetes erecta* L.

Phenolic compounds, which are ubiquitous in the plant kingdom, have been recognized for their diverse biological activities, including antioxidant properties (Wojdylo et al. 2007).

The total concentration of phenolic compounds was observed to significantly increase when treated with 2 mM of boron in the foliage of tomato plants (Cervilla et al. 2012).

An alteration in the composition of phenolic compounds may serve as a potential indicator of abiotic stress within the plant (Cervilla et al. 2012).

Elguera et al. (2013) reported that CdCl₂ led to a reduction in the levels of free phenolics such as chlorogenic acid, ferulic acid, caffeic acid, and vanillic acid in the leaves of *L. sativum*, whereas exposure to sodium selenite resulted in an increase in the contents of chlorogenic acid and caffeic acid.

The administration of NiCl₂ was found to elevate the total phenolic content, as well as the levels of chlorogenic acid and caffeic acid, while it caused a decline in ferulic acid, with vanillic acid levels varying contingent upon the doses applied in the leaf rosettes of *Matricaria chamomilla* (Kovacik et al. 2009).

The inquiry was initiated by conducting an analysis of metal concentrations present in soil samples extracted from the specified industrial region. The findings indicated elevated levels of contamination within the soil, particularly attributed to zinc, followed by lead and copper. The concentrations of these analyzed metals within the soil were significantly higher than the

permissible limits established for soil contaminants according to the European Standard 2002. In accordance with the EU guidelines, the permissible limit for zinc and lead is established at 300 $\mu\text{g g}^{-1}$, while for copper, it is set at 140 $\mu\text{g/g}$ (European Union, 2002). Consequently, the necessity for soil remediation is imperative to avert further environmental degradation.

The concentration of metals accumulated within *N. biserrata* was assessed to elucidate its mechanisms of metal uptake. As anticipated, *N. biserrata* demonstrated substantial accumulation of metals within its tissues, reflecting the metal concentrations found in the soils of both contaminated and control locations. The levels of zinc, lead, and copper in *N. biserrata* samples obtained from both sites surpassed the safe thresholds outlined in the Malaysian Food Regulation 1985. This indicates that *N. biserrata*, notwithstanding its edible and therapeutic attributes, poses a risk to human health and/or medicinal applications if sourced from areas with heavy metal contamination. Consumers must exercise increased caution in selecting the origins of plants intended for dietary or medicinal utilization. Therefore, *N. biserrata* cultivated in contaminated environments may be more effectively employed as a phytoremediation agent for the extraction of metals from the soil. A study investigating the efficacy of various forage weeds in the removal of cadmium and lead near an automobile battery production facility in Nigeria revealed that *N. biserrata* serves as an effective accumulator of lead, regardless of the season (Adie et al., 2009).

Conclusion

The research indicates that the accumulation of lead (Pb) and zinc (Zn) in *Tagetes erecta* L. has influenced biochemical parameters to a certain level; however, the species demonstrated a notable proficiency in the bioaccumulation of both lead and zinc. Consequently, *Tagetes erecta* L. presents a viable option for the phytoremediation of soils contaminated with heavy metals such as lead and zinc.

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