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Research Article

An Impact Of Microplastic And Microplastic + Lead Induced Toxicity On Growth Parameters And Chlorophyll Content Of Tomato Plant: (Comparison Study)

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ABSTRACT:

The environmental damage caused by microplastics (MPs) and how it affects plants has become a global concern, but the synergistic effect of microplastic + heavy metal remains scarce. This research aim to gain insight into the comparison study of effect of (PE) microplastic and (PE) microplastic + lead nitrate with different concentration (control, 50mg/L, 100mg/L, 150mg/L, 200mg/L + Control, 0.05mg/L, 0.1mg/L, 0.15mg/L, 0.2mg/L) on the tomato plants: (parameters: growth parameters, fresh weight, dry weight and chlorophyll content). in this study we conclude that as the concentration of microplastic and lead increases the parameters like shoot length, fresh weight, dry weight and chlorophyll content decreases and root length increases. We also found that combined toxicity is more toxic than the single microplastic toxicity, as the concentration increases from control to 50mg/L in case of single microplastic toxicity the effect is quiet less but in case of combined toxicity there is huge difference in toxicity and at the concentration of 200mg/L + 0.2mg/L+ the germination is around negligible. In combined toxicity root length is also decreases as the shoot length. These findings suggest that bio-accumulation of lead nitrate by polyethylene microplastic is responsible for the suspension in growth parameters and huge effect in physiological parameters like chlorophyll content. As tomato crops are economically important plants, increase in polyethylene microplastic + uptake of lead nitrate in agricultural fields may have a negative influence on this economically important crop production that result into a loss of economy.

KEY WORDS: PE Microplastic, Synergistic effect, Lead nitrate, Tomato plant

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1. INTRODUCTION:

Growing volumes of plastic are being released into the atmosphere as a result of the extensive use and massive manufacturing of plastic goods. These polymers progressively break down into particles at both the micro and nano scales. Microplastics are found in marine habitats and terrestrial habitats all throughout the world, according to numerous studies (Xueying Zong *et al.*, 2021). Improper handling of plastic waste creates numerous environmental problems. MP can enter the environment directly or indirectly through primary emissions from the production of goods via secondary emission by degradation of plastic waste MP, which causes the release into the environment of a heterogeneous assortment of particle kinds, shapes, and sizes. Because of contemporary farming methods, agricultural systems are particularly vulnerable to plastic contamination. There is currently relatively little data on how microplastics affect the fauna of soils, particularly plants, given the possibility that these new contaminants will enter the agricultural system (Imran Azeem *et al.*, 2021). According to recent research, microplastics can build up in larger plants and hinder their ability to flourish. Given that plants are an essential living element

of terrestrial ecosystems, it is imperative to comprehend how microplastics interact with plant communities. The buildup of microplastics in plants may directly impact the environment and have consequences for food safety and agricultural sustainability. They can collect in these structures as a result of being absorbed by origins, fruits, and vegetables or deposited onto the root surface (Zhe-fu Yu *et al.*, 2021). Soil supplements, plastic mulching, irrigation, diffuse runoff from cities, water damage, and air fallout are some of the ways that microplastics can get into soils. In certain species, such as vegetation or crops growing as individual plants or in groups but not in an ensemble, the impact of microplastics on the growth of plants has been noted. For instance, crops like *Allium fistulosum* exposed to fibers demonstrated an opposite effect from crops like *Triticum aestivum* (wheat) subjected to films or *Lolium perenne* (grass) exposed to fibers (Yudi M. Lozano and Matthias C. Rillig 2023). These MPs pollute the soil and crops in human food chains, influencing physical variables including soil pH, aggregation, bulk density, and water-holding capacity that impact plant growth and physiology (Zhaolin Li *et al.*, 2023).

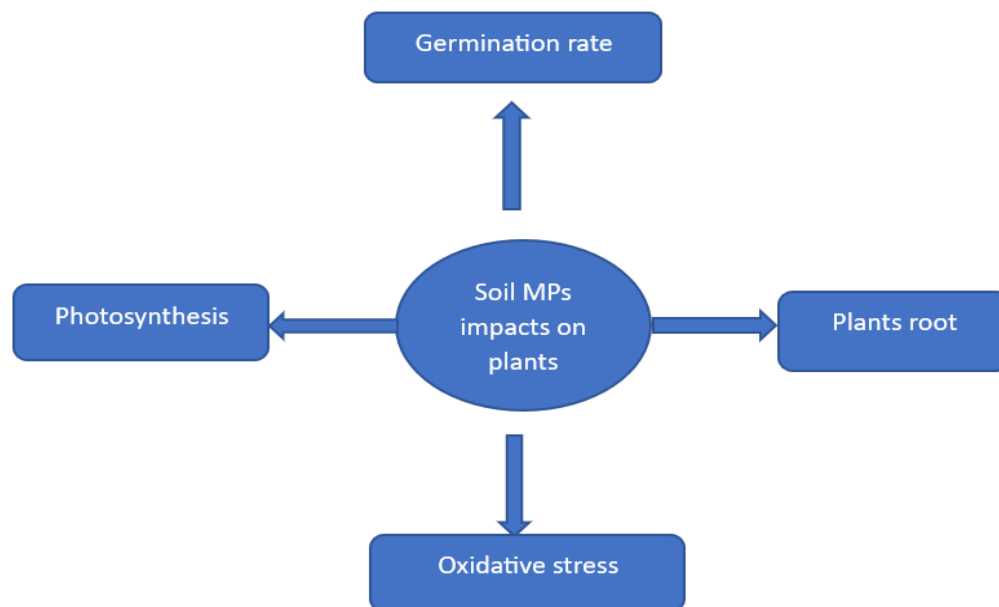


Figure 1. Impacts of soil microplastic on plants (Source: Zhaolin Li *et al.*, 2023).

The main causes of lead's existence in the atmosphere are a variety of ongoing processes and natural occurrences, including corrosion, soil erosion, volcanic eruptions, forest fires, and the byproducts of breakdown of nuclear metals, lead has been shown to have a wide range of harmful consequences on living things, including biochemical, physiological, and morphological risks. Crop growth, root length, seed germination, growth of the seedling transpiration, chlorophyll production, photosynthetic lamellar organization, and division of cells are all adversely affected (Aaliya Ashraf *et al.*, 2021). Lead toxicity same as in aquatic and in terrestrial environment, in terrestrial environment lead has a significant impact on the

development and growth of plants, lead pollution of soil has a number of detrimental impacts on plants, including decreased nutrient uptake, changes in plant water relations, and the production of ROS, which lowers photosynthesis and causes cell death, ultimately resulting in a significant drop in crop yield (Usman Zulfiqar *et al.*, 2019). Being exposed to lead in plants also significantly restricts growth of seedlings and germination. Lead hinders the growth of aerial parts of plants and roots at low doses. High levels of exposure to lead may also limit plant biomass. Photosynthetic plants typically suffer negative impacts on their adenosine triphosphate (ATP) levels and respiration when subjected to lead (Bertrand Pourrut *et al.*, 2011)

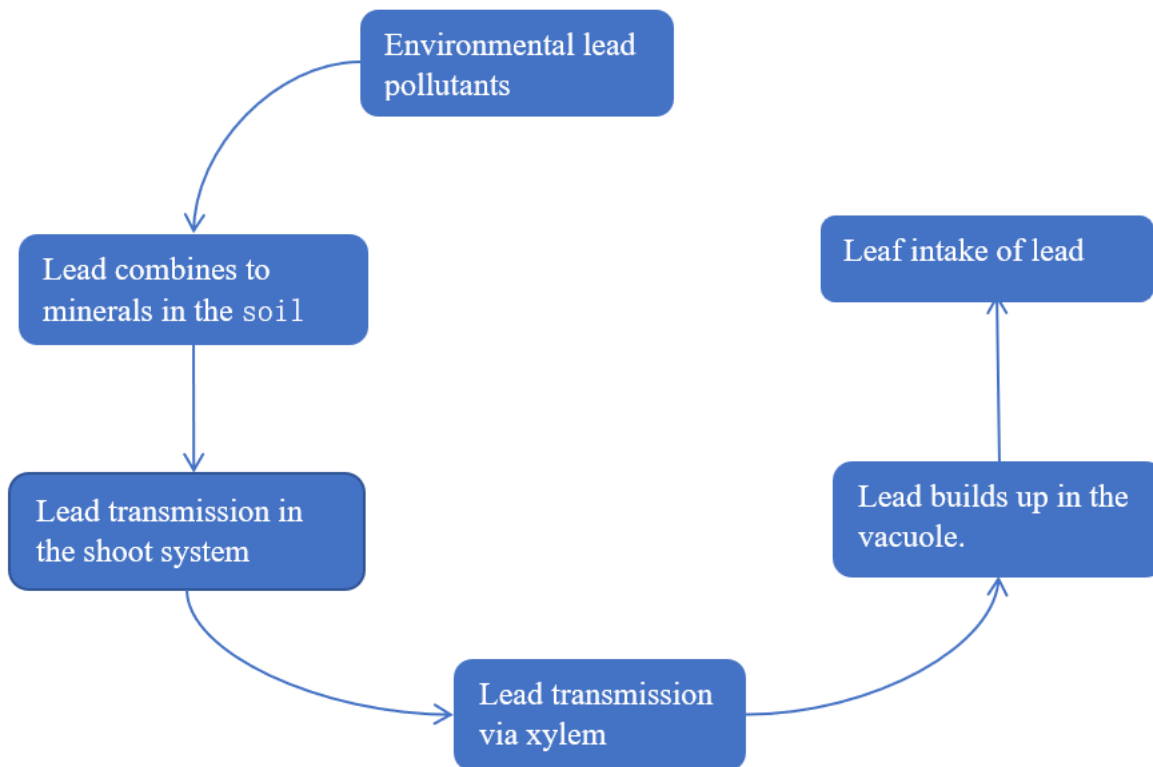


Figure 2. Bio - accumulation of lead into plants system through soil (Source: Samuel Collin *et al.*, 2022).

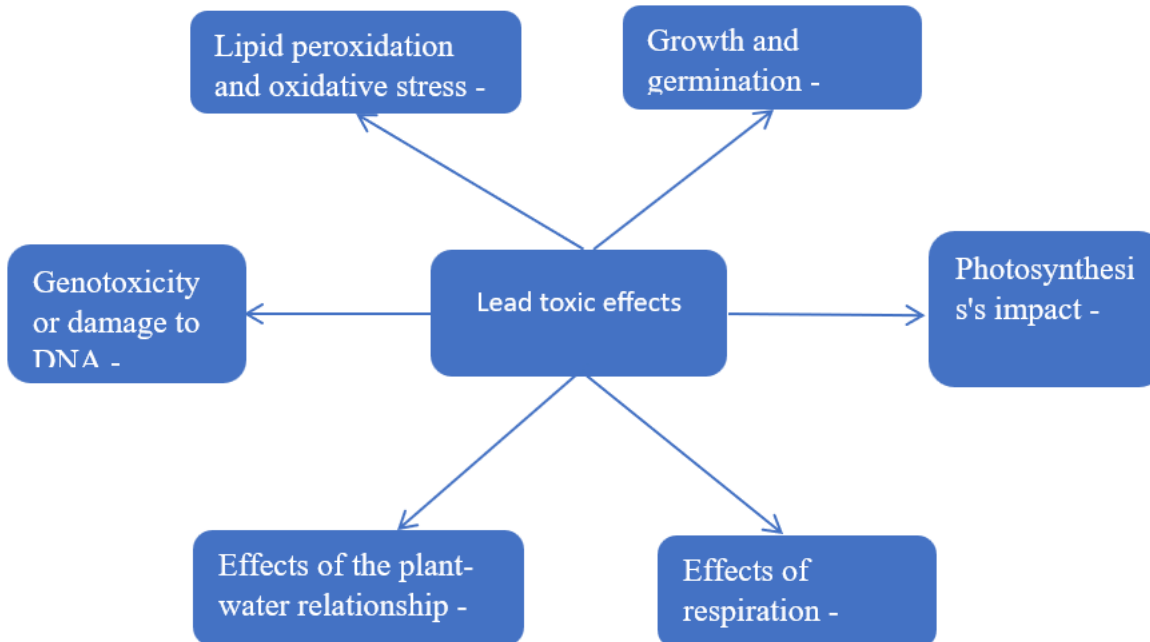


Figure 3. Effect of lead toxicity in plants (Source: Aaliya Ashraf *et al.*, 2021).

However the precise of the above research we get to know the effect of microplastic on the plants growth parameter, physiological parameters as well as get to know the lead toxicity on plants. So the aim of our present research to get the synergistic effect of microplastic + lead on tomato plant as tomato is an

economically important plant that is grown and consumed worldwide.

2. MATERIALS AND METHODS

i. Microplastic, Plant material and treatment

High density polyethylene microplastic with labelled rhodamine B fluorescent dye (HDPE microplastic) with a range of 1 -150 µm in diameter purchased from Green Nano, Udaipur, India. Tomato seeds were collected and authenticate from the IARI - Indian Agricultural Research Institute of India, and the variety of tomato collected for the research is (PUSA RUBY) (as it is perfect for the spring, summer, winter, and fall seasons). Tomato was chosen for this study because of its easy-to-measure parameters for examination, quick development cycle, sensitivity to external changes, and widespread usage.

This experiment is greenhouse pot experiment 5 - 5 pots used for both the experiment, the pots were filled with the ratio of 3 : 1 with garden soil and manure, and the pots were filled with the five different concentration of microplastic and lead. Five pots were filled with HDPE microplastic and five pots were filled with HDPE microplastic + lead nitrate with the concentration of (control, 50mg/L, 100mg/L, 150mg/L, 200mg/L + Control, 0.05mg/L, 0.1mg/L, 0.15mg/L, 0.2mg/L). control is taken in both the experimental setup for the comparison. The concentration of microplastic and lead were chosen based on the earlier experiment (Nuamzanei *et al.*, 2024 and Hirpa Abduro Ogo *et al.*, 2022).

Tomato seeds were surface sterilized and double distilled water (ddH₂O) was used for the soaking of tomato seeds for overnight. After the soaking process seeds were sowing into the experimental pots for the easy germination, the seedling should be uniformly. Distilled water (dH₂O) was used for the irrigation of pots on every alternative days.

ii. Growth parameters

Growth parameters: Root length, Shoot length, fresh weight and dry weight, for the measurement of these parameters the plants were randomly selected from the control group as well as from all the other concentration groups. To get rid of soil particles, the plants were removed and given a thorough wash. Both the root and shoot lengths were measured from the bottom to the longest root branch and from the bottom to the tallest leaf tip, respectively. Each plant's roots and shoots were removed at the root-shoot junction and evaluated with a digital balance to determine the plant fresh weight (PFW). In the meantime, the roots and shoots were oven-dried for 72 hours at 70 °C to determine the plant dry weight (PDW) (Nuamzanei *et al.*, 2024).

iii. Chlorophyll content

Chlorophyll was measured from the third and fourth leaves. Leaf material- Crushed in a pestle and mortar, adding 0.5g of MgCO₃ powder and 20 millilitres of 80% acetone, and then frozen for 4 hours at 40 degrees Celsius. Centrifuged for 5 minutes at 500 rpm and then

the supernatant was transferred to the 100 volumetric flask and absorbance were measured using a spectrophotometer. Using a spectrophotometer, the solution's color attenuation was measured at wavelengths of 645 and 663nm compared to the solvent, and the chlorophyll content calculated according to the formula of (Kamble *et al.* 2015).

Formula:

$$\text{Chl a} = 11.75 \times A_{662.6} - 2.35 \times A_{645.6}$$

$$\text{Chl b} = 18.61 \times A_{645.6} - 3.96 \times A_{662.6}$$

Where, A is absorbance.

3. RESULT AND DISCUSSION

□ Effect of HDPE microplastic on growth parameters (shoot length, root length, fresh weight, dry weight).

The current research concluded that the reduction in the shoot length of tomato plant with different concentration of HDPE microplastic (control, 50mg/L, 100mg/L, 150mg/L, 200mg/L) by 32%, which indicate the HDPE microplastic significantly decrease in the growth of tomato plant. This observation is consistent with past research on PVC on the growth of tomato plant and one more research on the observation of the effects of exposure to polyethylene (PE) and PE + PVC on *Lepidium sativum* and *Cucurbita pepo* L. growth after PVC exposure (Nuamzanei *et al.*, 2024 and Sara Pignattelli *et al.*, 2020).

As we see the length of the roots increases as the concentration PE microplastic increases, the root length increases by 20% from control to the 200mg/L concentration of microplastic. Therefore, the observed rise in primary roots could be a compensation mechanism for the secondary roots' decreased capacity to take up nutrients from the soil. This finding aligns with a comparable rise in root length noted in rice treated using polystyrene (PS) microplastics (Zhiqin Zhang *et al.*, 2022).

Plant fresh weight and plant dry weight also decreases compared to control as the concentration of PE microplastic increases. This observation is consistent with past research on PVC on the growth, plant fresh weight and plant dry weight of tomato plant.

□ Effect of HDPE microplastic on the chlorophyll content

As we have studied in our present research the increase in the microplastic concentration, decreases in the chlorophyll content as compared to the control. The reduction in sunlight harvesting for photosynthesis is indicated by the decrease in the amount of chlorophyll in the tomato plants treated with PE - MP. This observation we have studied in past research, When exposed to PE and PVC, *C. pepo* likewise showed same dose-dependent decrease in chlorophyll concentration (Ilaria Colzi *et al.*, 2022).

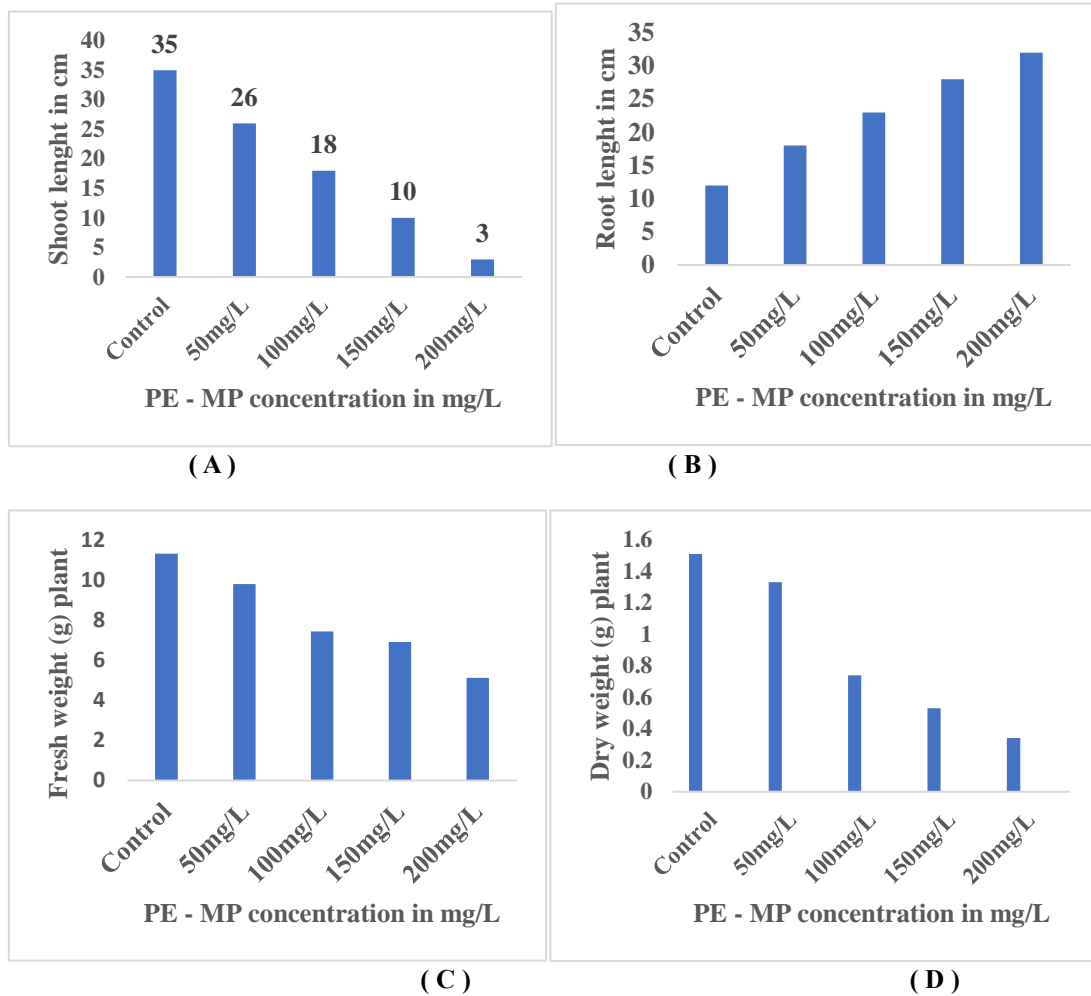


Figure 4. (A) Shoot length, (B) Root length, (C) Fresh weight, (D) Dry weight of tomato plant with different concentration of HDPE microplastic.

Table 1. Chlorophyll content of tomato plant with different concentration of HDPE microplastic.

S.NO	HDPE MICROPLASTIC CONCENTRATION	CHLOROPHYLL CONTENT
1.	Control	1.261
2.	50mg/L	1.099
3.	100mg/L	0.863
4.	150mg/L	0.693
5.	200mg/L	0.431

□ Effect of HDPE microplastic + lead nitrate on growth parameters (shoot length, root length, fresh weight, dry weight).

This current investigation concluded that at the concentration of 200mg/L + 0.2mg/L (HDPE microplastic + lead nitrate) tomato plant will stop germinating, at this concentration plant will not survive. And further studies at different concentration the shoot length decreases which indicates the HDPE microplastic + lead nitrate significantly stunted the growth of the plant. As we studied the length of the roots are also decreases as the concentration of these synergistic (microplastic + lead) increases (control, 50mg/L, 100mg/L, 150mg/L, 200mg/L) + (control, 0.05mg/L, 0.1mg/L, 0.15mg/L, 0.2mg/L). This shows that the bioaccumulation of lead nitrate makes the microplastic

more toxic. This observation is consistent with past research on PE microplastic on seed germination of tomato plant (Raveendra Gnana Keerthi Sahasa *et al.*, 2023). Plant fresh weight and plant dry weight also decreases compared to control as the concentration of (PE microplastic + lead nitrate) increases.

□ **Effect of HDPE microplastic + lead nitrate on the chlorophyll content**

As we have studied in our present research the increase in the microplastic + lead concentration, decreases in the chlorophyll content as compared to the control. The reduction in sunlight harvesting for photosynthesis is indicated by the decrease in the amount of chlorophyll in the tomato plants treated with PE -Microplastic + lead nitrate.

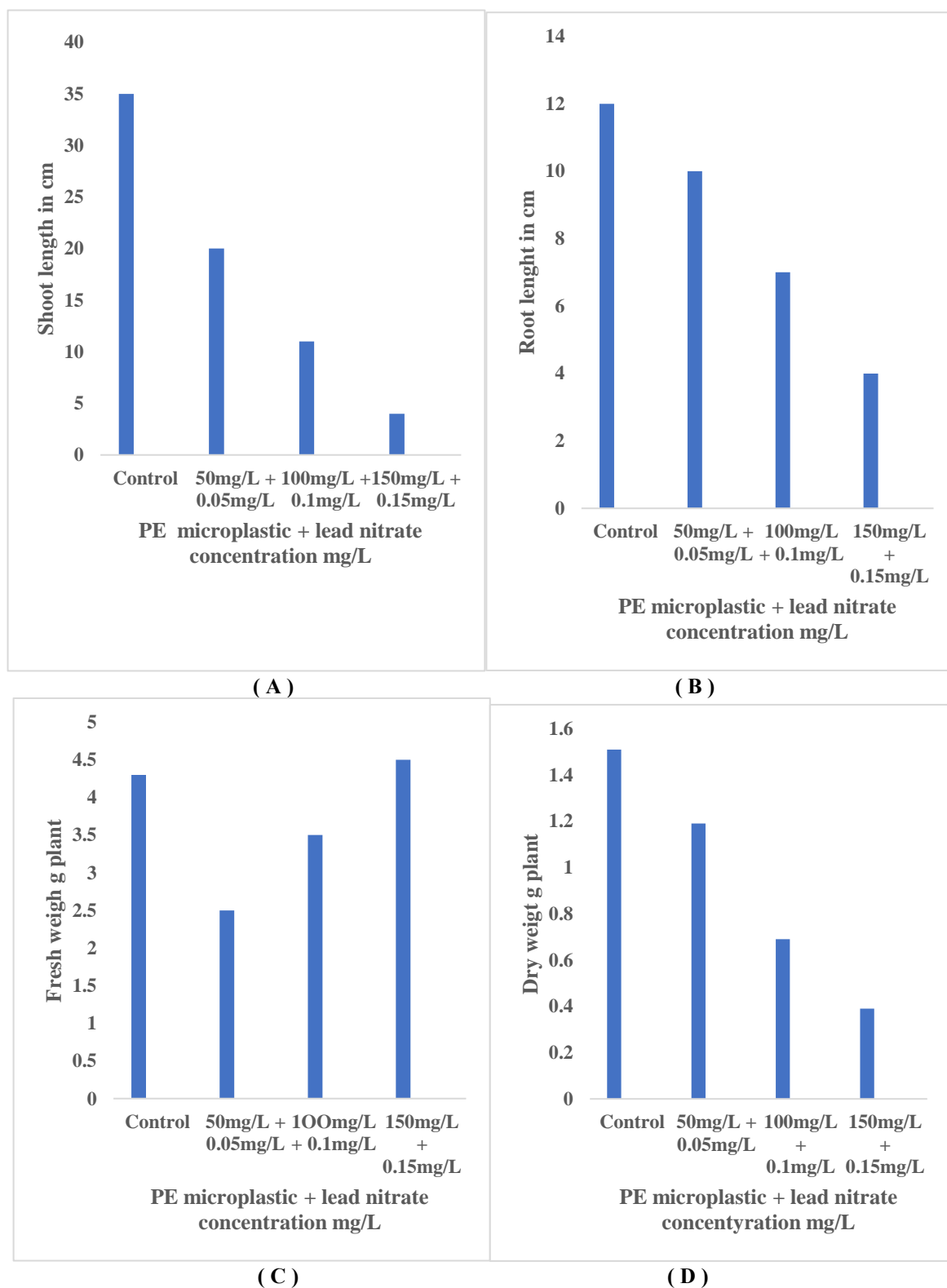


Figure 5. (A) Shoot length, (B) Root length, (C) Fresh weight, (D) Dry weight of tomato plant with different concentration of HDPE microplastic + lead nitrate .

Table 2. Chlorophyll content of tomato plant with different concentration of HDPE microplastic.

S.NO	HDPE MICROPLASTIC + LEAD NITRATE CONCENTRATION	CHLOROPHYLL CONTENT
1.	Control	1.261
2.	50mg/L + 0.05mg/L	0.891
3.	100mg/L + 0.1mg/L	0.593
4.	150mg/L + 0.15mg/L	0.215

4. CONCLUSION

This present study investigated the comparison of effect of HDPE microplastic and HDPE microplastic + lead nitrate on the growth and development of tomato plant and on chlorophyll content. The results revealed the presence of PE - MP and lead nitrate inside tomato roots suggesting that they can potentially accumulate into the food chain. The toxic effect of Microplastic decrease in the shoot length which stunted the growth of tomato plant, as well as increase in the root length of the plant. Therefore, the observed rise in primary roots could be a compensation mechanism for the secondary roots' decreased capacity to take up nutrients from the soil. Fresh weight and dry weight also decreases when the concentration of microplastic increases from control to 200mg/L. chlorophyll content decreases as compared to control. The reduction in sunlight harvesting for photosynthesis is indicated by the decrease in the amount of chlorophyll in the tomato plants treated with PE - MP.

As compared to microplastic the comparison study of microplastic + lead is more toxic than the single microplastic it decreases in the root length also, in synergistic effect the level of toxicity is much higher than the single effect. Synergistic toxicity stop the germination at the concentration of 200mg/L + 0.2mg/L. this study revealed that the bioaccumulation of lead nitrate by microplastic increase the toxicity of microplastic and heavy metal also.

The current investigation's generalizability is limited due to the use of a single plant species. The impact of microplastics on plants might exhibit significant variation depending on the specific microplastic composition and heavy metal and different plant species.

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