INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 05, Issue 09, September 2025, pp : 01-05
www.ijprems.com
editor@ijprems.com
e-ISSN : 2583-1062
Impact Factor : 7.001

COMPARATIVE ANALYSIS OF HEAVY METAL EFFECTS ON THE PHENOLOGICAL TRAITS OF LETTUCE (*LACTUCA SATIVA* L.) AND AMARANTH (*AMARANTHUS VIRIDIS* L.)

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ABSTRACT

Heavy metal contamination of agricultural land is detrimental to food safety, ecosystem and human health. This study assessed the impact of heavy metal contamination on phenological parameters of lettuce (*Lactuca sativa*) and amaranthus (*Amaranthusviridis*) under greenhouse condition. Plants were grown in soil contaminated with varying concentrations (10, 20 and 30 mg/kg) of lead (Pb), Copper (Cu), and Nickel (Ni). Completely randomized design with three replicates was used for the experiment. The phenological parameters (leaf number, plant height, leaf length, and stem diameter) were measured at 3 days, 2 weeks, and 3 weeks after sowing. The results revealed that heavy metal exposure significantly (P≤5%) affected the phenological traits of both plant species. Amaranthus recording the highest leaf number under Pb (9.33±1.53 at 30 mg/kg) followed by Cu (8.00±2.00cm at 20 mg/kg 2WAS) while the least (2.00±1.10cm) was found with Cu also at 10 mg/kg, 3DAS. The highest (6.33±0.58cm) and least (2.20±0.26cm) plant height for lettuce were recorded Ni and Cu (30 and 10mg/k) at 3WAS and 3DAS respectively. Stem diameter was highest under Ni (5.67±1.15 cm) with Amaranthus at 30mg/kg and lowest (1.27±0.46cm) in Lettuce at 10mg/kg at 3WAS and 3DAS respectively, as compared to the control.The findings suggest that low-moderate heavy metal concentrations possessed the growth and developmental effects on the plants examined.

Keywords: Lead, Nickel, Phenological Parameters, Lettuce, Amaranths, And Heavy Metals.

1. INTRODUCTION

Soil contamination by heavy metals has emerged as a critical environmental issue due to rapid industrialization, urban expansion, and the intensive use of agrochemicals (Khan *et al.*, 2015). These pollutants such as lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), and chromium (Cr) are not biodegradable and tend to persist in the environment, posing serious threats to food safety, soil health, and human well being (Alloway, 2013; Khan *et al.*, 2015). Heavy metal pollution has emerged due to anthropogenic activity which is the prime cause of pollution, primarily due to mining the metal, smelting, foundries, and other industries that are metal-based, leaching of metals from different sources such as landfills, waste dumps, excretion, livestock and chicken manure, runoffs, automobiles and roadwork (Ranald, 2021).Metals such as lead (Pb), copper (Cu), and nickel (Ni) are persistent, non-biodegradable, and toxic when accumulated in food crops, posing risks to human health (Alloway, 2013).

Conventional remediation methods, including excavation and chemical treatments, are often costly and ecologically disruptive, prompting interest in more sustainable alternatives such as phytoremediation (Khan *et al.*, 2015). Phytoremediation—the use of plants to remove or stabilize contaminants—offers a sustainable alternative to costly conventional remediation approaches (Ali et al., 2013). Leafy vegetables such as lettuce (*Lactuca sativa*) and amaranthus (*Amaranthus viridis*) are promising phytoremediators because of their rapid growth, high biomass, and reported ability to accumulate heavy metals (Cui et al., 2004; Muchuweti et al., 2006). However, comparative data on their growth responses under Pb, Cu, and Ni stress remain limited, particularly in Nigeria. This study thus, assessed the effects of Cu, Pb, and Ni on the phenological traits of lettuce and amaranthus under greenhouse conditions to determine their phytoremediation potential.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out at screen house, department of Agriculture of AbdullahiFodio University of science and technology Aliero. Aliero local government area is located at approximately latitudes 11° 03' S, 12 47N and longitudes 3° 6'W and 4° 27E (Singh, 2013).

2.2 Contaminants Selection

Three heavy metals viz: copper (Cu), lead (Pb), and nickel (Ni) were chosen based on their prevalence in agricultural soils, potential toxicity to humans and plants, and their capacity for bioaccumulation in edible crops. They are also commonly associated with anthropogenic activities like industrial emissions, use of phosphate fertilizers, and wastewater irrigation, which can lead to their accumulation in soils used for food production. The metals are also on the priority pollutants list of United State Environmental Protection Agency (USEPA) (He *et al*., 2015).

2.3 Plant Selection and Experimental Design

Lettuce (*Lactuca sativa* L.) and Amaranthus (*A. viridis*) L.) were selected for their short growth cycles, high consumption rates, and capacity to accumulate heavy metals in edible tissues (Ghosh et al., 2022). Certified seeds from the Research Institute, Bagwada Kano, were procured, sterilized and planted in 5 kg of metal-spiked soils, with one seedling per bucket arranged in a completely randomized design (Raza et al., 2022). Copper (Cu), lead (Pb), and nickel (Ni) were applied at 10, 20, and 30 mg/kg using $CuCl_2$, $Pb(NO_3)_2$, and $NiSO_4$, respectively, alongside a control (0 mg/kg). This gave 18 treatment combinations replicated three times, totaling 57 experimental plots. Greenhouse conditions were maintained at 22–28 °C, 60–70% relative humidity, and a 12–14 h photoperiod. Plants were irrigated with deionized water, with no fertilizers or pesticides applied (Singh et al., 2024).

2.4 Assessing the Effects of Heavy Metals on the Phenological Traits of the Plants

The plant phenological parameters were recorded from the plants in the experimental pots a week after Planting. The parameters assessed were plant height (cm) using a measuring tape, leaf area (cm^2) using the formula, F (L x W) (F = constant factor (0.75), L = leaf length and W = width of the leaf), Stem diameter (cm, using caliper), and number of leaves/plant by counting (Drew et al., 2023).

2.5 Data Analysis

Data were generated analyzed in SPSS (Version 26) using one-way ANOVA, at $p \leq 0.05$ level of significance.

3. RESULTS

3.1 Effect of Heavy Metals on the Morphological Parameters of the Plants

3.2. Effect on the Number of Leaf

Heavy metals significantly increased leaf number compared to control (Table 3.1). Amaranthus had the highest number of leaves under Pb at 30 mg/kg (9.33 ± 1.53), while lettuce performed best under Ni at 30 mg/kg (6.33 ± 0.58). Control plants consistently had the lowest leaf counts.

3.1 Effect on the Number of Leaf in Amaranthus and Lettuce plants

Heavy	Amaranthus			Lettuce		
Metals	3DAS	2WAS	3WAS	3DAS	2WAS	3WAS
	10mg/kg	20mg/kg	30mg/kg	10mg/kg	20mg/kg	30mg/kg
Cu	2.00 ± 1.10^{bc}	8.00 ± 2.00^{a}	7.13 ± 4.97^{a}	2.20 ± 0.26^{a}	3.93 ± 0.90^{a}	5.67 ± 0.58^{ab}
Pb	4.00 ± 1.10^{a}	6.00 ± 2.00^{ab}	9.33 ± 1.53^{a}	2.33 ± 0.89^{a}	3.50 ± 0.50^{a}	5.00 ± 1.00^{ab}
Ni	3.67 ± 1.15^{ab}	7.67 ± 1.53^{a}	7.07 ± 5.90^{a}	2.53 ± 0.50^{a}	4.00 ± 1.00^{a}	6.33 ± 0.58^{a}
Control	1.33 ± 0.58^{c}	3.67 ± 0.58^{b}	5.67 ± 0.58^{a}	1.33 ± 0.57^{b}	3.17 ± 0.29^{a}	4.73 ± 0.46^{b}

Different superscript letters denote significant differences ($p < 0.05$). Keys: 3DAS= 3 days after sowing; 2WAS= 2 weeks after sowing; 3WAS= 3 weeks after sowing

3.3 Effect on the Plant Height

As shown in Table 3.2, plant height increased progressively across all treatments over time. For Amaranthus, Ni at 30 mg/kg resulted in the highest height (8.50±2.18cm at 3WAS), followed by Pb and Cu with 7.87±1.96cm and 7.87±1.96cm respectively. In Lettuce, similar trends were observed, with the tallest plants found under Ni at 30 mg/kg (7.27±2.11cm).

3.2 Effect on plant of Amaranthus and Lettuce plant

Heavy	Amaranthus			Lettuce		
Metals	3DAS	2WAS	3WAS	3DAS	2WAS	3WAS
	10mg/kg	20mg/kg	30mg/kg	10mg/kg	20mg/kg	30mg/kg
Cu	$2.10{\pm}0.36^{b}$	$4.47{\pm}0.47^{ab}$	$7.67{\pm}2.08^{a}$	$1.83{\pm}0.15^{ab}$	$2.83{\pm}0.76^{b}$	$4.17{\pm}1.28^{b}$
Pb	$4.17{\pm}1.04^{a}$	$5.87{\pm}1.96^{b}$	$7.87{\pm}1.96^{a}$	$1.87{\pm}0.23^{ab}$	$2.96{\pm}0.47^{b}$	$3.57{\pm}0.81^{b}$
Ni	$4.30{\pm}1.01^{a}$	$6.50{\pm}2.18^{a}$	$8.50{\pm}2.18^{a}$	$3.17{\pm}1.61^{a}$	$5.50{\pm}1.80^{a}$	$7.27{\pm}2.11^{a}$
Control	$1.17{\pm}0.29^{b}$	$2.07{\pm}0.06^{b}$	$2.99{\pm}0.0^{b}$	$0.82{\pm}0.28^{b}$	$1.67{\pm}0.58^{b}$	$2.67{\pm}0.58^{b}$

Different superscript letters denote significant differences ($p < 0.05$). Keys: 3DAS= 3 days after sowing; 2WAS= 2 weeks after sowing; 3WAS= 3 weeks after sowing

3.4 Effect on the Leaf Length

Amaranthus treated with heavy metals recorded higher leaf heights than the control, especially at 30 mg/kg (Table 3.3). The highest (at 30 mg/kg) and less (at 10 mg/kg) values were observed with Pb (2.73±0.38 and 1.03±0.06 cm) at 3WAS and 3DAS respectively. In Lettuce, although the leaf height improved across treatments, differences were minimal, with the control performing comparably to treated groups. The highest (3.33±0.58cm) was recorded with Ni at 30 mg/kg while the lowest (1.27±0.46cm) was found with Pb at 10 mg/kg on 3WAS and 3DAS respectively.

3.3 Effect on the leaf length of Amaranthus and Lettuce plants

Heavy	Amaranthus			Lettuce		
Metals	3DAS	2WAS	3WAS	3DAS	2WAS	3WAS
	10mg/kg	20mg/kg	30mg/kg	10mg/kg	20mg/kg	30mg
Cu	$1.07{\pm}0.12^{a}$	$1.93{\pm}0.31^{a}$	$2.43{\pm}0.51^{a}$	$1.23{\pm}0.25^{a}$	$2.27{\pm}0.74^{a}$	$3.00{\pm}1.00^{a}$
Pb	$1.03{\pm}0.06^{a}$	$1.80{\pm}0.10^{a}$	$2.73{\pm}0.38^{a}$	$1.27{\pm}0.46^{a}$	$1.93{\pm}0.41^{a}$	$2.76{\pm}0.68^{a}$
Ni	$1.07{\pm}0.12^{a}$	$1.93{\pm}0.29^{a}$	$2.67{\pm}0.58^{a}$	$1.50{\pm}0.62^{a}$	$2.33{\pm}0.58^{a}$	$3.33{\pm}0.58^{b}$
Control	$0.67{\pm}0.26^{b}$	$1.23{\pm}0.23^{b}$	$1.30{\pm}0.17^{b}$	$0.86{\pm}0.05^{a}$	$1.70{\pm}0.60^{a}$	$2.20{\pm}0.17^{a}$

Different superscript letters denote significant differences ($p < 0.05$). Keys: 3DAS= 3 days after sowing; 2WAS= 2 weeks after sowing; 3WAS= 3 weeks after sowing

3.5 Effect on Stem Diameter

Table 3.5 present the result on effect of heavy metals on the steam diameter of Amaranthus and Lettuce plants, measured at 3DAS, 2WAS, and 3WAS. Stem diameter increased over time in both *Amaranthus* and lettuce across all treatments.

Nickel (Ni) produced the largest stem diameters in *Amaranthus* (2.00–5.67 cm), highest at 30 mg/kg.In lettuce, Ni also gave the greatest values (1.00–2.50 cm), especially at 2 WAS and 3 WAS.All heavy metal treatments enhanced stem growth compared to the control.

3.5. Effect of Heavy Metals on Stem Diameter in Amaranthus and Lettuce

Heavy	Amaranthus			Lettuce		
Metals	3days AS	2WAS	3WAS	3daysAS	2WAS	3WAS
	10mg/kg	20mg/kg	30mg/kg	10mg/kg	20mg/kg	30mg/kg
Cu	$1.47{\pm}0.92^{ab}$	$3.17{\pm}0.76^{a}$	$3.33{\pm}2.02^{ab}$	$0.67{\pm}0.21^{bc}$	$1.07{\pm}0.40^{b}$	$1.53{\pm}0.61^{bc}$
Pb	$1.67{\pm}0.58^{ab}$	$3.33{\pm}1.15^{a}$	$5.33{\pm}2.08^{a}$	$1.07{\pm}0.12^{ab}$	$1.50{\pm}0.52^{ab}$	$2.33{\pm}0.58^{ab}$
Ni	$2.00{\pm}1.00^{a}$	$3.67{\pm}0.58^{a}$	$5.67{\pm}1.15^{a}$	$1.27{\pm}0.46^{a}$	$1.93{\pm}0.12^{a}$	$2.50{\pm}0.50^{a}$
Control	$0.27{\pm}0.02^{b}$	$1.03{\pm}0.06^{b}$	$1.13{\pm}0.12^{b}$	$0.25{\pm}0.04^{c}$	$0.97{\pm}0.06^{b}$	$1.27{\pm}0.06^{c}$

Different superscript letters denote significant differences ($p < 0.05$). Keys: 3DAS= 3 days after sowing; 2WAS= 2 weeks after sowing; 3WAS= 3 weeks after sowing

4. DISCUSSION

The current study assessed the potentials effects different concentrations (10, 20, 30 mg/kg) of copper (Cu), lead (Pb), and nickel (Ni), on phonological parameters of *Lactuca sativa* (lettuce) and *Amaranthus viridis* (Amaranthus). The experiment was carried in soils artificially spiked with these metals to to determine their phytoremediation capacity under greenhouse conditions.

The exposure of the experimental plants to Cu, Pb, and Ni at varying concentration have been observed to influenced their main phenological traits, including plant height, leaf number, leaf length, and stem diameter of the experimental plants. Amaranthus exhibited the highest leaf number (9.33±1.53) under Pb at 30 mg/kg, while lettuce achieved its maximum (6.33±0.58) under Ni. Both species recorded high plant height under Ni treatment, with Amaranthus reaching 8.50±2.18 cm and lettuce 7.27±2.11 cm respectively. These values were significantly higher ($p < 0.05$) than those in control plots. However, leaf length showed minimal variation ($p > 0.05$).

This growth stimulation pattern aligns with the concept of hormesis, where low to moderate levels of some heavy elements cause positive growth responses (Ali et.al 2021). Cu and Ni, as essential micronutrients, support metabolic functions at sub-lethal concentrations (Alloway, 2013). The higher growth performance of Amaranthus could be linked to its deeper root system and higher metal tolerance, as demonstrated by (Etuk et al. (2024) and Liu and Wang (2024). Similarly, the enhanced uptake in lettuce may be due to its shallow roots, facilitating greater contact with bioavailable metals.

Previous studies (El-Gendy*et al.,* 2022**;** Huang et al., 2025; Rahman et al., 2023) also reported growth stimulation in leafy vegetables under controlled metal stress. The findings of Alamet al. (2020) and Garba et al. (2019) found that heavy metals in agricultural soils can lead to variable but often enhanced plant responses, depending on species and soil conditions. These findings affirm that the phenological changes observed in this study are consistent with established physiological responses to low-level heavy metal exposure.

From the observed data in the present study, the higher concentration of selected heavy metals in Amoathus can probably be due to the nature of its extensive vascular architecture compared to that in lettuce with dense-leaved heads and shallow roots. The current results thus, suggest that lettuce is more suitable for phytoremidiation of Cu and Pb, while Amaranthus demonstrates potential for Ni remediation via translocation. This distinction emphasizes the importance of plant selection in phytoremediation strategies, especially in soils contaminated with mixed metal pollutants.

5. CONCLUSION

Lettuce and amaranthus exhibited distinct responses to heavy metal stress. Lettuce showed higher accumulation and tolerance for Cu and Pb, while amaranthus responded better to Ni. Moderate levels of heavy metals enhanced growth traits, suggesting hormetic stimulation. Both crops demonstrate phytoremediation potential: lettuce for Cu and Pb, and amaranthus for Ni.

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