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Effects of environmental stressors on morphological traits of *Glycine max* (L.) Merr. and microbial diversity of soil treated with cadmium and lead

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ABSTRACT

This study was carried out to investigate the effects environmental stressors of cadmium and lead on the germination and morphological traits (growth properties) of *Glycine max*. The experiment was carried out with four treatments and control with four replicates per treatment. Five (5) seeds were planted per bag in order to carry out germination study. Prior to planting the seed, the soil was treated with Cadmium chloride ($CdCl_2 \cdot H_2O$) and Lead nitrate [$Pb(NO_3)_2$] in the following proportions; 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg and laid out on the prepared site in a completely randomized setting. Data for germination percentage were collected from day 3 to day 6 and data for growth parameters (height, number of leaves, number of branches, and stem girth) were collected from 2 weeks after planting (2WAP) to 6 weeks after planting (6WAP). The values recorded for germination in control, 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg for day six (6) were 84.00±16.73, 75.00±30.00, 95.00±10;00, 70:00±25:82, and 55.00±25.17 percent respectively. There was no significant difference between the various levels of treatment and control in percent germination of *Glycine max*. However, germination was apparently enhanced in the 100mg/kg treatment. For morphological traits, height of *Glycine max*, the values of control, 50mg/kg, 100mg/kg, 150mg/kg and 200mg/kg at 6WAP were 34.13±6.01, 32.75±4.03, 34.43±7.18, 25.18±4.29 and 31.00±6.38 cm respectively. There was no significant difference ($p<0.05$) in height of *Glycine max* observed between the various levels of treatment and control. Similar results were obtained for number of leaves, number of branches, and stem girth. The results on microbial analysis distribution showed that *Bacilius cereus* was found in all the treatments and control. However, the 150mg/kg and 200mg/kg treatments had less species diversity. Fungal population was adversely affected by heavy metals. For cadmium (cd) and lead (pb) content of the soil and seed samples that were analyzed, values recorded shows that metals present in the soil and seeds increased along the concentration gradient.

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INTRODUCTION

The presence of heavy metals in the environment is of great ecological concern owing to their toxicity at certain levels in living organisms and their non-biodegradability attribute which is responsible for their bio-accumulation in living organisms (Tariq *et al.,* 2025). The accumulation of heavy metals in agricultural soils is of increasing concern due to food safety and potential health risks as well as its detrimental effects on soil ecosystems (Ede *et al.,* 2025). The dangers these pose are even more relevant to us owing to our indecent and indiscriminate disposals of heavy metal containing materials and equipment. The increasing influx of heavy metals into water bodies from industrial, agricultural, and domestic activities is of global concern because of their well-documented negative effects on human and the ecosystem (Mataka *et al.*, 2006).

Pollutants in the soil and environmental variables can occasionally affect how plants germinate and thrive. The pollutant in the soil leads to soil degradation which is a significant issue in both agriculture and everyday life (Lal, 2025).

Soil life plays a major role in many natural processes that determine nutrient and water availability for agricultural productivity. In addition to serving as a medium for plant development and waste disposable, soil also acts as a pollutant transmitter for air, groundwater, surface and food. Soil contamination can also have a negative impact on the quality of air (Adam and Nganje, 2010). The accumulation of toxic elements in soil in various regions of Nigeria and around the world is largely due to improper waste and sewage disposable, agricultural and production practices like unauthorized mining of solid minerals, excessive use of fertilizers and pesticides, oil spells and industrial dumping (Adeyi *et al.*, 2014). The accumulation of chemicals, salts, radioactive materials, toxins or disease-causing agents in the soil that have negative impact on the pants and animal health constitutes soil pollution.

The term Heavy metal includes transition metal, metalloids, lanthanides and actinides, which has been proposed based on their density, atomic number and the chemical properties or toxicity (John, 2002). Several studies have been conducted in order to evaluate the effects of different heavy metals on plants (Reeves and baker, 2000). Heavy metals have been reported to interfere in biochemical reactions of plant and induce physiological disorders like reduction in leaf chlorophyll. Although, some heavy metals such as copper (Cu), zinc (Zn), magnesium (mg), iron (Fe), etc. are essential in plant nutrition, many heavy metals do not play significant role in the plants physiology. Cadmium, in its purest form, is a soft silver white metal that is found naturally in the earth's crust. Cadmium rarely exists in its pure form. In the environment, it is usually in combination with other elements. Such compound includes cadmium oxide, cadmium nitrates and cadmium sulfide. Human activities such as irrigation of farmland with industrial effluent or sewage water and the use of fertilizer have largely contributed to cadmium load in the environment. Among all heavy metals, cadmium appears to be one of the most dangerous elements to all kinds of organs (Wojcik and Tukiendorf, 2005). Cadmium has been identified as one of the most photogenic heavy metal (Pilom-Smits, 2005). It has a highly toxic contaminant that affects many plants metabolic processes (Like *et al.,* 2008). Cadmium has shown to inhibit enzymatic activities in plants. High concentration of Cadmium in the soil represents a potential threat to human health because it is incorporated in the food chain mainly by plant uptake.

In Nigeria, lead is one of the toxic heavy metals which are naturally present in low quality fossil fuel, and it pollutes roadside soils and industrial environments all over the world (Edegbai and Anoliefo, 2016). Lead is one of the most available trace elements. The Agency for Toxic substances and Disease Registry ranks it second among all hazardous substances (ATSDR, 2007). Lead has become ubiquitous in the soil and the environment as a result of natural deposit and increased human activity. Given the pervasiveness of lead toxicity, it is critical to determine whether vegetables, fruits and food chops are safe for human consumption (Edegbai and Anoliefo, 2016).

Socio-economic crops serve as means of export to countries around the world and Soybean (*Glycine max*) provides about 64% of the world's oilseed meal supply as well as 28 % of total vegetable oil production (USDA, 2000). Soybean is a major economic crop. In some developing countries like Nigeria, particularly in rural regions, soybean symbolizes the unsurpassed source of non-meat protein accessible for enhancing the dietetic value of most traditional food. The preference of soybean as a better alternative for meat proteins is predicated on the fact that the seeds contain high protein levels, and its amino acid composition is approximate to the composition of animal proteins (Sikorski, 2007). Cultivation of soybean seeds from farms, the availability of essential mineral elements, including favorable environmental conditions must be guaranteed; among such mineral elements is nitrogen. Among mineral elements, nitrogen is required by plants in large amounts. It acts as a constituent of several plant cell components, including amino acids as well as nucleic acids. Thus, the deficiency of nitrogen inhibits plant growth rapidly. When such a deficiency persists, most species display chlorosis (yellowing of the leaves), that is seen in older leaves close to the base which ultimately fall off the plant. However, younger leaves may not display these symptoms initially because nitrogen can be mobilized from older leaves. Due to the high dependence on cash crops such as soybean (*Glycine max*) to man, animal and the generation of income, this research was therefore carried out to investigate the effects of cadmium and lead on Glycine max.

Cadmium (Cd) and Lead (pb) are among the most common heavy metals in agricultural soils. Cadmium and lead are considered as environmental hazards, as they are toxic for human being and other organisms (Wang *et al.,* 2006; Ekmekyapar *et al.,* 2012; Ghaderi *et al.,* 2012). There is a culture of impunity in the way society manages its wastes. Literature has shown that organ failures are directly or indirectly linked to pollution (mostly heavy metals). The average life expectancy of a Nigerian is placed at 45 years and this is reducing by the second. *Glycine max* through biological nitrogen fixation, meets most of the nitrogen requirements of the soil. *Glycine max* is cultivated worldwide and is consumed as food and animal feed. Therefore, the aim of this study was to investigate the effects of environmental stressors of cadmium and lead contaminants on soil microbial diversity and morphological traits of *Glycine max* L.

MATERIALS AND METHODS

Study area

The study was conducted at the undergraduate project site behind the life science complex, in the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Edo State, Nigeria which lies within humidity tropical vegetation (6°23`50"N and 5°37'23"E).

Collection and preparation of plants seeds and soil samples

The seeds of *Glycine max* used for this study were obtained from local market in Benin City, Nigeria. Soil samples were collected from undisturbed site at the University of Benin premises. Top soil (0-10cm), was collected from the plot. Thereafter, five (5) kg soil was placed into 20pieces of planting bags which was labeled and perforated.

The experiment was carried out using an adopted method from the study of Ede *et al.* (2025). The site used for this experimental layout was properly weeded and the surface covered with black cellophane to confine the root to the soil within the nylon bags. The soil collected was dried and thoroughly mixed. Five (5) kg dried soil was weighed, each into 20 perforated planting bags which were marked for proper identification. One control set was used for the entire experiment.

Heavy metal

Two heavy metals; cadmium (cd) and lead(pb) used for the study were obtained from their soluble salts, Cadmium Nitrate ($Cd(NO_3)$ $4H_2o$) and Lead Nitrate Pb (NO^3) and quantities of the heavy metals corresponding to the various treatments were then calculated.

Viability studies

Viability study was done to determine good seeds for planting and it was done by floatation method. The seeds of *Glycine* max were soaked in a bowl of water and left for two (2) minutes, the bad ones floated to the top of the bowl which were removed, then the ones that sunk below which were the good seeds were used for planting.

Experimental setup and treatment

The experiment was carried out with four treatments and one control with four replicates per treatment. The soil was treated with Cadmium chloride ($CdCl_2 \cdot H_2O$) and Lead nitrate [$Pb(NO_3)_2$] in the following proportion; 50mg/kg, 100mg/kg, 150mg/kg, 200mg/kg and control. Each planting bag was filled with 5kg of treated soil and the planting bags which were perforated for proper drainage, were identified and laid out on the prepared site in a completely randomized setting (Erhenhi *et al.,* 2019a). The soils were left for four days, following which the seeds were planted at a depth of 3cm. Five (5) seeds were planted per planting bag in order to carry out germination studies. Watering was done regularly; early in the morning or late in the evening. Germination readings were taken till the 6th day of planting.

Determination of morphological traits

The plants were thinned to one per bag so as to effectively carry out morphological analysis. The various readings of the plant (*Glycine max*) height, girth, leaf area, number of branches and number of leaves were taken at a 7 days interval (Erhenhi *et al.,* 2019b).

Soil analysis

Soil microbial analyses, physicochemical analyses and heavy metal analyses in both soil and plant parts were done according to established procedures as adopted by Iyama *et al.* (2022).

RESULTS

Effect of cadmium and lead on germination and morphological traits of *Glycine max*

Germination of *Glycine max* was assessed from day 3 to day 6 under different concentrations of cadmium and lead. By day 6, percentage germination ranged from 55.00 ± 25.17% at 200 mg/kg to 95.00 ± 10.00% at 100 mg/kg, while the control recorded 84.00 ± 16.73%. Although no statistically significant difference (p< 0.05) was observed among treatments, germination appeared enhanced at 100 mg/kg, whereas higher concentrations (200 mg/kg) showed reduced germination (Fig. 1).

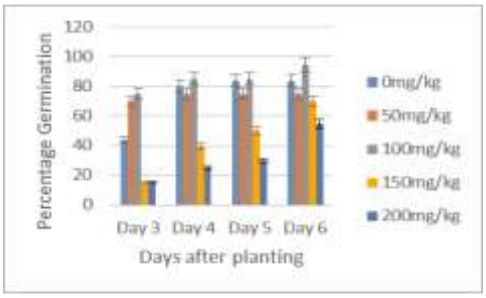

Fig. 1. Percentage germination of *Glycine max* treated with cadmium and lead

Plant height

Plant height measured at 2 and 6 weeks after planting (WAP) showed no significant differences across treatments. At 6 WAP, values ranged from 25.18 ± 4.29 cm at 150 mg/kg to 34.43 ± 7.18 cm at 100 mg/kg, compared to 34.13 ± 6.01 cm in the control. This indicates that cadmium and lead had minimal impact on plant height within the tested concentrations (Fig. 2).

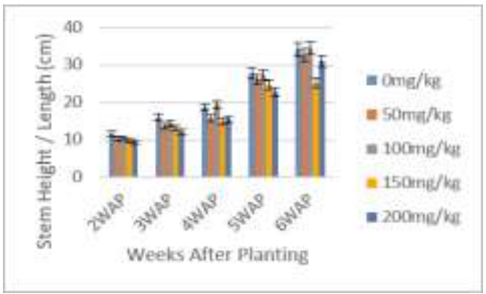

Fig. 2. Effect of cadmium and lead on the height/length of the stem (Cm) of *Glycine max*

Stem girth

Stem girth at 6 WAP ranged from 1.63 ± 0.50 cm to 1.80 ± 0.14 cm across treatments, with no significant difference (p< 0.05) observed. This suggests that stem thickness was not markedly affected by heavy metal exposure (Fig. 3).

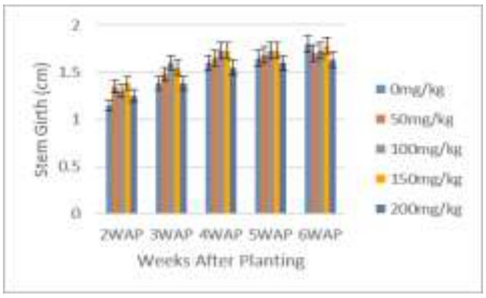

Fig. 3. Effect of cadmium and lead on the stem girth (cm) of *Glycine max*

Number of leaves

Leaf production at 3 WAP showed similar values across all treatments, ranging from 9.00 ± 1.41 to 10.50 ± 1.29 leaves. No significant variation was observed, indicating that leaf development was not significantly influenced by cadmium and lead concentrations (Fig. 4).

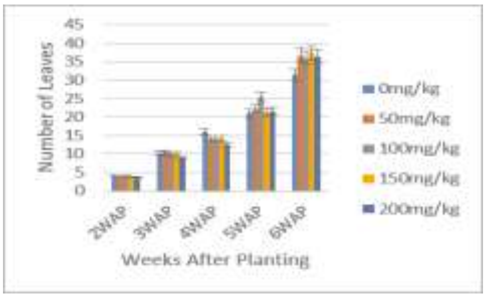

Fig. 4. Effect of cadmium and lead on the number of leaves of *Glycine max*

Number of branches

No branching was observed at 2 WAP across all treatments. At 6 WAP, branch numbers ranged from 9.25 ± 2.36 to 13.50 ± 4.43, with the control showing the highest value. However, there was no significant difference ($p < 0.05$) among treatments (Fig. 5).

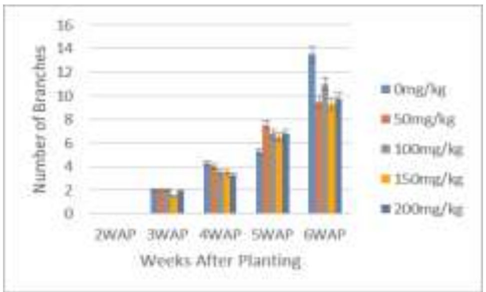

Fig. 5. Effect of cadmium and lead on the number of branches of *Glycine max*

Microbial distribution in soil samples

Microbial analysis revealed variations in bacterial and fungal species across treatments. *Bacillus cereus* was the most frequently occurring bacterium, present in the control and higher treatment levels (100–200 mg/kg). Other bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus mirabilis* showed treatment-specific occurrence. Fungal diversity was highest at 50 mg/kg, with species including *Aspergillus niger*, *Mucor* spp., *Penicillium notatum*, and *Aspergillus fumigatus*. Heavy metal exposure negatively affected fungal populations, although some species demonstrated tolerance at higher concentrations. A significant difference was observed in fungal distribution across treatments (Table 1).

Table 1. Microorganism species present in the soil samples

Sample code (mg/kg)	Bacteria isolates	Fungal isolates
Control (0)	*Bacillus cereus* *Staphylococcus aureus*	*Mucor* spp *Aspergillus niger*
50	*Bacillus subtilis* *Staphylococcus aureus*	*Aspergillus niger* *Aspergillus fumigatus* *Mucor* spp
100	*Bacillus cereus* *Proteus mirabilis*	*Aspergillus niger* *Mucor* spp
150	*Bacillus cereus*	*Penicllium notatum* *Aspergillus niger* *Mucor* spp
200	*Bacillus cereus*	*Aspergillus fumigatus*

Bacterial enumeration

Bacterial counts ranged from 1.0 × 10^5 ± 28.284 at 200 mg/kg to 2.2 × 10^5 ± 84.852 at 150 mg/kg. The control and 50 mg/kg treatments showed similar counts (2.2 × 10^5 ± 28.284), while a decline in bacterial population was observed at higher concentrations, particularly at 200 mg/kg, indicating inhibitory effects of heavy metals on microbial growth (Table 2).

Heavy metal concentration in soil and seed

Analysis of cadmium (Cd) and lead (Pb) concentrations showed a consistent increase with treatment levels. Cadmium concentration in soil was highest at 200 mg/kg (0.037) and lowest at 100 mg/kg, while it was not detected at 50 mg/kg. In seeds, lead accumulation was highest at 200 mg/kg (0.072) and lowest at 50 mg/kg (0.028). Cadmium was not detected in seeds at 50 mg/kg. These results indicate increased uptake and accumulation of heavy metals in both soil and plant tissues with increasing treatment concentration (Table 3).

Table 2. Microbial counts of the soil samples

Treatment (mg/kg)	Bacteria counts ($\times 10^5$ cfu/g ± SD)	Fungal counts ($\times 10^3$ cfu/g ± SD)
Control	2.2×10^5 ± 28.284	7.0×10^3 ± 1.414
50	2.2×10^5 ± 28.284	1.3×10^3 ± 3.535
100	1.8×10^5 ± 28.284	1.5×10^3 ± 1.414
150	2.2×10^5 ± 84.852	1.1×10^3 ± 1.414
200	1.0×10^5 ± 28.284	1.7×10^3 ± 1.414

Table 3. Heavy metal analysis for soil and seed concentration in mg/kg

Treatment (mg/kg)	Soil		Seed	
	(Cd)	(Pb)	(Cd)	(Pb)
50	ND	0.011	ND	0.041
100	0.0005	0.014	0.005	0.028
150	0.027	0.022	0.027	0.072
200	0.037	0.025	0.048	0.036

ND – Not detected

DISCUSSION

The findings of this study indicate that cadmium and lead contamination did not significantly affect the germination of *Glycine max* across most treatment levels, although slight inhibition was observed at the highest concentration (200 mg/kg). This suggests that soybean seeds exhibit a degree of tolerance to heavy metal exposure during early developmental stages. Similar observations have been reported by Soyingbe *et al.* (2007), and Azmat *et al.* (2005), who found no significant variation in germination under comparable conditions. The minimal effect at lower concentrations may be attributed to protective mechanisms such as seed coat selectivity, soil pH buffering, and nutrient interactions. However, reduced germination at higher concentrations aligns with previous reports indicating that excessive heavy metals can interfere with water uptake and hormonal regulation, particularly gibberellic acid activity, thereby suppressing germination.

In terms of growth performance, no significant differences were observed in plant height, girth, leaf number, or branching across treatments when compared to the control. This finding contrasts with studies such as Fatoba *et al.* (2012), which reported growth inhibition under heavy metal stress, but agrees with Eshghi *et al.* (2010), suggesting that plant response may vary depending on environmental conditions and exposure duration. The absence of early growth effects may indicate that cadmium and lead toxicity manifests more prominently at later developmental stages or under prolonged exposure, influenced by processes such as metal chelation and soil chemical interactions.

The accumulation of cadmium and lead in both soil and seed samples followed a concentration-dependent trend, confirming that metal uptake by plants is largely governed by availability in the soil. This supports the linear accumulation model described by Benzarti *et al.* (2008). The presence of these metals in seeds raises significant public health concerns due to the potential for bioaccumulation and biomagnification in consumers. Chronic exposure to cadmium and lead has been associated with severe health outcomes, including organ damage, neurological disorders, and increased mortality, as highlighted by global health reports. Notably, some measured concentrations exceeded recommended safety limits, indicating that such contaminated crops may be unsafe for consumption.

Furthermore, heavy metal contamination significantly impacted soil microbial communities. A marked reduction in microbial biomass and diversity, particularly among fungi, was observed with increasing metal concentrations. This suggests that heavy metals exert toxic effects on soil biological activity, disrupting ecological balance and nutrient cycling. Although some fungi demonstrated tolerance under extreme conditions, overall microbial suppression confirms the detrimental influence of cadmium and lead on soil health. The variation in microbial sensitivity across studies highlights the complexity of soil ecosystems and the influence of environmental factors such as pH, organic matter, and exposure duration.

Overall, the study demonstrates that while early plant growth parameters may not be significantly affected by moderate heavy metal contamination, there are substantial risks associated with metal accumulation in edible plant parts and the degradation of soil microbial integrity. These findings underscore the importance of monitoring and managing heavy metal pollution in agricultural soils to safeguard crop quality, environmental sustainability, and public health.

CONCLUSION

This study demonstrates that cadmium and lead contamination had no statistically significant effect on the germination and early growth parameters of *Glycine max*, although slight reductions were observed at higher concentrations. Germination was highest at 100 mg/kg and lowest at 200 mg/kg, while growth indices such as plant height, girth, leaf number, and branching showed no significant variation across treatments when compared with the control. These findings suggest that soybean exhibits a degree of tolerance to moderate levels of heavy metal exposure during early developmental stages. In conclusion, while soybean growth may not be markedly impaired by moderate heavy metal contamination, the associated risks of metal accumulation in edible tissues and disruption of soil microbial ecology highlight the need for strict monitoring and management of contaminated agricultural soils to ensure food safety and environmental sustainability.

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