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Assessing the Toxicity of Heavy Metals and Potential Tolerance of Common Bean (*Phaseolus vulgaris*) while Monitoring the Population Dynamics of the Associated Rhizobia

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Abstract

Microbially-assisted phytoremediation (MAP) is increasingly recognized as the feasible alternative for removing hazardous heavy metals (HMs) from contaminated environments. However, the dynamics of rhizobial-plant interactions during phytoremediation remain unclear. This study investigated the toxicity of some selected heavy metals (Cobalt, Nickel, and Manganese), the potential tolerance of Phaseolus vulgaris grown in the HMs-rich effluents, and the population dynamics of the associated Rhizobia within the Katsina metropolis. After 80 samples of P. vulgaris collected from Lambun Sarki garden were exposed to 10 mL of 0.5-2g/L of Ni and Co and 5-20 g/L Mn, respectively, in mesocosms, and the plants treated with 10 mL HMs solutions daily. After three weeks treatment and then Indices of HMs toxicity on seeds and plants (4 and 3, respectively) were monitored in all the treatments. Weekly rhizobial counts on Congo Red Yeast Extract Mannitol Agar (CRYEMA) were taken to monitor rhizobial population dynamics. Pure isolates obtained after three iterations were identified biochemically. One-way ANOVA was employed for statistical analyses using AnalyStat (version 1.6.50). Generally, Ni exerts the highest toxicity, with Mn having less toxicity. Average rhizobial counts increased weekly, with high counts obtained in Ni and Mn treatments. However, they did not differ significantly between weeks (p = 0.061). Thus, longer time intervals (>2 weeks) are required to observe significant shifts in population dynamics. Moreover, HMs concentration did not affect the colony counts (p = 1.00). Metabolism profile of the preliminarily identified Rhizobium sp. and Sinorrhizobium melliloti evidenced HMs removal and plant growth promotion ability. The research demonstrated the phytoremediation ability of P. vulgaris and how rhizospheric population dynamics change during phytoremediation and contributed towards understanding HMs impact as environmental stressors on rhizospheric plant-microbe interactions. Future research targeting the hyperaccumulation capacity of the plants and heavy metals tolerance of the identified rhizobia are recommended, as this may help in knowing the BCF, TF, and BAC of the plants as well as the tolerable amount of the heavy metals to the bacteria.

Keywords: Heavy metals, Phytoremediation, Phaseolus vulgaris, Rhizobia.

INTRODUCTION

Phytoremediation is an eco-friendly, sustainable, cost-effective, non-invasive, and aesthetically pleasing emerging technology that employs plants and associated microbes to accumulate, detoxify, and stabilise contaminants in soil and water (Sharma *et al.*, 2023). It encompasses four main mechanisms: phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration, with phytoextraction being the most efficient and commercially viable (Sharma *et al.*, 2023). While heavy metals naturally occur in soil, their accumulation beyond certain thresholds poses a global environmental hazard, impacting soil fertility, water quality, agricultural productivity, and human health (Lebrazi and Fikri, 2018; Umar *et al.*, 2020; Yahaya *et al.*, 2021). Given the environmental damage caused by heavy metals and their threats to the ecosystem, removing them to ensure a safe environment is imperative (Lebrazi and Fikri, 2018). Phytoremediation offers advantages such as increased efficiency, cost-effectiveness, and environmental friendliness compared to other removal strategies (Mohamed *et al.*, 2015). However, not all plants can accumulate heavy metal pollutants, and even cultivars within the same species vary in their capabilities (Lone *et al.*, 2008).

Although microbially-assisted phytoremediation (MAP) is recognized as a feasible alternative for removing hazardous heavy metals from contaminated environments, research on the toxicity of the heavy metals to the plants, tolerability of the plants to the heavy metals as well as the population dynamics of rhizobial-plant interactions during the phytoremediation process is still unclear. Understanding these interactions can elucidate the impact of heavy metals as plant growth inhibitors and the potential of the plant to overcome the metals' toxicity in the presence of the associated Rhizobia, thus contributing to a better understanding of the scenarios involved in the phytoremediation processes.

This research, therefore, aims to address these knowledge gaps by assessing the toxicity of the HMs and the potential tolerance of *Phaseolus vulgaris* to the selected heavy metals (Ni and Co at concentrations of 0.5-2g/L, and Mn at concentrations of 5-20 g/L) to examine the effects of these metals on the plant. Additionally, the study explores the role of specific Plant Growth Promoting Rhizobacteria (PGPR) known to contribute to phytoremediation processes (Li *et al.*, 2021) through weekly microbial counts and cellular identification of predominant isolates.

MATERIALS AND METHODS

Description of the Study Area

The sampling site was Lambun Sarki Garden, a major vegetable source in the centre of the Katsina metropolis (Abubakar *et al.*, 2016). The garden is being irrigated by one of the largest wastewater flows in Katsina, which is being illegally used as a dumping site, where the refuse of different origins is discarded, including wastes from the neibouring iron bending workers, making it an important target for this experiment.

Metal Solutions

Stock solutions of the selected heavy metals were prepared according to the method suggested by Al-Mamun *et al.* (2013) with modifications. Cobalt and Nickel,0.5g/L, 1g/L, 1.5g/L, and 2g/L were used, while 5g/L, 10g/L, 15g/L and 20g/L concentrations of Manganese were prepared.

Bacteria Isolation and characterizations

Enumerations of rhizobacteria in the plant mesocosms were carried out weekly using CREYEMA (Congo Red Yeast Extract Mannitol Agar) medium through pour plate methods.

After 21 days of the experiment, the plant roots were cut, washed, and homogenised using a clean mortar and pestle, after which serial dilution was carried out. The Spread plate method was applied for the initial isolation, and the resulting colonies were further cultured via the streak plate method on the CREMA to obtain their pure culture (Ankur *et al.*, 2017; Umar *et al.*, 2018). Based on standard protocols, Biochemical characterization involving Indole, Methyl-Red, Citrate utilisation, urease, catalase, oxidase, and triple sugar iron agar tests were conducted (Cheesbrough, 2010).

Plant Materials

Forty seeds of *Phaseolus vulgaris* were collected from Lambun Sarki garden within the Katsina metropolis using plant polythene bags. After that, botanical identification and authentication were done at the Biology department of Umaru Musa Yar'adua University. They were then transferred to the Microbiology laboratory of Umaru Musa Yar'adua University for the experiment. The seeds were sterilized using 4% (v/v) sodium hypochlorite after which they were soaked in distilled water for 12 hours to allow dormancy breakage, as modified from the methods of Costa and Sharma (2016). Using a polythene bag containing approximately 100g of the Lambun sarki's garden soil, a total of forty healthy growing *Phaseolus vulgaris* plants were transferred to the Biology Department of the Umaru Musa Yar'adua University for botanical identification and authentication, after which they were transferred to the Microbiology laboratory for the experiments (Shakir *et al.*, 2020)

Experimental Treatments

The seeds were placed in Petri dishes containing filter paper moistened with 10ml of each concentration of the selected heavy metals. The plates were left for seven days with the germination observation at 24 hour intervals. Similarly, the plants' microcosms were watered with 10 mL of varying concentrations of the selected heavy metals daily for three weeks (Faizan *et al.*, 2012).

Assessment of *P. vulgaris* growth under Heavy Metals stress

The growth analysis was conducted to ascertain the germination percentage and mean germination time were measured based on the method of Mavi *et al.* (2010). The leaves' morphological appearance was also observed daily for characteristics such as necrosis, leaf loss, and occasional death (Abderrahmane *et al.*, 2018).

Tolerance Index

To measure the plant capacity to grow in the presence of higher concentrations of the Nickel, Cobalt, and manganese, tolerance indices (TI) of the plants at three different concentrations of the heavy metals (Co & Ni = 1g/L, 1.5g/L and 2g/L, Mn = 10g/L, 15g/L and 20g/L) were measured according to the method of Shakir *et al*. (2020). The harvested plants, including two controls, were oven-dried at 60°C for 72 hours, after which they were weighed accurately using the standard laboratory weighing balance.

Calculations and Statistical Analysis

The following formulas were employed for the calculation of germination percentage (GP), mean germination time (MGT), and tolerance indices (TI):

i. Mean germination time (MGT) =Σ dn / Σ n. *(1)*

ii. Germination percentage (GP) =No. of germinated seeds/ total no. of seeds x 100*(2)*

Where: d= germination period in days, n= seed germinated on day d

Tolerance index (TI) = Dried matter in the metal treatments/Dried matter in the control × 100.

For the statistical analysis, one-way ANOVA, using AnalyStat (version 1.6.50) was employed.

RESULTS

Germination percentage of *Phaseolus vulgaris* seeds

Table 1 below shows the germination percentage of the *P. vulgaris* seeds within the first week of the experiment across each treatment. The lowest germination percentage was observed in Cobalt treatment (12.5%). The highest percentage was observed in manganese, which has a 50% germination percentage. Likewise, 50% germination was observed in control plates.

Table 1: Germination percentages of *Phaseolus vulgaris* seeds in varying concentrations of Co, Mn and Ni

S/N	Heavy Metal	Germination Percentages (%)							Metal conc. in g/l (seed germinated)
		D1	D2	D3	D4	D5	D6	D7	
1	Co	0	0	12.5	12.5	12.5	12.5	12.5	0.5 (1 seed)
2	Mn	37.5	37.5	50	50	50	50	50	5, 10, 15 (2, 1, 1 seeds)
3	Ni	0	0	25	25	25	25	25	1, 1.5 (1 seed each)
4	Control	0	0	25	25	50	50	50	2/4 seeds

Key: D 1 to D 7 represent day 1 to day 7

Mean Germination Time

Table 2 presents the average germination time of the *P. vulgaris* seeds among the treatments. The manganese treatment observed the fastest germination, with a mean germination time of 1 to 1.5 days. A similar germination time was observed in Nickel, Cobalt and the negative treatments.

Table 2: Mean Germination times of *Phaseolus vulgaris* seeds in varying concentrations of Co, Mn, and Ni

S/No	Heavy Metal	Mean Germination (days)
1	Cobalt	3.0 ± 0.00
2	Manganese	1.5 ± 0.41
3	Nickel	3.0 ± 0.00
4	Negative control	3.0 ± 0.00

Rhizobia population dynamics during the experiment

Table 6 shows the data obtained from the enumerations of the rhizobia cells during the phytoremediation process. Rhizobial counts of plants treated with Co (0.5 to 2g/L) and Mn (5 to 20g/L) increased between the first and second week. For the Ni, all the populations decreased across all the concentrations (0.5 to 2 g/L), while in the control plant, there was a sudden rise (Table 6).

Additionally, the number of colonies in Yeast Extract Mannitol Agar (YEMA) medium obtained from 1mL of each dilution of the roots of the *P. vulgaris* treated with the maximum concentrations of the heavy metals was shown in Table 7. The highest population was seen in isolates of the control plants (2.16 $\times 10^9$CFU), followed by Mn and Co treated plants (Table 7). The lowest population was observed from the roots having the highest concentration of Nickel (2g/L), which was 1.68 $\times 10^9$CFU.

Table 3: Colony counts of the bacteria isolates obtained from the *phaseolus vulgaris* roots

S/N	Heavy metal	Concentration (g/L)	Average Colony Count ($\times 10^9$ CFU/g of soil)	
			Two weeks incubation	Three weeks incubation
1	Co	0.5	2.65	34.60
		1.0	4.12	20.23
		1.5	6.77	17.77
		2.0	6.38	12.20
2	Mn	5	31.75	39.20
		10	29.20	31.00
		15	9.28	22.48
		20	2.99	4.68
3	Ni	0.5	70.30	16.55
		1.0	61.80	13.78
		1.5	52.10	11.70
		2.0	48.60	4.68
4	Control	NC 1	137.60	191.0
		NC 2	148.80	155.8
		NC 3	99.60	220.6
		NC 4	144.60	149.6

Key: NC1 to NC4 represent negative control 1 to negative control 4

Table 4: Bacteria counts obtained at the highest concentrations of heavy metals in CREYEMA

S/N	Heavy Metal (concentration)	Rhizobacterial counts (CFU/ml)
1	Cobalt (2.0g/L)	1.81×10^9
2	Manganese (20g/L)	1.82×10^9
3	Nickel (2.0g/L)	1.68×10^9
4	Positive control (Distilled water)	2.16×10^9
5	Positive control (Distilled water)	2.00×10^9

Bacteria identification

Table 8 shows the results of the identification of the isolates after growth on CREYEMA, the selective medium for the growth of *Rhizobium* species, and subjecting the isolates to the biochemical tests earlier mentioned.

Table 5: Characterization of *Rhizobacteria* associated with *Phaseolus vulgaris*

S/N	Identification	Isolate A	Isolate B	Isolate C
1	Colonies on CREYEMA	Positive	Positive	Positive
2	Colony appearance	Round, whitish, dried small colonies	Round, whitish, dried small colonies	Whitish, flat colonies with irregular margins
3	Gram reaction	Gram-negative coccobacilli arranged singly	Gram-negative coccobacilli arranged singly	Gram-negative rods arranged singly and in pairs
4	TSI	A/A, H_2S^-, Gas^+	A/A, H_2S^-, Gas^+	K/A, H_2S^-, Gas^+
5	Catalase	+	+	+
6	Oxidase	+	+	+
7	Indole	+	+	+
8	MR	+	+	+
9	VP	-	-	-
10	Citrate	+	+	+
11	Urease	-	-	-
	Organism	*Rhizobium* sp.	*Rhizobium* sp.	*Sinorhizobium meliloti*

Key: CREYEMA = Congo Red + Yeast Extract Mannitol Agar, + = Positive, - = negative

Average Shoot Length

The average shoot length of *P. vulgaris* across the heavy metals concentrations was shown in Table 3, where nickel treatment showed the most rapid decline in shoot length. The decline in Cobalt is relatively intermediate, and the least declination of the shoot length was observed in manganese (Table 3).

Table 6: Average Shoot lengths of *Phaseolus vulgaris* grown in varying concentrations of Co, Mn, and Ni

S/N	Heavy metal (g/L)	Average Shoot Length (mm) across the study period (2 weeks)									
		D8	D9	D10	D11	D12	D13	D14	D15	D16	D17-21
1	Co (0.5)	80.00	78.33	50.00	50.00	43.33	41.67	38.33	35.00	35.00	35.00
	Co (1.0)	75.00	63.33	60.00	50.00	21.67	35.00	31.67	30.00	10.00	10.00
	Co (1.5)	60.00	43.33	23.31	21.67	18.33	15.00	15.00	13.33	0.00	0.00
	Co (2.0)	59.67	40.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	Mn (5)	91.33	73.33	73.33	73.33	73.33	68.33	61.67	60.00	50.00	50.00
	Mn (10)	83.33	70.00	63.33	53.33	41.67	41.67	40.00	36.67	38.33	38.33
	Mn (15)	79.33	61.67	50.00	48.33	45.00	40.00	36.67	36.67	33.33	33.33
	Mn (20)	66.67	61.67	40.00	28.33	23.33	25.00	21.67	21.67	20.00	20.00
3	Ni (0.5)	83.33	43.33	38.33	36.67	30.00	30.00	25.00	25.00	13.33	0.00
	Ni (1.0)	80.30	45.00	36.67	36.67	26.67	26.67	20.00	16.67	0.00	0.00
	Ni (1.5)	75.00	53.33	48.33	16.67	23.33	23.33	0.00	0.00	0.00	0.00
	Ni (2.0)	91.67	50.00	30.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	Control	76.00	72.50	66.71	62.86	62.86	62.86	62.86	62.86	62.86	62.86

Key: D8 to D21= Day 1 to day 21, Co = Cobalt, Ni = Nickel, Mn= Manganese

Tolerance index

The maximum tolerance capacity of *P. vulgaris* against different concentrations of heavy metals is shown in (Table 4). Manganese treatment appeared more tolerable across all the selected concentrations, having 87%, 60%, and 51% at 10g/L, 15g/L, and 20g/L, respectively. Cobalt displayed intermediate tolerance, possessing 78.51%, 55%, and 42% at 1.0g/L, 1.5g/L and 2.0g/L relatively. However, less tolerance was observed in Nickel treatment, where 1.0g/L, 1.5g/L, and 2.0g/L treatments appeared to have TI values of 65%, 49%, and 38.2%, respectively.

Table 7: Tolerance index of *Phaseolus vulgaris* against three different concentrations of Co, Mn, and Ni

SN	Cobalt (g/L)			(Nickel (g/L)			Manganese (g/L)			Control
	1.0	1.5	2.0	1.0	1.5	2.0	1.0	1.5	2.0	-
%	78.51%	55%	42%	65.7%	49%	38.2%	87%	60%	51%	105%

Toxicity of the heavy metals on the *P. vulgaris* plants

The toxic effects of higher concentrations of Co, Mn, and Ni against *P. vulgaris* plants are shown in Table 5. The effects ranged from Very Partial Necrosis (VPN), Partial Necrosis (PN), Total Necrosis (TN), Leaf loss, and the eventual death of the plant. Co and Ni treatments died between day 10 and day 11 from the beginning of the observation. However, the Manganese treatment resisted the toxicity until the end of the experiment (Table 5).

Table 8: Effects of heavy metals on *Phaseolus vulgaris* plants for three weeks

Days	Heavy Metals								
	Cobalt (2.0g/L)			Manganese (20g/L)			Nickel (2.0g/L)		
	Necrosis	Leaf loss	Death	Necrosis	Leaf loss	Death	Necrosis	Leaf loss	Death
D8	TN3L, PN2L	-	0/3	PN2L	2 leaves	0/3	TN5L	-	0/3
D9	TN3L, PN2L	-	0/3	VPN2L, TN1L	1	0/3	-	3	0/3
D10	-	-	3/3	VPN1L, TN1L	-	1/3	TN3L, TN3LS/3	-	0
D11	-	-	3/3	VPN2L, TN2L	-	1/3	-	-	3/3
D12	-	-	3/3	TN2L, NLS	-	1/3	-	-	3/3
D13	-	-	3/3	N2LS/3	-	1/3	-	-	3/3
D14	-	-	3/3	TN2LS/3	-	1/3	-	-	3/3
D15	-	-	3/3	N2LS/3			-	-	3/3
D16	-	-	3/3	TN2LS/3			-	-	3/3
D17-21	-	-	3/3	-			-	-	3/3

Key: PN1L &PN2L = Partial necrosis of 1 and 2 leaves, TN1L, TN3L, &TN5L = Total necrosis of 1, 3 and 5 leaves, NLS&N2LS = Necrosis of 1 and 2 leaves and shoot, TN2LS&TN3LS = Total necrosis of 2 and 3 leaves and shoots.

DISCUSSION

The germination of the *P. vulgaris* seeds appeared unaffected by the heavy metals at lower concentrations, having at least one germinated seeds across each heavy metal treatment. However, as the concentrations increased, the seeds germination was relatively inhibited. Previous studies by Ain *et al.* (2016) and Siddique *et al.* (2011) reported similar observations, in which the germination of wheat and *Vigna mungo* (L) seeds were not affected by lower concentrations of Ni, Co, and Mn. However, at higher concentrations, the seed germination was significantly inhibited. This may be due to the suppression of the activities of hydrolytic enzymes and penetration of the metal ions into the embryonic tissue of the seeds, as reported by Sengar *et al.* (2006). Among the treatments, manganese appeared to have the highest germination percentage (50%). This finding is consistent with the previous experiment by Thummala *et al.* (2016), who recorded a higher germination percentage in horse gram seeds treated with manganese. Similarly, Roy and Bera (2000) observed that 100 ppm manganese favors 100% seed germination, and attributed it to the fact that manganese is among the essential metals required for various physiological processes essential for the seed germination.

The results of rhizobacterial population dynamics obtained in this study (Table 3) showed that the aerobic mesophilic heterotrophic bacterial count of weeks one and two did not differ significantly when compared against each other ($p = 0.061$) and also across the various concentrations ($p = 1.00$), which means that the bacterial populations are affected in virtually the same way by the different heavy metals. This result differed from the one obtained by Makia (2011) when she evaluated the effect of Nickel and Manganese on *Sinorrhizobium meliloti* in which a decrease in the rhizobial population was observed. This result variation may be due to the nature of the exposure of the microbes to the heavy metals or arise from differences in the duration of the experiments.

Regarding the identification and biochemical characterization of the isolates, all the isolates were capable of growing on YEMA medium supplemented with Congo red (CREYEMA), which is the selective medium for the growth of *Rhizobium sp.* (Faisal *et al.*, 2012).

In terms of average shoot length (Table 6), in which a decline in the *P. vulgaris* shoot length was observed, previous research by Rai *et al.* (2005) also reported a significant reduction in *Phyllanthus amarus* shoot length under cadmium stress. Furthermore, the tolerance indices of *P. vulgaris*, as presented in Table 4, were slightly higher than the previously observed tolerance by Borcelo *et al.* (1986), who studied the *P. vulgaris* tolerance under cadmium toxicity, which could be resulting from the differences in terms of the heavy metals toxicity to the plants.

Several heavy metals are considered a source of micronutrients to plants. Their presence in low concentrations may improve plant growth (Haruna *et al.*, 2014; Murad *et al.*, 2020). However, at higher concentrations, the toxicity of the metals is manifested in the plant biomass, which includes necrosis, chlorosis, leaf loss, and eventual death (Shakir *et al.*, 2020).

Similar effects were observed in this study, as presented in Table 5. Regarding nickel toxicity, results from a previous study by Maheshwari *et al*. (2012) also showed that at higher concentrations, the toxicity of Nickel inhibits the hydrolysis of proteins and RNA by suppressing the performance of RNase and protease. Also, the activity of ribonuclease decreased in roots and shoots. Greater Ni^{2+} concentrations led 22-24% and 37-39% reduction in protease activity in rice seedlings' shoot and root parts in the first 20 days of seedlings growth. In most species of plants, a decrease in ribonuclease, protease, and amylase enzyme activity is the cause of seed germination, growth retardation, and eventual death (Ahmed *et al*., 2012).

Regarding the Cobalt treatment, Mohamed (2008) reported that Cobalt at high concentrations suppressed the seedling growth and dry weight of the ragi and paddy seedlings. Cobalt at higher levels may directly inhibit root growth by inhibiting cell division, cell elongation, or a combination of both, resulting in the limited uptake and translocation of nutrients and water and induced mineral deficiency. Similar results were also reported on the effect of Cobalt on *V. mungo* (L) by Jayakumar and Vijayarengan (2006) and *P. americanum* (L) and *P. Aculeata* (L) by Burhan *et al*. (2001). Furthermore, Bhupendra *et al*. (2014) proved that a higher concentration of manganese could affect the roots and shoots of green and black gram.

CONCLUSION

This research showed that P. *vulgaris* displayed tolerance to various degrees on Ni, Co, and Mn, in the order Nickel > Cobalt > Manganese. Average rhizobial counts increased weekly, with high counts obtained in Ni and Mn treatments. However, they did not differ significantly between weeks (p = 0.061). Thus, longer time intervals (>2 weeks) are required to observe significant shifts in population dynamics. Moreover, HMs concentration did not affect the colony counts (p = 1.00). Metabolism profile of the preliminarily identified *Rhizobium* sp. and *Sinorrhizobium melliloti* evidenced HMs removal and plant growth promotion ability. The research demonstrated the tolerability of *P. vulgaris* and how rhizospheric population dynamics change during phytoremediation and contributed towards understanding HMs impact as environmental stressors on rhizospheric plant-microbe interactions.

RECOMMENDATIONS

As the present study assessed the tolerability of *P. vulgaris* exposed to different concentrations of heavy metals, Co, Ni, and Mn, future research targeting the hyperaccumulation capacity of *P. vulgaris* against the heavy metals through analysis of its Bioconcentration Factor (BCF), Translocation Factor (TF) and Bioaccumulation Factor (BAF) is highly recommended, as it may help in knowing the maximum amounts of the heavy metals the plant can accumulate as well as its ability to distribute the heavy metals to the aboveground biomass.

Furthermore, assessing the tolerability of the identified rhizobia to higher concentrations of heavy metals is highly recommended, as the concentrations used in the current study did not significantly affect the rhizobial population. Hence, the research did not detail the maximum tolerable amount of the metals to the rhizobia despite its significance in selecting appropriate bacterial species augmenting the microbially-assisted phytoremediation under the appropriate level of the heavy metals contamination.

REFERENCES

Abderrahmane, Z., Hishamuddin, O., Mohd, H. I. & Musihazli, M. (2018). Influence of lead on in vitro seed germination and early radicle development of Acacia auriculiformis Cunn. Ex Benth species. Annual Research & Review in Biology, 28(1), 1-12.

Abubakar, S., Latiff, A., Lawal, I. M., Jagaba, A. H. (2016). Aerobic treatment of kitchen wastewater using sequence batch reactor (SBR) and reuse for irrigation landscape purposes. *American Journal of Engineering Research* (AJER) 1, 23-31.

Ahmad, M.S.A., &Ashraf, M. (2012).Essential roles and hazardous effects of Nickel in plants. *Springer, NewYork*, 125-167.

Ain, Q., Akhtar, J., Amjad, M., Haq, M.A., Saqib, Z.A. (2016). Effect of Enhanced Nickel Levels on Wheat Plant Growth and Physiology under Salt Stress. *Communications in Soil Science and Plant Analysis*. 47, 2538-2546

Al-mamun, M., Poostforush, M., Mukul, S., Parvez, K., & Subhan, A. (2013). Biosorption of As (III) from aqueous solution by *Acacia auriculiformis* leaves. *Iran Computer Science, Engineering & Electrical Engineering* 20: 1871-1880.

Ankur, T., Vijay, K. P. &Akash, T. (2017). Isolation, identification, biochemical and antibiotic characterization of *Rhizobium* strains from *Vigna mungo* (L) *Helped, Cover aretinum L* and*Vigna*

radiata (L). *Wilczek in Muzaffarnagar* 6(5): 24-35.

Barceló, J., Poschenrieder, C., Andreu, I., Gunsé, B. (1986). Cadmium-Induced Decrease of Water Stress Resistance inBush Bean Plants (*Phaseolusvulgaris* L. cv. Contender) I. Effects of Cd on Water Potential, Relative WaterContent, and Cell Wall Elasticity. *J. PlantPhysiol*, 125, 17-25.

Bhupendra, Kiran and Rizvi G (2014) Effect of arsenic, manganese and chromium on in vitro seed germination of black gram (*Vigna mungo* L.) and green gram (*Vigna radiata* L.). *Journal of Chemical and Pharmaceutical Research* 6(5) 1072-1075.

Burhan, S. S., and A. Tahira (2011) Effect of zinc and Cobalt on germination of and seedling growth of Pennisetum americanum (L), Schumann and Parcunsenia aculeata (L.). *Pakistan journal of biological sciences* 4(5):575-580.

Chessbrough, M. (2010). *District Laboratory Practice in Tropical Countries, Volumesl &II*(2nd ed.), Cambridge University Press, 143-155.

Corradi, M.G., A. Blanchi and A. Albasini, (1993) Chromium toxicity in *Salvia sclarea*. Effect of hexavalent chromium on seed germination and seedling development. *Environmentsl Botany*,147: 439-441. 33(3): 405-413.

Costa, D., & Sharma, P. (2016). Effect of copper oxide nanoparticles on growth, morphology, photosynthesis and antioxidant response in *Oryza sativa*. *Photosynthetica*, 54, 110-119.

Faisal, S., Mohammad, M. T., Muhammad, S., and Zafar A (2012) Isolationandbiochemicalcharacterizatio nof*Rhizobiummeliloti* fromrootnodulesof*Alfalfa* (*Medicosativa*).*The Journal of Animal & Plant Sciences*, 22(2): 522-524

Faizan, S., Kausar, S., & Perveen, R. (2012). Variation in growth, physiology and yield of four chickpea cultivars exposed to cadmium chloride. *J. Environ. Biol*. 33: 1137-1242.

Haruna, M.Y., Khan, A.H., and Umar, Z.D. (2014). Effect of acid rain on growth of papaya (*Carica papaya*) and castor (*Ricinus communis*) plants. *e-Journal of Science and Technology*. *10*(1)9, 43-47.

Jayakumar, K., Jaleel, C.A., Vijayarengan, P. (2007). Changes in growth, biochemical constituents, and antioxidant potentials in radish (*Raphanussativus* L.) under cobalt stress. Turkish Journal of Biology. 31, 127-136.

Lebrazi, S. &Fikri-Bebrahim, K. (2018). Rhizobium-Legume symbioses: Heavy metal effects and principal approaches for bioremediation of soil in legumes for health and sustainable management. *Springer: Heidelberg, Germany*. 205-233

Li, Y., Pan, H. D., He, L., Wang, Q., & Sheng, X. F. (2017). Cd immobilization and reduced tissue Cd accumulation of rice (*Oryzasativawuyum*) in the presence of heavy metal-resistant bacteria. *Ecotoxicology and Environmental safety*, 138, 56-63.

Lone, M., Iqbal, H., Zhen-li, S., Peter, J., & Yang, X. (2008). Phytoremediation of heavy heavy metals polluted soils and water: Progress and perspectives. *Journal of Zhejian University Science* 9(3): 210-220.

Maheshwari, R., Dubey, R.S. (2012) Nickel toxicity inhibits ribonuclease and protease activities in rice seedlings: protective effects of proline. Plant Growth Regulation. 51, 231-243.

Makia Al-Hujeju (2011) Effects of silver, mercurycobalt and nickel ions on *Sinorrhizhobiummellilot*ibacteria.Basrs h journal of Agricultural Science. 24 (3)2011.

Mavi, K., Demir, I., &Mathew, S. (2010). Mean germination time estimates the relative emergence of seed lots of three cucurbit crop under stress conditions. *Seed Science and Technology* 38(14) 25-43.

Mohamed, M.Azooz (2008) Impact of Cobalt on Germination and Seedling Growth of *Eleusinecoracana* L.and*Oryzasativa* L. Under Hydroponic Culture. Global Journal of Molecular Sciences 3 (1): 18-20, 2008ISSN 1990-9241.

Mohamed, O. A., Kong, Z. Y., Deng, Z. S., Liu, X. D., Glock, B. R., & Wei, G. H. (2015). Rhizobial symbiosis effect on the growth, metal uptake, and antioxidant responses of 44 Medicago lupulina under copper stress. Environmental Science and Pollution Research, 22 (44), 12479 - 12489.

Murad, A., K., Adnan, A., and Naji Ullah (2020) EffectofNickelonthegerminationandbioc hemicalparametersoftworicevarieties. Fresenius Environmental BulletinVolume 29 – No. 02/2020 pages 956-963.

Rai, V., Khatoon, S., Bisht, S., Mehrotra, S. (2005). Effect of cadmium on growth, ultramorphology of leaf andsecondary metabolites of *Phyllanthusamarus*

Schum. and Thonn. *Chemosphere*, 61, 1644–1650.

Roy SB and Bera AK (2000). Effect of mercury and manganese on seed germination, seedling growth, fresh weight and dry weight of mungbean seedling. *Environmental Ecology* 18: 844-847.

Sengar, R.S., Gupta, S., Gautam, M., Sharam, A., Sengar, K. (2008). Occurance, Uptake, Accumulation and Physiological Responces of Nickel in Plants and its Effects on Environment. *Research Journal of Phytochemistry*. 2, 44-60.

Shakir, U., Jafar, K., Khizar, H., Ahmed, A. E., Uzma, S., Bofan, Y., Yuehua, M., Hongzheng, W., & Zhong, H. T. (2020). Comparative study of growth, cadmium accumulation and tolerance of three chickpea (*Cicerarietinum* L.)cultivars.*MPPIPlants* 9(310) 2-18

Sharma JK, Kumar N, Singh NP andSantal AR (2023) Phytoremediationtechnologies and their mechanism forremoval of heavy metal fromcontaminated soil: An approach for asustainable environment.*Front. Plant Sci.* 14:1076876.

Siddiqui, M.H., Al-Whaibi, M.H., Basalah, M.O. (2011). Interactive effect of calcium and gibberellin on nickel tolerance in relation to antioxidants systems in *Triticum aestivum*. *Protoplasma*. 248, 503-511.

Thummala, C., Duddela, V., Meruva, K., and Kurva, P (2016). Impactsofcadmiumandmanganese on*invitro*seedgerminationandseedlinggrowthofhorsegram. *Indian Journal of Plant Sciences* ISSN: 2319–3824.

Umar, Z.D., Aminu, M., and Yahaya, Y.R. (2020). Survival response of Consortium isolates from Diesel contaminated soil within Katsina State, Nigeria. *International Journal of Environment*, 9(2), 51-66

Umar, Z.D., Aziz, N.A.A., Zulkifli, S.Z., and Muskhazli, M. (2018). Efficiency of effective polycyclic aromatic hydrocarbons (PAHs) degrading consortium in resisting toxic heavy metals during PAHs biodegradation. *International Journal of Environment*, *7*(1), 14-27.

Yahaya, Y.R. and Umar, Z.D. (2021). Effective Microbial Bioremediation via the Multi-omics Approach: An Overview of Trends, Problems and Prospects. *UMYU Journal of Microbiology Research*, 6(1), 1-10.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., and Chen, Z. (2020).Phytoremediation: a promising approach for revegetation of heavy metalpolluted land. *Front. Plant Sci.* 11, 359.