https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 2 (2025); 140-151
e-ISSN:3092-8214 p-ISSN:3092-8206
DOI: https://doi.org/10.33003/chemclas-2025-0901/147

Enhanced Phytoremediation of Copper Contaminated Soil Using Plant Growth Regulators (IAA and GA3) with *Helianthus annus*

Fatimah Muhammad Kntapo*, Yagana Baba Abba & Rukaiyat Mohammed Said

[1]*Department of Applied Chemistry, School of Applied Science, College of Science and Technology (CST), Kaduna Polytechnic, Kaduna*

(*)Corresponding Author's: fm.kntapo@kadunapolytechnic.edu.ng; 07068360166

Abstract

Human anthropogenic activities, such as industrialization and urbanization has led to the introduction of heavy metals (HMs) into the ecosystem. These HMs are nonbiodegradable which lead to their bioaccumulation and biomagnification within the food chain. They are considered toxic when they exceed their threshold limit which is peculiar to each element. Most methods used to get rid of these inorganic pollutants tend to be harsh and expensive, an environmentally-friendly and cost-effective alternative is phytoremediation. Although there are limitations associated with phytoremediation technology which include slow growth, small biomass and plant low tolerance to stress. These limitations can be minimized by the use of plant growth regulators (PGRs). This study made use of different concentration (low, medium and high) of two types of PGRs; indole acetic acid (IAA) and gibberellic acid (GA3) to enhance phytoremediation of Cu using *Helianthus annus (Ha).* Concentration of HMs in soil, root and shoot was determined using Atomic Absorption Spectrophotometer (AAS), the values obtained were used to determine the phytoremediation efficiency. Significant differences between treatments were identified by one-way analysis of variance (ANOVA). The highest bioaccumulation factor (BAF) was 3.628 found with 200 mg/L GA3, the most effective translocation factor (TF) was 1.194 with 200 mg/L IAA, while enrichment factor (EF) had highest value of 1.569 with 200 mg/L GA3. Combination of IAA and GA3 at controlled doses might optimize both metal uptake and translocation at the same time. Field trials should validate laboratory findings for large-scale application; this can serve as a long term and eco-friendly remedy to HMs polluted farmlands.

Key words: Bioaccumulation factor, Enrichment factor, Heavy metals, Nonbiodegradable, Phytoremediation, Translocation factor.

Introduction

Heavy metals are inorganic nonbiodegradable pollutants that can be toxic when they exceed their threshold limit, they can be found in the environment through various means and due to their non-biodegradable nature, they are accumulated in food chains leading to metal toxicity which is harmful to living things [1].

Phytoremediation is the use of plants such as: shrubs, trees, aquatic plants, and grasses to detoxify or get rid of pollutants, in order to restore the ecosystem [2,3]. It involves the use of plants to clean up the environment (water, air, soil or sediments). It has several advantages over other methods, because it is easier, more effective, novel, cost effective and ecofriendly [4,5,6].

Cu finds applications in alloying like bronze and brass, copper wires, coins, pipes, barrier cream, preservation of wood and fabric. So, Cu can be introduced into the environment through wastes from agriculture, industry and metal production other sources include landfills and waste disposals. Also, Cu is an essential nutrient for both plants and animal growth and developments [7]. In plants Cu plays a major role in photosynthesis, reproduction, seed production, secretion of respiratory enzymes, water regulation, chlorophyll production. It increases sugar content, intensifies colour, disease resistance, improves flavour in fruits and vegetables. Despite being essential, Cu is considered toxic when its concentration exceeds a certain threshold hold in plants it induces stress and causes injury to plants, leaves become streaked, stalk becomes soft and limp, there is necrosis of older leaf edges. Excess Cu decreases root growth in Chloris gayana (rhodes grass) and reduces biomass and seed production in Polygonum convolvulus (black bindweed). There was reduction in root malformation in Phaseolus vulgaris (bean) as a result of Cu accumulation in the root. In animals excess Cu causes anemia, liver and kidney damage, eyes, mouth cavity, stomach and intestinal irritations, diarrhea, insomnia, anxiety, agitation, restlessness, Wilson's disease, lack of appetite, fatigue, Jaundice, speech impairment, difficulty in swallowing, brain damage, demyelination, hepatic cirrhosis, death [8]. Research work reported Cu from the soil being remediated by the phytoremediation potential of *Lactura sativa*, *Boehmeria nivea* as well *Alternathera bettzickiana* [9, 10].

Helianthus annuus (Sunflower) is an annual plant from the family of Asteraceae and can be grown on different kind of soil. It is an inflorescence specie on a stem of 3 m high. Its flowers have a diameter of 30 cm hosting its big seed. Various studies have indicated the phytoremediation potential of Sunflower [11]. Alaboudi, et al. [12] indicated that *Helianthus annuus L* has the tendency to accumulate Pb and Cd in contaminated soil. Forte & Mutiti [13] reported the accumulation of Cu and Pb by *Helianthus annuus* from contaminated soil. There was accumulation of abnormal level of metals (Cd, Cr, Cu and Zn) by the roots of *Helianthus annuus* [14].

Growth and physiology of plants are influence by signaling and regulating substances known as phytohormones or plant growth regulators (PGRs), which react to external and internal signals. Production and composition of root exudates is regulated by the activity of phytohormones, these phytohormones are organic substance release by plants to increase rate of growth and developments in plants, both activities enhance nutrient

mobilization in the soil that leads to metal uptake via the root system. PGRs promote cell activation and also regulate intracellular processes thereby improving growth which increases HMs accumulation within plant tissues [15].

PGRs enhances phytoremediation potential by promoting growth in the shoot and root, increase biomass yield and encourages the potential of antioxidation in the plants [16]. Signaling potential of Phytohormone can influence heavy metal tolerance leading to detoxification and/or accumulation [17]. There are various classes of PGRs which include auxins e.g indole-3-acetic acid (IAA) (Fig. 1), indole-3-butyric acid (IBA) and phenylacetic acid (PAA), cytokinins e.g Kinetin, Zeatin , Benzyladenine and BAP, gibberellins include GA1- GA7 (Fig. 2), ethylene (Ethephon), and abscisic acid (Dormins, Phaseic Acid) [19]. Effect of phytoremediation of Cd in *Bidens Pilosa L* was increased by 15.36%, 32.33% and 64.38% after using 6-BA, SA and 24 – EBB respectively [18]. Au was increased by 6,48 fold in *Brassica juncea* after adding IBA [19].

Therefore, this study reported enhanced phytoremediation of copper contaminated soil using plant growth regulators (IAA and GA3) with *Helianthus annus*.

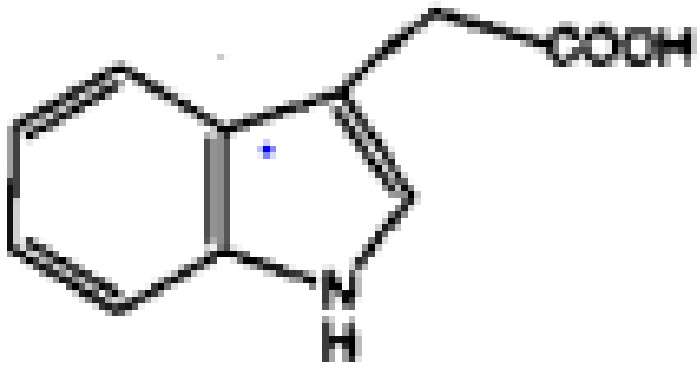

Figure 1: Indole -3 -acetic acid (IAA). Source: [20]

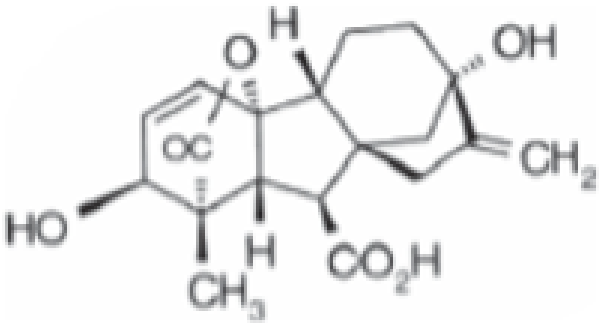

Figure 2: Gibberellic Acid (GA3) Source: [20]

Materials and Methods

Soil sample collection

Soil samples was collected from the surface layer (0-30 cm), the soil sample was pulled together and mixed uniformly to give a composite sample. And was used for phytoremediation analysis.

Seed collection, identification and authentication

Seeds of *Helianthus annuus* with voucher number ABU01079 was supplied by a herbarium expert of the Department of Botany, Ahmadu Bello University, Zaria, Nigeria in December 2023.

Nursery for seeds

Portion of the soil collected was used to nurse the seed. The seed planting was carried out in the late afternoon when the temperature was cool. *Helianthus annuus* seed was planted 1 to 2 cm from the edge. Watering was done based on weather condition twice or once daily, in the morning and late afternoon. The seeds were allowed to germinate and grow for 10 days, then three heathy seedlings obtained were replanted in each experimental pot for the analysis [21, 22].

Experimental design

The pots used for the analysis were plastic with a 22 cm upper diameter, 18 cm lower diameter and 22 cm height, 21 pots were labeled, 18 pots for the treatment and 3 for control. Soil samples were spiked with Cu in form of $CuSO_4$ (450 $mgkg^{-1}$) and mixed thoroughly. Then 5 kg each of this contaminated soil was stored in labeled plastic pots with no drainage pathway, in order to prevent leakage. After equilibrating the soil for 2 weeks, three healthy seedlings obtained from the nursery were replanted in each experimental pots and watering was not allowed to exceed the water holding capacity of the soil [21,22].

Procedure for application of Plant growth regulators (PGRs)

After 2 days of replanting of seedlings, 100 cm^3 of IAA and GA3 in different concentrations (50, 100 and 200 mg/L) was applied to the plant around the

roots area (at once) [23,24,25]. Plants were then harvested after 60 days of growth.

Plant's harvest and sample preparation

After harvesting, each plant was gently washed with deionized water and sorted according to plants tissues (roots and shoot). Each was dried and ground to powder, each was label separately in a zip lock polythene bags and stored for the analysis. Soil from each sample pot was collected separately, dried, crushed to powder using a mortar and pestle, and then stored in labelled zip lock polythene bags.

Digestion of soil and determination of HMs concentration

One gram (1g) of each powdered soil was placed into a 50 cm^3 beaker, 10 cm^3 of acid mixture of HNO_3 and HCl (2:1) was added and digested for 2 hours on a hot plate. After cooling it was filtered using Whatman filter paper in a 25 cm^3 volumetric flask and then diluted to the mark with deionized water, the filtrate was analyzed for Cu, using Atomic Absorption Spectrophotometer (AAS) [26].

Digestion of plant and determination of HMs concentration

Each prepared labeled plant sample (500 mg) was placed in a 50 cm^3 conical flasks and 10 cm^3 of HNO_3 and HCl (3:1 ratio) was added and heated in a water bath for 30 minutes. After cooling 5cm^3 of HNO_3 was added and heated for 1 hour, the digest was filtered into a 25 cm^3 volumetric flask. Deionized water was used to top up to the mark, Cu was determined after the digestion using Atomic Absorption Spectrophotometer [26].

Data Analysis

The data were analyzed using SPSS Statistical Package, Significant differences between treatments were identified by one-way analysis of variance (ANOVA). When the analysis of variance indicated a significant effect, differences among means were determined by the Tukey comparison test ($p < 0.05$).

Results and Discussion

The effect of PGRs; IAA and GA3 on the phytoremediation potential of *Ha* was evaluated using three key indices; Bioaccumulation Factor (BAF), Translocation Factor (TF), and Enrichment Factor (EF).

The result of effect of IAA and GA3 in enhancing phytoremediation of Cu using *Ha* is represented in Table 4.1. the result indicated the highest BAF value of 3.628 with 200 GA3, then 2.447 with 50 IAA. Followed by 2.196 (100 GA3), 1.950 (100 IAA), 1.190 (200 IAA), 1.024 (control) and finally 1.021 (50 GA3). TF levels from the highest as seen in table 4.1 above is; 1.194 (200 IAA) >0.787 (100 IAA) > 0.767 (200 GA3) > 0.673 (50 IAA) > 0.563 (control) > 0.513 (50 GA3) > 0.462 (100 GA3).

Table 1: Effect of indole-3-acetic acid (IAA) and gibberellic acid (GA3) on phytoremediation potential of Cu using *Helianthus annuus (Ha)* (Concentration of Cu in mg/kg)

Amendment (mg/L)	Cu in soil	Cu in Root	Cu in Shoot	BAF	TF	EF
Control	3.908 ± 0.375	2.484 ± 0.240	1.431 ± 0.012	1.024 ± 0.025	0.563 ± 0.066	0.651 ± 0.035
50 IAA	2.415 ± 0.094	3.419 ± 0.192	2.461 ± 0.340	2.447 ± 0.027	0.673 ± 0.040	0.984 ± 0.042
100 IAA	2.457 ± 0.308	2.731 ± 0.563	2.060 ± 0.350	1.950 ± 0.383	0.787 ± 0.190	0.839 ± 0.100
200 IAA	3.814 ± 0.159	2.089 ± 0.173	2.495 ± 0.248	1.190 ± 0.146	1.194 ± 0.025	0.651 ± 0.085
50 GA3	3.406 ± 0.431	1.988 ± 0.189	1.017 ± 0.137	1.021 ± 0.036	0.513 ± 0.071	0.328 ± 0.051
100 GA3	2.214 ± 0.130	3.347 ± 0.539	1.513 ± 0.034	2.196 ± 0.189	0.462 ± 0.090	0.685 ± 0.057
200 GA3	1.780 ± 0.228	3.634 ± 0.284	2.766 ± 0.319	3.628 ± 0.403	0.767 ± 0.063	1.569 ± 0.246 t

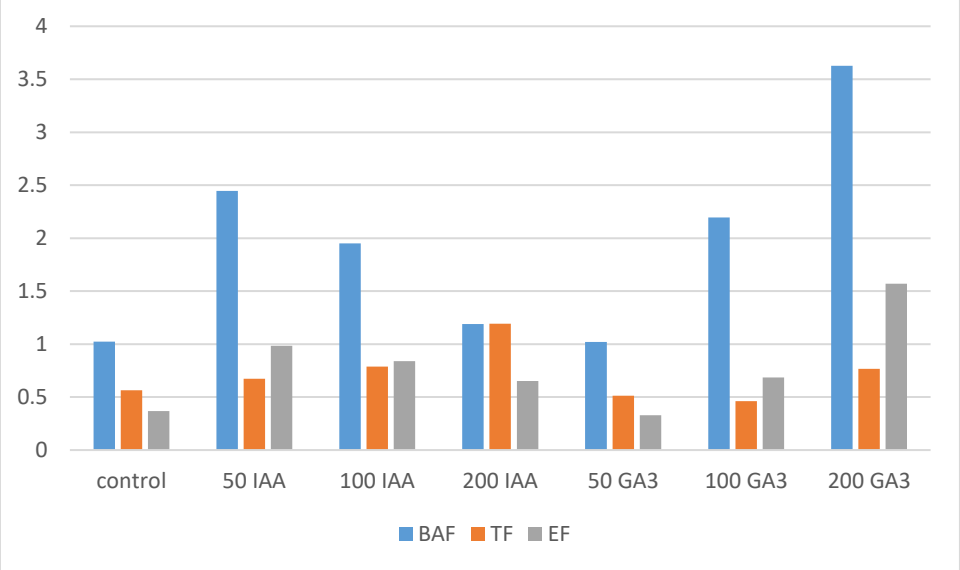

Figure 1: BAF, TF and EF of Cu enhanced with IAA /GA3 using *Ha*

The highest EF value was 1.569 (200 GA3), then 0.984 (50 IAA), 0.839 (100 IAA), then 0.685 (100 GA3), 0.651 (200 IAA), 0.651 (control) and then 0.328 (50 GA3). Figure 1 shows a representation of BAF, TF and EF of Cu after enhancing with different concentration of PGRs. Analysis of variance (ANOVA) indicated a P value of 0.0001 for all factors, BAF had a F value of 51.13 and R squared values was 0.9564. At P< 0.05, Tukey multiple comparison test showed statistically significant difference (SD) between control and all samples except 200 IAA and 50 GA3. There was statistically no significant difference (ND) between 50 IAA with 100 IAA and 100 GA3, 100 IAA with 100 GA3, as well as between 200 IAA with 50 GA3. There was statistical SD between other treatment.

TF had 21.94 for F value and R squared value was 0.9039, Tukey multiple comparison at P < 0.05 indicated statistical SD between control and 200 IAA, another SD was found with 50 IAA and 200 IAA, then 100 IAA with 200 IAA,100 GA3 and 200 GA3. 200 IAA was statistically SD with all GA3 treatments, 100 GA3 was also SD with 200 GA3.

EF for Cu enhanced with PGRs showed F and R squared of 39.98 and 0.9449 respectively, at P < 0.05 Tukey multiple comparison showed statistical SD between control with 50 and 100 IAA as well as 200 GA3. There was also statistical SD between other treatments except between 50 IAA with 100 IAA and 100 GA3, and also between 100 IAA with 200 IAA and100 GA3, then between 200 IAA with 200 GA3.

Bioaccumulation Factor (BAF)

BAF evaluates a plants ability to accumulate HMs, values higher than one (1) shows a high accumulation potential. BAF is calculated by dividing the metal in plants (mg kg^{-1}) by total metal in soil (mg kg^{-1}) [27] all values in this study (control included) have a value > 1, which indicate the hyper accumulation ability of Ha. Highest BAF was observed with 200 mg/L GA3 (3.628) while the lowest BAF value was with 50 GA3 (1.021) which is lower than the control value (1.024), the effects of PGRs depends on their concentration [28].

Translocation Factor (TF)

TF evaluates the root-shoot transfer value that's the ability of plant to translocate metals from the roots to the shoots., It is calculated by dividing the metal concentration in shoot (mg kg^{-1}) by metal concentration in root (mg kg−1). The highest TF was observed with 200 mg/L IAA (1.94) then 100 mg/L IAA (0.784) indicating that IAA is highly effective in transferring metals from roots to shoots. GA3 on the other hand shows TF values lower than control value (0.563) especially with 50 and 100 GA3 (0.513 and 0.462) suggesting that GA3 promotes root accumulation rather than translocation. The control value suggest that natural translocation is relatively limited

Enrichment Factor (EF)

EF depict the relationship between the concentration of HMs in the plant to that in the soil. The EF is calculated as HMs in shoot in relation to HMs in soil [29]. GA3 at 200 mg/L significantly enhances metal uptake with the highest EF (1.569), IAA at 50 and 100 IAA shows moderate EF values of 0.954 and 0.839 respectively, indicating it is less effective in total metal uptake than GA3. The control EF (0.651) is lower than the enhanced samples except 50 GA3 (0.328).

Effect of Indole-3-acetic acid (IAA) on phytoremediation

IAA is the most biodegradable auxin easily degraded by light and plant enzymes (IAA-oxidase), IAA improves Cell division and Elongation, Promotes Growth, increase Root formation which leads to large biomass for HMs accumulation [30, 31]. This study indicated decreasing BAF value with concentration; 50mg/L IAA (2.447) > 100 mg/L (1.950) > 200 mg/L(1.190) this indicates that the effects of PGRs depends on their concentration, and there is an optimum concentration for significant effect [28].

The highest TF was with 200 mg/L IAA (1.194) indicating phytoextraction potential. while all treatments had EF < 1. Since all treatments values are above control value, this indicates the enhancing effect of IAA on phytoremediation. Similar studies have identified the enhancing effect of IAA on phytoremediation; [32] indicated that IAA enhances the phytoremediation of Cu and Cd in Ha by 64% and 25%.. Pb was increased by 2.7 and 2.8 in *Zea mays L* after adding GA3 and IAA respectively [15].

Effect of Gibberellic acid (GA3) on phytoremediation

GA3 is the most common type of gibberellin, is a tetracyclic di-terpenoid compound GA3 stimulate seed germination, promote cell division and triggers growth providing more biomass for HMs accumulation [30]. Contrary to IAA, GA3 had increasing level of BAF with increasing concentration; 50mg/L (1.021) < 100mg/ L (2.196) < 200 mg/L (3.628), signifying there is optimum concentration for each PGR. All TF and EF were < 1 except EF with 200 mg/L GA3 (1.569). High BAF indicates phytostabilization potential of GA3, as seen from other researches; [33] reported that Cd concentration as well as root and shoot biomass was elevated by enhancing with cytokinins and GA3. There was improvement in the effect of phytoremediation of Zea mays with different concentration of GA3 [34]. GA3 enhances Cd uptake in *Tagetes patala* by 12- 50% [23]. There was improvement in the effect of phytoremediation of Zea mays with different concentration of GA3 [34].

Conclusion

This study utilized IAA and GA3 at three concentrations (50, 100, and 200 mg/L) to assess their influence on Cu uptake in soil, root, and shoot. The results indicated that 200 mg/L GA3 significantly increasing BAF and EF, while 200

mg/L IAA had the highest TF, making them ideal for Phyto stabilization and phytoextraction respectively.

Recommendations

1. Combination of IAA and GA3 at controlled doses might optimize both metal uptake and translocation at the same time.

2.Field trials should validate laboratory findings for large-scale application.

Conflict of Interest

The authors declare that they have no conflict of interest

References

1. Ashraf, S., Ali, Q., Zahir, Z.A., Ashraf, S. & Asghar, H.N. (2019) Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. Ecotoxicology Environ Saf 174:714–727
2. Tangahu, B.V., Abdullah, S.R., Basri, H., Idris, M., Anuar, N. & Mukhlisin, M., (2022) Lead (Pb) removal from contaminated water using constructed wetland planted with Scirpus grossus: Optimization using response surface methodology (RSM) and assessment of rhizobacterial addition. Chemosphere 291 (P3), 132952.
3. Wiefe, S., Baden, C. & Banddi, T (2024) Enhanced Phytoremediation of heavy metals by soil- applied organic and inorganic amendment. Heavy metal Phyto availability, accumulation ` and mental recovery. Soil and sediment contamination
4. Zhang, Q., Kong, W., Wei, L., Wang, Y., Luo, Y., Wang, P. & Jiang, G. (2020) Uptake, phytovolatilization, and interconversion of 2, 4-dibromophenol and 2, 4-dibromoanisole in rice plants. Environmental International. 142, 105888
5. Kristanti, R.A., Mardarveran, P., Almaary, K.S., Elshikh, M.S., AbdelGawwad, M.R., Tang, D.K.H., 2023. Phytoremediation of bauxite wastewater potentiality by Jatropa curcas. Bioprocess Biosyst. Eng. 46, 373–379.
6. Paewa-ploaniczak, M., Kumar, A.,Rukauczan, M., Sinkkonen, A., Roshund, M. & `Plociniezak, T.(2024). The potential of enhanced phytoremediation to clean up multi – `contaminated soil – Insights from met transcriptomics. Microbiological Research. 284 `(127738)
7. Arif, N.V. Yadav, S., Singh, S., Singh, P., Ahmad, R.K., Mishra, S., Sharma, D.K., Tripathi, N., ` Dubey, K. & Chauhan,D.K. (2016). Influence of High And Low Levels of Plant- ` Beneficial Heavy Metal Ions on Plant Growth and Development. Frontiers in `Environmental Science 4:69.

8. Tchounwou, P.B, Yedjou, C.G.,Patlolla, A.K. & Sutton, D.J.(2012) Heavy Metals Toxicity and the Environment. EXS 101: 133–164.
9. Rahman, Z. & Singh, V. P. (2019) The Relative Impact of Toxic Heavy Metals (THMs)(Arsenic (As), Cadmium (Cd), Chromium (Cr)(VI), Mercury (Hg), and Lead (Pb)) on the Total Environment: An Overview. Environmental Monitoring and Assessment 191 (7):419.
10. Khalid, A., Farid, M., Zubair, M., Rizwan, M., Iftikhar, U., Ishaq, H.K., Farid, S., Latif, U., Hina, K. & Ali, S. (2020) Efficacy of Alternanthera bettzickiana to remediate copper and cobalt contaminated soil physiological and biochemical alterations. International Journal Enviroment Res 14:243–255
11. Chirakkara, R.A. & Reddy, K.F. (2015). Plant Species Identification for Phytoremediation of Mixed Contaminated Soils journal of Hazard. Toxic Radioact. Waste 04015004:1-10.
12. Alaboudi, K.A., Ahmed, B. & Brodie, G. (2018) Phytoremediation of Pb and Cd contaminated soils by using sunflower (Helianthus annuus) plant. Ann. Agric. Sci. 63:123–127.
13. Forte, J. & Mutiti, S. (2017) Phytoremediation potential of Helianthus annuus and Hydrangea paniculata in copper and leadcontaminated soil. Water Air Soil Pollut 228:77
14. Tang Y-T, Deng T-H-B WQ-H (2012) Designing cropping systems for metal-contaminated sites: a review. Pedosphere 22:470–488
15. Hadi, F., Bano, A. & Fuller, M.P (2010). The improved phytoextraction of lead (Pb) and the growth of maize (Zeamays L.): The role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. Chemosphere 80: 457–462.
16. Liu, S., Yang, B., Liang, Y., Xiao, Y. & Fang, J. (2020) Prospect of phytoremediation combined with other approaches for remediation of heavy metal-polluted soils. Enviromental Science Pollution Res 27:16069–16085
17. Fathy, M., Saad, S. M. E., Naseem, M., Dandekar, T. & Othman, E. M. (2022) Cytokinins:Wide-Spread Signaling Hormones from Plants to Humans with High Medical Potential. Nutrients 14: 1495
18. Yang, Q., Jing, H..& Xue, K.(2023).Effect of exogenous plant hormone spraying on the `phytoremediation by Bidens Pilosa Lin cadmium contaminated soil. PubmedYang,
19. Kulkarni, M. G., Stirk, W. A., Southway, C., Papenfus, H. B., Swart, P.A. ,Lux, A., Vaculík, M., Martinka, M. and Staden, J.V. (2012) Plant growth regulators enhance gold uptake in Brassica juncea. International journal of phytoremediation,15(2):117-126.

20. Jamil, M., Saher, A., Javed, S., Farooq, Q., Shakir, M., Zafar, T., Koma, L., Hussain, K., Shabir, A., Javed, A. and Huzafa, M. (2021) A review on potential role of auxins in plants, current applications and future directions. J. Bio. & Env. Sci. 18 (1): 11-16
21. Ebrahimi, M (2014). Effect of EDTA and DTPA on Phytoremediation of Pb-Zn Contaminated Soils by Eucalyptus camaldulensis Dehnh and Effect on Treatment Time. Desert 19 (1): 65-73
22. Beavers, A., Koether, M., Thomas, M. & Greipsson, S. (2021) Effects of Exogenous Application of Plant Growth Regulators (SNP and GA3) on Phytoextraction by Switchgrass (Panicum Virgatum L.) Grown in Lead (Pb) Contaminated Soil. Sustainability 13: 10866
23. Sun, Y., Xu, Y., Zhou, Q., Wang, L., Lin, D., & Liang, X. (2012) Thepotential of gibberellicacid3 (GA3) and Tween-80 induced phytoremediation of co-` contamination of Cd and Benzo [a] pyrene (B[a]P) using Tagetes patula. Journal of `environmental management,114: 202-208.
24. Ahmad, A., Hadi, F. & Ali, N. (2014) Effective phytoextraction of cadmium (Cd) with increasing concentration of total phenolics and free proline in Cannabissativa (L) plant under various treatments of fertilizers, plant regulators and sodium salt. International journal of phytoremediation 17(1):56-65.
25. Makarova, A., Elena, N.,Tatiana, A. & Ksenia, P. (2021) The Improved Phytoextraction of Heavy Metals and the Growth of Trifolium repens L.: The Role of K2HEDP and Plant Growth Regulators Alone and in Combination. Sustainability 13:2432.
26. Fan, M., Xiao, X., Guo, Y., Zhang, J., Wang, E., Chen, W., Lin, Y. & Wei, G. (2017) Enhanced phytoremediation of Robinia pseudoacaciain heavy metal-contaminated soils with rhizobia and the associated bacterial community structure and function. Chemosphere 197:729–740
27. Pérez-Hernández, H., Yakelín, A. P., Méndez-López. A. &Fernández-Luqueño, F (2024). Efect ` of ZnO Nanoparticles During the Process of Phytoremediation of Soil Contaminated `with As and Pb Cultivated with Sunfower `(Helianthus annuus L. Int J Environ Res ` 18:7
28. Farman, S., Mushtaq, A. & Waqar, M.A. (2019) Plant growth regulators (PGRs) and their applications: A review. International Journal of Chemical and Biochemical Sciences (ISSN 2226-9614) IJCBS, 15: 94-103
29. Magaji, Y., Ajibade, G.A., Yilwa ,V. M. Y., Appah, J., Haroun, A. A., Alhaji, I., Namadi, M. M. and Sodimu, A. I. (2018) Concentration of heavy metals in the soil

and translocation with phytoremediation potential by plant species in military shooting range. World Scientific News, An international scientific journal, 92(2): 260-271

30. Small, C. C. & Degenhardt, D (2018). Plant growth regulators for enhancing revegetation success `in `reclamation: a review. Ecological engineering. 118: 43-51.
31. Lin, H. R., Shu, H. Y. and Lin, G. H (2018). Biological roles of indole-3-acetic acid in Acinetobacter baumannii. Microbiological research. 216: 30-39.
32. Shah, N., Irshad1, M., Murad, W., Hamayun, M., Qadir, M., Hussain, A., Ara, H., Fahad, A., Almutairi, M., Ahmad, A & Ali,S. (2024) IAA is more effective than EDTA in enhancing phytoremediation potential for cadmium and copper contaminated soils.
33. Yang, W., Dai,H., Wei, S. & Skuza, L (2024 b). The effect of exogenous plant growrh regulators `on elevated Cd Phytoremediation by Solanum nigrum Lin contaminated soil. `Environmental science pollution research. 31(3):396443925
34. Yang, Q., Yu, H., Zhao , Z. & Ju, Z (2024 a) Effects of Enhanced Phytoremediation `Techniques on Soil Aggregate Structure. Agriculture 14: 1-22.