Journal of
Plant Science & Research

Open Science Publications

Volume 11, Issue 2 - 2024

www.opensciencepublications.com

Enhancing Phytoremediation of CCA-Contaminated Soils using NPK Fertilizers in *Acacia auriculiformis* and *Casuarina equisetifolia* Seedlings

Research Article

Rathna Kumari BM, and Nagaraja N

[1]*Department of Botany, Government First Grade College, Vijayanagar, Bengaluru, India*

[2]*UGC-Malaviya Mission Teacher Training Centre, Bangalore University, Jnana Bharathi Campus, Bengaluru, India*

***Corresponding author:** Rathna Kumari BM, Department of Botany, Government First Grade College, Vijayanagar, Bengaluru, India Email: bmrathnakumari@gmail.com

Article Information: Submission: 18/04/2024; **Accepted:** 04/06/2024; **Published:** 08/06/2024

Abstract

The soil amendments are known to reduce the toxicity of heavy metals in soil. A pot experiment was conducted to evaluate the effect of four different concentrations (220:117:140, 440:117:140, 220:117:280 and 440:117:280) of Nitrogen, Phosphorous and Potassium (NPK) and their effects on *Acacia auriculiformis* and *Casuarina equisetifolia* in phytoremediation of CCA compounds. Results showed that the growth and biomass of the seedlings treated with CCA (1000 mg kg^{-1} soil) were significantly increased with an increase in NPK concentrations. Similarly, the amounts of Cu, Cr, and As found in the roots of *A. auriculiformis* at 440:117:2080 mg $g^{-1}$of NPK were 3.577, 3.070, and 0.094 mg g^{-1} respectively. Similarly, the quantity of Cu, Cr, and As found in stem and leaves in similar concentrations of NPK were 1.683 and 2.220 mg g^{-1}, 2.600 and 1.913 mg g^{-1}, and 0.077 and 0.085 mg g^{-1} respectively. Furthermore, the amount of Cu, Cr, and As found in the roots of *C. equisetifolia* at similar concentrations of NPK were 1.670, 3.320 and 0.240 mg g^{-1} respectively. Nevertheless, the amount of Cu, Cr, and As found in stem and leaves were 0.350 and 0.398 mg g^{-1} and 0.110 and 1.530mg g^{-1}, 2.540 and 0.154 mg g-[1] at 440:117:2080 mg of NPK respectively. The higher concentrations of NPK significantly ($p \leq 0.05$) increased the biochemical parameters of both the test plants. The maximum amount of total carbohydrates, proteins, and chlorophyll content found in *A.auriculiformis* and *C. equisetifolia* were 4.06, 1.61 and 0.28 mg g^{-1} and 3.575, 1.073 and 0.369 mg g-[1] at NPK ratio of 220:117:280 mg kg^{-1} soil respectively. The present study advocates that the higher concentrations of NPK improve soil properties and plant productivity by reducing heavy metal solubility and the NPK can greatly enhance phytoremediation in CCA-contaminated soils using *A. auriculiformis* and *C. equisetifolia* tree species.

Keywords: *Acacia auriculiformis; Casuarina equisetifolia*; CCA; NPK; Biochemical parameters

Introduction

Heavy metals remain persistent in the soil for a long period by posing a long-term threat to the environment [1, 2]. These are biologically magnified and affect soil fertility thereby reducing crop production [3]. Chromated copper arsenate (CCA) is a wood preservative, used in the protection of timber from microbial decay and insect damage [4]. The CCA-treated woods are widely used as construction material resulting in the release of its components viz Cr. Cu and As in to agricultural fields, which initiates concern for food safety [5].

Phytoremediation is an effective technology in mitigating heavy metal contamination of the soil [6]. Plants used in phytoremediation produce relatively high biomass, tolerant to metal toxicity and exhibit high metal absorption capacity [7]. *Acacia auriculiformis* and *Casuarina equisetifolia* are moderate-sized fast-growing tree species widely distributed in India. They have a shallow root system and produce huge biomass in short

ISSN: 2349-2805

periods even in dry conditions [8]. These plant species are potential candidates for the production of huge biomass and are well adapted to even poor soil conditions[9]. Furthermore, these species are found to accumulate huge amounts of metals in their tissues [10]. Since these plant species show better tolerance to heavy metal stress, they are used in the remediation of CCA-contaminated sites [11].

The chemical enhanced technology helps the uptake of heavy metals in plants [12]. The immobilization of heavy metals using different soil amendments is one of the most effective ways to remediate contaminated soil [13]. Several chemical amendments have been used for the enhancement of phytoextraction process[14].The EDTA and DTPA are known to increase biochemical constituents in seedlings of *A auriculiformis* and *C equisetifolia* treated with chromated copper arsenate[15]. Nitrogen is constituent of amino acids and is widely applied in nurseries. The optimization of N, P, and K doses for improved seedling growth in Eucalyptus species has been reported [16]. The application of 100 ml NPK stimulated 45% shoot biomass and 26% Cr uptake compared with the non-fertilized cultures[17]. Surprisingly, information on the influence of inorganic fertilizers viz NPK which are required for healthy growth and development of plants in phytoremediation of CCA in tree plant species is meagre. The objective of the study was to investigate the influence of NPK on the growth, biochemical changes and uptake of CCA components viz Cu, Cr and As in the seedlings of *A. auriculiformis* and *C. equisetifolia* tree species in pot conditions.

Materials and Methods

Plant material

Experiments on the effect of four levels of inorganic fertilizers viz. Nitrogen, Phosphorus, and Potassium (NPK) treated with chromated copper arsenate (1000 mg kg^{-1} soil) were conducted in the seedlings of *Acacia auriculiformis* and *Casuarina equisetifolia* species in Bengaluru, India. The healthy seeds of *A. auriculiformis* and *C. equisetifolia* were procured from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, India. The seeds were surface sterilized with bavistin (50% WP) followed by a double wash with deionized water. The CCA (1000 mg kg^{-1} soil) concentration was prepared from commercially available C-type CCA with a proportion of Cr (CrO_3-47.5%), Cu (CuO-18.5%), and As (As_2O_5- 35%). Four levels of NPK viz 220:117:140 mg, 440:117:140 mg, 220:117:280 mg, and 440:117:280 mg kg^{-1} soil were prepared and were applied separately.

Pot experiments

The healthy seedlings of *A. auriculiformis* and *C. equisetifolia* were transplanted from root trainers in plastic pots (2000cc) filled with a sufficient quantity of air-dried soil mixed with compost. All the pots were arranged in the greenhouse in four replications. The potting medium (sand and soil in 2:1, v/v) was prepared, mixed thoroughly with CCA (1000 mg kg^{-1} soil) and four levels of NPK (220:117:140 mg,440:117:140 mg, 220:117:280 mg,and 440:117:280 mg kg^{-1} soil). The potting medium consisted of sand and soil (2:1 v/v) without NPK was used as control. Regular management practices, including sufficient irrigation and weed control, were performed during the experiments.

Quantification of CCA compounds

After six months, the shoots (stem and leaves) and roots of the seedlings of each test tree species were harvested and washed thoroughly with deonized water. The seedling growth indices in terms of height were measured with measuring tape and collar diameter was measured with a calliper. The fresh and dry weights of the seedlings were recorded for each sample using electronic balance. The dry weights of the seedlings were measured after oven drying at 65-75^{o} C till the weight becomes constant. The washed plant samples were separated into shoots and roots dried and powdered. One gram of the root and shoot (stem and leaves) were digested in 10 ml acid mixture of 60% HNO_3 and 2 mL 30 % H_2O_2 in a microwave oven drying at 200±5^{0} C for 20 min. Furthermore, the concentrations of Cu, Cr, and As accumulated in roots, stems, and leaves were analyzed using an atomic absorption spectrometer (Shimadzu, AA-6880, Japan).

Biochemical analysis

The amount of total carbohydrates, protein, and chlorophyll content found in the 6-month-old seedlings of *A. auriculiformis* and *C. equisetifolia* was estimated by spectrophotometric methods. The leaves separated from each treatment and were cut into fine pieces and then ground with mortar and pestle. 0.5 g of fresh leaf powder from each treatment was homogenized with 5 µL of 80% acetone, incubated overnight at room temperature, and centrifuged at 5000 rpm for 5 min. The supernatant was made up to 5 µL with 80% acetone and the optical densities were measured at 645 and 663 nm wavelengths using UV-VIS Spectrophotometer (Shimadzu, UV-1900, Kyoto, Japan). The amount of total carbohydrates was analyzed by the Anthrone method [18]. Similarly, the total protein contents of the seedlings were determined by Lowry's method [19]. Furthermore, the amount of total chlorophyll was estimated using Arnon's method [20] and the results obtained in different treatments were compared with the control samples.

Statistical analysis

The experimental data on seedling growth, biomass, accumulation of Cu, Cr and As compounds in root, stem and leaves, and changes in total carbohydrates, proteins, and chlorophyll content of *A. auriculiformis* and *C. equisetifolia* on exposure to different NPK concentrations treated with CCA (1000 mg kg^{-1} soil) were statistically analyzed by one-way analysis of variance (ANOVA). The data are presented as mean, standard error and coefficient of deviation (CD) (0.05). Furthermore, different treatments of the study were analyzed statistically for significant differences at $p \leq 0.05$ by Duncan's multiple range test (DMRT).

Results

Fertilizers on growth and biomass of the seedlings

The growth and biomass of the seedlings of *A. auriculiformis* and *C. equisetifolia* treated with four different concentrations of NPK along with CCA (1000 mg kg^{-1} soil) were varied (Table 1).

Citation: Rathna Kumari BM, Nagaraja N. Enhancing Phytoremediation of CCA- Contaminated Soils using NPK Fertilizers in *Acacia auriculiformis* and *Casuarina equisetifolia* Seedlings. J Plant Sci Res. 2024;11(2): 264

Table 1. Effect of various levels of NPK treated with 1000 mg chromated copper arsenate (CCA) on growth and biomass of *Acacia auriculiformis* and *Casuarina equisetifolia* seedlings.

(NPK in mg/pot)	*Acacia auriculiformis*				*Casuarina equisetifolia*			
	Height (cm)	Collar diameter (cm)	Fresh weight (g)	Dry weight (g)	Height (cm)	Collar diameter (cm)	Fresh weight (g)	Dry weight (g)
0:0:0	44.00^{e}	5.10^{d}	16.15^{d}	10.29^{c}	44.25^{e}	3.37^{c}	20.23^{e}	8.40^{e}
220:117:140	61.00^{c}	5.26^{c}	24.78^{b}	14.81^{ab}	57.25^{c}	3.93^{b}	27.87^{c}	11.59^{c}
440:117:140	67.00^{a}	5.36^{a}	26.06^{ab}	15.39^{a}	67.50^{ab}	4.31^{ab}	31.69^{ab}	12.82^{ab}
220:117:280	68.75^{a}	5.38^{a}	27.39^{a}	16.17^{a}	69.75^{a}	4.38^{a}	32.82^{a}	13.61^{a}
440:117:280	64.25^{b}	5.33^{b}	25.15^{b}	14.94^{ab}	65.00^{b}	4.18^{ab}	30.10^{b}	12.49^{bc}
$SE_{(0.05)}$	1.296	2.017	1.225	1.409	2.57E+00	0.244	1.31E+00	2.57E+00
$CD_{(0.05)}$	2.242	3.49	2.12	2.437	4.448	0.422	2.263	4.448
p-value	2.24E-07	0.4131	8.44E-10	0.000122	1.59E-11	1.58E-05	4.05E-11	1.59E-11

Data are means of four replications in each experiment. Data with the same letter are not significantly different. Different letters indicate significant differences at p≤0.05 level according to the Duncan's multiple range test

The height and collar diameter of the seedlings were significantly (p≤0.05) increased with an increase in NPK concentrations. The maximum and minimum height and collar diameter of *A. auriculiformis* recorded were 68.75 and 44 cm and 69.75 cm and 5.38 and 5.10 cm at NPK concentration of 440:117:280 and control respectively. Similarly, the highest and lowest height and collar diameter of *C. equisetifolia* recorded were 69.75 and 44.25 cm and 4.38 and 3.37 cm at NPK ratio of 440:117:280 and control respectively. Furthermore, the maximum and minimum fresh and dry weights of *A. auriculiformis* were found to be 27.39 and 16.15 mg and 16.17 and 10.29 g in NPK concentration of 220:117:280 25.15 and control respectively. Similarly, the maximum and minimum fresh and dry weight of *C. equisetifolia* were found to be 32.82 and 20.23 mg and 13.61 and 8.40 g in NPK concentration of 220:117:280 25.15 and control respectively.

Fertilizers on biochemical parameters of seedlings

The soil rich in sufficient nutrients are required for the healthy growth and development of plants. However, the soil contaminated with toxic heavy metals may affect the morphological and biochemical parameters of plants. The influence of different concentrations of NPK on the biochemical parameters of the seedlings of *A. auriculiformis* and *C. equisetifolia* is presented in (Table 2). The results showed a gradual increase in total carbohydrate, protein, and chlorophyll content with an increase in NPK concentrations. The minimum and maximum amount of total carbohydrates, proteins and chlorophyll content recorded in the seedlings of *A. auriculiformis* were 3.04 and 4.06 mg g^{-1}, 0.95 and 1.61mg g^{-1} and 0.20 and 0.28 mg g^{-1} in control and 220:117:280 mg kg^{-1} soil concentrations of NPK respectively. Similarly, the minimum and maximum amount of total carbohydrates, proteins and chlorophyll content recorded in the seedlings of *C. equisetifolia* were 2.475 and 3.575 mg g^{-1}, 0.826 and 1.073mg g^{-1} and 0.210 and 0.369 mg g^{-1} in control and 220:117:280 mg kg^{-1} soil concentrations of NPK respectively.

Fertilizers on bioaccumulation of CCA compounds in the seedlings

Plants respond differently to fertilizers depending on their ability to accumulate heavy metals in shoots and root. Accumulation of CCA in the form of its components viz Cu, Cr, and As amended with different concentrations of NPK were recorded in the root of six months old *A. auriculiformis* seedlings (Table 3). The results revealed a significant (p≤0.05) increase in accumulation of CCA compounds with an increase in concentration of NPK in both root and shoot. The highest amounts of Cu, Cr, and As found in the root were 5.027, 4.207, and 0.147 mg g^{-1} respectively at 220:117:280 NPK level. Similarly, the maximum amount of Cu, Cr, and As recorded in stem and leaves were 2.333, 3.623, and 0.117 mg g^{-1} and 3.343, 3.650, and 0.134 mg g^{-1} respectively in the similar concentration of NPK.

The amount of CCA components viz Cu, Cr, and As amended with different concentrations of NPK were recorded in the root, stem, and leaves of the seedlings of *C. equisetifolia* (Table 4). The observations showed a significant (p≤0.05) increase in the amount of CCA compounds with an increase in concentration of NPK in both stem and leaves. The maximum amount of Cu, Cr, and As found in the root were 3.650, 4.230, and 0.329 mg g^{-1} respectively at 220:117:280 NPK level. Similarly, the maximum amount of Cu, Cr, and As recorded in stem and leaves were 0.645, 0.874, and 0.198 mg g^{-1} and 2.870, 3.870, and 0.242 mg g^{-1} respectively in the similar concentration of NPK.

Discussion

The addition chemical amendments to soil contaminated with heavy metals enhance the phytoremediation process, and fertilizers are the most widely used amendments [21]. Reports show that the mobility of metals in the soil can be considerably increased by the addition of chelating agents. A number of natural and synthetic chelating agents are available, although their effectiveness varies with plant and soil types. The results of the study showed an increased growth and biomass of the seedlings treated with NPK in CCA-contaminated soils. It is apparent that fertilizers enhance the plant growth, and heavy metal extraction capacity of plants [22]. The increase in seedling height on application of nitrogen might lead to increased production of photosynthesis [23]. There was an increase in the mean plant biomass and tolerance, on treated with fertilizers in Pb, Cd and

Table 2. Effect of various levels of NPK treated with 1000 mg chromated copper arsenste (CCA) on carbohydrate, protein and chlorophyll content of *Acacia auriculiformis* and *Casuarina equisetifolia* seedlings.

(NPK in mg/pot)	*Acacia auriculiformis*			*Casuarina equisetifolia*		
	Carbohydrate (mg g^{-1})	Protein (mg g^{-1})	Chlorophyll (mg g^{-1})	Carbohydrate (mg g^{-1})	Protein (mg g^{-1})	Chlorophyll (mg g^{-1})
0:0:0	3.04^{c}	0.95^{c}	0.20^{b}	2.475^{c}	0.826^{c}	0.210^{b}
220:117:140	3.55^{b}	1.35^{b}	0.20^{b}	2.848^{bc}	0.916^{bc}	0.245^{b}
440:117:140	4.06^{a}	1.60^{a}	0.26^{a}	3.493^{a}	1.043^{ab}	0.358^{a}
220:117:280	4.06^{a}	1.61^{a}	0.28^{a}	3.575^{a}	1.073^{ab}	0.369^{a}
440:117:280	4.05^{a}	1.61^{a}	0.26^{a}	3.313^{ab}	1.033^{ab}	0.354^{a}
$SE_{(0.05)}$	2.20E-02	1.222	0.04	0.267	1.13E-01	2.20E-02
$CD_{(0.05)}$	0.029	2.115	0.07	0.463	0.197	0.039
p-value	2.41E-09	0.00058	0.00249	4.00E-05	0.0171	2.42E-09

Data are means of four replications in each experiment. Data with the same letter are not significantly different. Different letters indicate significant differences at p≤0.05 level according to the Duncan's multiple range test

Table 3. Effect of various levels of NPK treated 1000 mg chromated copper arsenate (CCA) on accumulation Copper, Chromium and Arsenic content in Stem, Root and Leaves of *Acacia auriculiformis* seedlings at six month age.

NPK (mg/pot)	Copper (mg g^{-1})			Chromium (mg g^{-1})			Arsenic (mg g^{-1})		
	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root	Leaves
0:0:0	0.453^{e}	1.030^{f}	0.620^{e}	0.503^{e}	0.623^{c}	0.523^{e}	0.021^{e}	0.040^{f}	0.033^{e}
220:117:140	1.520^{c}	3.253^{d}	1.947^{d}	1.730^{d}	2.810^{b}	1.460^{d}	0.051^{d}	0.076^{d}	0.064^{d}
440:117:140	2.123^{b}	4.703^{b}	3.063^{b}	3.143^{b}	3.730^{a}	2.737^{b}	0.094^{b}	0.130^{b}	0.118^{b}
220:117:280	2.333^{a}	5.027^{a}	3.343^{a}	3.623^{a}	4.207^{a}	3.650^{a}	0.117^{a}	0.147^{a}	0.134^{a}
440:117:280	1.683^{c}	3.577^{c}	2.600^{c}	2.220^{c}	3.070^{b}	1.913^{c}	0.077^{c}	0.094^{c}	0.085^{c}
$SE_{(0.05)}$	1.01E-01	1.37E-01	0.1194	9.90E-02	2.58E-01	0.0707	3.19E-03	0.0035	0.0068
$CD_{(0.05)}$	0.176	0.237	0.207	0.171	0.447	0.122	0.006	0.006	0.018
p-value	4.24E-17	8.86E-21	9.32E-19	1.26E-20	1.37E-14	7.15E-23	2.29E-20	2.92E-21	1.85E-15

Data are means of four replications in each experiment. Data with the same letter are not significantly different. Different letters indicate significant differences at p≤0.05 level according to the Duncan's multiple range test

Table 4. Effect of various levels of NPK treated with 1000 mg chromated copper arsenate (CCA) on accumulation of Copper, Chromium and Arsenic content in Stem, Root and Leaves of *Casuarina equisetifolia* seedlings at six month age.

NPK (mg/pot)	Copper (mg g^{-1})			Chromium (mg g^{-1})			Arsenic (mg g^{-1})		
	Stem	Root	Leaves	Stem	Root	Leaves	Stem	Root	Leaves
0:0:0	0.210^{c}	0.340^{d}	0.232^{e}	0.230^{d}	0.390^{e}	0.430^{e}	0.023^{b}	0.035^{e}	0.026^{e}
220:117:140	0.420^{c}	0.860^{c}	0.632^{d}	0.435^{c}	2.214^{d}	0.745^{d}	0.098^{ab}	0.118^{d}	0.099^{d}
440:117:140	0.533^{b}	1.870^{b}	1.980^{b}	0.658^{b}	3.540^{b}	2.980^{b}	0.123^{ab}	0.288^{b}	0.182^{b}
220:117:280	0.645^{a}	3.650^{a}	2.870^{a}	0.874^{a}	4.230^{a}	3.870^{a}	0.198^{a}	0.329^{a}	0.242^{a}
440:117:280	0.350^{c}	1.670^{b}	1.530^{c}	0.398^{c}	3.320^{c}	2.540^{c}	0.110^{ab}	0.240^{b}	0.154^{b}
$SE_{(0.05)}$	0.105	0.127	0.127	0.124	0.641	0.471	0.015	0.021	0.097
$CD_{(0.05)}$	0.184	0.223	0.223	0.218	0.12	0.828	0.026	0.036	0.170
p-value	7.29-08	2.10E-09	0.0040	5.11E-07	5.64E-06	4.71E-04	8.9E-03	5.17E-04	3.45E-05

Data are means of four replications in each experiment. Data with the same letter are not significantly different. Different letters indicate significant differences at p≤0.05 level according to the Duncan's multiple range test

Zn-contaminated soils [24]. The observations of [25] showed that the addition of NPK fertilizers significantly increased the biomass of *Leersia hexandra* with higher Cu extraction efficiency. Nitrogen plays an important role in plant growth since N is a component of all plant structures such as proteins, enzymes, and chlorophyll. Appropriate fertilization could increase the plant biomass content and may enhance their ability to uptake and tolerate heavy metals [26].

Nutrient solutions were applied to the soil to improve the growth of plants for phytoremediation.Furthermore, nutrient availability is an important factor governing the success of

Citation: Rathna Kumari BM, Nagaraja N. Enhancing Phytoremediation of CCA- Contaminated Soils using NPK Fertilizers in *Acacia auriculiformis* and *Casuarina equisetifolia* Seedlings. J Plant Sci Res. 2024;11(2): 264

phytoremediation and can be regulated through the addition of fertilizers.Several studies showed that the application of NPK significantly increase heavy metal uptake in plants. Phosphorus contributes to root expansion and plant nutrient uptake [27].The increased accumulation of CCA in the seedlings could be attributed to the increased solubility of CCA compounds in the soil. Urea was found to enhance the Cd phytoaccumulation in *Carpobrotus rossii* [28]. Furthermore, nitrogen application reduces the production of a large number of reactive oxygen species (ROS) induced by biotic and abiotic stress [29]. Nitrogen fertilizers improve the resistance and tolerance to plants exposed to Cd stress [30]. Similarly, Cd phytoremediation efficiency of *Solanum nigrum* was found to be improved with the addition of nitrogen fertilizer [31]. The N, P, and K are important components of plant amino acids, proteins, genetic material, and enzymes [32].

It is evident from the studies that, the NPK enhances the biochemical composition of *A. auriculiformis* and *C. equisetifolia* seedlings. Results of the study showed increased amount of carbohydrate content, proteins and chlorophyll content with an increase in the concentrations of NPK in the seedlings of test species. The reactive oxygen species (ROS) causes severe changes in protein structure, altering the proteins' functions, with subsequent effects on metabolic pathways [33]. The results of our study showed an increase in the chlorophyll content in the seedlings exposed to different concentrations of NPK. Accumulation of excess amounts of Cu damages the ultra-structure of the chloroplast, which directs changes in the composition of the thylakoid membrane [34].

Conclusion

The studies reveal that the NPK plays a pivotal role in the enhancement of the plant defense system and alleviation of CCA phytotoxicity by enhancing the plant biomass of the seedlings of tree species. The NPK also improved the accumulation of Cu, Cr, and As in the root, stem, and leaves of *Acacia auriculiformis* and *Casuarina equisetifolia.* These plants could be used as phytoaccumulators for remediation of CCA compounds on application of NPK. However, further studies are required on the evaluation of the phytoremediation efficiency of these tree species in field conditions.

Acknowledgement

The authors are grateful to Bangalore University, Bengaluru, India for extending research facilities for conduct of these studies.

References

1. Suman J, Uhlik O, Viktorova J, Macek T (2018) Phytoextraction of heavy metals: A promising tool for clean-up of polluted environment? Front Plant Sci. 16: 1476.
2. Arunakumari K, Begum, F, Surinaidu L, Nandan MJ, Alapati U (2023) Persistence of heavy metals and human health risk assessment in the south Indian industrial area, AQUA-Water Infrastructure, Ecosystems and Society 72: 898-913.
3. Danovaro R, Montanara AC, Corinaldesi C, Dell Anno A, Illuminata S, et al. (2023) Bioaccumulation and biomagnifications of heavy metals in marine micro-predators. Commn Biol. 6: 1206.
4. Morais S, Fonseca HMAC, Oliveira SMR, Oliveira H, Gupta VK, et al. (2021) Environmental and health hazards of chromated copper arsenate treated wood. A review. Int. J. Environ. Res. Public Health, 18: 5518.
5. Rathna Kumari BM (2022) Effects of copper bioaccumulation on growth and biochemical constituents of the seedlings of *Casuarina equisetifolia* L. Curr. Bot. 13: 8-11.
6. Yan A, Wang Y, Tan SN, Yusof MLM, Gosh S, Chen Z (2020). Phytoremediation: A promising approach for revegetation of heavy metal polluted land. Front. Plant Sci 11: 359.
7. Shabani N, Sayadi MH (2012) Evaluation of heavy metals accumulation by two emergent macrophytes from the polluted soil: an experimental study. Environmentalist 32: 91-98.
8. Luo J, Qi S, Peng, L, Wang J (2016) Phytoremediation efficiency of CD by *Eucalyptus globules* transplanted from polluted and unpolluted sites. Int. J. Phytoremed. 18: 306-314.
9. Rathna Kumari BM, Nagaraja N (2023) Studies on phytoremediation of chromated copper arsenate (CCA) using Acacia plant species (Fabaceae), Int. J. Phytoremed. 25: 1669-1675.
10. Ratha Kumari BM, Nagaraja, N (2023) Effect of chromated copper arsenate on protein, carbohydrate, and chlorophyll content of tropical Eucalyptus and Acacia species. Asian J Trop Biotechnol 20: 56-61.
11. Rathna Kumari BM, Raveesha HR (2021) Phytoremediation of soil contaminated with chromated copper arsenate (CCA) using Eucalyptus species. Int J Ecol Environ Sci 3: 24-28.
12. Jiang Y, Jiang S, Li Z, Yan X, Qin Z, et al. (2019) Field scale remediation of Cd and Pb contaminated paddy soil using three mulberry (*Morus alba* L.) cultivars, Ecol. Eng. 129: 38-44.
13. Ok YS, Kim SC, Kim DK, Skousen JG, Lee JS, et al. (2011) Ameliorants to immobilize Cd in rice paddy soils contaminated by abandoned metal mines in Korea. Environ Geochem Health 1: 23-30.
14. Zakari S, Jiang X, Zhu X, Liu W, Allakonon MGB, et al. (2021) Influence of sulphur amendments on heavy metals phytoextraction from agricultural contaminated soils: A meta-analysis. Environ Pollut. 288: 117820.
15. Rathna Kumari BM, Nagaraja N (2024) Studies on the effect of chromated copper arsenate (CCA) and chelating agents on biochemical parameters of *Acacia auriculifotmis* and *Casuarina equisetifolia* tree species. J Soil Sci Plant Nutr 24: 1362-1368.
16. Sundaraju R, Chinnathurai AK, Vijayakumar R (1991) Application of fertilizer and micronutrients on *Eucalyptus teretocornis* and *Eucalyptus camaldulensis* nurseries. Indian Forester 117: 1021-1028.
17. Hedge JE, Hofreiter BT (1962) Carbohydrate Chemistry 17 (Eds. Whister, R.L ,Be Miller, J.N.), Academic Press, New York.
18. Lowry OH, Rosebrough NJ, Faar AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265-275.
19. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.
20. Xu G, Shao H, Zhang Y, Junna S (2018) Nonadditive effects of biochar amendments on soil phosphorus fractions in two contrasting soils. Land Degrad Dev29: 2720-2727.
21. Wang J, Li R, Zhang H et al (2020) Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. BMC Microbiol. 20: 38.
22. Linquist BA, Liu L, van Kessel C, van Groenigen KJ (2013) Enhanced efficiency nitrogen fertilizers for rice systems: Meta-analysis of yield and nitrogen uptake. Field Crops Res. 154: 246-254.
23. Barrutia O, Epelde L, Garcia-Plazaola JI,Garbisu C, Becerril JM (2009) Phytoextraction potential of two *Rumex acetosa* L. accessions collected from metalliferous sites: effect of fertilization. Chemosphere 74: 259-264.

24. Liu G, Yates MD, Cheng S, Call DF, Sun D, et al. (2011). Examination of microbial fuel cell start-up times with domestic wastewater and additional amendments. Bioresource Technol. 102: 7301-7306.

25. Lin H, Zhang C, Zhang X, Liu L Chhuon K (2020) Effects of phosphorous fertilizers on growth, Cu phytoextraction and tolerance of *Leersia hexandra* swartz under different Cu stress levels. Int J Phytoremediation, 22: 578-584.

26. Li Z, Guo F,Cornelis JT,Song Z, Wang X, et al. (2020) Combined Silicon-Phosphorous fertilization affects the biomass and phytolith stock of rice plants, Front Plant Sc 11: 67.

27. Shehu HE, Kwari JD,Sandabe MK (2010) Effects of N, P and K fertilizers on yield, content and uptake of N, P and K by sesame (Sesamun indicum), Int J Agric Biol 12: 1560-8530.

28. Liu WX, Zgang CJ, Hu PJ, Luo YM, Wu LH, et al. (2016) Influence of nitrogen form on the phytoextraction of cadmium by a newly discovered hyperaccumulator, *Carpobrotus rossii*. Environ. Sci Popput Res 23: 1246-1253.

29. Abid AA, Zhang Q, Cai M, et al. (2019) Nitrous oxide emission and identification of N_2O production pathways using ^{15}N stable isotope under alternate wetting-drying conditions in rice paddy soils. Appl Ecol Environ Res 11: 35-47.

30. Jalloh MA, Chen J, Zhen F, Zhang G (2009) Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress. J. Hazard Mater, 162: 1081-1085.

31. Yang W, Dai, H, Skuza L, Wei S (2022). Enhanced Cd phytoextraction by *Solanum nigrum* L. from contaminated soils combined with the application of N fertilizers and double harvests. Toxics 10: 266.

32. Ihtisham M, Liu S, Shahid MO, et al. (2020) The optimized N, P, and K fertilization for Bermudagrass integrated turf performance during the establishment and its importance for the sustainable management of urban green spaces, Sustainability, !2: 10294.

33. Hänsch R, Mendel R.R (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), Curr. Opin. Plant Biol. 12: 259-266.

34. Rahman Z, Singh VP (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. Environ Monit Assess. 191: 419.