



# Evaluation of ecological potency in bamboo species for phytoremediation and eco-rejuvenation of fly ash-degraded land: a two-year field study

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## Abstract

Ecological restoration of fly ash-degraded soils is a major concern for developing countries like India. So far, various physicochemical techniques have been employed to restore these polluted lands, but the limitations of cost, inefficiency, and secondary pollutant generation have forced ecologists to look for alternative approaches. Phytoremediation has been widely employed to replace these techniques, and to produce an economic asset along with the restoration of fly ash-degraded land, through bamboo species which serve as a parallel factor increasing the overall efficiency of bio methods, becomes very interesting. Four bamboo species, *Bambusa balcooa*, *B. vulgaris* ‘wamin’, *B. bambos*, and *B. vulgaris*, were planted at abandoned fly ash dumpsite. After two years of plantation, there was a significant improvement in all the physicochemical characteristics of the fly ash dumpsite. Phytoremediation indices in terms of bioconcentration factor (BCF) (1.26 and 1.72) and translocation factor (TF) (1.98 and 1.25) presented that *B. bambos* is the ideal species for the phytoextraction of Cr and Zn, while *B. balcooa* is an ideal species for the phytostabilization of lead (Pb), arsenic (As), and zinc (Zn), *B. vulgaris* ‘wamin’ for the phytostabilization of copper (Cu), nickel (Ni), Zn, and As and *B. vulgaris* for phytostabilization of Cu, chromium (Cr), and Ni, respectively. There was a significant improvement in biomass production in all the bamboo species being highest in *B. balcooa* (1087 kg ha<sup>-1</sup>) after two years of establishment on the fly ash dumpsite. Further, *B. balcooa* was found as the ideal bamboo species for sequestration of atmospheric carbon dioxide (CO<sub>2</sub>) (8217 tonnes C ha<sup>-1</sup> year<sup>-1</sup>). This work further suggests that *B. balcooa* is an excellent species for eco-restoration with a reliable air pollution tolerance index (APTI) (16.65). Principal component analysis demonstrated that *B. bambos* is the most potent bamboo species for phytoremediation of fly ash dumpsites owing to its high biomass capacity, positively correlated with hyperaccumulation of heavy metals. However, the fate of heavy metals after litter decomposition needs to be examined in detail to make this study a widespread approach.

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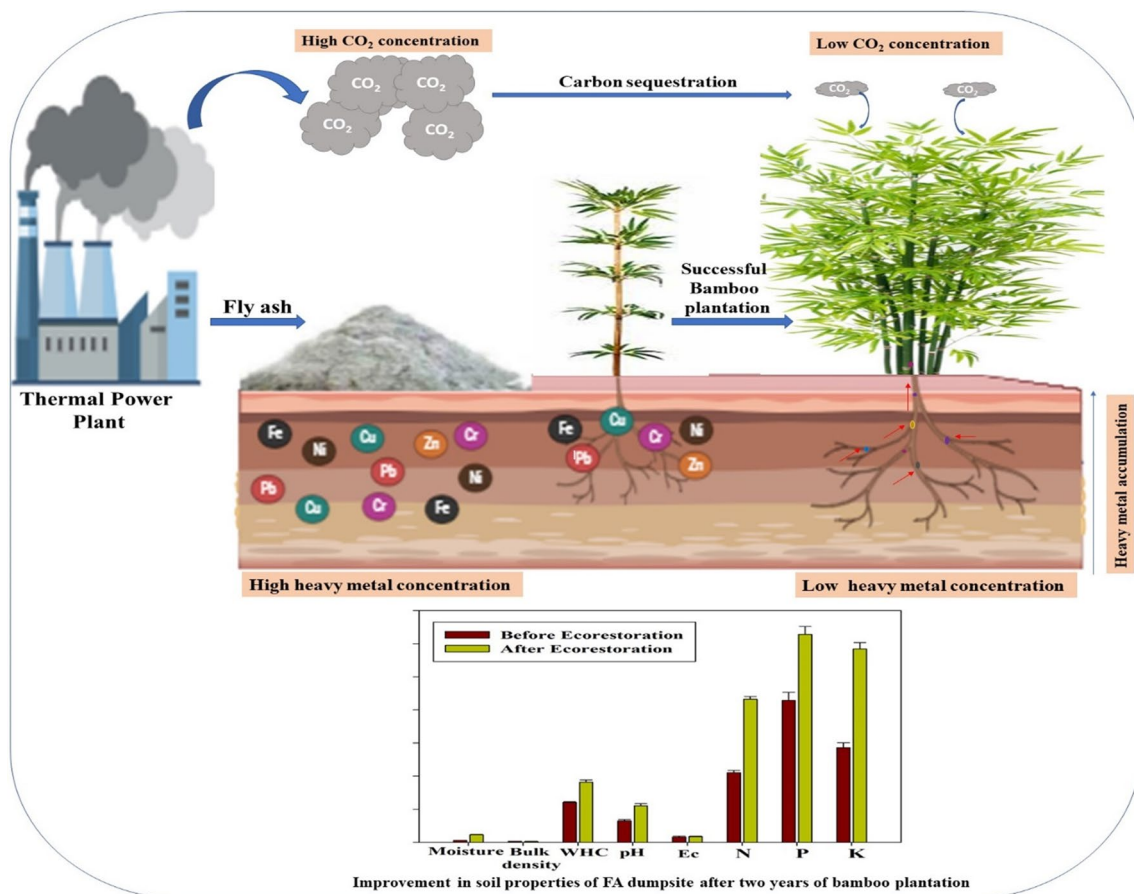
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## Graphical Abstract



**Keywords** Phytoremediation · Fly ash · Carbon sequestration · Eco-restoration · Heavy metal pollution

### Abbreviations

AGB	Above ground biomass
APTI	Air pollution tolerance index
BCF	Bioconcentration factor
BGB	Below ground biomass
CEC	Cation exchange rate
CRMs	Certified reference materials
CSR	Carbon sequestration rate
DAP	Days after planting
EC	Electrical conductivity
FYM	Farmyard manure
RWC	Relative water content
TF	Translocation factor
WHC	Water holding capacity

### Introduction

Coal combustion residues such as fly ash, boiler slag, bottom ash, and flue gas desulfurization materials are produced in large quantities by thermal power plants around the world that use coal as a primary source of electricity generation (Heidrich et al. 2013). India, due to its huge energy requirements, ranks 2nd globally in coal burning and consumes about 600 million tonnes of coal from which about 239 MTs fly ash is generated annually as a by-product (CEA 2020-2021). This voluminous amount of fly ash has occupied and degraded about 63,000 hectares of land throughout the sub-continent (Sangita et al. 2016). The presence of heavy metals and organic pollutants in fly ash raises serious environmental



and health concerns (Gordana et al. 2018). The harsh conditions induced by fly ash deposition restrict the biological activities of soil and consequently lead to its degradation (Shakeel et al. 2022). Additionally, the disposal of fly ash results in the degradation of air and water quality, almost complete extinction of vegetation, and adverse effects on both fauna and human health (Akhtar et al. 2022). However, due to the presence of some essential plant elements like calcium (Ca), potassium (K), iron (Fe), Zn, molybdenum (Mo), and Cu, it has some plant growth-promoting potential as well (Shakeel et al. 2019). Also, it has been shown to improve soil characteristics at lower concentrations (Shakeel et al. 2020). Despite various approaches by government agencies and non-government organizations (NGOs) to address and enhance fly ash utilization, measures for the restoration of already degraded lands are still lacking.

Phytoremediation of fly ash-degraded soils through eco-rejuvenation is gaining attraction as a self-sustaining, cost-effective, and environmentally friendly alternative to traditional physicochemical methods (Cameselle et al. 2019; Liu et al. 2022). The standard physicochemical methods for soil remediation include soil replacement, isolation, thermal treatment, stabilization, soil flushing, electrokinetics and vitrification (Yao et al. 2015; Song et al. 2017). Most of these methods are laborious, expensive, need sophisticated instruments, alter the soil functionality, and release secondary pollutants into the environment (Daniel et al. 2022). Therefore, the efficacy of phytoremediation technology is principally reliant on the selection of suitable plant species that can quickly and efficiently accumulate metals from contaminated soils and reduces toxicity (Wan et al. 2020; Bhanshe et al. 2022). In India, various plant species have been evaluated for their role in the phytoremediation of fly ash-degraded soils (Dwivedi et al. 2008; Pandey et al. 2015; Kumari et al. 2016). Most of these studies have employed weed species that pose a threat of invasion to native crops and have no significant economic benefits (Ma and Gao 2014). Singh et al. (2020) evaluated two weed species, *Saccharum munja* and *Saccharum spontaneum*, for the phytoextraction of heavy metals present in fly ash dumpsite. Similarly, Pandey et al. (2016) evaluated three weed species, *Lantana camara*, *Ipomea carnea*, and *Solanum surattense*, for their phytoremediation potential of fly ash dumpsite. Maiti et al. (2021) evaluated the remediation potential of *Cynodon dactylon* and *Saccharum spontaneum* on a fly ash dump. All these studies reported that weeds are excellent candidates for the phytoremediation process, but they cannot be used for any other socio-economic purpose. Furthermore, these weed species also pose the threat of biological invasion to the nearby agricultural croplands. Therefore, there must be

some plant species, which can withstand the harsh conditions of fly ash, remediate it, attain good biomass, and have an economic value as well. All these conditions refer to the candidature of bamboo which has a quick biomass capacity, efficient phytoremediation potential and many economic benefits (Fuke et al. 2021).

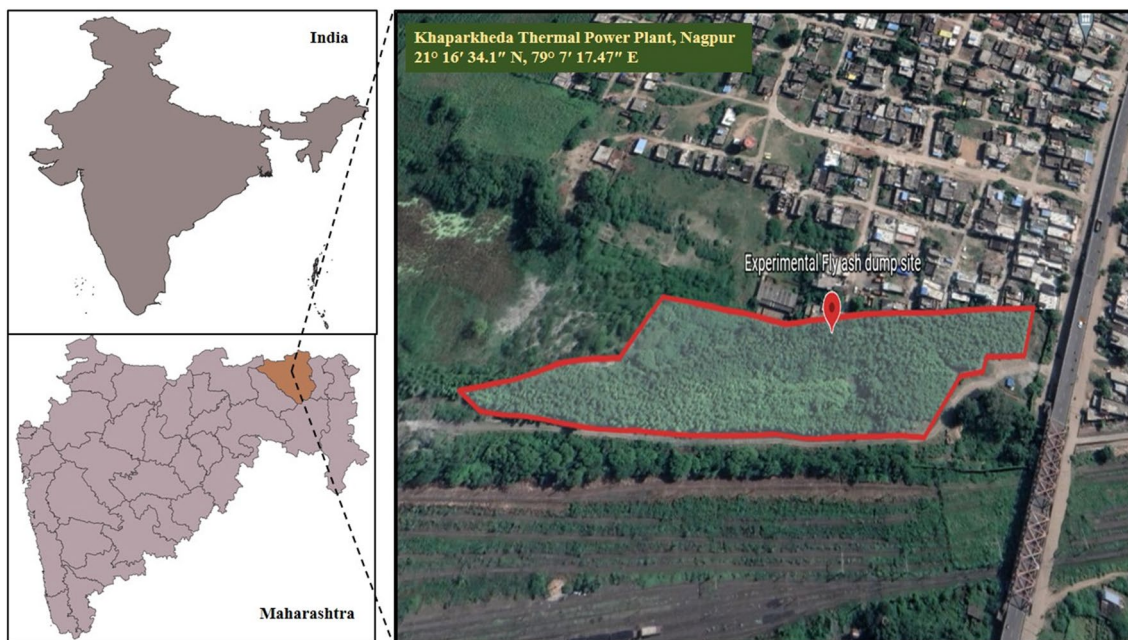
For the bioremediation and ecological regeneration of heavy metal-degraded soils, bamboo is an underutilized plant species (Bian et al. 2020). Recent studies have indicated that some bamboo species have a great potential to absorb toxic metals and adapt to metalliferous conditions, even though there have only been a limited number of studies on bamboo for bioremediation of fly ash-contaminated soils (Singh et al. 2022). In addition to its phytoremediation capacity, it is potentially a good alternative for timber wood from forests, an efficient CO<sub>2</sub> sequester, an excellent plant species to check soil erosion and control particulate air pollution (Rathour et al. 2022). With all these uses, bamboo is now a multipurpose plant species, and its products can be extremely beneficial for the economy and sustainability of agriculture. Four bamboo species were chosen for this study due to their non-edible nature and wide distribution in the region. Most commonly, these species are used for construction purposes and have the least chances of entering the food chain (Sawarkar et al. 2022). Therefore, the objective of this study was to screen various bamboo species and categorize them as phytostabilizers or phytoextractors based on the determination of bioconcentration factor (BCF) and translocation factor (TF). This study also entails at evaluating the growth potential of different bamboo species on the contaminated site along with their CO<sub>2</sub> sequestration capacity to restore the overall environment at the fly ash dumpsite. Further, this study brings novel insights into eco-restoration by combining phytoremediation with the air pollution tolerance index (APTI) of different bamboo species on the FA dumpsite.

## Materials and methods

### Study area

The study was carried out in the abandoned fly ash dumpsite of Khaparkheda thermal power station Nagpur in the Maharashtra state of India (Fig. 1). The site's coordinates lie 21.276138° N, 79.121518° E to 21.229927° N, 79.092980° E. This thermal power station has a total capacity of 1340 MW. On a daily basis, it consumes about 14,800–15800 tonnes of coal and generates about 2.87 million tonnes FA annually (CEA 2021). The physicochemical





**Fig. 1** Experimental site of the Khaperkheda thermal power plant, Nagpur, Maharashtra, India

characteristics of the study area were observed at two stages, i.e., before plantation, and two years after plantation.

### Design and sampling of experiments

Four varieties of bamboo, *Bambusa bambos*, *B. balcooa*, *B. vulgaris* ‘wamin’ and *B. vulgaris* were obtained from CSIR-NEERI Nagpur, India, which were then transplanted into the treated pits at the experimental field site in a completely randomized block design (CRBD). The 2-year-old seedlings were planted by following the rectangular system of plantation in 3 m x 4 m pits treated with farmyard manure (30 kg pit<sup>-1</sup> FYM) and (10 kg pit<sup>-1</sup> press mud). Regular inspections were done to check waterlogging, and replacement of dying seedlings with fresh seedlings. Before planting, the initial growth metrics of seedlings were assessed. Plant samples were taken to determine heavy metals and carbon sequestration. Three randomly chosen samples from each bamboo variety were taken for analysis before and after the plantation.

### Physicochemical characteristics

Moisture content of the soil was determined by the gravimetric technique of Reynolds (1970). 10 g of the soil sample was placed in an aluminium moisture box after removing the cover and placing it in the oven. The sample

was heated at 105 °C until it reached a constant weight. After cooling the sample in a desiccator, the weight of the cooled moisture box was taken. Moisture content was expressed in terms of percentage (%). The Keen-Raczowski box approach was used to calculate the porosity (%), bulk density (g cm<sup>-3</sup>), and WHC (%) (Viji and Prasanna 2012).

pH and EC: A pH meter of the make phc101, HANNA instruments, Woonsocket, RI, USA, was used to determine the acidity and alkalinity of the soil. 10 g of soil sample was taken in a 100-mL beaker and diluted to 25 mL with distilled water (1: 2.5). After stirring the mixture thoroughly, an electrode was placed in the suspension and pH was recorded. EC was determined by a conductivity metre (HANNA Instruments, HI76310, Woonsocket, RI, USA).

Organic carbon: For the determination of organic carbon, the Walkley and Black (1934) method was used. 1 g soil sieved through a 0.5 mm sieve was taken in a conical flask. Then 20 mL of conc. H<sub>2</sub>SO<sub>4</sub> and 10 mL of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> were added to the mixture, which was vigorously stirred for a minute before being incubated at room temperature for 30 min. Before titration, 4 to 5 drops of ferroin indicator were applied, and the sample was titrated against 0.5 N ferrous ammonium sulphate until the colour changed to brownish red.

Total nitrogen (N): Total N was calculated by using Kjeldahl's technique (1883). In a digestion tube, 0.5 g of the





sample was added together with 1 g of the digestive solution ( $K_2SO_4$ :  $CuSO_4$ ) and 10 mL of concentrated  $H_2SO_4$ , and the tube was heated up to 420 °C. At this point, the sample changed to light green colour. After cooling, 4%  $H_3BO_3$  and an indicator were placed onto one side of the distillation equipment with a digestive tube on the other. Mechanical adjustments were made to the distillation unit to accommodate 40% sodium hydroxide. In an Erlenmeyer flask carrying boric acid, the samples were steam-treated, and the resultant  $NH_3$  was extracted. After being titrated with 0.02N  $H_2SO_4$ , the green distillate's colour returned to its natural shade (pinkish colour). Using the values from the blank and sample titrate, the total N proportion in the sample was calculated (mL).

**Available N:** The procedure established by Subbaiah and Asija (1956) was used to determine the amount of available N. In a 1-L round bottom flask, a 5-g soil sample was placed. Liquid paraffin was used to prevent frothing and bumping of the sample. Then 25 mL potassium permanganate and 25 mL sodium hydroxide solution were put into a flask containing 100 ml of the sample. Samples were filtered in  $H_3BO_3$ , and the filtrate was collected in a 150-mL beaker, titrated with 0.02N  $H_2SO_4$  until the colour changed to red. Blank was prepared in the flask without a soil sample.

**Total phosphorous (P):** Total P content was determined through Jackson's (1967) approach. 0.5 g of the sample was put into a flask, and 30 mL of the digested solution (consisting of 9:4 ratios of  $HNO_3$  and  $HClO_4$ ) was added till the sample turned colourless. After that, to the 10 mL of filtered sample, 10 mL ammonium vanadomolybdate was added in a volumetric flask. Absorbance was taken at 440 nm. By using a standard calibration curve, the content of phosphate in the sample was determined.

**Available P:** The amount of available P was calculated using Olsen (1954). A 2.5 g soil sample was taken in a 150-mL conical flask to which a pinch of activated charcoal was added. Then 50 mL of Olsen reagent was added, mixed thoroughly, and filtered. At 660 nm, the absorbance was measured, and the mixture without sample served as blank.

**Total potassium (K):** To determine the total K content, the flame photometer of make, 1027-Systronics photodiode autoignition, was employed. 0.5 g of soil was taken and put in a bottom flask (150 mL), to which 30 ml of the digested solution ( $HNO_3$ :  $HClO_4$ ) were added till the sample turned colourless and diluted to a given volume using distilled water.

**Available K:** Available K was calculated using Schollenberger et al. (1945) method. In a 250-ml conical flask, 5 g of air-dried and sieved soil was placed and 1N 50 ml ammonium acetate. The mixture was shaken for 30 min and

left at room temperature overnight for proper leaching, then filtered through Whatman No 40. The available Na and K in soil were measured through the flame photometer.

**Cation exchange capacity (CEC):** The CEC was determined by following the method of Lorenz et al. (1999). For 60 min, 25 g of oven-dried, and the sieved soil sample was mixed with 1N  $NH_4OAc$ , and then the mixture was left overnight at room temperature. An empty flask without any soil sample was used as the control. The collected distillate was titrated with 0.02N  $H_2SO_4$  until the colour changed to red. It is then filtered through Whatman paper 42 after incubation in a Buchner funnel. The leachate was treated with a pinch of ammonium chloride salt, which was then washed with isopropyl alcohol. The filtrate's chloride content was checked using silver nitrate. The amount of ammonical N was assessed by washing in a 10% KCl solution. Then a distillation flask was filled with 20 mL of the above extract, a few drops of phenolphthalein indicator and 40% NaOH were added until the contents turned alkaline. In a 4% boric acid solution, the distillate was collected and tested for ammonia by titrating with 0.02N  $H_2SO_4$ .

## Assessment of heavy metal accumulation

For the analysis of heavy metals, both plant and soil samples were air dried, mashed, sieved, and acid digested. A digestion mixture of  $HNO_3$  and  $HClO_4$  (9:4) were utilized for soil digestion, and  $HNO_3$  was used for plant components by following the method of Estefan et al. (2013). In a 250-mL conical flask, 1 g air-dried sample was placed, and 30 mL digesting mixture was added. The suspension was stirred carefully and subjected to heat in a fume hood at a temperature of 145 °C for 1 h. The suspension was kept at room temperature and passed through Whatman No.42 filter paper to get a volume of 50 mL. After digestion, the samples were submitted to Inductive Coupled Plasma-Optical Emission Spectroscopy of make ICP-OES 6000, iCap 6300 DUO, Thermo fisher, England, for the analysis of different heavy metals.

## Quality control/quality assurance

By analysing a CRM-HC87632955 standard (National Institute of Standards and Technology, Gaithersburg, USA), the accuracy and precision of the heavy metal analysis in soil and fly ash samples was examined. The recovery rates of all analysed heavy metals obtained for the CRM ranged from 1001 to 992  $mg\ kg^{-1}$  (Al = 997, Co = 997, Cr = 992, Cu = 993, Fe = 1000, Ni = 993, Mn = 994, Zn = 993, Pb = 1001, As = 987, and Ba = 998).



The recovery rates of all analysed heavy metals in soil and fly ash samples were found ( $\text{mg kg}^{-1}$ ) for Al 12,414.70, Co 4.85, Cr 21.18, Cu 1.95, Fe 11,123.98, Ni 10.33, Mn 181.38, Zn 17.78, Pb 1.80, As 0.55 and Ba 97.38 respectively. The measuring values were not significantly different from the certified values or within the range of certified values.

### Assessment of bioconcentration and translocation factor

To determine the plants' metal phytoremediation capacity, BCF and TF were determined. The BCF was calculated to assess how effectively plants can take up heavy metals from the soil. While TF was tasked with assessing plants capacity to move heavy metals from root to shoot. The formulas given by Mohanty et al. (2012) were used to calculate phytoremediation indices.  $\text{BCF} = \text{MC}_{\text{root}} / \text{MC}_{\text{fly ash}}$  and  $\text{TF} = \text{MC}_{\text{shoot}} / \text{MC}_{\text{root}}$ , where MC is metal concentration.

### Determination of plant growth

Using a measuring tape and a vernier caliper, the rate of growth in the four bamboo plant species was determined (height, No. of culms, diameter, and leaf litter). Plants species inside the quadrats were evaluated for estimating the above-ground biomass (AGB) through a quadrat technique. Formulas for AGB and BGB were followed according to Prayogo et al. (2021).

Above ground biomass ( $\text{tonnes ha}^{-1}$ ) =  $0.131 \times \text{DBH}^{2.28}$  (DBH = Diameter at breast height).

Below ground biomass ( $\text{tonnes ha}^{-1}$ ) =  $0.26 \times \text{AGB}$ .

### Assessment of carbon sequestration rate

Trees, grasses, and other plants absorb atmospheric carbon dioxide through photosynthesis, a process known as carbon sequestration and store it as organic carbon in biomass. By multiplying the total biomass with a factor of 0.5, the total carbon content contained in the selected bamboo species was calculated by following the approach of Freibauer (2003).

Carbon sequestration rate ( $\text{Tonnes C ha}^{-1} \text{ year}^{-1}$ ) =  $(\text{AGB} + \text{BGB}) \times 0.5$

### Assessment of biochemical stress attributes in bamboo species

The photosynthetic pigment chlorophyll was measured by using the Maclachlan and Zalik (1963) standard procedure. After centrifuging the 0.5 g plant leaf samples, they were macerated in 80% alcohol, at which point absorbances at 663 nm, 645 nm, and 510 nm were measured. Chlorophyll content by calculated by following the formula:

$$\text{Total chlorophyll} (\text{mg g}^{-1}) = ((20.2 \times A_{645}) + (8.02 \times A_{663})) \times V / 1000 \times w$$

where  $A_{645}$  = absorbance at 645 nm;  $A_{663}$  = absorbance at 663 nm;  $V$  = volume;  $w$  = weight of the leaf sample.

To evaluate the ascorbic acid concentration, a fresh leaf sample weighing 500 mg was homogenized in 20 mL of extraction media (0.5 g of oxalic acid and 75 mg EDTA in 100 mL of distilled water). After centrifuging for 15 min, the homogenate was combined with 5.0 mL of dichlorophenol indophenols (20 g mL). Using a spectrophotometer after shaking, the optical density was found to be 520 nm (Shimadzu, UV-1800, Kyoto, Japan). A sample of 0.5 g of fresh leaf material was pulverized and macerated, then mixed with 50 mL of distilled water to determine the pH of the leaves following the method suggested by Prasad and Rao (1982). The pH of the supernatant was then determined using a (hach-intellical phc101, HANNA instruments Woonsocket RI USA). Gathered leaf samples were immersed in distilled water for an entire night, bolted dry, and the weight was collected to estimate the turgid weight (TW) to determine the relative water content (RWC). These leaves were reweighed after drying in an oven at 70 °C to determine their dry weight (DW). Then, following the technique described by Mir et al. (2021), the relative water content was calculated.

$$\text{RWC} (\%) = [(FW - DW) / (TW - DW)] \times 100$$

where RWC = relative water content; FW = fresh wt.; DW = dry wt.; TW = turgid water.

### Determination of Air pollution tolerance index (APTI) in bamboo species

APTI was determined in this study, and the methodology described by Mondal et al. (2022) was followed. It is calculated by using four crucial factors relating to these plants' leaves, which include leaf chlorophyll, leaf pH,



**Table 1** Physicochemical properties of fly ash dumpsite before and after 2 years of bamboo plantation

Properties	Before Plantation	FYM + Press mud	2 years after plantation
<i>Physical characteristics</i>			
Moisture content (%)	1.63 ± 0.2	60.11 ± 0.04	23.6 ± 1.11
Bulk density (gm/cm <sup>3</sup> )	1.23 ± 0.12	0.27 ± 0.01	0.97 ± 0.07
Porosity (%)	51.57 ± 1.63	81.02 ± 0.01	66.6 ± 3.73
Water holding capacity (%)	37.49 ± 2.88	300.02 ± 0	55.24 ± 1.87
<i>Chemical characteristics</i>			
Ph	7.52 ± 0.66	8.2 ± 0.04	8.29 ± 0.38
EC (μS/cm)	112.32 ± 14.31	3001 ± 1.48	318.6 ± 31.1
Organic carbon (%)	0.08 ± 0.04	32.92 ± 1.33	1.02 ± 0.26
Organic matter (%)	0.14 ± 0.07	56.62 ± 2.29	1.74 ± 0.45
Total N (mg kg <sup>-1</sup> )	177.33 ± 58.29	5404 ± 39.6	1295 ± 150.57
Available N (mg kg <sup>-1</sup> )	10.27 ± 5.83	322 ± 3.96	40.6 ± 4.85
Total K (mg kg <sup>-1</sup> )	1224 ± 477.91	8200 ± 140.01	1787 ± 62.28
Available K (mg kg <sup>-1</sup> )	44.6 ± 12.22	195 ± 1.13	128.93 ± 45.46
Total P (mg kg <sup>-1</sup> )	315.74 ± 30.69	14,800.6 ± 19.03	479.86 ± 52.44
Available P (mg kg <sup>-1</sup> )	11.45 ± 2.5	198 ± 0.28	21.47 ± 4.08
<i>Exchangeable cations</i>			
Sodium (mEq/100 g)	0.82 ± 0.04	3.09 ± 0.06	16.85 ± 1.24
Potassium (mEq/100 g)	1.06 ± 0.17	6.82 ± 0.03	2.85 ± 0.57
Calcium (mEq/100 g)	1.4 ± 0.3	26.7 ± 0.42	2.63 ± 0.84
Magnesium (mEq/100 g)	2.12 ± 1.16	6.1 ± 0.14	2.58 ± 0.7
Cation exchange capacity (mEq/100 g)	5.27 ± 0.3	7.72 ± 0.01	21.27 ± 2.49

Data is provided as the mean ± SD of three random independent replicates

ascorbic acid content, and relative water content, total chlorophyll content. It was calculated by using the following formula:

$$\text{APTI} = A(T + P) + R/10$$

where  $A$  = ascorbic acid content;  $T$  = total chlorophyll content;  $P$  = leaf pH;  $R$  = relative water content.

### Statistical analysis

A one-way variance analysis (ANOVA) was performed on data in triplicates to assess whether the results were significant at  $p \leq 0.05$ . Data present average of three random independent replicates ± standard deviation. For the correlation of variables, principal component analysis and Pearson correlation were determined through Origin 2021b software.

## Results and discussion

### Physicochemical characteristics of fly ash dumpsite before and after 2 years of bamboo plantation

The changes in physicochemical characteristics of fly ash dumpsite are demonstrated in Table 1. Initially, the dumpsite soil was degraded, with all the characteristics unfavourable for plant growth. However, after 2 years of bamboo plantation, all the characteristics were enhanced considerably as compared to the unplanted site. The physical characteristics of fly ash dumpsite were improved markedly in terms of moisture content (1347%), porosity (29.14%), WHC (47.34%) and bulk density (21.13%) after 2 years of bamboo plantation. Similarly, the chemical characteristics were also enhanced considerably, such as pH (10.23%), EC (183.65%), organic matter (1142%), organic carbon (1175%), total N (630%), available N (295%), total P (52.1%), available P (87.51%), total K (45.99%), and available K (189%). The exchangeable cations in terms of Na<sup>+</sup> (1954%), K<sup>+</sup> (168.87%), Mg<sup>+</sup> (21.70%), Ca<sup>+</sup> (87.8%) were improved significantly. Consequently, the CEC was improved by 303.6% two years

**Table 2** Concentration of heavy metals at the fly ash dumpsite before and after two years of bamboo plantation

Heavy metal	Before plantation (mg/kg)	2 years after plantation (mg/kg)
Al	17,137.37 ± 4380.17	12,414.7 ± 3812.28
Co	9.33 ± 5.78	4.85 ± 1.36
Cr	24.03 ± 14.16	21.18 ± 7.43
Cu	13.8 ± 10.64	1.95 ± 1.72
Fe	14,838.03 ± 6144.05	11,123.98 ± 1464.24
Ni	12.97 ± 6.37	10.33 ± 1.96
Mn	267.13 ± 121.88	181.38 ± 45.16
Zn	36.6 ± 17.36	17.78 ± 2.68
Pb	2.73 ± 0.21	1.8 ± 1.23
As	0.93 ± 0.5	0.55 ± 0.39
Ba	112.33 ± 40.96	97.38 ± 32.95

Data is provided as the mean ± SD of three random independent replicates

after bamboo plantation in comparison with the initial degraded dumpsite.

Due to the limitations of high cost, inefficiency, and introduction of secondary pollutants, the physio-chemical techniques for the restoration of fly ash-polluted soils are being taken out of consideration (DalCorso et al. 2019). Therefore, phytoremediation, which is an emerging, sustainable, cost-effective and reliable, eco-friendly technology for the rejuvenation and remediation of fly ash-contaminated soils, becomes a promising technique in this field. Owing to its quick biomass and hyperaccumulation activity, bamboo is a potential plant species for the phytoremediation of fly ash-contaminated sites (Kumar et al. 2021a, b). This study finds a considerable improvement in the physicochemical attributes of the fly ash dumpsite after two years of the bamboo plantation as compared to the initial site without plantation. The moisture content, porosity and WHC increased while bulk

density decreased in comparison with the initial observations at the dumpsite. This happened probably due to the penetration of the bamboo root system into the soil, which increased the porosity in soil, WHC and consequently, the moisture content of soil (Stirzaker et al. 1996; Colombi et al. 2019). The bulk density decreased because the bamboo establishment reduced the soil compaction, the higher the bulk density, the more compact and less porous will be the soil (Tracy et al. 2012). The pH and EC of the dump site were improved significantly by bamboo plantation because the plants uptake anions and cations from the soil, which determine these two important characteristics (Teng and Rengel 2003). Further, there was a marked improvement in the organic content of dumpsite after two years of bamboo plantation. This occurs because of the deposition of litter and its microbial decomposition (Giweta 2020). The improvement in NPK content can be attributed to the supplementation of press mud (Ahyango et al. 2022). Also, the litter decomposition may have contributed to the enhancement in NPK content (Yadav et al. 2008). The improvement in the CEC can be attributed to the increased content of various cations and anions such as  $K^+$ ,  $Ca^+$ ,  $Mg^+$  and  $Na^+$  (Matschonat and Vogt 1997; Santoso and Blair 2022).

### Heavy metal concentration in fly ash dumpsite before and after 2 years of plantation

The changes in concentration of various heavy metals present at the fly ash dumpsite are given in Table 2. Before plantation, the content of heavy metals varied as Al 17,136.37 mg kg<sup>-1</sup>, Co 9.33 mg kg<sup>-1</sup>, Cu 13.8 mg kg<sup>-1</sup>, Cr 24.03 mg kg<sup>-1</sup>, Ni 12.97 mg kg<sup>-1</sup>, Fe 14,838.03 mg kg<sup>-1</sup>, Mn 267.13 mg kg<sup>-1</sup>, Zn 36.6 mg kg<sup>-1</sup>, Pb 2.73 mg kg<sup>-1</sup>, As 0.93 mg kg<sup>-1</sup>, Ba 112.3 mg kg<sup>-1</sup>. All the heavy metals were considerably reduced at the fly ash dumpsite after two years of bamboo plantation. The average concentration of Al was reduced to 12,424.7 mg kg<sup>-1</sup> (27.49%), Co to 4.85 mg kg<sup>-1</sup>

**Table 3** BCF and TF of bamboo species grown on fly ash dumpsite

Bamboo Species	Heavy Metals											
		Al	Co	Cr	Cu	Fe	Ni	Mn	Zn	Pb	As	Ba
<i>B. balcooa</i>	BCF	1.33	1	1.27	0	1.21	1.28	0.97	1.52	10.5	6.5	1.01
	TF	0.02	0.03	0.85	0.64	0.06	0.03	0.14	0.77	0.19	0	0.61
<i>B. vulgaris</i> 'wamin'	BCF	0.93	1.3	4.77	7.18	1.01	4.21	1.13	2.15	0.5	2	1.1
	TF	0.02	0.01	0.24	0.28	0.08	0.01	0.1	0.78	0.5	0.13	0.07
<i>B. bambos</i>	BCF	1.71	3.32	1.26	0	1.1	1.28	2.27	1.72	0.61	1.2	1.42
	TF	0.04	0.03	1.98	0.78	0.08	0.15	0.18	1.25	0.5	0.17	0.11
<i>B. vulgaris</i>	BCF	1.14	1.27	4.58	4.26	1.3	5.47	1.17	1.3	0.32	0.82	1
	TF	0.02	0.02	0.43	0.46	0.05	0.03	0.14	0.88	0.9	0	0.79



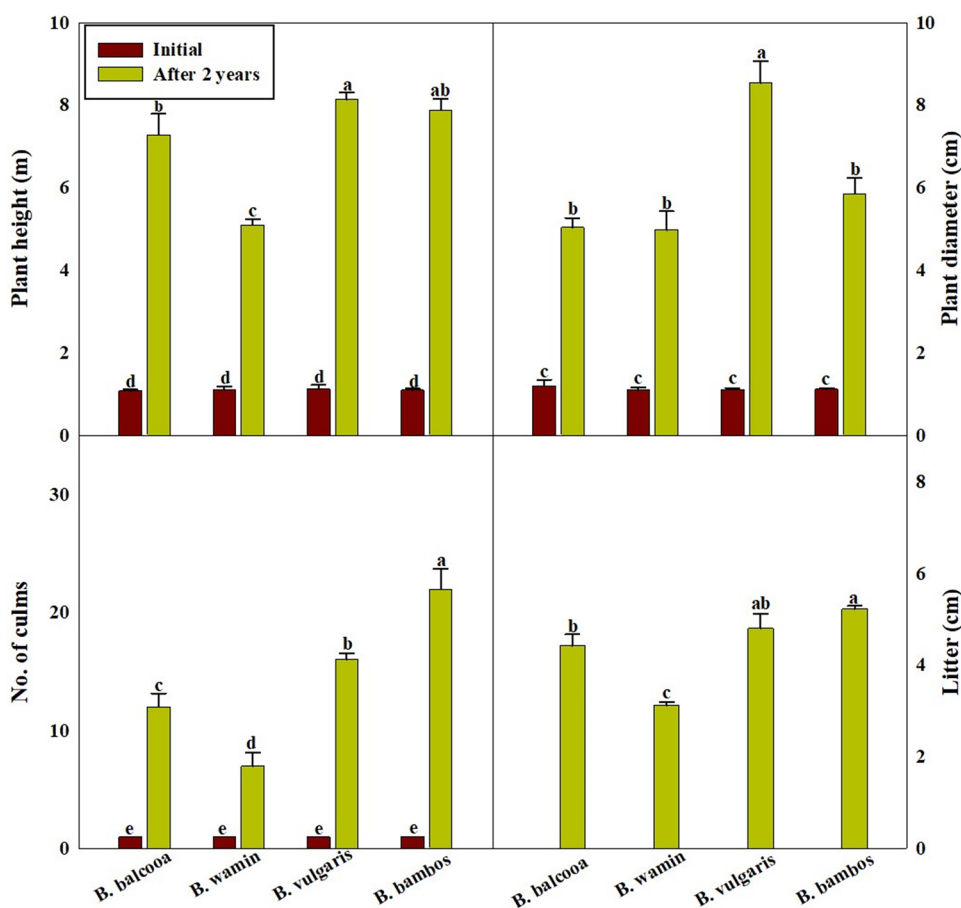


(48.01%), Cr to 21.18 mg kg<sup>-1</sup> (11.86%), Cu to 1.95 mg kg<sup>-1</sup> (85.01%), Fe to 11,123.98 mg kg<sup>-1</sup>, (25.03%), Ni to 10.33 mg kg<sup>-1</sup> (20.35%), Mn to 181.38 mg kg<sup>-1</sup> (32.20%), Zn to 17.78 mg kg<sup>-1</sup> (51.42%), Pb to 1.8 mg kg<sup>-1</sup> (34.06%), As to 0.55 mg kg<sup>-1</sup> (40.86%), Ba to 97.38 mg kg<sup>-1</sup> (13.30%) respectively.

The pattern of heavy metal accumulation, bioconcentration and translocation varied significantly among the four bamboo species. After the plantation of bamboo on the fly ash dumpsite for two years, the content of several heavy metals was significantly reduced. This possibly occurs through the uptake of heavy metals by plants via passive diffusion or symplastic, a pathway involving specific carrier proteins (Peer et al. 2005; Yan et al. 2022). Different species of bamboo showed variance in BCF and TF. BCF determines the potential of plants to concentrate heavy metals in their roots from the soil. A BCF of > 1 indicates that the species is potentially capable of phytostabilization. While TF determines the ratio of heavy metal transfer from root to aerial parts. A TF of > 1 indicates that the species is potentially capable of

phytoextraction (Yoon et al. 2006; Kumar et al. 2021a, b). The observations from this work demonstrated that *B. bambos* is the potential candidate species for phytoremediation of fly ash-contaminated soils owing to its > 1 BCF and TF indices. The other three bamboo species are potential phytostabilizers of various heavy metals owing to their < 1 TF. Phytoextraction is a process through which plants uptake and remove the toxic heavy metals from the soil, making it clean of toxicity, while phytostabilization is a process which enables plants to stabilise metals and reduce their presence in soil matrices (Suman et al. 2018). This limits the presence and accumulation of toxic and dangerous leachates into the environment in accordance with the aims of phytoremediation (Radziemska 2018). The findings of this study are further strengthened by the PCA plot (Fig. 4), which clearly illustrates that *B. bambos* is the suitable species with all the growth characteristics enhanced along with hyperaccumulation of heavy metals at the fly ash contaminated site.

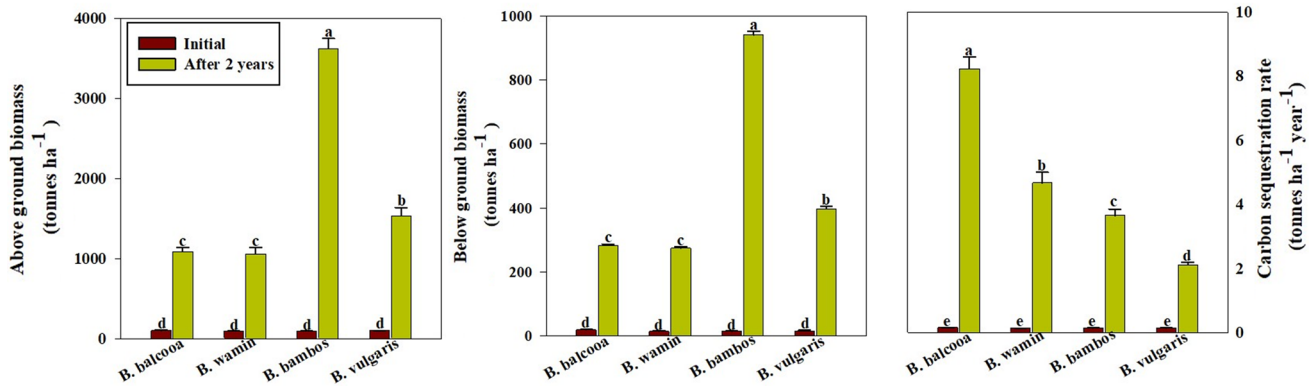
**Fig. 2** Plant growth of 2-year-old bamboo seedlings initially and after two years of plantation on fly ash dumpsite. Data is provided as the mean ± SE of three random independent replicates



### Phytoremediation index of bamboo species after two years of plantation at fly ash dumpsite

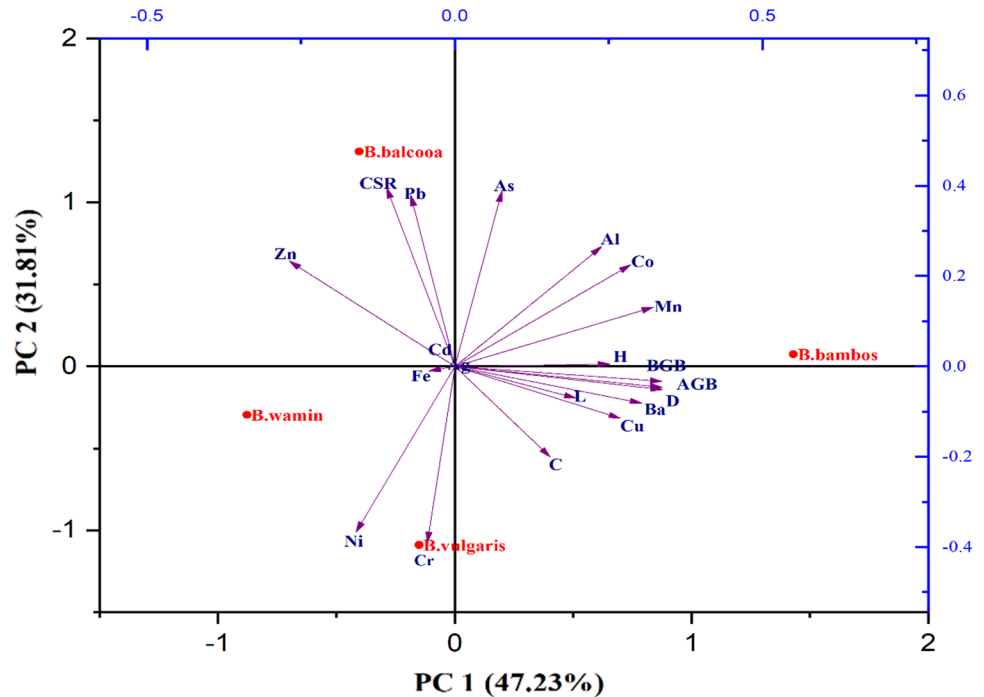
To assess the phytoremediation index of bamboo species, BCF and TF were determined for all four bamboo species (Table 3). The results demonstrate that *B. balcooa* is an excellent phytostabilizer of Pb with BCF (10.5), followed by As (6.5), and Zn (1.52) but not a good phytoextractor of these metals because its TF is < 1 for all the heavy metals studied. The *B. vulgaris* ‘wamin’ is an efficient phytostabilizer of Cu with BCF (7.18) followed by Cr (4.77), Ni (4.21), and Zn (2.15) but not a good phytoextractor

of these heavy metals as its TF value is < 1. The *B. bambos* is an excellent phytostabilizer of Co with a BCF of (3.32) followed by Mn (2.27) and Zn (1.72). Notably, *B. bambos* is an excellent phytoextractor of Cr and Zn as the TF is > 1 for the two heavy metals. The *B. vulgaris* is an efficient phytostabilizer of Ni with a BCF of (5.47) and Cr (4.58) but not a good phytoextractor of these heavy metals as TF < 1. Hyperaccumulators represent a fascinating group of plant species for phytoremediation, which can accumulate 100-folds more heavy metal concentrations in comparison to other species growing in the same environment (Cappa and Pilon-Smits 2014). In addition to higher metal accumulation, these plants show a faster rate



**Fig. 3** Biomass and carbon sequestration rate of 2-year-old bamboo seedlings initially and after two years of plantation on fly ash dumpsite. Data is provided as the mean ± SE of three random independent replicates

**Fig. 4** PCA plot of plant growth attributes (H=height; D=diameter, C=No. of culms, L=litter); biomass (AGB=above ground biomass, BGB=below ground biomass), CSR=carbon sequestration rate and different heavy metals: Al, Fe, Co, As, Cu, Ni, Mn, Zn, Pb, Cr, Ba



of biomass production (Sura-de Jong 2015). This work demonstrates that bamboo has a strong phytoremediation potential with higher biomass yields which corroborates the findings of Bian et al. (2020).

### Plant growth of different bamboo species after two years of plantation on fly ash dumpsite

The growth of all four bamboo species was increased considerably at the fly ash-degraded site after two years of plantation (Fig. 2). In *B. balcooa*, the plant height was enhanced by 574%, diameter by 320%, No. of culms by 1100%, and litter deposition by 4320%. Likewise, in *B. vulgaris* ‘wamin’, the plant height was enhanced by 364%, diameter by 353%, No. of culms by 600%, and litter deposition by 3020%. In *B. bambos*, the plant height was enhanced by 627%, diameter by 668%, No. of culms by 1500%, and litter deposition by 4700%. Similarly, in *B. vulgaris*, the plant height was enhanced by 616%, diameter by 423%, No. of culms by 2100%, and litter deposition by 5120%. This implies that the best increment in growth was observed in *B. bambos* followed by *B. balcooa*, *B. vulgaris* and *B. vulgaris* ‘wamin’.

This study finds that hyperaccumulation potential in bamboo varies according to the species. Among the four bamboo species, *B. bambos* was identified as the best species for phytoremediation of fly ash dumpsites because, along with the hyperaccumulation, it showed significant improvements in plant growth in terms of diameter, height, culms, litter, and biomass production in terms of AGB and BGB. This possibly occurs due to the chelation and compartmentation of heavy metals into the vacuoles or other insensitive plant parts to protect the photosynthetic cells and other sensitive cell organelles from heavy metal toxicity (Hasan et al. 2017). By doing so, bamboo uptakes heavy metals in higher concentrations from the contaminated soil without showing any interference in growth and development.

### Biomass and carbon sequestration in different bamboo species after 2 years of plantation on fly ash dumpsite

The plant biomass in terms of AGB and BGB, and the CSR in all four bamboo species were assessed before and after two years of plantation at the fly ash dumpsite (Fig. 3). Results showed that in *B. balcooa*, AGB and BGB were increased by 2554% and 2620%. Likewise in *B. vulgaris* ‘wamin’ 3141% and 3200% enhancements were observed in AGB and BGB. In *B. bambos*, 10,646% and 11,200% improvements were observed in AGB and BGB. Similarly, in *B. vulgaris*, AGB and BGB were improved by 4584% and 4675%, respectively. A significant improvement was observed in the CSR of different bamboo species after two years of bamboo establishment on the fly ash dumpsite as *B. balcooa* > *B. vulgaris* ‘wamin’ > *B. bambos* > *B. vulgaris* (Fig. 3).

Carbon sequestration through the plantation of fly ash-contaminated soils can be crucial in preventing the concentration of atmospheric CO<sub>2</sub> from rising. This study demonstrates that there was a significant enhancement in the CSR of all four bamboo varieties after two years of plantation on the fly ash dumpsite. The PCA plot (Fig. 4) illustrates that *B. balcooa* has the maximum CSR among all the bamboo species under consideration in this work. The improvement in CSR can be attributed to the enhanced growth characteristics, which could possibly have increased the photosynthetic rate of plants (Farrelly et al. 2013). Through photosynthesis, plants uptake huge amounts of CO<sub>2</sub>, which is the principal greenhouse gas in the atmosphere and capture it within organic forms (Toochi 2018). This way sequestration of carbon occurs by green plants, which helps to mitigate the harmful effects of climate change.

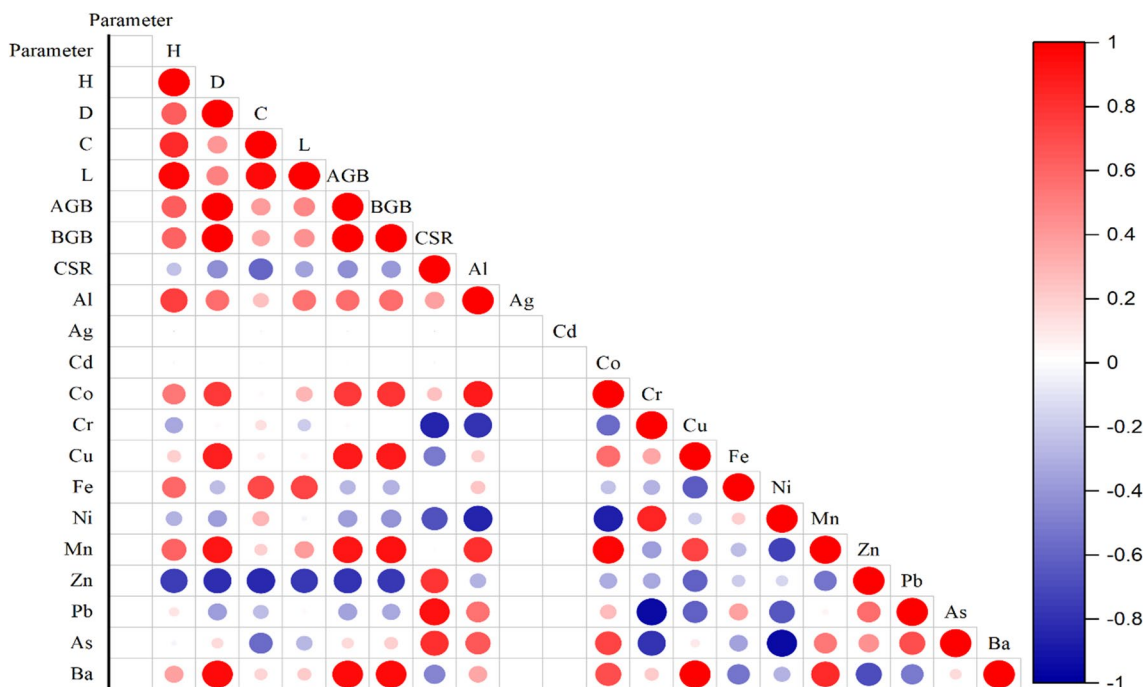
### APTI of different bamboo species after two years of plantation at fly ash dumpsite

All four species of bamboo, *B. balcooa*, *B. wamin*, *B. vulgaris*, and *B. bambos* were examined for their APTI capacity two years after being planted at the fly ash

**Table 4** Stress tolerance parameters and APTI of different bamboo species after two years of plantation at fly ash dumpsite

Plant sample	pH	Ascorbic acid (mg/100 g)	Relative water content (%)	Total chlorophyll (mg/g)	APTI
<i>B. balcooa</i>	6.21 ± 0.02	9.48 ± 0.84	96.97 ± 5.25	1.36 ± 0.14	16.65
<i>B. vulgaris</i> ‘wamin’	6.07 ± 0.11	7.95 ± 0.38	85.01 ± 2.91	1.34 ± 0.19	14.39
<i>B. bambos</i>	6.03 ± 0.01	4.72 ± 0.20	81.45 ± 3.31	1.21 ± 0.17	11.56
<i>B. vulgaris</i>	6.01 ± 0.02	5.27 ± 0.19	83.33 ± 0.00	1.13 ± 0.16	12.21

Data is provided as the mean ± SD of three random independent replicates



**Fig. 5** Pearson's correlation plot of plant growth attributes (H=height; D=diameter, C=No. of culms, L=litter); biomass (AGB=above ground biomass, BGB=below ground biomass),

CSR=carbon sequestration rate and different heavy metals: Al, Fe, Co, As, Cu, Ni, Mn, Zn, Pb, Cr, Ba

dumpsite (Table 4). The findings showed that *B. balcooa* has the highest values for stress tolerance in terms of ascorbic acid ( $9.48 \pm 0.84$ ), pH ( $6.21 \pm 0.02$ ), total chlorophyll ( $1.36 \pm 0.14$ ), and RWC ( $96.97 \pm 5.25$ ). All these stress tolerance parameters significantly enhance the APTI (16.65) in *B. balcooa* as compared to the other species. Least stress tolerance values were observed in *B. wamin*, with ascorbic acid values of ( $7.95 \pm 0.38$ ), pH ( $6.07 \pm 0.11$ ), total chlorophyll ( $1.34 \pm 0.19$ ), RWC ( $85.01 \pm 2.91$ ), and APTI (14.39); *B. bambos* was the second lowest, with ascorbic acid values of ( $4.72 \pm 0.20$ ), pH ( $6.03 \pm 0.01$ ), RWC ( $81.45 \pm 3.31$ ), total chlorophyll ( $1.21 \pm 0.17$ ), APTI (11.56). Similarly, for *B. vulgaris*, the values were ascorbic acid ( $5.27 \pm 0.19$ ), pH ( $6.01 \pm 0.02$ ), RWC ( $83.33 \pm 0.00$ ), total chlorophyll ( $1.13 \pm 0.16$ ), and APTI (12.21).

APTI is a reliable method to evaluate the pollution tolerance in plant species under atmospheric stress conditions (Brar et al. 2013). There are four important components to determine the APTI of plant species, which include leaf chlorophyll content, RWC, ascorbic acid and leaf pH (Patel et al. 2023). This study showed that all these components exhibited maximum values in *B.*

*balcooa* corresponding to its highest APTI (Table 4). A key measure of a plant's tolerance to air pollution is the pH of its leaves which declines under acidic environmental conditions and leads to inhibitory effects on photosynthesis (Molnar et al. 2020). To cope with acidic circumstances, tolerant plant species keep the pH of their leaves near to 8.8 (Goswami et al. 2018a, b). In this study, *B. balcooa* sustained this pH range in its leaves, showing significant levels of photosynthetic activity and biomass. The RWC, which assesses the water status in plants under stress, is another key physiological aspect (Bharti et al. 2018). Atmospheric pollution slows transpiration, which leads to less water absorption, low RWC, and consequently, reduced plant growth. Tolerant plant species maintain a RWC between 58 and 73% (Goswami et al. 2018a, b). In this study, the RWC of *B. balcooa* falls within this range and therefore, exhibited maximum stress tolerance. Ascorbic acid has antioxidant activity and is crucial for preventing the oxidative burst under stress-induced circumstances in plants (Imelda et al. 2022). As a result, ascorbic acid concentrations in the tolerant plant species are higher than those in sensitive plant species (Zahedi et al. 2022). In this research, *B. balcooa* has the





maximum ascorbic acid concentration and, therefore, the highest level of stress tolerance compared to the other species of bamboo. Chlorophyll is a crucial factor in determining the APTI in plants (Punit et al. 2021). Acidic and alkaline impurities can degrade stomata through stomata obstruction and the production of pheophytin (Yadav et al. 2020). To sustain the essential photosynthetic activities under stressful circumstances, resistant plant species exhibit high chlorophyll contents. (Khayatnezhad and Gholamin 2021; Sawarkar et al. 2022). In this study, *B. balcooa* maintained its chlorophyll content as compared to other bamboo species leading to its highest APTI, plant biomass, and rate of carbon sequestration after two years. The cumulative result of these four components clearly demonstrated that *B. balcooa* is the most effective species for eco-restoration.

### Principal component analysis and Pearson's correlation

To simplify and summarize the complex data of this study, principal component analysis was employed (Fig. 4). It showed a total of 79.04% data variability (PC 1 = 47.23% and PC 2 = 31.81%), which is significant according to Hasan and Abdulazeez (2021), which stated that the data should exhibit more than 70% variability through PCA analysis. Among the four bamboo species, *B. bambos* exhibited a significantly positive correlation between biomass and hyperaccumulation of As, Al, Ba, Cu, and Co. *B. Balcooa* showed a markedly positive correlation between CSR and hyperaccumulation of Pb and Zn. *B. vulgaris* presented the highest hyperaccumulation of Ni and Cr, while *B. vulgaris* 'wamin' showed maximum hyperaccumulation of Fe, respectively. Further, Pearson's plot (Fig. 5) illustrated the correlation ( $P \leq 0.05$ ) between plant growth parameters and the accumulation of different heavy metals. Plant diameter exhibited a positive correlation with Co, Cu, Mn and Ba, No. of culms exhibited a positive correlation with Zn, ABG/BGB exhibited a positive correlation with Co, Cu, Mn, and Ba, and CSR exhibited a positive correlation with Pb and As.

### Conclusion

Bamboo plantations can be a sustainable, eco-friendly, and economical method to restore and remediate fly ash dumpsites. Not only can it provide restoration services but also many useful bamboo products. Plants' potential to adapt and hyperaccumulating heavy metals in fly ash dumpsites differ. Therefore, this work screened out four bamboo species, *B.*

*balcooa*, *B. vulgaris* 'wamin' *B. bambos* and *B. vulgaris* and found *B. bambos* as the most suitable plant species with the highest biomass production and phytoremediation potential of fly ash dumpsites. In addition to phytoextraction and phytostabilization of various heavy metals, bamboo plantation significantly sequestered the CO<sub>2</sub> from the atmosphere, which is the leading global greenhouse gas and, therefore, this approach can be a significant contribution to meet the goals of sustainable development. Further, this study evaluated the APTI of different bamboo species and found that *B. balcooa* exhibited strong stress tolerance leading to excellent APTI as compared to the other bamboo species. Therefore, suitable bamboo species can be planted around the thermal power plants and other industrial areas to remediate and restore the overall polluted environment for sustainable and eco-friendly community development.

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**Authors' contribution** AS and RS: Equal contribution in manuscript writing, methodology, experimental data collection, statistical analysis, and revision; PA: Laboratory instrumentation, analysis, and resource management; MK: Validation, project administration, and revision; LS: Conceptualization, resource management, and supervision.

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### Declarations

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Ethical approval** This study does not need ethical approval as no human or animal research is involved.

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