(Supplementary material)

Title: Effect of the Co-Application of *Eucalyptus Wood* Biochar and Chemical Fertilizer for the Remediation of Multimetal (Cr, Zn, Ni, and Co) Contaminated Soil

Materials and Methods

Study area

The study area is known as Bera project and is situated at an elevation of 218 m above mean sea level. Bera project operates in a leasehold area of 209.56 hectares having total geological coal reserves of 17.9 million tonnes. The leasehold area of the Bera project mostly has the geological stretch of ancient Archean rocks and receives an annual average rainfall of 115 mm. The project area is surrounded by an annually flowing water stream tributary, which finally connects to the Damodar River (primary-River in Dhanbad district). The opencast project has two active overburden dumps formed due to the continuous dumping of the overburden materials using end dumping method. The dumps were two years old having an overall height of 50 m and slope of 42˚.

Chemical characterization of biochar

Elemental analysis of biochar was done using a CHNS analyzer (Euro EA3000 CHNS-O analyser, HEKAtech HmbH, Germany) using the method described in an earlier study (Wu et al., 2012). The proximate analysis was done using the Thermogravimetric analyzer (TGA) (NETZSCH, STA 449 F3, USA) following the thermal programme as described by the method (ASTM D5142–02, 2009). The pH and EC of the biochar were determined using the previously reported methods [3,4]. The pH at the point of zero charge was determined using the pH drift method [5]. The exchangeable cations viz., Na, K, Ca, and Mg were determined by extracting biochar with 1 M ammonium acetate solution followed by analysis using ion chromatography (IC) (Model: Dionex ICS 2100, Thermo Scientific, USA) [6]. The cation exchange capacity (CEC) of the biochar was determined using the modified ammonium acetate method as reported in the earlier studies [7,8]. The heavy metals in the biochar were determined using the method reported in an earlier study [9], followed by analysis using AAS (AAS ICE 3000 series, Thermo Scientific).

The surface functional groups on the biochar were determined using a Fourier transform infrared spectroscopy (Nicolet i650 FTIR spectrometer, Thermo Fisher Scientific, USA). The surface morphology of the biochar samples was determined using the scanning electron microscope (Carl-Zeiss MERLIN (GEMINI-2), Germany) followed by EDX analysis. The Brunauer-Emmett-Teller (BET) surface area and pore size analysis of the biochar were done using surface area analyzer (ULTRAPYC 1200e, Quantchrome instruments, USA) via N_2 adsorption at 77 K and the N_2 gas adsorption-desorption process at 77 K in an Autosorb iQ (Quantchrome instruments, USA), respectively [10]. The X-ray diffraction (XRD) analysis of the biochar samples was done using the PANalytical X'Pert Pro diffractometer through Cu Kα radiation (λ = 1.5406 Aº) generated at a voltage of 40 kV and 30 mA current. The samples were scanned using a step size of 0.1 degrees from a 2θ angle of 0 to 80˚ to determine the mineralogical content in the biochar.

Pot-culture study details

The *A. auriculiformis* was selected for the reclamation study based on its properties like a dense root system, drought tolerance, large canopy cover as reported in the earlier studies, where it was used for the reclamation of degraded lands. Before the plantation, the *A. auriculiformis* seeds were sowed in the biochar-soil mixtures (biochar mixed at the rate of 0.5, 1, 2, 5, and 10 % w/w ratios with the mine-soil) in the petri-dish and maintained at a minimum moisture level of 10 – 15 per cent. The soil mixture containing seeds were incubated in an incubator maintained at a temperature of 22 ˚C under a continuous flow of air and illuminated with artificial light (4100 lux) during eight hours a day. After ten days, as the saplings started coming out of the seeds, the saplings were transferred into the individual pots containing the mixture of mine-soil, biochar (400 and 600) and biochar-fertilizer mixture (400 and 600). The pot culture study was conducted for a duration of 15 months and, for the entire duration; water was added to the pots on every third day to maintain the average moisture content of 6 – 8 per cent. The mean temperature inside the poly-house during the rainy season (July – October 2019) was around 29 ˚C, and relative humidity was 79 %. During the winter (November – February) and summer season (March – June), the mean temperature was 23.5 ˚C and 33˚C, respectively, and the relative humidity was 65 % and 71 %, respectively.

Figures

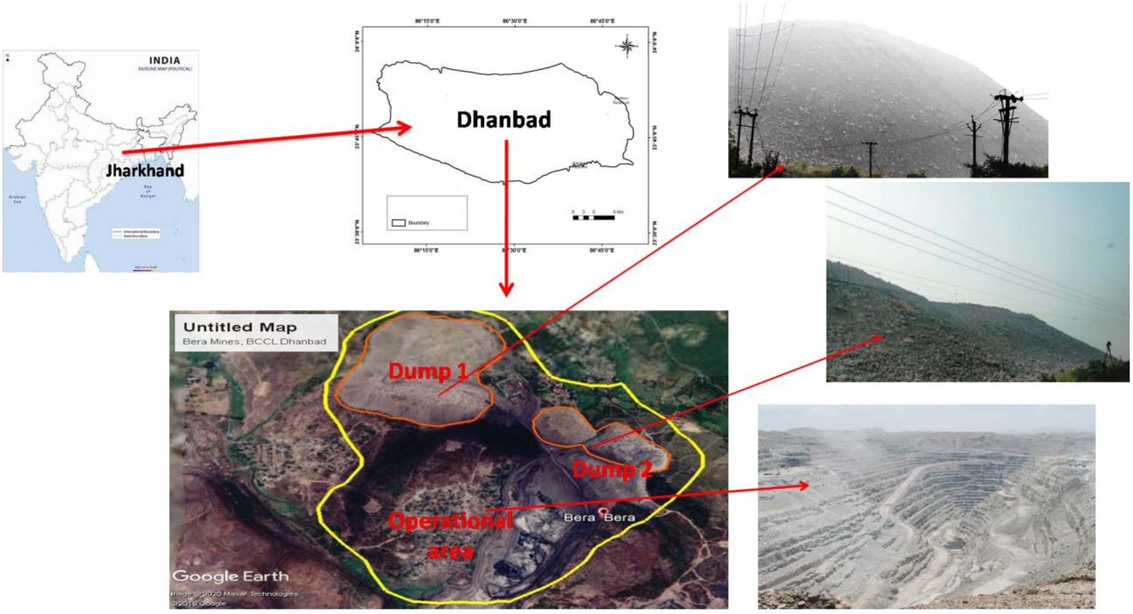


Figure S1: Bera opencast coal mines, Bastacolla area, Dhanbad, Jharkhand, India

Figure S2: Pot-culture study in a poly-house using biochar and fertilizer as the soil amending material in the soil planted with *Acacia Auriculiformis*

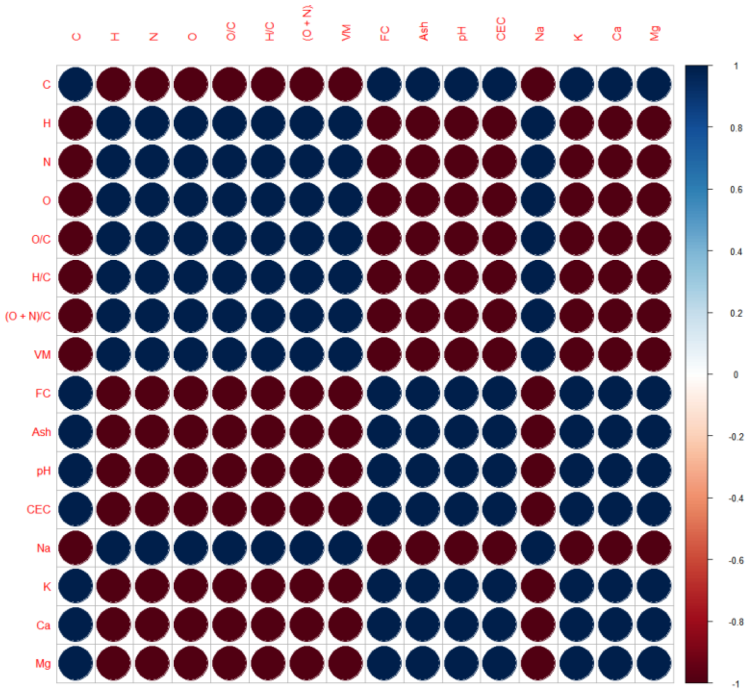


Figure S3: Correlation matrix for the physicochemical properties of the *Eucalyptus* wood biochar

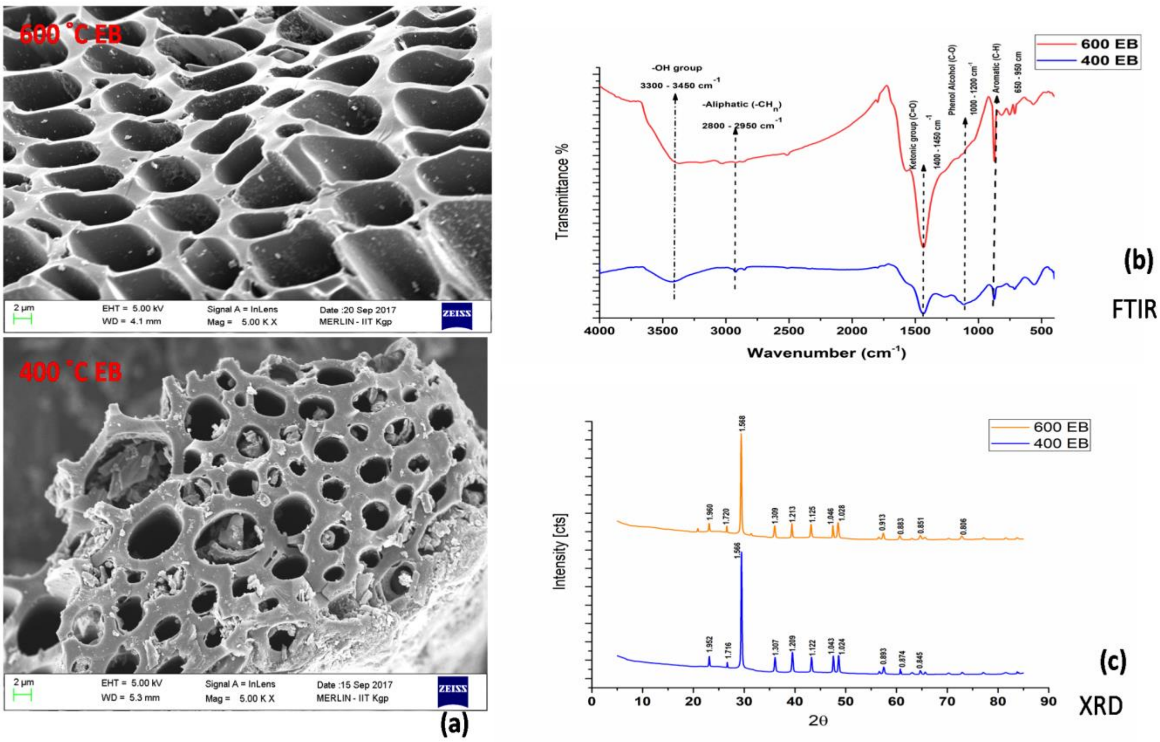


Figure S4: (a) SEM image of the EB; (b) FTIR curve of the EB; (c) XRD curve of the EB produced at 400 and 600 ˚C

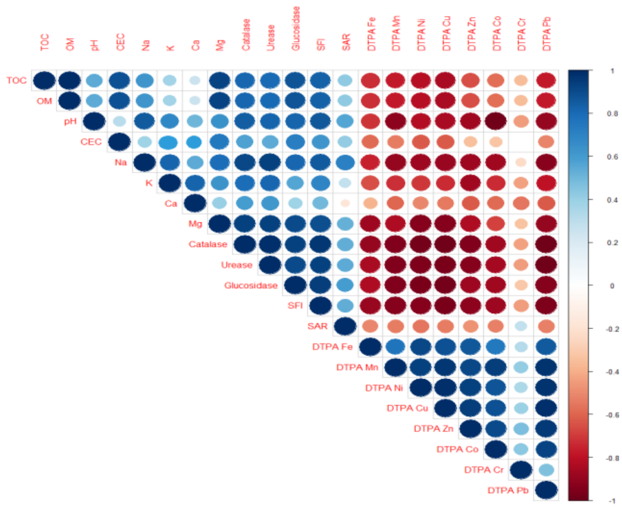


Figure S5: Correlation matrix among the soil physicochemical properties

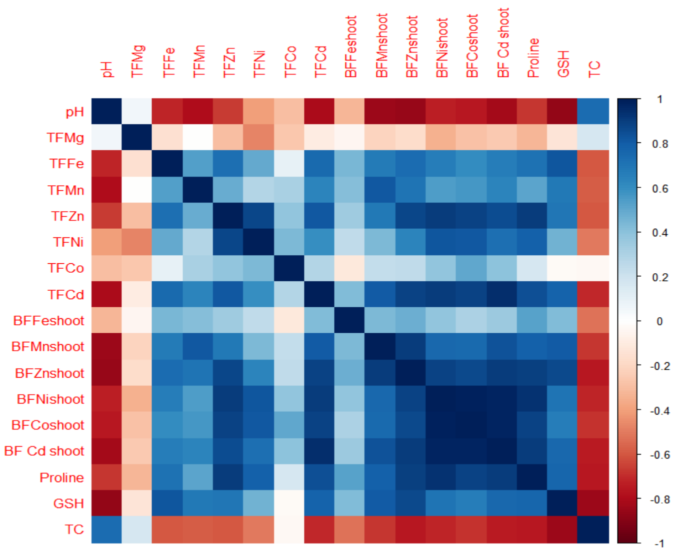


Figure S6: Correlation matrix among proline, GSH, chlorophyll content, TF, BAC, and soil pH

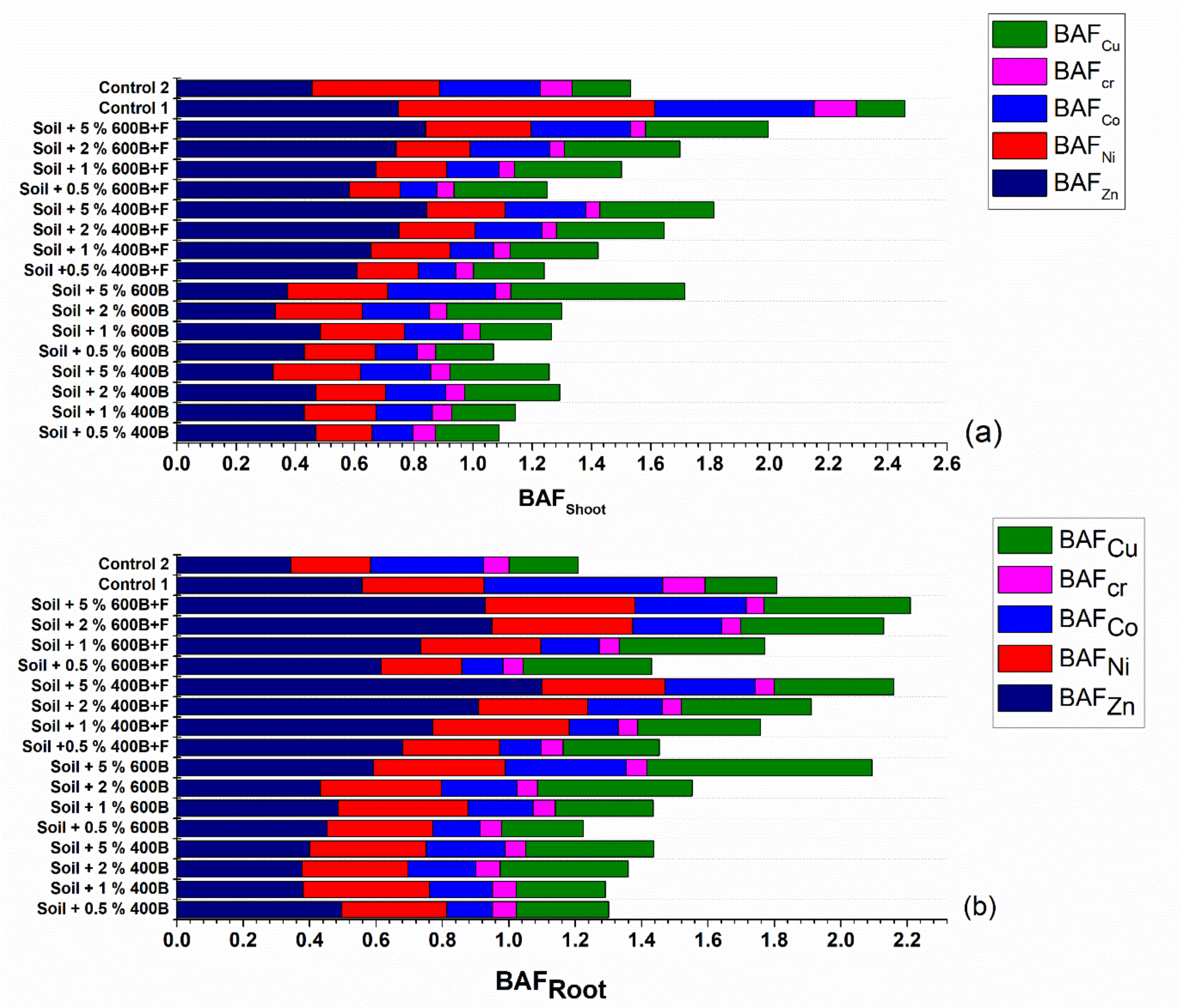
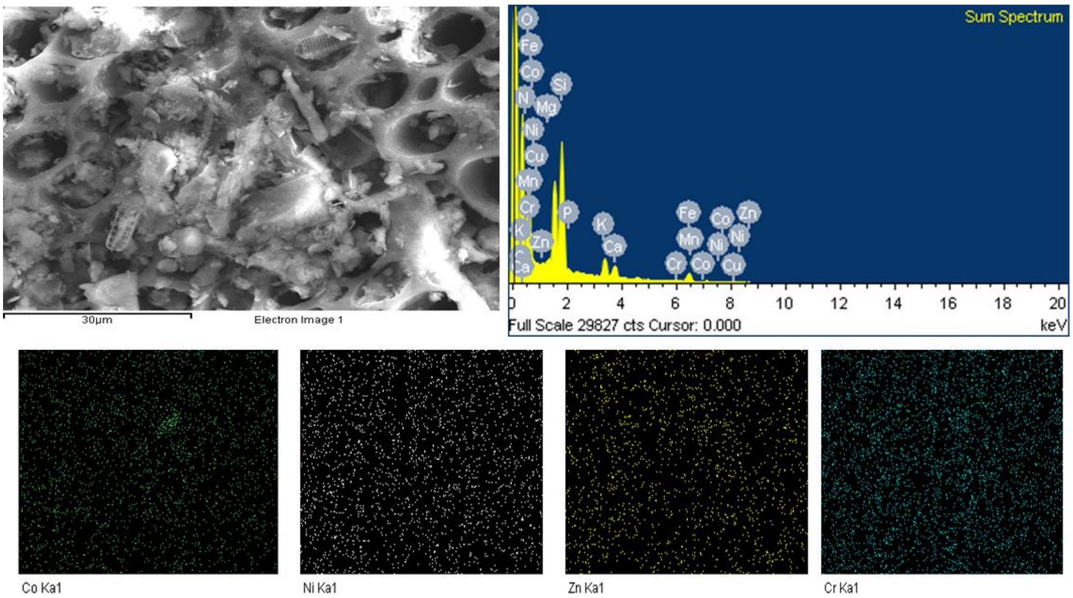
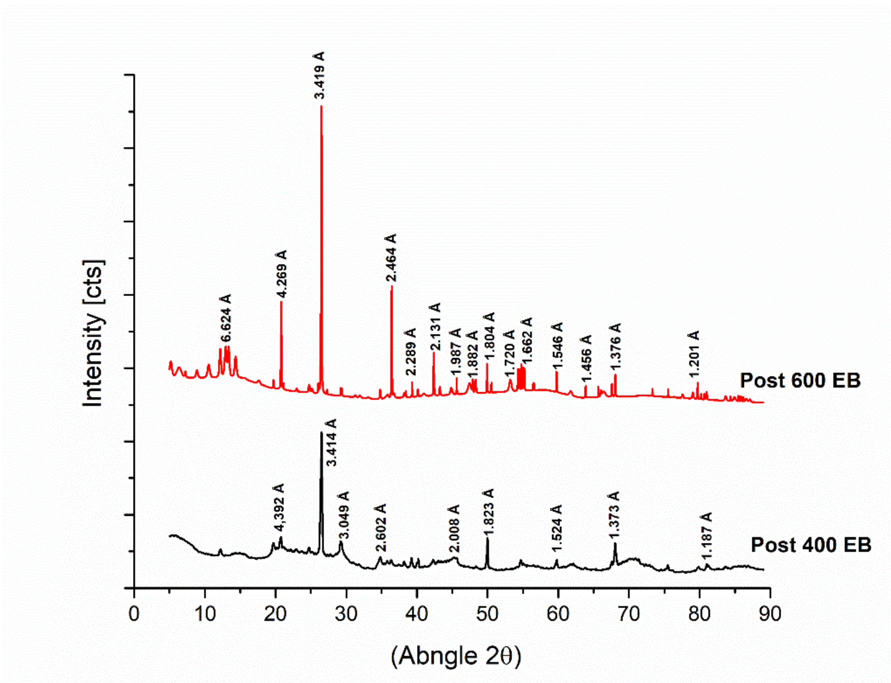


Figure S7: Bioaccumulation factor (BAC) of the heavy metals in the shoot and root part of the *A. Auriculiformis*



(a)



(b)

Figure S8: Post *Eucalyptus wood* Biochar (EB) characterization: (a) SEM Image and heavy metals mapping adsorbed onto the EB surface; (b) XRD analysis of the post 400 and 600 EB

Table S1: Elemental and proximate analysis results of Eucalyptus wood biochar produced at 400 and 600 ˚C

Sample ID	C %	H %	N %	O %	O/C	H/C	(O+N)/C	VM %	FC %	Mineral matter %	pH_{pzc}
400 EB	70.46	2.39	4.11	23.04	0.24	0.40	0.295	72.57	21.32	6.11	7.52
600 EB	79.59	1.93	2.89	15.59	0.15	0.29	0.177	63.24	28.46	8.30	7.94

Table S2: Physicochemical characteristics of eucalyptus wood biochar (mean ± S.D, n = 3)

Sample ID	pH	CEC (cmol/kg)	Exchangeable (mg/kg)			
			Na	K	Ca	Mg
400 EB	9.03±0.23	11.29±0.28	96.32±2.27	1693.21±36.23	1281.34±38.50	119.32±3.76
600 EB	9.95±0.21	13.38±0.42	51.39±1.06	2493.86±60.33	1421.87±41.10	159.27±5.03

Table S3: Heavy metals content in the eucalyptus wood biochar (mean ± S.D, n = 3)

Sample ID	**Ni (µg/kg)**	**Co (µg/kg)**	**Zn (µg/kg)**	**Cu (µg/kg)**
400 EB	72.05±16.62	21.97±0.63	764.66±26.12	32.91±1.06
600 EB	112.64±21.16	38.42±1.05	967.83±29.59	39.62±1.12

Table S4: BET surface area and pore volume of the eucalyptus wood biochar

Sample ID	**BET surface area (m^2/g)**	**Total pore volume (cc/g)**	**Average pore diameter (Å)**	**Micropore area (m^2/g)**
400 EB	83.47	0.0968	29.92	n.d.
600 EB	294.81	0.166	18.41	306.42

Table S5: DTPA extractable heavy metals in the mine soil (n = 3, mean ± S.D.) after pot-culture study

	Application rate (% (w/w)	DTPA-Ni (mg/kg)	DTPA-Cu (mg/kg)	DTPA-Zn (mg/kg)	DTPA-Co (mg/kg)	DTPA-Cr (mg/kg)
400 °C EB	0.5	6.11 ± 0.42^{b}	2.56 ± 0.49^{c}	5.04 ± 0.37^{b}	2.54 ± 0.51^{c}	2.02 ± 0.40^{c}
	1	4.50 ± 0.64^{c}	1.98 ± 0.32^{d}	4.39 ± 0.35^{c}	2.29 ± 0.45^{cd}	1.72 ± 0.25^{cd}
	2	$3.28 \pm 0.45d^{e}$	$1.70 \pm 0.51d^{e}$	3.79 ± 0.23^{de}	2.21 ± 0.44^{cd}	1.51 ± 0.27^{def}
	5	2.17 ± 0.33^{f}	$1.55 \pm 0.60d^{e}$	3.34 ± 0.63^{fg}	2.02 ± 0.33^{cd}	1.33 ± 0.31^{defg}
400 °C EB + Fertilizer	0.5	3.46 ± 0.35^{c}	1.96 ± 0.37^{c}	4.23 ± 0.53^{c}	2.18 ± 0.32^{c}	2.09 ± 0.35^{c}
	1	2.99 ± 0.45^{cd}	1.79 ± 0.52^{cd}	3.56 ± 0.49^{d}	1.97 ± 0.46^{cd}	1.85 ± 0.62^{cd}
	2	2.12 ± 0.40^{de}	1.45 ± 0.47^{def}	2.98 ± 0.35^{e}	1.60 ± 0.40^{cd}	1.50 ± 0.49^{def}
	5	1.71 ± 0.41^{e}	1.32 ± 0.27^{ef}	2.45 ± 0.46^{f}	1.42 ± 0.42^{d}	1.27 ± 0.25^{efg}
600 °C EB	0.5	5.51 ± 0.42^{b}	2.43 ± 0.58^{c}	4.10 ± 0.39^{cd}	2.40 ± 0.47^{c}	1.57 ± 0.33^{de}
	1	3.79 ± 0.16^{d}	2.00 ± 0.45^{d}	3.61 ± 0.42^{ef}	1.99 ± 0.32^{cd}	1.21 ± 0.27^{efg}
	2	$3.52 \pm 0.49c^{d}$	1.59 ± 0.43^{de}	3.19 ± 0.49^{gh}	1.91 ± 0.28^{cd}	1.08 ± 0.25^{fg}
	5	$2.21 \pm 0.42e^{f}$	1.34 ± 0.47^{e}	2.59 ± 0.31^{ij}	1.68 ± 0.31^{d}	0.98 ± 0.20^{g}
600 °C EB + Fertilizer	0.5	2.89 ± 0.43^{de}	1.92 ± 0.4^{c}	3.31 ± 0.54^{de}	2.22 ± 0.46^{c}	1.61 ± 0.42^{de}
	1	2.30 ± 0.37^{de}	1.71 ± 0.40^{cde}	2.88 ± 0.59^{e}	1.92 ± 0.57^{cd}	1.49 ± 0.49^{def}
	2	1.49 ± 0.52^{e}	1.36 ± 0.45^{def}	2.33 ± 0.52^{fg}	1.77 ± 0.45^{cd}	1.10 ± 0.25^{fg}
	5	1.43 ± 0.48^{e}	1.19 ± 0.39^{f}	1.97 ± 0.63^{g}	1.63 ± 0.42^{cd}	0.94 ± 0.31^{g}
Fertilizer [@ 75 kg/ha N, 30 kg/ha P, 90 kg/ha K]	Control 1	5.91 ± 0.70^{b}	4.19 ± 0.33^{b}	5.35 ± 0.38^{b}	6.05 ± 0.53^{b}	4.06 ± 0.55^{b}
Only mine soil	Control 2	8.62 ± 0.66^{a}	5.65 ± 0.42^{a}	6.90 ± 0.49^{a}	8.26 ± 0.38^{a}	4.61 ± 0.34^{a}

Table S6: Acid extractable heavy metals in the mine-soil after pot-culture study

	Application rate (% (w/w)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Co (mg/kg)	Cr (mg/kg)
400 °C EB	0.5	18.30±1.88^{c}	7.01±0.76^{cd}	20.92±2.03^{c}	13.53±0.42^{c}	79.23±2.06^{bc}
	1	13.90±0.70^{d}	6.55±0.67^{d}	18.46±1.09^{d}	10.07±0.94^{e}	74.14±2.73^{bcd}
	2	12.37±1.84^{f}	4.12±0.70^{f}	15.13±1.47^{e}	8.90±0.77^{f}	68.26±2.28^{d}
	5	9.30±0.68^{g}	3.82±1.46^{f}	12.67±1.38^{f}	7.19±0.86^{h}	66.18±1.58^{d}
400 °C EB + Fertilizer	0.5	15.26±1.05^{d}	7.50±0.79^{c}	18.30±1.61^{d}	12.63±1.18^{d}	81.33±1.34^{b}
	1	11.00±0.57^{e}	5.72±0.34^{e}	15.04±2.09^{e}	9.16±0.87^{f}	75.28±2.05^{bcd}
	2	10.19±0.48^{f}	3.33±0.55^{f}	12.27±2.91^{f}	7.87±0.69^{g}	70.09±2.95^{cd}
	5	8.34±0.54^{g}	2.11±0.67^{g}	9.30±2.32^{g}	4.53±0.81^{i}	67.16±2.21^{d}
600 °C EB	0.5	12.86±0.92^{e}	6.08±0.63^{c}	15.63±1.47^{c}	12.68±0.72^{c}	73.43±2.31^{c}
	1	9.47±1.2^{g}	4.49±0.57^{d}	11.69±1.90^{e}	10.67±0.32^{e}	72.11±1.16^{d}
	2	9.05±0.62^{g}	3.52±0.62^{e}	8.68±1.36^{g}	7.28±0.51^{g}	69.32±1.52^{e}
	5	8.18±0.38^{h}	3.20±0.59^{ef}	6.35±2.20^{i}	5.97±0.62^{h}	66.24±2.57^{g}
600 °C EB + Fertilizer	0.5	16.97±1.02^{c}	4.42±0.60^{d}	13.61±1.48^{d}	11.90±0.66^{d}	69.74±2.19^{e}
	1	10.85±1.22^{e}	3.71±0.44^{de}	10.59±1.54^{f}	8.19±1.28^{f}	67.62±2.39^{f}
	2	8.52±1.18^{g}	3.15±0.69^{ef}	7.69±1.69^{h}	4.86±0.78^{i}	65.08±2.07^{h}
	5	6.91±1.24^{h}	2.56±0.42^{f}	5.84±2.13^{i}	3.58±0.81^{j}	62.51±1.95^{i}
Fertilizer [@ 75 kg/ha N, 30 kg/ha P, 90 kg/ha K]	Control 1	22.68±1.14^{b}	14.91±0.96^{b}	27.16±2.36^{b}	25.33±0.95^{b}	105.19±3.07^{b}
Only mine soil	Control 2	32.20±1.37^{a}	23.32±1.06^{a}	32.45±1.64^{a}	33.28±1.65^{a}	114.83±2.57^{a}

Table S7: Statistical analysis to test the normal distribution of the data

Parameter	Number of observations (N)	Skewness	Standard Error	Z-Value	Shapiro-Wilk P-value
Soil Fertility Index	32	0.370	0.572	0.646	0.316
pH	32	0.342	0.582	0.587	0.442
Organic Matter	32	0.270	0.472	0.572	0.330
Ns	32	0.232	0.473	0.471	0.513
K	32	0.379	0.472	0.806	0.290
Ca	32	0.307	0.472	0.650	0.384
Mg	32	0.125	0.472	0.264	0.136
PO_4^{3-}	32	0.149	0.472	0.315	0.169
NH_4-N	32	0.312	0.472	0.661	0.955
CEC	32	0.320	0.472	0.678	0.871
Soil Catalase	32	0.421	0.472	0.892	0.906
β – glucosidase	32	0.288	0.472	0.610	0.944
Urease	32	0.293	0.472	0.621	0.947
DTPA Ni	32	0.394	0.472	0.834	0.643
DPTA Co	32	0.459	0.472	0.972	0.523
DPTA Cu	32	0.433	0.472	0.917	0.304
DTPA Zn	32	0.454	0.472	0.961	0.598
DTPA Cr	32	0.462	0.472	0.978	0.346

Table S8: Heavy metals in the plant shoot and root parts (n = 3, mean ± S.D)

	Application rate (%)	**Zn_{shoot}**	**Ni_{shoot}**	**Co_{shoot}**	**Cr_{shoot}**	**Cu_{shoot}**
400 EB	0.5	9.81±1.06	3.47±0.44	1.86±0.32	6.06±0.23	1.51±0.02
	1	7.94±0.65	3.36±0.47	1.91±0.51	4.89±0.35	1.41±0.02
	2	7.11±0.56	2.89±0.31	1.82±0.43	4.32±0.32	1.32±0.03
	5	4.81±0.76	2.74±0.40	1.71±0.41	4.28±0.45	1.28±0.01
600 EB	0.5	7.86±0.77	3.67±0.32	1.78±0.28	5.12±0.22	1.46±0.03
	1	7.28±0.74	3.12±0.45	1.80±0.37	4.46±0.35	1.38±0.03
	2	4.07±0.75	2.99±0.36	1.80±0.43	4.11±0.32	1.29±0.04
	5	3.48±0.59	2.81±0.28	1.65±0.45	3.42±0.37	1.24±0.02
400 EB + Fertilizer	0.5	9.49±0.57	2.67±0.35	1.59±0.23	4.44±0.36	1.45±0.04
	1	7.66±0.46	2.52±0.27	1.59±0.26	4.01±0.42	1.34±0.04
	2	6.50±0.40	2.33±0.33	1.64±0.37	3.40±0.47	1.28±0.02
	5	5.36±0.45	2.15±0.39	1.63±0.44	3.16±0.53	1.23±0.02
600 EB + Fertilizer	0.5	7.91±0.41	2.92±0.23	1.47±0.42	4.05±0.25	1.39±0.3
	1	7.11±0.39	2.60±0.28	1.44±0.51	3.55±0.28	1.34±0.02
	2	5.69±0.57	2.13±0.36	1.31±0.37	3.28±0.31	1.23±0.02
	5	4.90±0.50	2.46±0.31	1.20±0.28	3.24±0.42	1.06±0.01
Control 1		20.30±0.93	19.62±0.72	13.65±0.72	14.91±0.72	2.46±0.04
Control 2		14.88±0.88	13.84±1.08	11.35±1.09	12.07±0.65	4.60±0.05

		Zn_{root}	Ni_{root}	Co_{root}	Cr_{root}	Cu_{root}
400 EB	0.5	10.38±1.13	5.79±0.78	2.08±0.25	5.74±0.11	1.95±0.04
	1	7.02±1.10	5.29±0.86	1.67±0.19	5.28±0.13	1.76±0.06
	2	5.71±0.98	3.94±1.12	1.84±0.15	5.00±0.18	1.59±0.06
	5	5.07±0.90	3.26±0.89	1.35±0.23	4.15±0.19	1.47±0.04
600 EB	0.5	8.27±0.93	4.88±0.49	2.04±0.21	5.27±0.11	1.85±0.04
	1	7.30±0.66	4.30±0.76	1.37±0.35	5.02±0.12	1.69±0.03
	2	5.30±0.84	3.71±0.65	1.35±0.28	4.37±0.18	1.55±0.05
	5	5.51±1.06	3.31±0.68	1.22±0.27	4.14±0.15	1.43±0.04
400 EB + Fertilizer	0.5	10.62±1.14	3.76±0.77	1.73±0.15	4.85±0.22	1.76±0.02
	1	9.01±1.10	3.89±1.16	1.55±0.19	4.29±0.17	1.66±0.04
	2	7.89±1.09	2.96±0.50	1.42±0.16	4.01±0.13	1.38±0.03
	5	6.98±1.12	3.03±0.77	1.23±0.26	3.77±0.21	1.15±0.04
600 EB + Fertilizer	0.5	8.36±0.75	4.14±0.64	1.81±0.12	4.28±0.17	1.71±0.03
	1	7.78±0.97	3.92±0.60	1.33±0.30	4.06±0.29	1.63±0.02
	2	7.29±1.01	3.62±0.56	1.18±0.22	3.71±0.27	1.36±0.04
	5	5.42±1.16	3.12±0.55	1.03±0.11	3.34±0.24	1.13±0.03
Control 1		15.14±1.17	8.33±0.78	7.37±0.65	13.39±0.84	3.23±0.04
Control 2		11.13±1.23	7.73±0.70	4.82±0.69	8.77±0.66	4.85±0.05

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