

Title: Elucidating the effect of Biochar-bentonite composite-based Seed balls for the Remediation of coal mining impacted Heavy metals contaminated Soil

Supplementary Material

Figures:

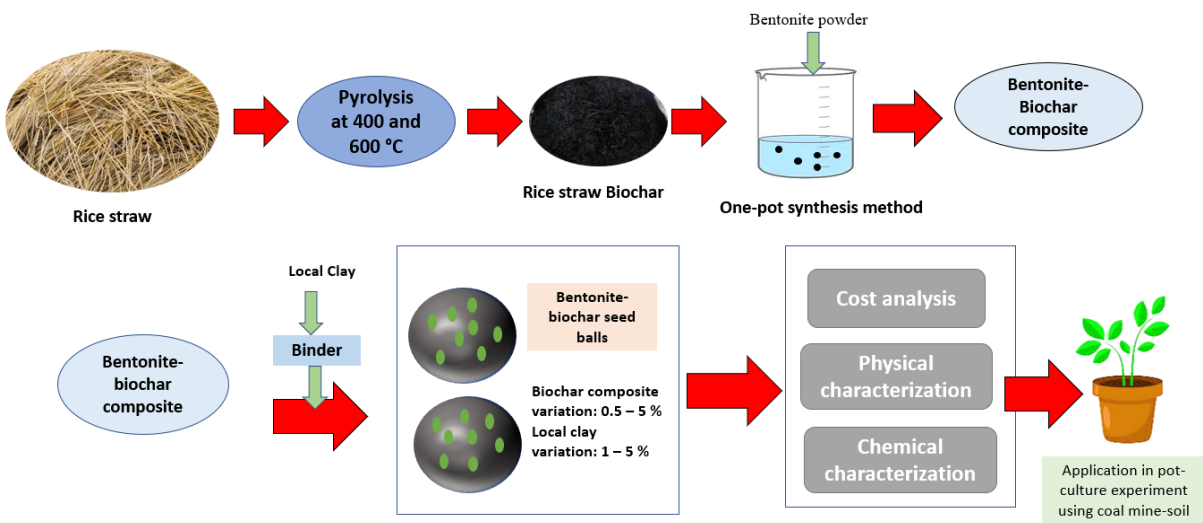


Figure S1. Methodology for the development of seed balls from rice-straw waste derived bentonite biochar composite and kaolinite to be implemented in the pot-culture study.

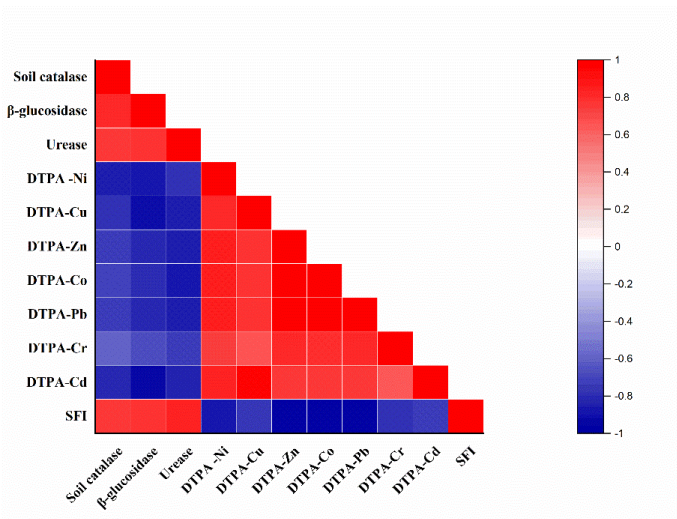


Figure S2. Correlation matrix among soil enzymes, DTPA-extractable heavy metals, and SFI.

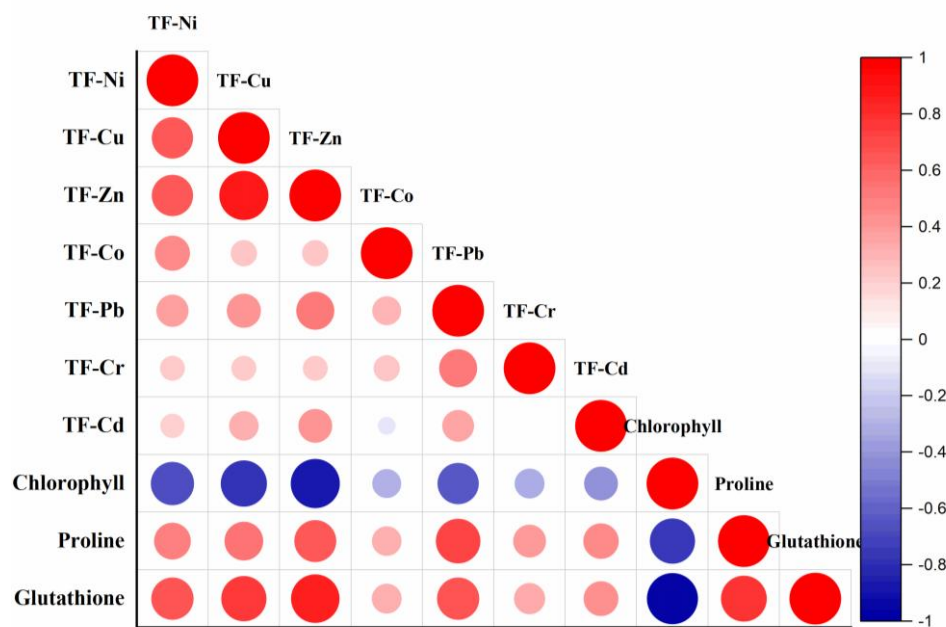


Figure S3. Correlation diagram among total chlorophyll, proline, glutathione, and translocation factor of heavy metals.

Soil Texture Analysis

Sand: 52 %
Silt: 40 %
Clay: 8 %

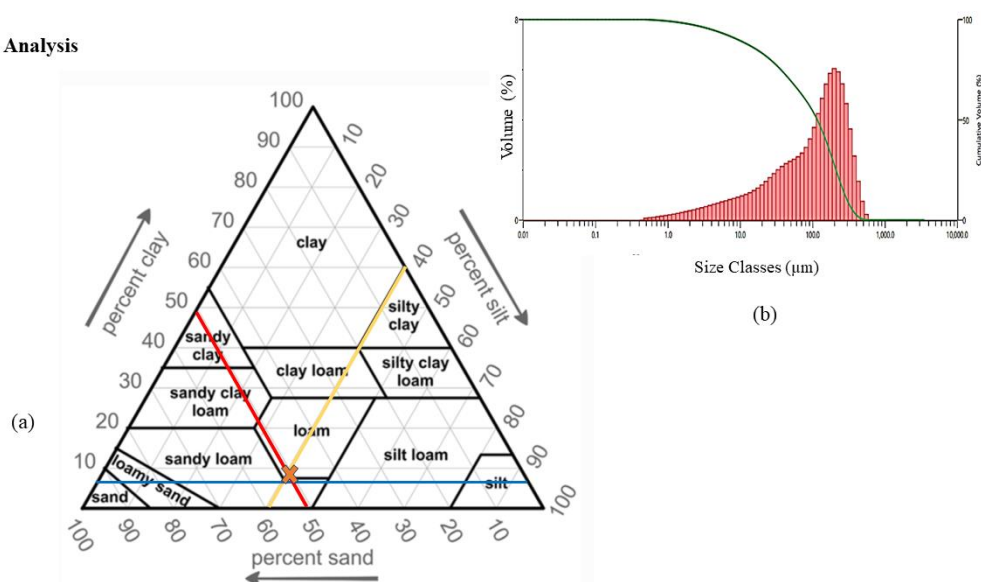


Figure S4. Soil granulometry and texture analysis.

Tables

Table S1. Physicochemical characteristics of the kaolinite clay.

Parameters	Values
pH	7.78
Cation exchange capacity (CEC) (cmol kg ⁻¹)	13.89
Zn (mg kg ⁻¹)	47.85
SSA (m ² g ⁻¹)	28
Organic Matter %	0.89
Exchangeable Mg (mg kg ⁻¹)	38.39
Exchangeable K mg kg ⁻¹)	59.68
Exchangeable Ca (mg kg ⁻¹)	118.42
Available P (mg kg ⁻¹)	6.89

Table S2. Physicochemical characteristics of bentonite-biochar composite.

Parameter	Values
Carbon (%)	41.67
Hydrogen (%)	2.12
Nitrogen (%)	2.16
Oxygen (%)	17.87
Volatile Matter (%)	47.58
Fixed Carbon (%)	11.48
Mineral Matter (%)	32.12
BET Surface area (m ² g ⁻¹)	126.87
pH	8.50
CEC (cmol kg ⁻¹)	56.46
Exchangeable K (g kg ⁻¹)	7.62
Exchangeable Ca (g kg ⁻¹)	3.18
Exchangeable Mg (mg kg ⁻¹)	213.60
Exchangeable Na (mg kg ⁻¹)	344.20

Table S3. Physicochemical characteristics of the seeds balls developed through the combination of biochar-bentonite composite and kaolinite.

Items	pH	Exchangeable-K (mg kg ⁻¹)	Exchangeable-Ca (mg kg ⁻¹)	Exchangeable-Mg (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Organic carbon %	CEC (cmol kg ⁻¹)
0.5B1C	7.29	83.46	91.53	30.27	7.83	1.53	14.66
1B1C	7.68	90.28	97.26	35.56	9.29	1.89	16.43
3B1C	8.24	98.44	107.39	37.44	10.35	2.34	18.29
5B1CB	8.76	108.21	118.24	40.21	12.36	2.68	23.22
0.5B3C	7.69	94.76	102.38	33.47	8.31	1.77	17.24
1B3C	8.13	104.33	114.76	36.51	9.58	2.31	19.43
3B3C	8.42	110.81	122.39	41.39	11.32	2.42	24.73
5B3C	8.89	123.21	129.72	44.57	13.46	2.83	26.89
0.5B5C	8.06	98.77	108.63	37.82	10.11	2.04	18.33
1B5C	8.42	109.43	116.29	43.72	14.26	2.38	22.32
3B5C	8.84	118.37	124.78	48.24	17.29	2.56	24.47
5B5C	9.07	128.38	133.43	54.39	19.88	2.88	26.44

Table S4. Post-pot-culture concentrations and variations of total heavy metals content in the amended soil (n = 3, mean \pm S.D.).

Ni	Cu	Zn	Co	Pb	Cr	Cd
(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)
40.29 \pm 4.71	17.24 \pm 0.59	56.24 \pm 1.81	19.32 \pm 0.79	21.49 \pm 0.61	124.38 \pm 3.55	1.02 \pm 0.026
38.23 \pm 1.37	16.43 \pm 0.67	52.35 \pm 1.28	16.44 \pm 0.41	18.76 \pm 0.66	120.66 \pm 4.16	0.96 \pm 0.018
35.14 \pm 1.35	13.2 \pm 0.37	46.33 \pm 1.33	13.86 \pm 0.53	16.28 \pm 0.70	115.29 \pm 3.21	0.93 \pm 0.029
33.19 \pm 1.37	11.16 \pm 0.48	41.57 \pm 1.29	11.29 \pm 0.35	13.14 \pm 0.51	111.32 \pm 3.98	0.88 \pm 0.022
36.47 \pm 1.33	16.12 \pm 0.54	52.16 \pm 1.18	17.48 \pm 0.63	17.26 \pm 0.66	121.39 \pm 3.53	0.95 \pm 0.026
32.26 \pm 0.83	13.25 \pm 0.36	47.38 \pm 1.63	14.54 \pm 0.47	15.44 \pm 0.50	116.44 \pm 2.46	0.89 \pm 0.024
27.45 \pm 0.72	11.69 \pm 0.43	42.64 \pm 1.10	10.19 \pm 0.38	11.16 \pm 0.31	111.28 \pm 2.20	0.82 \pm 0.015
21.38 \pm 0.67	8.67 \pm 0.21	37.31 \pm 1.24	8.76 \pm 0.24	8.35 \pm 0.24	106.73 \pm 1.91	0.74 \pm 0.013
33.28 \pm 1.31	14.24 \pm 0.52	49.36 \pm 1.33	15.44 \pm 0.33	14.48 \pm 0.46	116.81 \pm 2.47	0.87 \pm 0.018
28.64 \pm 1.10	11.23 \pm 0.35	43.38 \pm 1.59	11.38 \pm 0.30	10.33 \pm 0.39	111.39 \pm 2.30	0.81 \pm 0.19
23.19 \pm 0.72	7.64 \pm 0.24	38.44 \pm 1.26	7.28 \pm 0.23	7.14 \pm 0.21	105.24 \pm 1.98	0.74 \pm 0.017
20.33 \pm 0.67	5.22 \pm 0.14	32.17 \pm 0.84	5.26 \pm 0.14	4.22 \pm 0.16	99.77 \pm 1.79	0.66 \pm 0.019
49.97 \pm 1.93	20.3 \pm 0.79	62.19 \pm 2.40	22.11 \pm 0.86	24.82 \pm 0.82	133.82 \pm 3.82	1.14 \pm 0.033

Table S5. Heavy metals in shoot and root parts of the Shorgham grass.

Samples	Ni ^{shoot}	Cu ^{shoot}	Zn ^{shoot}	Co ^{shoot}	Pb ^{shoot}	Cr ^{shoot}	Cd ^{shoot}
	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)
0.5B1C	2.04	2.31	5.24	1.68	2.14	7.21	0.096
1B1C	1.9	2.15	4.89	1.53	1.83	6.42	0.083
3B1C	1.72	1.89	4.67	1.32	1.61	4.76	0.065
5B1CB	1.55	1.64	4.33	1.12	1.36	3.21	0.044
0.5B3C	1.91	2.03	4.91	1.52	1.96	6.44	0.078
1B3C	1.68	1.77	4.62	1.28	1.64	4.89	0.061
3B3C	1.51	1.52	4.18	1.1	1.32	3.26	0.042
5B3C	1.24	1.28	3.91	0.84	1.06	2.54	0.03
0.5B5C	1.16	1.71	4.43	1.21	1.52	4.38	0.063
1B5C	1.21	1.33	3.95	0.94	1.19	3.17	0.036
3B5C	0.94	1.12	3.45	0.62	0.85	2.34	0.024

5B5C	0.76	0.82	2.92	0.48	0.54	1.59	0.016
Control	3.97	3.26	7.43	2.2	2.76	10.28	0.15
Heavy metals in the Root part							
	Ni_{root}	Cu_{root}	Zn_{root}	Co_{root}	Pb_{root}	Cr_{root}	Cd_{root}
	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)
0.5B1C	2.88	2.59	6.11	2.06	2.44	11.43	0.126
1B1C	2.61	2.29	5.73	1.73	2.17	9.77	0.115
3B1C	2.53	2.1	5.54	1.63	1.92	7.24	0.097
5B1CB	2.39	1.95	5.26	1.41	1.76	6.32	0.073
0.5B3C	2.62	2.36	5.84	1.77	2.29	9.45	0.11
1B3C	2.31	2.11	5.5	1.48	1.94	7.33	0.092
3B3C	2.11	1.85	5.17	1.31	1.7	6.21	0.065
5B3C	1.92	1.64	4.86	1.02	1.42	5.19	0.054
0.5B5C	2.34	2.17	5.42	1.51	1.79	6.74	0.087
1B5C	1.95	1.78	5.1	1.06	1.53	5.22	0.063
3B5C	1.56	1.61	4.69	0.82	1.17	4.16	0.042
5B5C	1.32	1.48	4.2	0.66	0.88	3.34	0.02
Control	3.76	3.13	7.68	2.65	3.31	28.26	0.2