**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

**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) | 47.85 |
| SSA (m2/g) | 28 |
| Organic Matter % | 0.89 |
| Exchangeable Mg (mg/kg) | 38.39 |
| Exchangeable K (mg/kg) | 59.68 |
| Exchangeable Ca (mg/kg) | 118.42 |
| Available P (mg/kg) | 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 (m2/g) | 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 ± S.D.)

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

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Samples** | **Nishoot** **(mg kg-1)** | **Cushoot (mg kg-1)** | **Znshoot****(mg kg-1)** | **Coshoot (mg kg-1)** | **Pbshoot** **(mg kg-1)** | **Crshoot** **(mg kg-1)** | **Cdshoot (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 |
|  | **Niroot** **(mg kg-1)** | **Curoot** **(mg kg-1)** | **Znroot** **(mg kg-1)** | **Coroot****(mg kg-1)** | **Pbroot** **(mg kg-1)** | **Crroot** **(mg kg-1)** | **Cdroot** **(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 |