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Evaluation of the bioavailability and translocation of selected mobile chemical species by *Brassica juncea* and *Spinacea oleracea L* for a South African power utility coal fly ash

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Abstract: This study evaluated the physicochemical, mineralogical properties, mobile chemical species' bioavailability and translocation in *Brassica juncea* and *Spinacea oleracea L* plants of a South African coal fired power utility. Coal fly ash (CFA) disposal is associated with various environmental and health risks including air, soil, surface and ground water pollution due to the leaching of toxic chemical species; these ends up in food webs affecting human health, while repeated inhalation causes bronchitis, silicosis, hair loss and lung cancer. The morphology, chemical, and mineralogical composition of CFA were determined using Scanning Electron Microscopy (SEM), X-ray fluorescence (XRF) and X-ray Diffraction, respectively. In pot culture experiments, *S. oleracea L* and *B. juncea* plants were grown in three sets of pots containing CFA (set 1), soil (set 2) and a mixture of CFA plus soil at ratio 1:1 (50% CFA: 50% soil) (set 3), while no plants were grown in set 4 as a control for the leachate samples. SEM showed that surface morphology of CFA has a lower degree of sphericity with irregular agglomerations of many particles. The XRF results revealed that CFA contains 43.65 %, 22.68 % and 10.89 % of SiO_2, Al_2O_3 and Fe_2O_3 respectively which indicate that the CFA is an alumino-silicate material. While XRD showed that the coal CFA contains mullite as a major phase followed by quartz mineral phases. Chemical species such as Fe, Mn, B, Ba and Zn were accumulated highly in most parts of the plant species. However, *B. juncea* showed higher potential to accumulate chemical species as compared to *S. oleracea L*. The bioconcentration and translocation factors (BF and TF) showed that *B. juncea* was the most effective in terms of bioconcentration and translocation of most of the chemical species. This indicates that *B. juncea* has potential in application for phytoremediation of CFA dumps and could contribute to remediation of CFA dumps and reduction of potential health and environmental impacts associated with CFA.

Keywords: coal fly ash, leachates, chemical species, pot culture experiments, translocation, bioconcentration.

1. Introduction

Coal fly ash dumps are major sources of chemical species contamination of the environment as a whole. Maiti and Prasad [1] mentioned that coal based thermal power plants generate coal fly ash

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(CFA) as the main industrial waste product, approximately 70 – 75% [2] and it has been recognized as an environmental hazard across the globe. In South Africa coal-fired power stations consume ±120 million tons of coal per annum, producing 30 million tons of CFA, to supply the bulk of South Africa's electricity. A modern coal fired power station with a total output of 3 600 MW was said to consume ±50 000 tons of coal every day by Eskom [3]. Eskom [3] mentioned that depending on the coal quality, the heat and ash content, stations can produce ±17 000 tons of ash per day. On the other hand, Sasol was producing about 7 million tons of ash, as reported by [4]. Many of South Africa's thermal power plants use wet ashing process while others use dry ashing processes to dispose ash. Usually CFA is transported from the power plant either damp or as slurry to a series of holding ponds where the solids are allowed to settle out of suspension. CFA is usually then stockpiled and used as landfill. In dry ashing systems, from the boilers CFA is transported by overland conveyors to ash disposal facilities and since the ash contains 12 % moisture, dust production is minimal[5].

Even though that is the case, agricultural lands have been continuously contaminated by direct discharge of industrial effluents, runoff wastewater from ash dumps, overflow of ash dykes during rainy seasons or through atmospheric fallout of CFA[6]. If soil and water get contaminated with industrial effluents containing high levels of chemical species, the chemical species find their way into food crops and vegetables and consequently enter the food web. Most of these chemical species at high concentrations are toxic and persistent, while some are toxic even at trace concentrations. They are regarded as environmental pollutants and pose a threat to the environment and human health [1]. Thus improper CFA dumping will continue to cause land degradation, water, air and soil pollution if preventive measures are not implemented. Dust emission of CFA causes air pollution and remains air-borne for a long period and causes health hazards for the local masses. Henceforth, repeated inhalation of CFA dust containing crystalline silica can cause bronchitis, silicosis (scarring of the lung), lung cancer and severe inflammation of the small airways of the lung and asthma-like symptoms. Exposure to some of the most common toxic contaminants in CFA like Ni causes allergic dermatitis known as nickel itch; inhalation can cause cancer of the lungs, nose, and sinuses; cancers of the throat and stomach have also been attributed to its inhalation; hematotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, and hepatotoxic; also causes hair loss [8].

Phytoremediation is a way to go about this pollution of air, soils and water sources caused by CFA where the chemical species also end up in food chain affecting human life; as a cost-efficient and ecologically benign process. This is because conventional remediation methods such as acid leaching, land-filling, and excavation process are very expensive and not eco-friendly [7]. Phytoremediation is aimed at providing an innovative, economical, environment-friendly approach for removing toxic chemical species from hazardous waste sites [6]. It involves techniques such as rhizofiltration, phytostabilization, phytovolatilization and phytoextraction. The emphasis is on phytoextraction because it involves chemical species translocation to shoots which is an important biochemical process and is advantageous in effective phytoremediation, in other techniques the harvest of root biomass is generally not feasible in many instances [8, 9 and 10]. Goswami and Das [11] studied phytoremediation of cadmium contaminated soil using *B. juncea* and the bioaccumulation was observed to be effective in decontamination of the soil. Other plant species like *Ipomea carnea* [12], *Jatropha curcas* [13], and *Azolla caroliniana* [14] are some of

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the plant species that have been evaluated for phytoremediation of CFA dumps. *B. juncea* and *S. oleracea L* were chosen for the current study because they have certain characteristics which make them suitable for phytoremediation application. This includes having high growth rate, production of more above-ground biomass, more accumulation of the target chemical species from soil, translocation of the accumulated chemical species from roots to shoots, tolerance to the toxic effects of the target chemical species, good adaptation to prevailing environmental and climatic conditions, and easy cultivation and harvest [15, 16, 17 and 8].

The aim of this study was to determine the potential of *S. oleracea L* and *B. juncea* in the phytoremediation of chemical species from CFA dumps. The specific objectives were therefore; (i) to evaluate the physicochemical and morphological composition of CFA from a selected South African coal fired power utility; (ii) to assess the bioavailability and translocation factor of chemical species in *S. oleracea L* and *B. juncea* over a specific period of time and their potential for field application in phytoremediation of such CFA dumps.

2. MATERIALS AND METHODS

2.1 Materials.

The dry CFA used was collected from a selected South African coal fired power utility. Good and viable seeds of *Spinacia oleracea L* and *Brassica juncea* were purchased from a local store and sown in seed trays and thereafter transplanted to the pots for growth experiments.

2.2 Physicochemical characterization of coal fly ash.

The chemical and mineralogical composition of CFA and soil were determined using X-ray fluorescence (XRF) and X-ray diffraction (XRD), respectively. The Thermo Fisher ARL Perform'X Sequential XRF with OXSAS software was used for analysis while for XRD analysis was done using a PANalytical X'Pert Pro powder diffractometer in θ–θ configuration with an X'Celerator detector and variable divergence- and fixed receiving slits with Fe filtered Co-Kα radiation (λ=1.789 Å). Scanning Electron Microscopy (SEM) was used to examine the morphology of CFA and a Zeiss 1450 fully analytical scanning electron microscope was used.

2.3 Experimental design.

Pot culture experiments were conducted for four successive months (April to July 2015). Prior to experiment, pots were washed thoroughly with Milli-Q water and filled up with different compositions of soil and CFA. Experiments were conducted in four sets. The first set was filled up with CFA, second set with soil only and the third set with a mixture of CFA and soil, the last set was for control with only CFA. *Spinacia oleracea L* and *Brassica juncea* plants were grown in three sets pots containing CFA (set 1), soil (set 2) and a mixture of CFA and soil at ratio 1:1 (50% CFA: 50% soil) (set 3). No plant was grown in set 4. Figure 1 shows the sets of growth media used. Seeds were sown in seed trays and irrigated daily until germination, then transplanted to pots. The pots were kept in a nursery to mimic the natural environment. The pots were monitored (measuring plant heights for growth performance) and leachates were collected daily, and finally heights of plants were recorded. Plants were irrigated daily and leachate collected was analyzed using ICP-MS for cationic chemical species. Harvesting of the plants was done in stages, the first

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harvest was of seedlings, the second harvest was done after 46 days of growth and the last harvest was done after 115 days of growth.

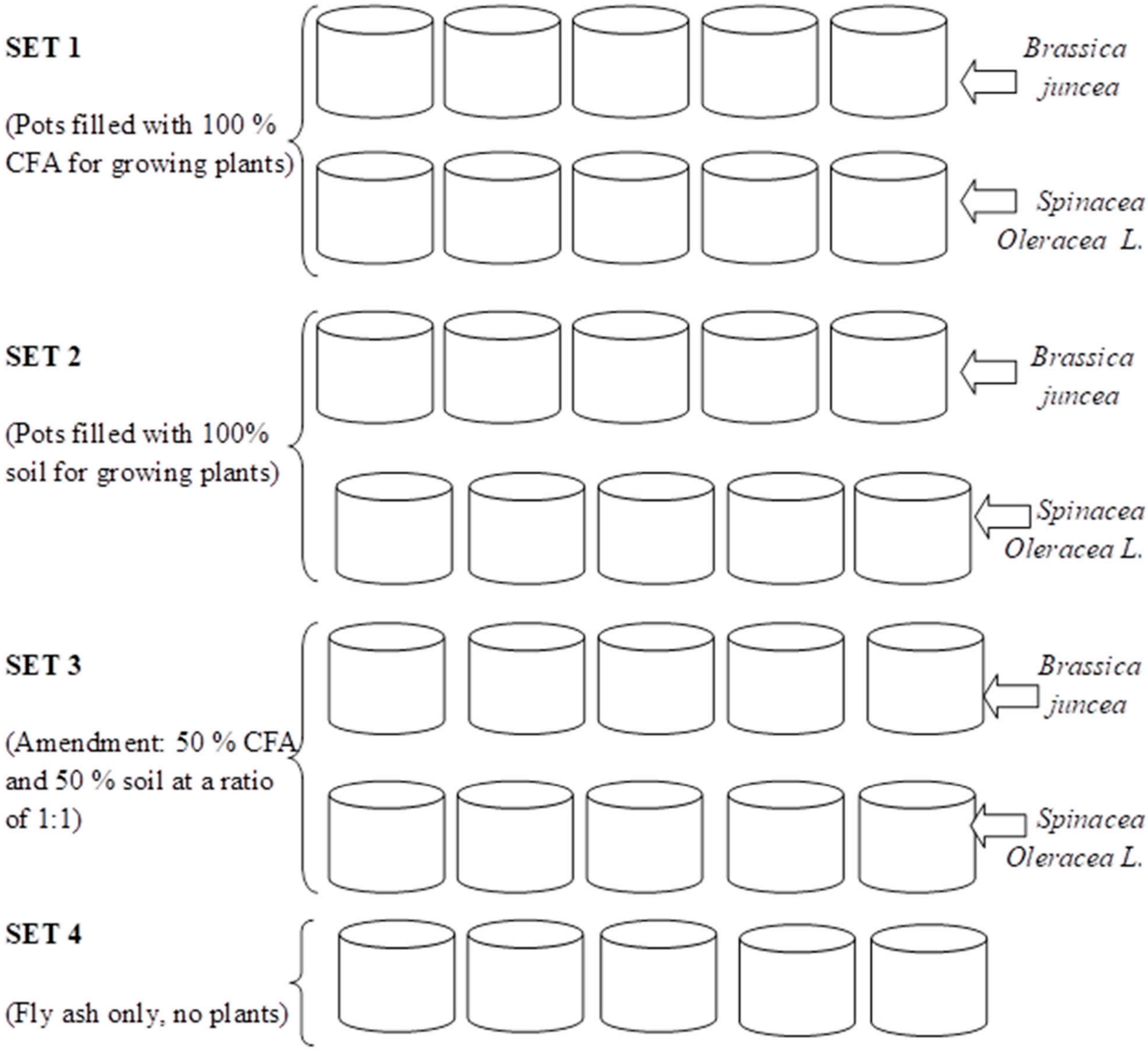

Figure 1: Illustration of set up of pots (materials used in each pot and ratio used, in case of combination is indicated and species grown in each pot is also shown).

2.4 Preparation of plants samples for bioaccumulation analysis, biomass estimation and chlorophyll content.

Samples of the whole plant were collected, then cut to separate parts: leaves, stems and roots in order to determine chemical species' concentrations in each part of the plant and for the estimation of biomass. . The biomass of the plants was estimated by weighing the mass (g) of plant sample after air drying. To estimate the chemical species bioaccumulation, dried samples were ground into a fine powder and 0.5 g was weighed for digestion through aqua regia ($HCl:HNO_3 = 3:1$ (v/v))

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to near dryness or until a white-coloured solution was formed. The acid digestion was carried out on a hotplate. After complete digestion of samples, 100 mL of MilliQ water (18.2 MΩ/cm) was added and left to cool down. Samples were then filtered through 0.45 µm pore membrane. Samples were then analyzed using Inductively Coupled Plasma- Mass Spectrometry (ICP-MS). Blanks and internal standards were set for quality assurance. Growth performance was obtained by measuring heights of plant species in pot culture experiments.

For chlorophyll analysis, Leaves of *B. juncea* and *S. oleracea L* were accurately weighted and 0.5 g of each fresh plant leaf sample was taken. This was sufficiently homogenized in a blender with 10 ml of the extracting solvent (90 % ethanol). The homogenized sample mixture was centrifuged at 10,000 rpm for 15min at 40 ^{0}C. The supernatant was separated and 0.5 ml of it was mixed with 4.5 ml of ethanol. The solution was transferred to a corresponding cell and put in cell compartment and analysed for Chlorophyll-a, Chlorophyll-b and carotenoids content using spectrophotometer (SQ Pharo 100). The chlorophyll contents were then determined using the following equations [18]:

$$Ch-a = 13.36A_{664} - 5.19A_{649} \quad (1)$$
$$Ch-b = 27.43A_{649} - 8.12A_{664} \quad (2)$$
$$C\,x + c = (1000A_{470} - 2.13Ch - a - 97.63Ch - b)/209 \quad (3)$$

Where A = Absorbance, Ch-a = Chlorophyll a, Ch-b = Chlorophyll b, C x+c = Carotenoids

2.5 Bioconcentration Factor (BCF) and Translocation Factor (TF)

Bioconcentration Factor (BCF) was calculated for each plant part (root, stem, leaf). BCF was calculated for each plant part (root, stem, leaf) using the following equations for the CFA and soil:

$$BCFa = \ Metal\ in\ leaves\ /\ Metal\ in\ FA\ or\ soil. \quad (4)$$

$$BCFb \ = \ Metal\ in\ stem\ /\ Metal\ in\ FA\ or\ soil. \quad (5)$$
$$BCFc \ = \ Metal\ in\ roots\ /\ Metal\ in\ FA\ or\ soil. \quad (6)$$

Where BCFa is the bioconcentration Factor of the stem, BCFb is the bioconcentration Factor of the roots, and BCFc is the bioconcentration Factor of the leaves.

Then translocation Factor (TF) which is an asset to assess a plant's potential for phytoremediation purpose was also calculated. TF is based on the ratio of metal concentration in plant stem as compared to that of the plant root and leaves [19].

Thus: $TF \ = \ BCFa\ /\ BCFb \quad i.e. leaf/stem.$ (7)

$TF \ = \ BCFb\ /\ BCFc \quad i.e. stem/root$ (8)

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3 RESULTS AND DISCUSSION

3.1 Physicochemical characterization of a selected South African coal fired power utility.

Figure 2 depicts the morphology of CFA as determined by SEM at different magnification levels. It is observed that morphology of CFA has a lower degree of sphericity with irregular agglomerations of many particles while there were dominant spherical particles and smaller sharp needle-like particles.

Figure 2: SEM micrographs of a selected South African coal fired power utility CFA at (a) x20 000 and (b) x30 000 magnifications.

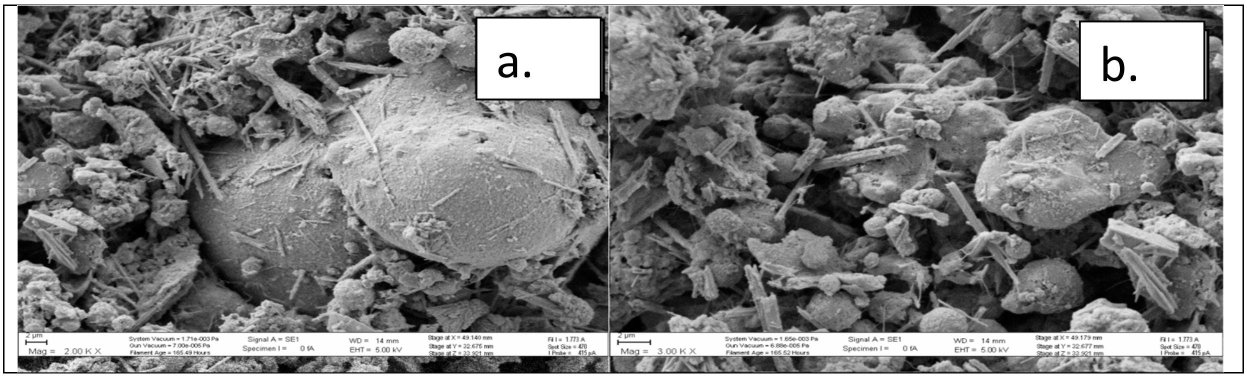

Figure 3 presents the XRD spectrum of the CFA. The spectra showed the presence of mullite, quartz, calcite, hematite, magnetite and albite as mineral phases in the CFA. The quantitative results from XRD showed that mullite is the dominant mineral (48.14 %) followed by quartz (28.51 %). Other mineral phases were at trace levels.

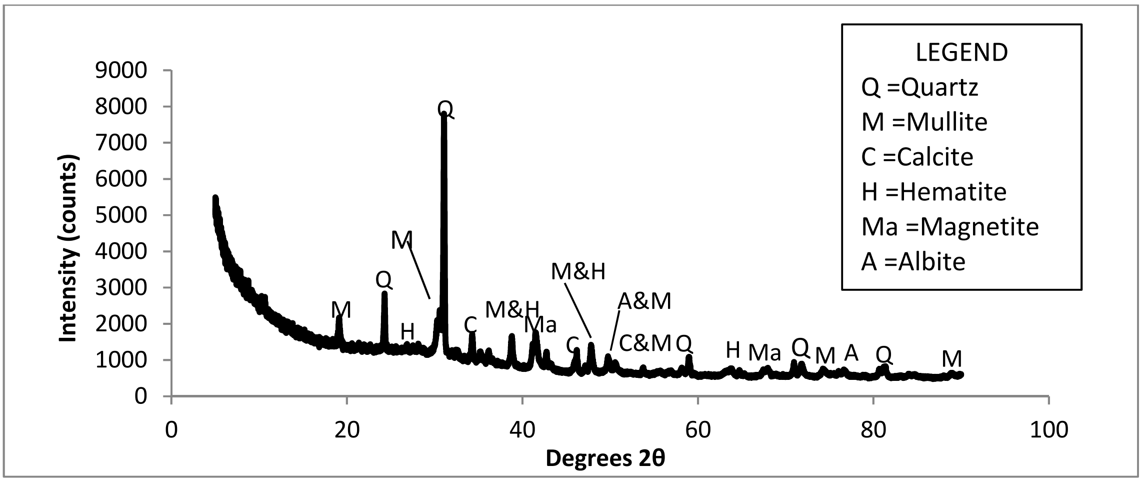

Figure 3: XRD spectrum of the CFA.

Table 1 shows the chemical composition of CFA and soil as determined by XRF. The analysis revealed that CFA consists of Fe_2O_3 (10.89%), SiO_2 (43.65 %) and Al_2O_3 (22.68 %). The total percentage of these three oxides were >70% indicating that this South African coal fly ash can be

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categorized as class F (SiO_2-rich), which is either derived from anthracitic or bituminous coals [20]. High concentration of Fe_2O_3, SiO_2 and Al_2O_3 confirms that this CFA is an alumino-silicate material [21]. The soil had SiO_2 (82.9 %) as the main component, while Fe_2O_3 and Al_2O_3 were available in small amounts. Elements such as Ni, Cu, Zn, Zr, W, Sr, Ni, As, Rb and Mo were observed at trace levels in both CFA and soil.

Table 1: Comparison of the chemical composition of CFA and soil samples.

Coal Fly ash (CFA) sample				**Soil sample**			
Major elements (as oxides)	(w/w) %	Trace elements	mg kg^{-1}	Major elements (as oxides)	(w/w) %	Trace elements	mg kg^{-1}
SiO_2	43.65	**As**	22	**SiO_2**	82.90	**As**	24
TiO_2	1.23	**Cu**	57	**TiO_2**	0.54	**Cu**	17
Al_2O_3	22.68	**Ga**	35	**Al_2O_3**	7.33	**Ga**	9
Fe_2O_3	10.89	**Mo**	9	**Fe_2O_3**	3.31	**Mo**	8
MnO	0.06	**Nb**	31	**MnO**	0.03	**Nb**	9
MgO	1.88	**Ni**	63	**MgO**	0.25	**Ni**	20
CaO	7.04	**Pb**	29	**CaO**	0.36	**Pb**	6
Na_2O	0.19	**Rb**	39	**Na_2O**	0.71	**Rb**	51
K_2O	0.81	**Sr**	2001	**K_2O**	1.09	**Sr**	46
P_2O_5	0.54	**Th**	41	**P_2O_5**	0.05	**Th**	13
Cr_2O_3	0.18	**U**	28	**Cr_2O_3**	0.01	**U**	5
NiO	0.01	**W***	48	**NiO**	0.00	**W***	235
V_2O_5	0.02	**Y**	65	**V_2O_5**	0.01	**Y**	10
ZrO_2	0.07	**Zn**	40	**ZrO_2**	0.07	**Zn**	21
CuO	<0.01	**Zr**	507	**CuO**	0.00	**Zr**	405
SO_3	0.36			**LOI**	3.02		
LOI	9.50			**TOTAL**	99.69		
TOTAL	99.11						

3.2 Assessment of biomass, growth performance and chlorophyll content of plant species in pot culture experiments.

The biomasses and growth performances of *B. juncea* and *S. oleracea L* over time in different growth media are presented in Figure 4 and 5, respectively. Results showed that in both growth media, roots, stems and leaves biomasses of *B. juncea* and *S. oleracea L* plants species increased with increasing number of days. Based on the roots and stem masses, *S oleracea L* showed a better tolerance in CFA as compared to *B. juncea* in CFA growth media. It is therefore anticipated that *S. oleracea L* will yield the better performance in phytoremediation because plants with extensive

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roots are capable of extracting more chemical species due to better exploration of growth media [13]. Furthermore, *S. oleracea L* showed better growth in the CFA media while *B. juncea* grew better in the soil as a growth media.

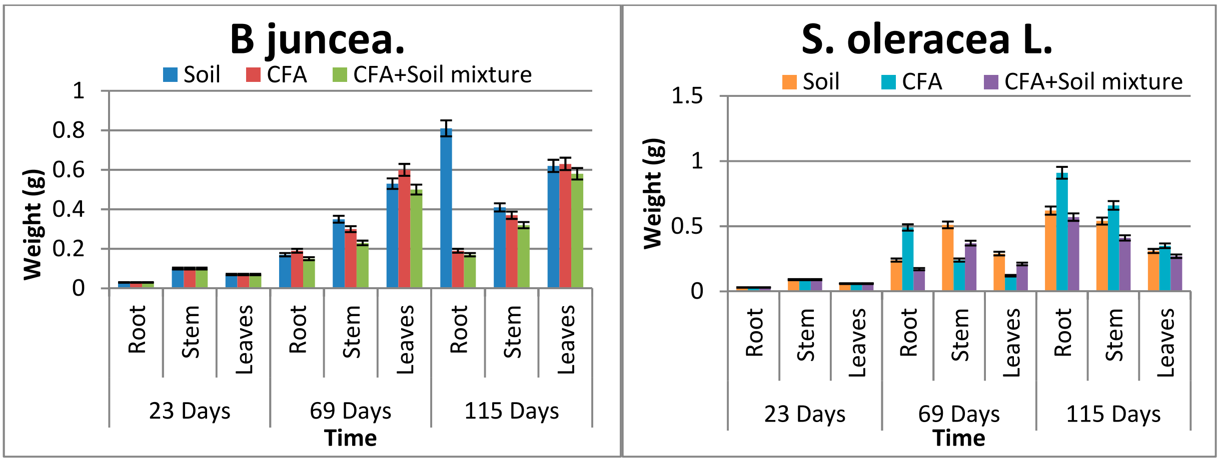

Figure 4: Above and below ground biomass for *B. juncea* and *S. oleracea L* in all growth media.

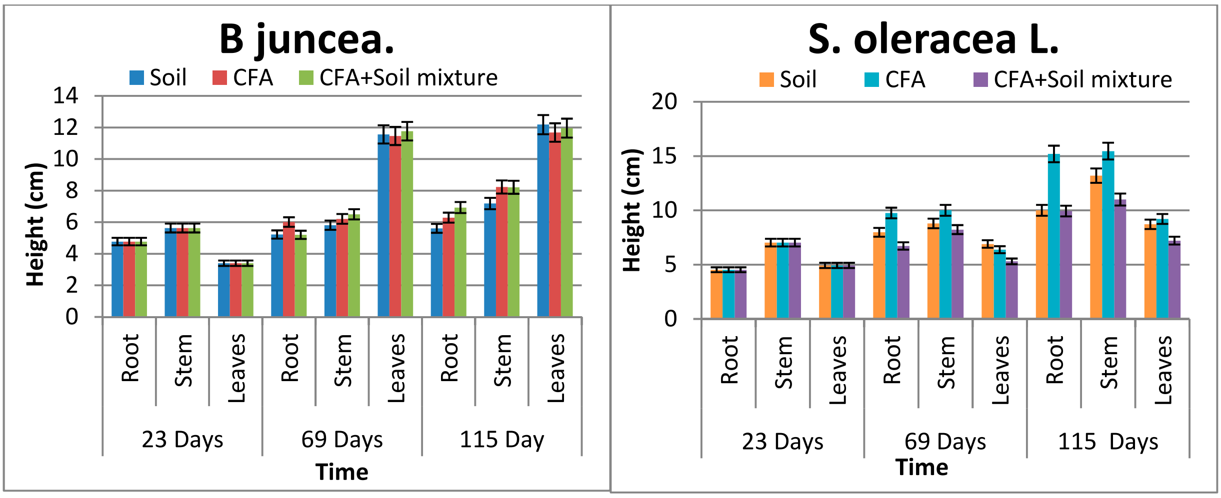

Figure 5: Growth performance of *B. juncea* and *S. oleracea L* in all growth media.

Table 2: Chlorophyll a and b content of *S. oleracea L* and *B. juncea* for different growth media with time.

It is observed that chlorophyll a and b increase during the first 69 days and decline in 115 days for most growth media (Table 2). This was observed in both plant species. Goswami and Das reported similar findings where there was a significant reduction in the total chlorophyll content of the young leaves of *B. juncea* and *B. napus* on exposure to 10 mM Cd for 15 days [11]. Compared to *B. juncea*, *S. oleracea L* shows the better content of chlorophyll b. Even though that is the case, *B juncea* showed to have more survival characteristics by having some carotenoid content while *S. oleracea L*'s was not detected (Table 2). This carotenoid content in *B. juncea* plants can help in the protection of the plant species and improve its tolerance to harsh environments including high

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amounts of chemical species found in CFA, hence it can promote its effectiveness in phytoremediation than *S. oleracea L*. But, statistically there was no significant difference between the two plant species in terms of growth performance and biomass; even between the plant parts denoting similar growth performance for the two plant species under study.

Table 2: Chlorophyll a and b content of *S. oleracea L* and *B. juncea* for different growth media with time.

	Chlorophyll a for *B. juncea*			**Chlorophyll a for *S. oleracea L***		
	23 days	**69 days**	**115 days**	**23 days**	**69 days**	**115 days**
Soil	3.94	3.3	2.3	2.34	2.77	2.55
CFA	3.94	2.63	2.25	2.34	2.65	1.44
CFA + soil	3.94	4.11	3.94	2.34	2.64	2.75
	Chlorophyll b for *B. juncea*			**Chlorophyll b for *S. oleracea L***		
Soil	0.87	1	0.8	4.95	6.08	5.51
CFA	0.87	1.57	1.79	4.95	5.62	3.12
CFA + Soil	0.87	0.93	0.87	4.95	5.86	5.95
	Carotenoid content for *B. juncea*			**Carotenoid content for *S. oleracea L***		
Soil	0.92	0.59	0.69	ND	ND	ND
CFA	0.92	0.53	0.45	ND	ND	ND
CFA + Soil	0.92	0.76	0.92	ND	ND	ND

*NOTE

ND = Not Detected

3.3 The temporal evolution of the physicochemical characteristics of the leachates as plants were irrigated in the pot culture experiment.

The temporal variation of the chemical elements in the leachates from different growth media is based on the results for the leachates collected from the first week to the eleventh week of the pot culture experiments for different growth media. The full analysis results are presented in the supplementary information in Tables S1 to S7. The selected chemical species presented include B, Cr, Mn, Ni, Cu, Zn, Mo, Ba and Fe. They were chosen based on their higher concentrations in the leachates. Several authors have also indicated that these chemical species were present in CFA leachates in higher concentrations [22, 12 and 7].

Soil leachates had a neutral pH of 7.22 with very high amounts of Si. While CFA leachates had an alkaline pH of 10.62. In both soil and CFA, chemical species that were expected to be readily

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available to plants included Ca, Si, K, Ba, Mo, Na, Al, Mg, Sr and non-essential elements (Si, Ba, Na, Al, Sr), which, if uptaken by plants' roots can have negative impacts in plants. Physicochemical analysis of soil, CFA and CFA+soil leachates where plants were grown and where there were no plants showed that the alkalinity of the CFA changed over time (starting from an average of 9.4 to 7.5 pH level in the 11th week) and there was also a decrease in the EC (starting from an average of 411µS cm^{-1} to 224 µS cm^{-1} in the 11th week) due to the dissolution of soluble major oxides which was promoted by continuous irrigation with water. Chemical species like B, Cr, Mo and Ba (ranging from 4277.50 µg L^{-1} in the to 13.04 µg L^{-1}) were occurring at higher concentrations in leachates for most weeks for CFA and soil + CFA as a growth media, while Fe (starting from 10003.41 µg L^{-1} to 550.25 µg L^{-1} in the 11th week) was also high for soil as a growth media. It was observed that in the eleventh week of leachate collection all these chemical species plummet to very low concentrations. This suggests that these chemical elements can be reduced over time as plants are being irrigated which is either due to uptake by plants or washed off with water from the coal fly ash and soil. A similar trend was observed for CFA, soil and CFA+soil growth media. There was a significant difference in concentrations of different chemical species in leachates from different growth media for each plant species (*B. juncea* and *S. oleracea L)*.

3.4 Potential of the plant species for phytoremediation.

The phytoremediation potential of a plant is accessed by computing the bioconcentration Factor (BCF) and Translocation Factor (TF) of chemical species throughout the plant. The BCF value of more than 1 indicates that the plant is a potential accumulator of chemical species, while the TF value greater than 1 indicates that the plant is a potential translocator of chemical species

Table 2 shows BCF for chemical species accumulating in *B. juncea* and *S. oleracea L* in day 115 for all growth media. Higher BCF values were observed for Fe, Mn, Zn, Cu and Ni in plant parts of both plants. Conversely lower values were observed for Mo, B, Ba and Cr having BCF values less than 1 for most growth media over time. From the results it was concluded that *B juncea* is more suitable for the accumulation of many chemical species than *S. oleracea L* in CFA and soil as a growth media (Table 2). For the CFA + soil growth media, *S. oleracea L* accumulated many different chemical species than *B juncea* from 69 days to 115 days.

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Table 2: BCF for chemical species accumulating in both plant species in day 115 for all growth media.

	B. juncea								
Growth media	Soil			CFA			CFA + Soil		
	Leaf	**Root**	**Stem**	**Leaf**	**Root**	**Stem**	**Leaf**	**Root**	**Stem**
B	2,67	1,01	2,40	0,12	0,08	0,06	0,07	0,02	0,03
Cr	5,66	4,37	2,06	1,00	1,08	0,55	2,13	0,58	0,65
Mn	24,00	15,79	7,72	309,84	200,39	262,18	315,57	118,69	106,95
Ni	1,20	1,32	0,59	24,57	24,32	11,75	10,66	3,47	3,28
Cu	6,53	5,96	2,46	27,58	19,96	25,26	9,31	4,44	3,78
Zn	16,43	28,56	11,90	185,73	181,15	196,67	85,29	44,32	35,85
Mo	7,29	1,12	3,24	0,28	0,15	0,15	0,03	0,02	0,03
Ba	2,72	2,36	1,86	1,22	1,10	1,38	1,32	0,50	0,52
Fe	68,31	50,51	17,89	979,08	692,36	1098,15	4046,11	1128,22	1047,44
	S. oleracea L								
Growth media	Soil			CFA			CFA + Soil		
	Leaf	**Root**	**Stem**	**Leaf**	**Root**	**Stem**	**Leaf**	**Root**	**Stem**
B	0,11	0,11	0,11	0,01	0,01	0,01	0,01	0,01	0,01
Cr	1,13	1,13	1,13	0,38	0,38	0,38	1,24	1,24	1,24
Mn	6,92	6,89	6,90	97,36	97,28	97,32	8,01	8,00	8,00
Ni	0,51	0,51	0,51	11,24	11,23	11,23	6,10	6,10	6,10
Cu	1,94	1,93	1,93	17,62	17,61	17,61	9,36	9,36	9,36
Zn	5,67	5,65	5,66	42,96	42,93	42,95	91,92	91,91	91,92
Mo	0,24	0,24	0,24	0,01	0,01	0,01	0,01	0,01	0,01
Ba	0,34	0,34	0,34	0,48	0,48	0,48	0,35	0,35	0,35
Fe	0,22	0,22	0,22	957,95	957,33	957,31	257,23	257,19	257,20

Table 3 shows translocation factor of various chemical species over time (115 days) in different species of plants grown in different growth media with respect to translocation of chemical species from root to shoots of plant species under study. *B. juncea* species was observed to be an effective translocator of various chemical species (including Zn) for different growth media with. In most cases, TF values >1 were observed for *B. juncea* while *S. oleracea L* failed to translocate most chemical species (TF<1). Translocation was significant in stems to leaves of *B. juncea* in soil growth media, followed by CFA and then lastly CFA + soil growth media, hence could be a potential CFA dump phytoremediator. There was also significant chlorophyll and carotenoid content for *B. juncea* leading to better tolerance in the CFA media while *S. oleracea L* did not possess any carotenoid content which might have weakened its chemical species accumulation potential.

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Table 3: Translocation Factor (TF) of various chemical species for 115 days in different species of plants grown in different growth media. TF=BCFb /BCFc. i.e. stem/root and TF=BCFa /BCFb i.e. leaf/stem.

	B. juncea						***S. oleracea L.***					
	leaf/stem.			stem/root.			leaf/stem.			stem/root.		
	Soil	**CFA**	**CFA+Soil**	**Soil**	**CFA**	**CFA+Soil**	**Soil**	**CFA**	**CFA+Soil**	**Soil**	**CFA**	**CFA+Soil**
B	3.57	5.15	1.79	2.37	0.74	1.33	1.06	0.68	2.75	1.21	1.85	0.77
Cr	4.84	3.32	1.03	0.47	0.51	1.13	2.55	0.25	0.45	0.77	1.35	0.66
Mn	3.86	4.44	1.08	0.49	1.32	0.91	0.63	18.75	3.70	0.45	0.05	0.59
Ni	4.04	2.90	1.01	0.45	0.48	0.94	0.98	1.58	0.57	0.46	0.16	0.40
Cu	4.35	3.34	1.20	0.41	1.27	0.85	1.17	1.75	0.62	0.61	0.24	0.42
Zn	4.22	3.26	1.24	0.42	1.09	0.81	0.80	0.44	1.42	0.55	1.25	0.65
Mo	8.95	5.46	3.36	2.88	0.97	1.72	1.72	1.30	3.59	0.76	1.12	0.66
Ba	2.64	3.10	1.22	0.79	1.26	1.05	0.60	1.50	1.16	0.65	0.38	0.56
Fe	5.38	3.68	0.91	0.35	1.59	0.93	2.39	2.80	0.59	0.60	0.17	0.61

4. Conclusions and Recommendations.

The findings of the current study show that coal CFA is an aluminosilicate material with an alkaline pH. The CFA morphology showed that it has a lower degree of sphericity with irregular agglomerations of many particles while there were dominant spherical particles and smaller sharp needle like particles. There is a similarity in terms of the elemental composition of CFA and soil used in this study since SiO_2 is available in both mediums as a major oxide which gives an expectation of similar growth of the plants in both mediums. Chemical species such as B, Ba, Mo and Cr were occurring at higher concentrations in the leachates for most weeks in the pot culture experiments especially for CFA and soil + CFA growth media. Fe also dominated but only in soil as a growth media. In terms of the biomass estimation, growth performance and chlorophyll content of both plants, *S. oleracea L* showed better growth in the CFA media while *B. juncea* grew better in soil as a growth media. It is observed that chlorophyll a and b increase during first 69 days and decline in 115 days for most growth media. Furthermore there was some carotenoid content found in *B. juncea* while none was detected for *S. oleracea L*. Because carotenoid content is important in the protection of plant, this could have been attributed to better adaptability of *B. juncea* for growth in CFA and better bioaccumulator and translocator of chemical species hence could be more effective plant for phytoremediation than *S oleracea L*. The superiority of *B. juncea* was confirmed BCF and TF >1 for most chemical species as compared to *S. oleracea L.* This study observes that *B. juncea* is a potential plant species that can be applied at field scale for phytoremediation of coal FA dumps. This could significantly lead to reduction of environmental and health impacts associated with coal fly ash dumps especially for communities leaving near

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such coal fly ash storage facilities. However, there is still need for assessment of other plant species.

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Supplementary Materials

Table S1: Chemical elements concentrations in leachates obtained from soil pots growing *S. oleracea L.*

S. oleracea L.						
Values in mg L^{-1}						
Soil media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	14	7.38	8.22	6.22	3.14	1.28
Ca	25.78	23.30	4.74	109.90	3.26	1.05
K	29.44	27.45	9.30	48.88	7.52	0.69
Mg	15.22	8.85	2.39	60.79	2.45	0.33
Na	21.26	17.78	2.16	85.59	2.47	0.29
P	0.01	0.02	0.05	< 0.1	0.08	0.01
Si	14.65	16.13	11.25	167.90	32.96	0.95
Sr	0.14	0.37	0.03	0.75	0.03	0.01
Values in µg L^{-1}						
Li	<0.680	73.41	1.06	10.75	4.66	1.37
Be	0.01	0.11	0.07	1.34	0.42	<0.039
B	91.45	180.82	27.81	587.25	49.49	86.59
Ti	75.33	99.20	127.72	840.48	422.86	12.64
V	5.72	13.43	9.14	70.40	27.65	1.38
Cr	3.89	14.08	6.53	47.96	19.78	0.62
Mn	13.46	10.23	14.72	86.73	32.47	4.07
Fe	1997.88	3110.97	3564.41	39260.00	11790.00	297.21
Co	1.18	1.58	1.13	7.24	2.57	0.08
Ni	11.81	8.84	7.47	52.09	18.47	0.92
Cu	9.76	7.15	6.10	30.19	10.43	1.44
Zn	4.79	5.02	6.24	27.98	10.79	0.74
As	1.01	1.39	0.72	2.92	1.10	0.15
Se	1.48	1.84	0.66	2.06	<0.456	<0.456
Mo	0.22	14.16	0.24	0.52	0.21	0.21
Cd	0.03	0.03	0.00	0.03	0.02	0.01
Sb	0.12	0.34	0.09	0.22	0.11	0.04
Ba	136.54	92.90	54.80	402.76	133.97	5.50
Hg	0.01	0.01	<0.004	<0.003	0.00	0.00
Pb	0.40	0.49	0.55	6.56	1.70	0.05

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Table S2: Chemical elements concentrations in leachates obtained from CFA pots growing *S. oleracea L.*

S. oleracea L.						
Values in mg L^{-1}						
CFA media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	0.28	2.45	0.91	0.42	0.30	0.27
Ca	116.70	124.40	154.90	147.70	96.80	71.71
K	66.39	64.01	31.97	8.86	7.55	6.24
Mg	4.40	4.96	8.66	46.05	32.10	24.12
Na	60.37	50.95	16.64	9.53	7.95	6.42
P	0.03	< 0.005	< 0.005	0.03	0.03	0.03
Si	1.65	0.76	1.89	1.92	1.87	1.89
Sr	3.33	3.53	4.39	3.06	1.95	1.54
Values in µg L^{-1}						
Li	711.93	595.96	302.86	346.20	260.15	226.12
Be	0.01	0.00	0.01	<0.039	<0.039	<0.039
B	1377.09	1550.94	1492.71	6382.09	4936.09	4277.50
Ti	0.22	0.08	0.26	0.08	<0.070	<0.070
V	68.47	81.70	64.09	25.84	25.43	24.68
Cr	74.64	78.00	64.76	29.15	18.70	13.16
Mn	3.26	0.87	4.96	1.29	0.83	0.24
Fe	5.53	1.17	18	1.04	0.96	0.65
Co	0.51	0.50	0.37	0.31	0.25	0.21
Ni	1.13	0.82	1.08	0.67	0.39	0.45
Cu	0.89	1.15	3.09	0.72	0.56	0.69
Zn	0.69	0.74	2.24	<0.181	<0.181	<0.181
As	9.63	8.17	4.34	5.90	5.95	5.47
Se	5.71	9.84	8.48	13.11	9.70	8.79
Mo	126.45	122.56	109.19	73.12	47.39	32.61
Cd	0.02	0.03	0.03	0.04	0.03	0.04
Sb	3.78	4.53	17	9.85	8.94	8.55
Ba	132.43	108.06	143.88	69.18	65.46	63.00
Hg	0.01	0.01	0.02	0.02	0.01	0.01
Pb	0.04	0.02	0.06	0.01	0.01	0.00

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Table S3: Chemical elements concentrations in leachates obtained from CFA + soil pots growing *S. oleracea L.*

S. oleracea L.						
Values in mg L^{-1}						
CFA + soil media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	0.03	0.03	0.03	0.03	0.02	0.02
Ca	235.30	226.10	135.20	68.88	50.29	6.27
K	42.39	37.46	25.42	3.46	7.04	0.52
Mg	25.77	21.71	18.55	23.72	15.71	1.40
Na	32.15	31.00	8.37	7.99	6.06	0.41
P	0.05	0.04	0.08	0.13	0.37	0.06
Si	14	5.05	3.05	4.69	3.60	0.60
Sr	3.66	3.42	2.23	1.70	1.11	0.13
Values in µg L^{-1}						
Li	25.32	20.34	52.78	35.08	19.00	3.20
Be	0.00	0.01	0.00	<0.039	<0.039	<0.039
B	2155.13	1762.79	1591.22	3110.40	1809.38	177.83
Ti	0.18	0.17	0.48	0.41	0.34	1.26
V	15.36	12.41	13.67	31.27	22.91	4.85
Cr	25.62	34.31	11.95	8.75	3.60	0.41
Mn	0.40	1.18	136.78	0.12	0.17	0.67
Fe	1.87	14	8.44	7.14	6.63	22.36
Co	1.54	1.45	1.99	1.25	0.70	0.07
Ni	1.65	2.01	2.24	1.19	0.95	0.38
Cu	2.57	2.46	2.21	2.47	2.72	1.03
Zn	0.63	0.58	0.50	<0.181	<0.181	<0.181
As	10.16	8.18	7.76	9.63	6.95	1.33
Se	5.05	5	3.11	1.54	0.75	<0.456
Mo	132.64	121.16	86.08	40.06	18.36	1.16
Cd	0.03	0.04	0.02	0.02	0.01	0.00
Sb	6.00	4.66	4.00	6.72	4.38	0.32
Ba	205.63	237.13	170.99	96.47	70.92	10.63
Hg	0.01	0.02	0.01	0.02	0.01	0.00
Pb	0.03	0.03	0.03	0.00	0.01	0.03

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Table S4: Chemical elements concentrations in leachates obtained from soil pots growing *B. juncea*.

B. juncea						
Values in mg L^{-1}						
Soil media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	1.79	2.64	0.89	0.67	0.52	0.32
Ca	29.50	30.88	5.73	110.40	3.32	0.99
K	28.49	25.39	11.45	48.95	7.37	0.72
Mg	16.29	17.70	2.33	60.30	2.44	0.33
Na	20.62	17.65	2.35	81.25	2.47	0.32
P	0.01	0.01	0.02	< 0.1	0.07	0.02
Si	11.58	12.52	1.90	159.80	32.58	0.94
Sr	0.17	0.19	0.04	0.76	0.03	0.01
Values in µg L^{-1}						
Li	<0.680	1.75	0.89	12.13	4.81	0.92
Be	0.05	0.04	0.06	1.96	0.42	<0.039
B	79.81	88.07	23.49	922.35	29.30	10.74
Ti	29.81	38.39	16.47	722.26	4314	12.85
V	2.70	4.03	1.83	61.38	27.87	1.33
Cr	1.65	2.52	0.94	41.20	20.02	0.66
Mn	8.38	5.11	85.83	72.55	33.10	4.35
Fe	779.13	1140.13	437.07	36620.00	11640.00	287.61
Co	0.89	1.12	0.62	6.02	2.61	0.08
Ni	7.06	6.26	2.32	45.65	18.72	0.85
Cu	7.66	6.64	3.18	28.16	10.47	1.37
Zn	3.54	3.43	1.49	24.86	11.01	0.88
As	0.64	0.67	0.54	2.45	1.09	0.28
Se	1.10	1.26	0.45	1.05	<0.456	<0.456
Mo	1.07	0.75	0.32	0.97	0.18	0.14
Cd	0.03	0.03	0.00	0.03	0.01	0.01
Sb	0.11	0.09	0.05	0.22	0.11	0.03
Ba	139.06	146.75	17.96	367.56	135.07	5.42
Hg	0.00	0.00	<0.004	<0.003	0.00	<0.003
Pb	0.18	0.24	0.07	6.02	1.73	0.05

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Table S5: Chemical elements concentrations in leachates obtained from CFA pots growing *B. juncea*.

B. juncea						
Values in mg L^{-1}						
CFA media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	1.68	2.44	0.90	0.18	0.21	0.09
Ca	97.71	127.60	151.20	138.20	97.63	78.12
K	47.07	55.85	39.60	8.62	7.72	6.97
Mg	4.00	6.80	7.76	45.79	32.14	24.59
Na	31.67	40.40	19.22	9.22	8.10	7.20
P	< 0.005	0.01	< 0.005	0.03	0.04	0.03
Si	2.02	0.87	1.74	1.91	1.88	1.89
Sr	2.84	3.10	4.32	3.08	1.94	1.55
Values in µg L^{-1}						
Li	400.76	476.44	331.10	352.88	258.72	216.49
Be	0.00	0.01	0.00	<0.039	<0.039	<0.039
B	1365.75	1583.59	1491.62	6486.03	4901.60	4154.41
Ti	1.16	0.14	0.25	<0.070	<0.070	<0.070
V	54.04	71.46	66.39	25.92	25.28	25.03
Cr	62.75	64.82	74.56	29.15	18.61	13.04
Mn	0.92	0.44	0.89	1.07	0.75	0.48
Fe	35.56	3.12	1.56	1.13	0.93	0.62
Co	0.43	0.56	0.43	0.31	0.25	0.22
Ni	1.25	0.93	1.20	0.64	0.35	0.36
Cu	1.37	0.70	1.15	0.71	0.57	0.74
Zn	0.25	0.28	0.46	<0.181	<0.181	<0.181
As	3.42	8.56	5.42	6.06	5.86	5.17
Se	6.34	9.14	6.51	13.24	9.56	8.97
Mo	59.55	104.97	118.20	73.77	47.21	31.39
Cd	0.01	0.02	0.02	0.04	0.03	0.03
Sb	3.79	4.58	4.11	9.84	8.98	8.38
Ba	126.98	103.05	126.93	70.14	65.90	62.63
Hg	0.01	0.02	0.02	0.02	0.01	0.01
Pb	0.02	0.01	0.02	0.01	0.00	0.00

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Table S6: Chemical elements concentrations in leachates obtained from CFA + soil pots growing *B. juncea*.

B.juncea						
Values in mg L^{-1}						
CFA + soil media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	0.03	0.05	0.07	0.05	0.04	0.01
Ca	280.40	217.00	219.30	102.23	51.01	6.20
K	49.45	35.79	35.83	12.89	6.91	0.54
Mg	30.10	23.62	27.80	21.86	15.82	1.38
Na	49.82	29.15	19.87	15.22	6.13	0.44
P	0.03	0.06	0.04	0.04	0.04	0.04
Si	4.13	2.99	2.43	1.23	0.62	0.59
Sr	4.61	3.45	15	2.98	1.12	0.13
Values in µg L^{-1}						
Li	25.90	27.30	187.29	53.62	19.57	2.54
Be	0.01	0.00	0.01	0.04	<0.039	<0.039
B	1841.70	2140.25	2405.82	2100.15	1846.93	141.76
Ti	0.37	0.22	0.29	0.30	0.29	0.34
V	14.56	18.02	26.87	24.12	23.42	4.84
Cr	49.42	45.07	47.02	9.57	3.69	0.42
Mn	0.45	0.51	15.49	0.23	0.11	0.84
Fe	2.62	2.06	2.71	3.21	4.63	20.77
Co	1.78	1.51	2.90	1.23	0.74	0.06
Ni	2.78	1.29	2.64	2.03	0.93	0.32
Cu	4.67	1.60	4.11	3.20	2.78	0.98
Zn	1.42	0.30	0.81	0.62	<0.181	<0.181
As	11.04	10.36	7.83	8.21	7.20	1.33
Se	6.42	18	8.83	1.88	0.83	<0.456
Mo	189.41	132.89	163.92	68.25	18.74	1.07
Cd	0.03	0.02	0.03	0.02	0.01	0.01
Sb	5.24	6.15	6.89	6.32	4.46	0.32
Ba	235	180.79	208.23	126.24	71.63	10.94
Hg	0.02	0.02	0.03	0.01	0.01	0.00
Pb	0.04	0.02	0.04	0.02	0.01	0.03

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Table S7: Chemical elements concentrations in leachates obtained from CFA pots where no plants were grown.

No plants in the growth media						
Values in mg L^{-1}						
CFA media	**WEEK 1**	**WEEK 2**	**WEEK 5**	**WEEK 7**	**WEEK 9**	**WEEK 11**
Al	0.49	2.86	0.46	0.39	0.30	0.21
Ca	122.20	100.60	135.90	155.40	99.37	90.51
K	85.04	60.71	39.80	13.07	8.23	7.65
Mg	6.35	6.20	10.92	59.60	28.23	24.64
Na	78	49.17	21.15	11.11	6.36	5.76
P	0.01	0.01	0.00	0.03	0.01	0.03
Si	0.90	0.66	1.60	2.27	2.19	2.23
Sr	3.77	3.18	6	3.03	1.95	1.83
Values in µg L^{-1}						
Li	919.53	727.95	423.05	360.85	224.33	213.18
Be	0.00	0.00	0.00	<0.039	<0.039	<0.039
B	1544.72	1444.04	1826.02	6640.43	4556.76	4355.44
Ti	0.07	0.14	0.15	<0.070	<0.070	<0.070
V	75.56	82.59	75.19	30.13	25.87	26.84
Cr	111.31	98.10	112.31	24.13	15.19	12.91
Mn	0.96	0.67	1.24	7.32	39.83	0.17
Fe	0.59	1.15	0.73	0.92	0.55	0.52
Co	0.67	0.51	0.42	0.40	0.25	0.21
Ni	0.42	1.28	0.48	0.69	0.77	0.37
Cu	0.47	0.67	0.81	0.78	1.21	0.43
Zn	0.14	0.37	0.36	<0.181	<0.181	<0.181
As	8.47	9.39	6.42	10.79	6.63	6.05
Se	9.20	10.08	6.80	20.74	10.50	9.55
Mo	194.97	156	135.30	99.08	43.72	35.91
Cd	0.04	0.03	0.02	0.06	0.03	0.02
Sb	3.66	4.54	5.02	11.12	8.77	8.51
Ba	108.52	99.40	133.74	81.99	62.13	60.63
Hg	0.02	0.02	0.03	0.02	0.01	0.01
Pb	0.01	0.02	0.02	0.00	0.00	<0.002