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Phytoremediation potential of *Jatropha curcas* and *Pennisetum clandestinum* grown in polluted soil with and without coal fly ash: a case of BCL Cu/Ni mine, Selibe-Phikwe, Botswana

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Abstract

This study was conducted to identify plants which can be used for phytoremediation of the soils east and west of BCL Cu/Ni mine smelter [2.5 km east, 2.5 km west, 20 km west and 55 km west (control) of mine smelter]. Two ascensions of *Jatropha curcas* (J09 and J05) and *Pennisetum clandestinum* (Kikuyu) were raised in pots in the greenhouse in soil with and without coal fly ash. All plants failed to grow in soil from 2.5 km west of mine smelter without ash and addition of ash increased soil pH and enabled plants to survive. All species accumulated more metals when grown in soil without ash as compared to soil with ash thus translocation and bioaccumulation factors were higher in plants grown in soil without ash compared to soil with ash. Kikuyu was able to hyper accumulate Cu at 55 km west without ash while J05 and J09 failed to hyper accumulate any metal. Performance of species according to bioaccumulation factor followed the order Kikuyu> J05> J09. Heavy metal accumulation, translocation factor and bioaccumulation factor of heavy metals followed the order 55 km west> 2.5 km east> 20 km west of mine smelter. *Jatropha curcas* and *Pennisetum clandestinum* failed to hyper accumulate heavy metals so they are not viable candidates for the phytoextraction treatment of soils around the Selebi-Phikwe Cu/Ni mine. *Jatropha curcas* and *Pennisetum clandestinum* can be used for re-vegetation provided soil pH is increased and the heavy metal-contaminated soils are stabilized by coal fly ash addition.

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Introduction

There has been increasing concern over the accumulation of toxic heavy metals in the environment. Ekosse *et al.* (2003; 2004) and Vurayai *et al.* (2015) reported heavy metal pollution around the BCL Cu/Ni mine in Selebi-Phikwe, Botswana. Vegetation around Selebi-Phikwe mine has also been depleted due to the formation of dead zones especially on the western side of the mine smelter (Ekosse *et al.*, 2005) and this may also be a result of soil acidity (Vurayai *et al.*, 2015).

Heavy metals are dangerous to the environment due to their high level of toxicity to the biota (Alkorta *et al.*, 2004): they are non-degradable by neither microbial activity, chemical oxidation (Beiergrohslein, 1998) nor heat and thus accumulate readily to reach toxic levels (Bohn *et al.*, 1985). Heavy metal soil contamination may pose hazards to humans and the ecosystem through direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant-animal-human), drinking of contaminated ground water. The risks may include phytotoxi city, reduction in land usability for agricultural production causing food insecurity, and land tenure problems (McLaughlin *et al.*, 2000; Ling *et al.*, 2007). Polluted soils therefore require effective and affordable solutions.

Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance (Lichtfouse, 2014). Due to the expensive nature of these conventional remediation methods of heavy metals (Danh, 2009), biological treatments which employ use of living organisms to remove heavy metals from soil are continuously being researched. Presently, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil.

Phytoremediation is the use of metal accumulating plants to clean soil and water contaminated with toxic metals (Raskin *et al.*, 1997).

However the term phytoremediation encompasses several processes in plants: rhizofiltration, phytostabilization, phytodegradation and phytoextraction (Alkorta *et al.*, 2004) and in order to remove heavy metals from the soil phytoextraction is most preferred. Phytoextraction (also known as phytoaccumulation, phytoabsorption or phytosequestration) is the uptake of contaminants from soil or water by plant roots and their translocation to and accumulation in aboveground biomass (shoots) (Sekara *et al.*, 2005; Yoon *et al.*, 2006; Rafati *et al.*, 2011).

One of the approaches currently used to reach this goal is the use of plants with exceptional, natural metal-accumulating capacity, the so-called hyper accumulators. Hyper accumulators have the ability to grow on metalliferous soils and to accumulate extraordinarily high amounts of heavy metals in the aerial organs, far in excess of the levels found in the majority of species, without suffering phytotoxic effects (Rascio and Navari-Izzo, 2011). The success of the phytoextraction technique therefore depends on the identification and selection of appropriate plant species with hyper accumulation capacities for heavy metals and high biomass production and tolerance to pollution (González, 2012) hence screening and selection of plants species with superior remediation properties is important (Vara Prasad and de Oliveira Freitas, 2003).

Since plant properties favourable for use in phytoremediation include sufficient tolerance to site conditions, ability to accumulate multiple metal contaminants, fast growth and ability to be grown abundantly in large scale on wastelands, *Jatropha curcas* and *Pennisetum clandestinum* will be suitable for use in Selebi-Phikwe, Botswana. *Jatropha curcas* is known to bioaccumulate and bioconcentrate toxic heavy metals (Mohammad *et al.*, 2010). It is considered as a good accumulator of Cu, Zn, Fe, Pb, Al (Majid *et al.*, 2012), Cd, Ni, Zn, Cu, Cr, Pb (Chang *et al*, 2014) and Zn, Cu, Cr (Ahmadpour *et al.*, 2010). Apart from acting as an important alternative biofuel, *J. curcas* can grow in diverse types of soil conditions.

Studies by Madyiwa *et al.* (2002) also showed ability of Kikuyu grass to accumulate heavy metals such as Cu, Zn, Ni, and Pb above toxic levels without any signs of toxicity. It can accumulate Cr, Ni, Zn, Pb (Söğüt *et al.*, 2004) and As (Bech *et al.* 2002) in its upper parts. Previous studies demonstrated Kikuyu grass tolerance to salinity (Panuccio *et al.* 2002; Skerman and Riveros 1990), drought, and waterlogging (Whiteman 1990). The adaptability of kikuyu grass under multiple stress situations therefore suggests its possible utility in phytoremediation of Selebi-Phikwe's heavy metal contaminated soils.Soils around the mine have been reported to be acidic (Vurayai *et al.*, 2015) and for successful phytoremediation of these acidic soils, soil amendments like coal fly ash may change some physical-chemical properties of the soil, namely pH, redox potential, cation exchange capacity and texture. Coal fly ash has been reported as efficient for heavy metal stabilisation in contaminated areas thus enabling plants to grow (Mench *et al.*, 2000; Nachtegaal *et al.*, 2005).

This effect is attributed to the alkaline nature of coal fly ash which raises the soil pH reducing heavy metal bioavailability. Coal fly also contains almost all the essential plant nutrients (i.e. macronutrients including P, K, Ca, Mg and S and micronutrients like Fe, Mn, Zn, Cu, Co, B and Mo) and so improves soil fertility (Basu *et al.*, 2009).

The Morupule coal-fired power station in Palapye, Botswana produces 300t of coal fly ash per day and almost 70% of this is disposed of as a waste while only 30% is used in cement manufacturing (Sahu, 2008). Continuous disposal has reached alarming levels and use of this coal fly ash on polluted land around BCL Cu-Ni mine in Selebi-Phikwe might prevent an environmental catastrophe in both Selebi-Phikwe and Palapye.

The aim of the study was in-twofold: (i) to determine the heavy metal decontaminative capacity of *Jatropha curcas* and *Pennisetum clandestinum* and (ii) to investigate the effect of coal fly ash as an amendment in the remediation of polluted soil around Selebi-Phikwe Cu/Ni mine, Botswana.

Materials and methods

This experiment was carried out in October 2013-January 2014 and October 2014-January 2015. Soil was sampled from four different sites around the BCL Cu/Ni mine in Selebi-Phikwe, Botswana: 2.5km east, 2.5km west, 20km west and 55km west (control) of the BCL Cu/Ni mine smelter as described by Vurayai *et al.* (2015). Coal fly ash was collected from ash piles at the Morupule power station, Palapye, Botswana

Soil and heavy metal analysis

pH of coal fly ash was measured according to Hendershot *et al.* (1993) in five replicates. 7.5% coalfly ash per kg of dry soil was added to soils from the 4 different sites (2.5km east, 2.5km west, 20km west and 55km west of the BCL Cu/Ni mine smelter) and the pH of the soil and coal fly ash mixture was also measured in five replicates according to Hendershot *et al.* (1993). Heavy metal content of coal fly ash and soil mixed with coal fly ash was measured according to Vurayai *et al.* (2015).

Plant growth

Two ascensions of *Jatropha curcas* [GKM09-Tsamaya (J09) collected from Tsamaya, Botswana and GKM05-Mosetse (J05) collected from Mosetse, Botswana] were germinated in trays and raised in nursery beds for 2 weeks and transplanted into polyvinyl chloride (PVC) pots (210 mm × 230 mm) filled with soil (17 kg) collected from 2.5 km east, 2.5 km west, 20 km west and 55 km west of the mine smelter. The experiment was arranged in a 2 x 3 x 4 factorial design in a completely randomized block design with 5 replications. Plants were watered to reach 100% plant available water (Rosenthal *et al.*, 1987) every 2 days. Plants were also watered with half strength Hoagland solution once every week and the experiment ran for 90 days.

Determination of plant and soil heavy metal content

After plants were harvested, Cu, Ni, Fe, Mn, Zn, As, Cd, Co, Cr, Mo, Pb, Se, Sn, Li and Pt content in soil was measured using a Thermo Scientific™ iCAP™ 7400 ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry) as described by Vurayai *et al.* (2015).

Shoot and root samples were obtained per species per treatment per replication for all the plants. These were washed with deionized water and dried in an oven to constant weight. The samples were powdered by an electric grinder and 0.05g of each sample was used for digestion. 0.5 ml nitric acid, 2.5 ml hydrofluoric acid and 0.5 ml perchloric acid were added to the samples for the first cycle of digestion and were digested in a water bath at 50 °C for 2 hours. The acids were then dried on a hot plate at 150 °C. After drying only 3 ml nitric acid was added for the second cycle of digestion and the same procedure for digestion and drying was done as described on the first cycle.

After drying the acids 2 g of nitric acid was added to each sample and left to dissolve in a water bath. After dissolving, 8 g of deionized water was added to 2 g sample to make 10 g and this was used for heavy metal measurements. Heavy metal content was then measured using a Thermo Scientific™ iCAP™ 7400 ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometry) and Cu, Ni, Fe, Mn, Zn, As, Cd, Co, Cr, Mo, Pb, Se, Sn, Li and Pt were measured. Mean heavy metal content was expressed in mg/kg and relative standard deviation (RSD) was calculated and expressed as a percentage.

Quality control

For validating the analytical method standard reference material for plant samples BCR-670-Duckweed (*Lemna minor*) was used. Heavy metal limits of detections (LOD) and limits of quantification (LOQ) were calculated using according to Shrivastava and Gupta (2011).

$$LOD = yB + 3sB$$

$$LOQ = yB + 10sB$$

Where yB is the mean concentration of the blank and sB is standard deviation of the blank.

Translocation and bioaccumulation factors

Heavy metal translocation from root to shoot in plants was calculated using the formulae by Baker and Brooks (1989)

Translocation factor (TF) = Cs/Cr

Where

Cs = metal concentration in shoot (mg/kg)

Cr = metal concentration in roots (mg/kg)

TF>1 signifies that the plant effectively translocate heavy metals from roots to shoots.

Heavy metal Bioaccumulation factor was calculated according to Baker (1981) and Ma *et al.* (2001)

Bioaccumulation factor (BAF) = Cb/Cs

Where

Cb= heavy metal concentration in shoots (mg/kg)

Cs = heavy metal concentration in soil (mg/kg)

BAF is categorized as excluder (< 1), accumulator (1-10) and hyper accumulater (>10).

Statistical analysis

All the above experiments were repeated twice and pooled data is presented. The results were analysed using one-way ANOVA (IBM SPSS Statistics 22) and treatment means were compared using LSD at probability level of 0.05.

Results and discussion

Soil pH around Selebi Phikwe Cu/Ni minesmelter is acidic and it significantly increases ($p<0.05$) with distance from the mine smelter towards the west (Fig.1). Coal fly ash (100% ash) had a pH of 10.3 (alkaline) and application of coal fly ash caused a significant increase ($p<0.05$) in soil pH to above neutral 7 in all soils collected from various distances from the mine smelter.

Soil pH in soil collected from 2.5 km west of mine smelter increased from 3.36 to 7.01, 20 km west (5.63 to 7.31) 55 km west (6.28 to 7.64) (control) and 2.5 km east (4.3 to 7.45).

Similar types of results were observed by (Manoharan *et al.*, 2007) where coal fly ash also increased soil pH of acidic soils. According to Khan and Khan (1996), the increase in soil pH might be due to the neutralization of H^+ by alkali salts and also due to solubilization of basic metallic oxides of fly ash in soil. Coal fly ash can therefore be used as a soil buffering agent for polluted soils (Jalal and Goyal, 2006).

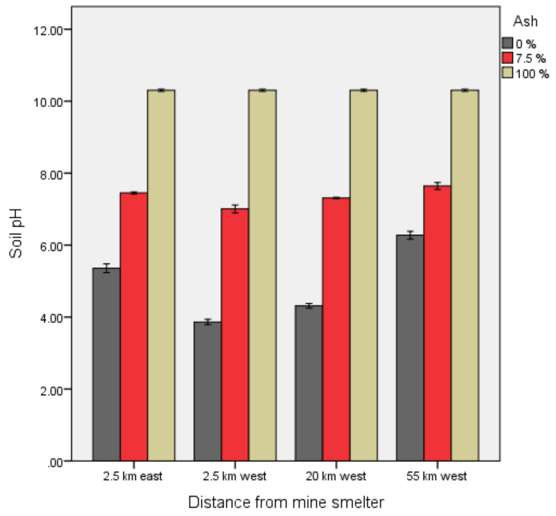

Fig. 1. Soil pH east and west of Selebi-Phikwe Cu/Ni mine smelter at different levels of coal fly ash. Error bars indicate ± standard error of the mean (n=5) (0% ash soil pH is according to Vurayai *et al.*, 2015).

Table 1 shows the elemental composition of fly ash and soil collected from sampling sites. The concentration of elements in 100% coal fly ash followed the order; from highest to lowest as Cr, Fe, Ni, As, Pb, Co, Li, Sn, Zn, Se, Mo, Cd, Mn and Cu. There were significant differences ($p<0.05$) between element content of 100% coal fly ash and soil without ash at all distances.

Addition of coal fly ash did not significantly increase ($p<0.05$) heavy metal content in soil for most metals. However it significantly increased Mo content at 55 km west and 2.5 km east, Se at 2.5 km east and Li at 2.5 km west. It has been reported by several authors that coal fly ash usually contains traces of heavy metals and may contaminate the soil (Adriano *et al.*, 1980; El-Mogazi *et al.*, 1988; Mishra *et al.*, 2007; Basu *et al.*, 2009). In order to reduce the risk of contamination Adriano *et al.* (1980) suggested that application of fly ash on agricultural soils should not exceed the 10% rate that's why 7.5 % coal fly ash was used in this study.

Table 2 shows the results of heavy metal concentrations on a plant certified standard reference material [Aquatic plant duckweed (*Lemna minor*) (BCR-670)] for As, Cd, Cr, Cu, Fe, Ni, Pb, Zn and Se. There was concordance between the results and certified concentrations and the recoveries obtained ranged from 91.4 % (As) to 134 % (Se).

Table 3 shows limit of detection (LOD) and limit of quantification (LOQ) of Cu, Fe, Mn, Zn, As, Cd, Co, Cr, Li, Mo, Ni, Pb, Pt, Se and Sn. LOD is expressed as the mean analyte concentration corresponding to the sample blank value plus three standard deviations of the blank and LOQ is expressed as the mean analyte concentration corresponding to the sample blank value plus ten standard deviations of the blank.

The mean heavy metal concentration in J05 (Table 4) showed that J05 accumulated more heavy metals in roots than shoots in all metals measured. J05 also accumulated more metals in soil without ash as compared to soil where ash was added. Overall heavy metal content in both roots and shoots followed the order 55 km west (control)> 2.5 km east > 20 km west > 2.5 km west of mine smelter except for Fe, Zn and Cd in J05.

Heavy metal concentration in J09 (Table 5) also showed that J09 roots accumulated more heavy metals than shoots in all metals measured. J09 also accumulated more metals in soil without ash as compared to soil where ash was added. Overall heavy metal content in both roots and shoots followed the order 55 km west (control)> 2.5 km east > 20 km west > 2.5 km west of mine smelter except for Ni and Pt.

Heavy metals accumulated more in roots as compared to shoots in Kikuyu (Table 6) except in Zn, As, Cr, Pt and Se which accumulated more heavy metals in shoots more than roots with and in soil without ash. Heavy metals content was however higher in soil without ash as compared to with ash and it followed the order 55 km west (control)> 2.5 km east > 20 km west > 2.5 km west of mine smelter except for Zn, As, Pt and Se. All plants did not manage to survive in soil collected 2.5 km west without ash this might be attributed to the plants' inability to overcome heavy metal toxicity and soil acidity. However all plants were able to survive in the same soil with coal fly ash.

This shows that heavy metal and low pH stress was alleviated by addition of coal fly ash. This might be attributed to coal fly ash increasing soil pH which reduces dissolution of heavy metals thus reducing heavy metal availability (Polat *et al.*, 2002; Su and Wong, 2003).

Table 1. Effect of coal fly ash (7.5 %) on heavy metal content (mg/kg) of soil collected from different directions from the BCL- Cu/Ni mine smelter in Selebi-Phikwe, Botswana. Error bars indicate ± standard error (n=5) [Metal content without ash (mg/kg) is according to Vurayai *et al.*, 2015).

Metal (mg/kg)	100% ash (mg/kg)	Distance from mine smelter							
		2.5 km west		20 km west		55 km west		2.5 km east	
		Without ash	With ash	Without ash	With ash	Without ash	With ash	Without ash	With ash
Cu	0.06±0.006	113 ± 0.63	114.3±0.71	14.39±0.17	14.4±0.21	0.91±0.05	0.93±0.07	12.6±0.71	13.2±0.76
Fe	9.41±0.11	4549±47.44	4560±38.9	1811±20.31	1831±22.4	665±9.92	692±9.4	1008±31.92	1010±30.5
Mn	0.58±0.04	36.3±1.11	37.1±1.44	13.5±0.16	13.56±0.11	16.15±0.64	17.3±0.8	15±0.33	15.2±0.37
Zn	1.65±0.06	3.23±0.12	3.2±0.11	3.36±0.05	4.4±0.07	2.48±0.14	2.49±0.08	2.69±0.05	3.2±0.07
As	6.36±0.15	3.23±0.08	3.1±0.13	4.09 0.06	4.12±0.05	1.75±0.08	1.9±0.07	2.8±0.14	2.76±0.09
Cd	0.66±0.07	0.48±0.01	0.5±0.07	0.2 5±0.01	0.27±0.03	0.36±0.02	0.35±0.03	0.28±0.01	0.3±0.02
Co	3.35±0.05	0.72±0.01	0.8±0.03	2.0 5±0.07	2.12±0.05	1.94±0.05	2.06±0.09	2.85±0.1	2.83±0.08
Cr	31.22±0.22	16.25±0.21	19.3±0.32	7.94±0.32	12.1±0.46	10.88±0.32	12.4±0.4	11.6±0.17	12.8±0.2
Mo	0.72±0.05	0.15±0.01	0.17±0.03	0.11±0.02	0.18±0.02	0.21±0.01	0.86±0.06	0.18±0.01	1±0.04
Ni	8.23±0.2	67.96±0.83	68.2±0.75	11.37±0.17	12.9±0.22	2.26±0.1	2.68±0.12	10.35±0.17	10.9±0.15
Pb	5.28±0.1	5.7±0.08	5.88±0.1	0.98±0.01	1.3±0.07	1.96±0.09	2.1±0.08	3.63±0.13	4±0.17
Se	0.79±0.03	1.04±0.05	1.2±0.06	0.87±0.03	0.89±0.02	0.29±0.02	0.28±0.01	0.86±0.01	1.71±0.06
Li	2.89±0.13	0.96±0.06	1.5±0.05	1.6±0.07	1.96±0.09	1.22±0.11	1.63±0.17	1.73±0.06	1.81±0.09
Pt	0±0.002	1.04±0.07	1.05±0.04	0.2±0.02	0.21±0.01	0.21±0.02	0.24±0.02	0.68±0.04	0.7±0.05
Sn	1.9 ± 0.06	0.75±0.06	0.8±0.77	1.06±0.04	1.4±0.03	0.52±0.04	0.61±0.05	0.62±0.05	0.9±0.05

Table 2. Heavy metal analysis of reference plant samples from an aquatic plant duckweed (*Lemna minor*) (BCR-670). Each value is the mean ± standard error.

Metal	Certified value (mg/kg)	Measured (mg/kg)	Recovery (%)
As	1.98	1.81±0.09	91.4
Cd	75.5	78.3±0.71	103.6
Cr	2.05	2.1±0.08	102.4
Cu	1.82	1.77±0.05	102.7
Fe	0.094-0.101	0.11±0.007	117-108.9
Ni	2.12-2.58	2.53±0.13	119.3-98.1
Pb	2.06	1.99±0.1	96.6
Zn	24	23.1±0.56	96.25
Se	0.149-0.273	0.18±0.009	120.8-134

Table 3. Heavy metal limit of detection and limit of quantification**.**

Metal	Limit of detection (mg/kg)	Limit of quantification (mg/kg)
Cu	0.0402	0.0872
Fe	0.0621	0.0903
Mn	0	0
Zn	0.197	0.3068
As	0.0534	0.0848
Cd	0	0
Co	0.1089	0.1763
Cr	0.1703	0.2644
Li	0	0
Mo	0.0414	0.0681
Ni	0.0134	0.0212
Pb	0.0467	0.0624
Pt	0.1424	0.148
Se	0	0
Sn	0.0267	0.0424

Table 4. Mean ionic concentration of roots and shoots of *Jatropha curcas* (J05). % RSD for all metals was < 3.6 % (n=5).

			Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km east	With ash	Roots	5.1	320	3	0.89	2.3	0.1	0.8	2.9	0.87	0.6	1.8	0.96	0.22	0.51	0.51
		Shoots	2.64	153	1.7	0.48	0.99	0.06	0.28	1.27	0.51	0.13	1	0.53	<LOQ	0.25	0.25
	Without ash	Roots	6.89	398	7	3.07	2.26	0.94	2.19	5.35	1.08	0.72	3.53	1.5	0.62	0.63	0.58
		Shoots	5.6	248	5.3	1.68	1.72	0.76	1.1	3.86	0.81	0.22	2.53	1.08	0.38	0.41	0.42
55 km west	With ash	Roots	6.21	348	4.47	1.01	2.74	0.12	0.7	3.2	0.91	0.8	2.19	1.45	0.4	0.24	0.69
		Shoots	3.9	201	3.13	0.58	1.52	0.09	0.31	1.47	0.6	0.15	1.32	0.92	0.22	0.13	0.4
	Without ash	Roots	10.1	517	9.5	3.22	2.46	1.42	2.54	6.46	1.1	0.97	3.8	1.42	0.74	0.51	0.86
		Shoots	9.34	422	9.2	2.16	2.15	1.27	1.86	5.7	0.98	0.46	3.52	1.38	0.63	0.45	0.73
20 km west	With ash	Roots	3.62	216	2.95	1.05	1.98	0.15	0.73	2.89	0.8	0.48	1.96	0.73	0.15	0.27	0.48
		Shoots	1.6	93	1.7	0.43	0.8	0.05	0.18	0.96	0.47	0.04	0.91	0.36	<LOQ	0.08	0.19
	Without ash	Roots	6.6	377	6.49	3.3	2.02	0.96	1.95	3.7	0.93	0.53	2.88	13.4	0.54	0.41	0.44
		Shoots	4.64	208	3.98	1.74	1.05	0.62	0.91	2.1	0.63	0.15	1.98	0.94	0.24	0.23	0.28
2.5 km west	With ash	Roots	3.2	230	2.7	0.91	1.8	0.12	0.38	1.92	0.68	0.19	1.91	0.64	<LOQ	0.22	0.36
		Shoots	1.2	83	1.1	0.31	0.5	0.03	0.07	0.55	0.3	0.02	0.73	0.21	<LOQ	0.04	0.11

Table 5. Ionic concentration of roots and shoots of *Jatropha curcas* (J09). % RSD for all metals was < 4.07 % (n=5).

			Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	Roots	4.2	302	3.6	1.78	2.1	0.08	0.8	3.3	0.92	0.6	1.8	0.86	0.19	0.33	0.45
		Shoots	2.14	135	1.84	0.78	1.2	0.05	0.28	1.34	0.48	0.14	1	0.4	0.07	0.13	0.18
	Without ash	Roots	6.89	256	7.4	2.7	2.1	0.99	2.19	5.12	1.28	0.7	3.53	1.58	0.51	0.5	0.5
		Shoots	5.6	132	5.8	1.98	1.51	0.73	1.1	3.54	0.78	0.24	2.53	1.4	0.37	0.37	0.32
55 km West	With ash	Roots	5.14	380	4.3	1.84	2.48	0.1	0.7	3.1	0.98	0.8	2	1.5	0.43	0.46	0.69
		Shoots	3.2	210	2.8	1.04	1.48	0.07	0.31	1.34	0.54	0.17	1.22	0.88	0.2	0.23	0.4
	Without ash	Roots	9.6	522	9.78	3.04	2.33	1.46	2.54	5.1	1.26	0.88	3.8	1.3	0.53	0.42	0.8
		Shoots	8.4	384	8.6	2.59	1.98	1.34	1.86	4.1	0.92	0.31	3.52	1.2	0.5	0.38	0.62
20 km West	With ash	Roots	2.9	220	3.6	1.53	1.9	0.09	0.73	2.2	0.91	0.38	1.96	0.8	0.13	0.2	0.32
		Shoots	1.14	84	1.46	0.64	0.92	0.04	0.18	0.88	0.37	0.02	0.91	0.27	0.04	0.08	0.1
	Without ash	Roots	6	350	5.7	2.1	2.7	0.87	1.95	3.42	1.2	0.5	2.88	1.45	0.6	0.3	0.36
		Shoots	3.8	198	3.28	1.39	1.4	0.53	0.91	1.91	0.54	0.12	1.98	0.83	0.31	0.17	0.2
2.5 km West	With ash	Roots	2.8	212	2.8	1.23	0.92	0.08	0.38	1.99	0.71	0.13	1.91	0.73	0.16	0.15	0.28
		Shoots	0.8	62	0.9	0.42	0.37	0.03	0.07	0.62	0.23	0.02	0.73	0.16	0.02	0.05	0.08

Table 6. Ionic concentration of roots and shoots of *Pennisetum clandestinum* (Kikuyu). % RSD for all metals was < 2.77 % (n=5).

			Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	Roots	5.31	382	3.62	0.98	0.89	0.25	0.22	1.37	0.3	0.97	5.68	1.81	0.07	0.58	0.31
		Shoots	3.4	288	2.63	1.75	1.42	0.07	0.14	1.98	0.12	0.15	3.21	1.2	0.12	0.88	0.18
	Without Ash	Roots	14.9	492	11.45	2.01	1.44	1.37	1.73	1.37	0.39	1.32	7.2	2.45	0.24	0.9	0.67
		Shoots	11.2	396	9.42	4.2	2.53	1.02	1.4	2.98	0.31	0.29	5.32	2.06	0.47	1.52	0.56
55 km West	With ash	Roots	5.46	426	4.29	1.1	1.11	0.3	0.3	2.35	0.38	1.08	6.79	2.1	0.09	0.7	0.46
		Shoots	4.1	318	3.67	2.15	1.86	0.13	0.21	1.7	0.2	0.16	4.39	1.53	0.17	1.16	0.29
	Without ash	Roots	14.96	626	11.63	2.2	1.57	1.75	1.89	1.69	0.65	1.89	8.1	1.11	0.37	1.02	0.63
		Shoots	14.78	552	11.34	5.24	2.87	1.46	1.77	4.8	0.57	0.59	7.61	2.4	0.86	1.9	0.61
20 km West	With ash	Roots	4.9	209	3.08	1.99	0.83	0.23	0.11	1.55	0.24	0.76	4.52	3.2	0.06	0.52	0.25
		Shoots	2.4	113	1.93	2.39	1.23	0.05	0.06	1.14	0.06	0.05	2.3	2.93	0.09	0.73	0.11
	Without ash	Roots	13.2	536	10.2	0.94	1.32	1.13	1.44	1.59	0.36	0.66	6.9	1.99	0.12	0.83	0.5
		Shoots	7.09	354	5.76	1.26	2.25	0.74	1.11	2.62	0.23	0.15	4.7	1.57	0.23	1.33	0.33
2.5 km West	With Ash	Roots	4.4	301	9.57	0.29	0.76	0.18	0.07	0.73	0.03	0.21	2.82	1.11	0.04	0.25	0.18
		Shoots	1.9	96	4.67	0.3	0.97	0.03	0.03	0.85	0.01	0.03	1.08	0.38	0.05	0.32	0.06

The main purpose of this study was to assess the ability of plants in accumulating heavy metals under heavy metal and low pH stress and all plants used in this study accumulated heavy metals in varying degrees. Similar studies have shown that kikuyu (Bech *et al.*, 2002; Madyiwa *et al.*, 2002; Sogut *et al.*, 2005) and *Jatropha curcas* (Ahmadpour *et al.*, 2010) accumulated heavy metals when grown in heavy metal contaminated soil. Heavy metal accumulation followed the order 55 km west (control)> 2.5 km east > 20 km west in soil without ash.

This may be attributed to heavy metal and low pH stress impacted the efficiency of plants to concentrate heavy metals into their biomass and also that heavy metals are generally more available at low soil pH (Pessarakli, 1999). Previous studies have shown that *Jatropha* plants in control medium soils accumulated more metals (Majid *et al.*, 2012).

However, Devi Chinmayee *et al.* (2014) indicated that *Jatropha curcas* plants grown in heavy metal contaminated soil accumulated enormous quantity of Pb, Cd and Cr as compared to control plant.

Application of coal fly ash resulted in a reduction in heavy metal accumulation in all plants. Similar results were reported by Gupta *et al.* (2007) and Gupta and Sinha (2009) where application of fly ash decreased the uptake of heavy metals. This may be attributed to the alkaline nature of the coal fly ash used (pH 10.3) which raised the soil pH (Fig. 1) making heavy metals unavailable and to the coexistence of constituents potentially capable of absorbing heavy metals. This therefore reduces the amount of heavy metals which can be accumulated by plants.

Translocation factor (TF) of J05 and J09 (Table 7 and 8, respectively) growing on polluted soil from 2.5 km east and west and 20 km west of the mine smelter was less than 1 for all metals while that of Kikuyu (Table 9) was < 1 for some metals and >1 for some. Previous studies have shown that metal accumulation in *Jatropha* follows the trend roots> shoots (Wu *et al.*, 2011; Majid *et al.*, 2012) as accumulation of heavy metals in *J. curcas* occurs mainly in the roots and to a lesser degree in the shoots. This is however not in line with (Ahmadpour *et al.*, 2010) who stated that *Jatropha curcas* accumulated highest levels of Pb and Cr in the leaves and stem, respectively.

Kikuyu exhibited the highest number of metals which had a TF> 1 [5 metals (Zn, As, Cr, Pt, and Se)] while J05 and J09 had 0 metals. The efficiency of phytoremediation is determined by the ability of the plant to translocate and concentrate heavy metals into their shoots (Rai *et al.*, 2004). TF which is defined as the ratio of metal concentration in shoots and roots is used to measure the effectiveness of a plant in translocating heavy metals from roots to shoots (Zhao *et al.*, 2003). Metal translocation to shoots is a crucial biochemical process and is desirable in an effective phytoextraction because the harvest of root biomass is generally not feasible (Zacchini *et al.*, 2009 and Tangahu *et al.*, 2011).

TF>1 signifies that the plant effectively translocate heavy metals from roots to shoots and so plants exhibiting TF values less than one are unsuitable for phytoextraction (Gupta and Sinha 2006).

The main aim of this study is to identify plants which can be used for phytoremediation of land around the Selebi-Phikwe Cu-Ni mine in Botswana and so plants which can phytoextract heavy metals and translocate them to the easily harvestable plant species (shoots) are most preferred (TF>1). Kikuyu can be considered for phytoremediation since it has a TF>1 for 5 metals.

The bioaccumulation factor (BAF) expressed as the ratio of metal concentration in plants to the concentration of metal in soil is used to measure the effectiveness of a plant in concentrating heavy metals into its biomass. In this regard, a plant is categorized as an excluder (<1), accumulator (1-10) and hyperaccumulator (>10) (Baker, 1981; Ma *et al.*, 2001). BAF (Table 10) shows that J05 is an excluder of most metals since it displayed a bioaccumulation factor (BAF) of <1. It is however an accumulator of Cd (2.5 km east without ash; 20 km west without ash), Mo (2.5 km east without; 20 km west without ash) and Pt (20 km west without ash). J09 is an excluder of most metals as they have a BAF of < 1 (Table 11). It is an accumulator of Cd (20 km without ash) and Se (20 km west without ash). J09 was however not able to hyper accumulate any metal.

Table 12 is showing that Kikuyu excludes most metals since their BAF is less than 1. It however accumulated Zn (2.5 km east without ash), Cd (2.5 km east without ash; 20 km west without ash), Mo (2.5 km east without ash; 20 km west without ash), Pb (20 km west with and without ash), Pt (20 km west without ash) and Se (2.5 km east without ash; 20 km west without ash). Kikuyu had a BAF >10 (16.24) with Cu at 55 km west without ash and J05 (10.26) with Cu at 55 km west in soil without ash. Both Kikuyu and J05 cannot be characterized as a hyper accumulator of Cu since the soil they were growing in is control soil (collected 55 km west of mine smelter) and was not polluted with heavy metals. For a plant to be classified as a hype accumulator despite having a BAF>10 it must also be growing insoils with exceptionally high heavy metal concentration (Milner and Kochian, 2008; Rascio and Navari-Izzo, 2011).

Table 7. Translocation factor from root to shoot of *Jatropha curcas* (05) grown on heavy metal contaminated soil amended with coal fly ash.% RSD for all metals was < 4.09 % (n=5).

		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	0.52	0.48	0.57	0.54	0.43	0.6	0.35	0.44	0.59	0.22	0.56	0.55	0.41	0.48	0.49
	Without Ash	0.82	0.62	0.76	0.55	0.76	0.81	0.5	0.72	0.75	0.31	0.72	0.72	0.61	0.65	0.72
55 km West	With ash	0.63	0.58	0.7	0.57	0.55	0.75	0.44	0.46	0.66	0.19	0.6	0.63	0.55	0.54	0.58
	Without ash	0.92	0.82	0.97	0.67	0.87	0.89	0.73	0.88	0.89	0.47	0.93	0.97	0.85	0.88	0.85
20 km West	With ash	0.44	0.43	0.58	0.41	0.4	0.33	0.25	0.33	0.59	0.08	0.46	0.49	0.27	0.3	0.41
	Without ash	0.7	0.55	0.62	0.53	0.52	0.65	0.47	0.57	0.68	0.25	0.69	0.70	0.44	0.57	0.64
2.5 km West	With Ash	0.38	0.36	0.41	0.34	0.28	0.25	0.18	0.29	0.44	0.11	0.38	0.33	0.15	0.18	0.31

Table 8. Translocation factor from root to shoot of *Jatropha curcas* (09) grown on heavy metal contaminated soil amended with coal fly ash.% RSD for all metals was < 5.12 % (n=5).

		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	0.51	0.45	0.51	0.49	0.57	0.63	0.35	0.41	0.52	0.23	0.56	0.47	0.37	0.39	0.4
	Without ash	0.81	0.52	0.78	0.74	0.72	0.74	0.5	0.69	0.61	0.34	0.72	0.89	0.73	0.74	0.64
55 km West	With ash	0.62	0.55	0.65	0.57	0.6	0.7	0.44	0.43	0.55	0.21	0.61	0.59	0.47	0.5	0.58
	Without ash	0.88	0.74	0.88	0.85	0.85	0.92	0.73	0.8	0.73	0.35	0.93	0.92	0.94	0.9	0.78
20 km West	With ash	0.39	0.38	0.41	0.42	0.48	0.44	0.25	0.4	0.41	0.05	0.46	0.34	0.31	0.4	0.31
	Without ash	0.63	0.57	0.58	0.66	0.52	0.61	0.47	0.56	0.45	0.25	0.69	0.57	0.52	0.57	0.56
2.5 km West	With ash	0.29	0.29	0.32	0.34	0.4	0.38	0.18	0.31	0.32	0.15	0.38	0.22	0.13	0.33	0.29

Table 9. Translocation factor (TF) from root to shoot of *Pennisetum clandestinum* (Kikuyu grass) grown on heavy metal contaminated soil amended with coal fly ash. % RSD for all metals was < 5.34 % (n=5).

		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	0.64	0.75	0.73	1.79	1.6	0.28	0.64	1.45	0.4	0.15	0.57	0.66	1.71	1.52	0.58
	Without Ash	0.75	0.8	0.82	2.09	1.76	0.74	0.81	2.17	0.79	0.22	0.74	0.84	1.96	1.69	0.84
55 km West	With ash	0.75	0.75	0.86	1.95	1.68	0.43	0.7	1.83	0.53	0.15	0.65	0.73	1.9	1.66	0.63
	Without ash	0.99	0.88	0.96	2.38	1.83	0.83	0.94	2.84	0.88	0.31	0.94	2.16	2.32	1.87	0.97
20 km West	With ash	0.49	0.54	0.63	1.2	1.48	0.22	0.55	1.36	0.25	0.07	0.51	0.92	1.5	1.4	0.44
	Without ash	0.54	0.66	0.56	1.34	1.7	0.65	0.77	1.65	0.64	0.23	0.68	0.79	1.92	1.6	0.66
2.5 km West	With Ash	0.43	0.31	0.49	1.03	1.28	0.17	0.43	1.16	0.33	0.14	0.38	0.34	1.25	1.28	0.33

Table 10. Bioaccumulation factor (BAF) of *Jatropha curcas* (J05). %RSD for all metals was < 6.98 % (n=5).

		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	0.2	0.15	0.11	0.15	0.36	0.2	0.1	0.1	0.28	0.13	0.09	0.13	0.13	0.07	0.28
	Without ash	0.44	0.25	0.35	0.62	0.62	2.71	0.39	0.33	0.47	1.22	0.24	0.3	0.56	0.48	0.68
55 km West	With ash	4.19	0.29	0.18	0.23	0.8	0.26	0.15	0.12	0.37	0.17	0.49	0.44	0.92	0.46	0.66
	Without ash	10.26	0.63	0.57	0.87	1.23	3.53	0.96	0.52	0.8	2.19	1.56	0.71	3	1.55	1.4
20 km West	With ash	0.11	0.05	0.13	0.1	0.19	0.19	0.08	0.08	0.24	0.22	0.07	0.28	0.19	0.09	0.14
	Without ash	0.31	0.11	0.29	0.52	0.26	2.5	0.44	0.26	0.39	1.18	0.17	0.96	1.2	0.26	0.26
2.5 km West	With ash	0.01	0.02	0.03	0.1	0.16	0.06	0.09	0.03	0.2	0.12	0.01	0.04	0.02	0.03	0.14

Table 11. Bioaccumulation factor (BAF) of *Jatropha curcas*(09). %RSD for all metals was < 7.03 % (n=5).

		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	0.16	0.13	0.12	0.27	0.43	0.17	0.1	0.1	0.27	0.14	0.09	0.1	0.1	0.08	0.2
	Without ash	0.44	0.13	0.39	0.74	0.54	2.6	0.39	0.31	0.45	1.33	0.24	0.39	0.54	0.43	0.52
55 km West	With ash	3.44	0.3	0.16	0.42	0.78	0.2	0.15	0.11	0.33	0.2	0.46	0.42	0.83	0.82	0.66
	Without ash	9.23	0.58	0.53	1.04	1.13	3.72	0.96	0.38	0.75	1.48	1.56	0.61	2.38	1.31	1.19
20 km West	With ash	0.08	0.05	0.11	0.15	0.22	0.14	0.08	0.07	0.19	0.11	0.07	0.21	0.19	0.09	0.07
	Without ash	0.26	0.11	0.24	0.41	0.34	2.12	0.44	0.24	0.34	1.13	0.17	0.85	1.55	0.2	0.19
2.5 km West	With ash	0.01	0.01	0.02	0.13	0.12	0.06	0.09	0.03	0.15	0.12	0.01	0.03	0.02	0.04	0.1

Table 12. Bioaccumulation factor (BAF) of *Pennisetum clandestinum*. %RSD for all metals was < 6.82 % (n=5).

		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5 km East	With ash	0.26	0.29	0.17	0.55	0.51	0.23	0.05	0.15	0.07	0.15	0.29	0.3	0.17	0.52	0.2
	Without ash	0.88	0.39	0.63	1.56	0.9	3.64	0.49	0.26	0.18	1.61	0.51	0.57	0.69	1.77	0.9
55 km West	With ash	4.41	0.46	0.21	0.87	0.98	0.37	0.1	0.19	0.12	0.19	1.64	0.73	0.71	4.14	0.48
	Without ash	16.24	0.83	0.7	2.11	1.64	4.01	0.91	0.44	0.47	2.8	3.37	1.22	4.1	6.55	1.17
20 km West	With ash	0.17	0.06	0.14	0.55	0.3	0.19	0.03	0.13	0.03	0.28	0.18	2.26	0.43	0.82	0.08
	Without ash	0.48	0.2	0.43	0.38	0.55	2.96	0.54	0.33	0.14	1.36	0.41	1.6	1.15	1.53	0.31
2.5 km West	With ash	0.02	0.02	0.13	0.09	0.31	0.06	0.04	0.04	0.007	0.18	0.02	0.07	0.05	0.27	0.08

Performance of species according to number of distances with BAF>1 follows the order kikuyu (10)> J05 and J09 (5) making Kikuyu the most suitable plant for phytoremediation of soil contaminated with various metals. It should be noted that none of the plants used in this study was able to hyper accumulate metals when grown in soil collected from the targeted areas for phytoremediation (2.5 km east and west and 20km west of mine smelter).

TF and BAF of most metals followed the order 55 km west > 2.5 km east > 20 km west. This may be attributed to the fact that heavy metals are more available at low soil pH and heavy metal and soil pH stress impacted the efficiency of plants to concentrate heavy metals into their biomass (Pessarakli, 1999).

Conclusions

Coal fly ash from Morupule power station, Palapye is alkaline and when added to soil collected east and west of Selebi-Phikwe Cu/Ni mine it increased the pH to above neutral. *Jatropha curcas* and *Pennisetum clandestinum* did not survive in soil collected from 2.5 km west of mine smelter (without ash) but survived in soil were ash was added (with ash). *Jatropha curcas* (J05 and J09) wasable to accumulate heavy metals but more so in roots than in shoots while *Pennisetum clandestinum* accumulated some metals in roots and some in shoots. Translocation factor of *Jatropha curcas* (J05 and J09) was less than 1 for all metals while that one of Kikuyu was <1 for some metals and >1 for some. Application of coal fly ash reduced heavy accumulation in plants and so these plants accumulated more heavy metals when grown in soil without ash as compared to soil with ash. Their TF and BAF were thus highest in soil without coal fly ash as compared to soil with ash.Heavy metal accumulation, TF and BAF in all plants followed the order 55 km west (control)> 2.5 km east> 20 km west of mine smelter. *Jatropha curcas* and *Pennisetum clandestinum* failed to hyperaccumulate heavy metals when grown in soil collected from the targeted areas for phytoremediation (2.5 km east and west and 20km west of mine smelter) therefore they are both not viable candidates for the phytoextraction treatment of soils closest to Selebi-Phikwe mine.

Kikuyu hyperaccumulated Cu when grown in control soil (collected 55 km west of mine smelter) which is not the targeted place for phytoremediation. *Jatropha curcas* and *Pennisetum clandestinum* therefore can be used for revegetating the area provided soil pH is increased and heavy metals are stabilized with coal fly ash.

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