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Evaluation of phytoremediation potential of *Moringa oleifera* and *Moringa stenopetala* when grown in polluted soil with and without coal fly ash

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Abstract

Phytoremediation potential of *Moringa oleifera* and *Moringa stenopetala* on polluted lands of east and west of Bamangwato Concessions Limited Cu/Ni mine smelter, Selebi-Phikwe, Botswana was evaluated. Plants were raised in greenhouse (pots) in soils collected 2.5 km east, 2.5 km west, 20 km west, and 55 km west (control) of the mine smelter, which were supplemented with and without coal fly ash. Without ash, both species did not survive in soils collected 2.5 and 20 km west. In soils from 2.5 km east, soil acidity and heavy metal stress reduced vegetative growth and total dry weight. Both species accumulated more metals in roots than in shoots, and failed to hyperaccumulate any metal. In soils collected 2.5 and 20 km west, coal fly ash enabled plants to survive, reduced accumulation of majority of heavy metals and increased vegetative growth and total dry weight. Heavy metal accumulation, translocation and bioaccumulation factors of plants grown in soils with ash generally followed the order 55 km west> 2.5 km east> 20 km west> 2.5 km west. *Moringa oleifera* and *Moringa stenopetala* are not good candidates for phytoextraction of heavy metals east and west of mine smelter, but have a potential for phytostabilisation with the help of coal fly ash.

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Introduction

The occurrence of Cu-, Ni- and Zn- sulphidic ores in Selebi-Phikwe, Botswana (*Barrie et al.,* 1993) and their mining and smelting by the Bamangwato Concessions Limited (BCL) Cu/Ni mine has resulted in soil pollution. Soils around mine are enriched with heavy metals and have very low pH (Ekosse *et al.*, 2003; 2004; Schippers *et al.*, 2007, Vurayai *et al.*, 2015). The soils are contaminated through deposition of particulate air matter (PAM), wash-offs, and other wastes due to mining and smelting activities (Ekosse *et al.*, 2005; Ekosse and Ngole, 2012 and Likuku *et al.*, 2013). The mineral contents identified in the PAM in Selebi-Phikwe are reflective of acidic nature and these minerals combine with H_2S, SO_2 and atmospheric moisture to release H_2SO_4. These reactions result in acid rain which lowers the pH of the soil and destroys vegetation. Vegetation around the mine, mainly on the western side, was depleted resulting on formation of dead zones (Ekosse *et al.*, 2005).

Heavy metals cannot be chemically degraded and hence persist in the soil for thousands of years and pose numerous health dangers to higher organisms (Roy *et al.*, 2005) and so the remediation and revegetation of areas around the mine appears to be the most suitable method for long term land reclamation. Low-cost and ecologically sustainable remedial options are required to restore contaminated lands and due to the expensive nature of conventional remediation methods of heavy metals (Danh, 2009), biological treatments which employs the use of living organisms to remove heavy metals from soil are continuously being researched. Phytoremediation has thus become an effective and affordable technological solution used to extract and remove heavy metals from the soil.

Phytoremediation is a process that uses plants to remove, transfer, or stabilize contaminants in soil, sediment, and ground water (Yu and Gu, 2007). It is also called green remediation, botano-remediation, agroremediation, or vegetative remediation (Pivetz, 2001). Heavy metal uptake by plants through phytoremediation technologies uses many mechanisms (phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization) and one of them is phytoextraction. The metals are taken up by the root system and transported to the stems and leaves (phytoextraction) without showing a toxicity syndrome (Cardwell *et al.*, 2002; Chatterjee and Chatterjee, 2000) thereby reducing the concentration of metals in contaminated soils to regulatory levels within a reasonable time frame. Phytoextraction seems to be the most promising technique and has received increasing attention from researchers since it was proposed by Chaney (1983). It depends on the ability of plants to grow and accumulate metals in harvestable tissues. The success of phytoextraction depends on the identification and selection of plants with exceptional, natural metal-accumulating capacity: the so-called hyperaccumulators. Screening and selection of plants species with superior remediation properties is therefore very important (Prasad and Freitas, 2003).

Another phytoremediation mechanism which can be used in areas around the BCL Cu/Ni mine is phytostabilisation. Phytostabilisation is the use of plants to immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing their migration in soil, as well as their movement by erosion and deflation (Erdei, 2005; Erakhrumen and Agbontalor, 2007). Phytostabilization is a less invasive, low-cost phytotechnology which in combination with amendments (i.e. aided phytostabilisation) has a potential to restore the physical, chemical, and biological properties of polluted soils (Bolan *et al.*, 2003; Kumpiene *et al.*, 2008). Amendments such as coal fly ash (alkaline) can improve phytostabilisation (Bolan *et al.*, 2003; Mench *et al.*, 2010) by increasing soil pH of acidic soils around mines. A lower soil pH and redox potential enhances the mobility of most metals, rendering them more bioavailable (Mench *et al.*, 2003) and coal fly ash increases soil pH immobilizing heavy metals thus reducing their availability to plants (Polat *et al.*, 2002; Su and Wong, 2004; Tsang *et al.*, 2014). This enables plants to survive, vegetate the area and stabilize the contaminants in the soil.

The tropical plants of the family of *Moringaceae* are used as a source of water purifying agents in developing countries. Seeds of *Moringa oleifera* are used to detoxify water by absorbing heavy metals like Cu, Pb, Cd, Cr, and Zn (Sajid *et al.*, 2005; Nand, 2012). Seed powder of *Moringa* is considered as capable of absorbing Cd and Pd from polluted water (Mataka *et al.*, 2006; Mataka *et al.*, 2010). Other studies have shown that *M. oleifera* (Amadi and Tanee, 2014; Offor *et al.*, 2014) and *Moringa stenopetala* (Yimer and Khan, 2016) accumulated heavy metals. Both species are characterized by high biomass yield and can tolerate unfavourable environmental conditions. They are drought tolerant and are found in locations with as little as 500 mm annual rainfall and can be grown in a wide range of soils. They also tolerate light frost and require little care after planting (Bosch, 2004; Jiru *et al.*, 2006). This makes them most suitable for use as phytoremedial plants in the semi-arid region of Selebi-Phikwe.

The aims of the presented study was (i) to determine the morphological responses of *M. oleifera* and *M. stenopetala* when grown in acidic and heavy metal contaminated soil, (ii) to determine the heavy metal decontaminative capacity of *M. oleifera* and *M. stenopetala* and (iii) to investigate use of coal fly ash in the remediation of polluted soil collected east and west of the BCL Cu/Ni mine smelter in Selebi-Phikwe, Botswana.

Materials and methods

Soil and coal fly ash collection

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

Plant growth

M. oleifera and *M. stenopetala* seeds were germinated at an average temperature of 28°c in trays. After radical and apical meristems emerged, seedlings were transplanted onto the nursery bed with potting soils and allowed to grow. After two weeks, healthy seedlings were selected as experimental plants, and transplanted into polyvinyl chloride (PVC) plastic pots (210 × 230 mm). The pots were filled with 17 kg of four different soils described above. The 7.5% coal fly ash was added per kg of dry soil. The experiment was arranged in a 2 × 2 × 4 factorial design in a completely randomized block design with 5 replications. There were 3 factors: Plant species (*M. oleifera* and *M. stenopetala*), fly ash (with and without fly ash) and distance from the mine smelter (2.5 km east, 2.5 km west, 20 km west and 55 km west-control). The planted pots were spaced 30 cm apart to preclude competition effects among treatments, and the 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 (Hoagland and Arnon, 1950) once every week and the experiment ran for 90 days.

Growth measurements

The total number of leaves was recorded 90 days after seedling transplanting per species per treatment per replication. Stem diameter was measured 20 cm from the soil surface with a Vernier calipers. Plant height was determined by measuring the distance from soil level to the top of plants with a millimetre ruler. For total dry weight measurement, plants were harvested (uprooted) 90 days after seedling transplantation, and washed with tap water to remove soil. The harvested tissues were allowed to dry in an oven to constant weight at 60°C, and their total dry weights were measured with an electric balance.

Plant and soil heavy metal analyses

Heavy metal content in soil after harvest was measured as described by Vurayai *et al.* (2015), while heavy metal content in plants was measured as described by Vurayai *et al.* (2017).

Bioaccumulation factor

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

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 hyperaccumulater (>10).

Translocation factor

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.

Statistical analysis

All the above experiments were repeated twice and pooled data is presented. Statistical analyses were performed by ANOVA using IBM SPSS Statistics 22. Treatment means were compared using LSD at probability level of 0.05.

Results and discussion

Plant growth

M. oleifera and *M. stenopetala* did not survive in soil collected from 2.5 km west and 20 km west of the mine smelter (Fig. 1 and 2). This might be attributed to an inability of the plants to overcome heavy metal toxicity and soil acidity. Vurayai *et al.* (2015) indicated that the soil is polluted with heavy metals and also has a low soil pH of 3.36 (2.5 km west) and 4.3 (20 km west). *Moringa* is said to grow well in soil pH between 6 and 7 (Jiru *et al.*, 2006).

Table 1. Ionic concentration (mg/kg Dw^{-1}) of roots and shoots of *M. oleifera*. The % RSD for all metals was < 4.58 % (n=5).

Distance from Mine smelter			Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5km East	With ash	Roots	2.3	156	1.44	1.1	0.94	0.09	0.8	2.99	0.15	0.95	2.1	0.6	0.11	0.24	0.28
		Shoots	1.2	393	3.37	1.42	0.6	0.04	0.51	1.69	0.09	0.2	1.31	0	0.05	0.11	0.16
	Without ash	Roots	5.38	540	4	1.86	1.1	0.47	1.2	4.7	0.37	1.87	3.1	0.99	0.32	0.46	0.43
		Shoots	3.8	341	2.87	0.87	0.78	0.33	0.91	3.24	0.31	0.41	2.3	0	0.21	0.3	0.3
55km West	With ash	Roots	3.53	202	2.2	1.29	1.1	0.14	1.02	3.3	0.27	1.12	2.78	0.9	0.17	0.32	0.43
		Shoots	2.24	590	4.07	1.85	0.8	0.08	0.74	2.3	0.19	0.24	1.92	0	0.09	0.23	0.28
	Without ash	Roots	6.58	607	4.2	2.52	1.2	0.83	1.48	4.85	0.85	2.05	3.33	1.16	0.58	0.48	0.57
		Shoots	5.39	492	3.99	1.36	1.1	0.76	1.33	4.02	0.68	0.72	3.13	0	0.45	0.39	0.48
20km West	With ash	Roots	1.94	122	1.1	0.98	0.83	0.04	0.24	2.2	0.14	0.82	1.95	0.47	0.08	0.2	0.3
		Shoots	0.78	227	2.2	1.07	0.56	0.2	0.11	0.97	0.06	0.07	1.04	0	0.02	0.08	0.09
2.5km West	With ash	Roots	1.3	94	0.9	0.42	0.9	0.04	0.28	1.7	0.09	0.46	1.85	0.29	0.05	0.09	0.2
		Shoots	0.42	120	1.2	0.58	0.44	0.01	0.03	0.41	0.02	0.03	0.79	0	0	0.02	0.04

In general, plant growth is limited in acid soils by a variety of factors including direct effect of pH (excess H^+ concentration), which if in excess causes cell death and consequent plant death. Moreover, the soil acidic pH induces heavy metal toxicity and causes plant death, since low soil pH enhances the mobility of heavy metals, rendering them more bioavailable (Mench *et al.*, 2003). If in excess, heavy metals affect various physiological processes, ultimately causing cell and plant death (Shahid *et al.*, 2015).

Both *M. oleifera* and *M. stenopetala* were able to grow in soil with coal fly ash (Figs 1-4). Coal fly ash used in this study was alkaline (pH 10.3) therefore was able to increase soil pH of both soils (2.5 km and 20 km west of mine smelter) to above neutral 7 (Vurayai *et al.*, 2017). Increase in soil pH after adding alkaline ash can be attributed to the neutralisation of H^+ by alkali salts and also due to basic solubilisation of basic metals oxides of fly ash in soil (Khan and Khan, 1996).

Table 2. Ionic concentration (mg/kg Dw^{-1}) of roots and shoots of *M. stenopetala*. The %RSD for all metals was <4.8 % (n=5).

Distance from Mine smelter			Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5km East	With ash	Roots	2.5	162	1.58	1.3	0.81	0.09	0.97	3.3	0.17	1.05	1.82	0.9	0.08	0.24	0.32
		Shoots	1.68	428	3.2	1.66	0.56	0.06	0.63	1.98	0.09	0.16	1.15	0	0.04	0.17	0.08
	Without ash	Roots	5.3	584	4.72	1.22	1.29	0.53	0.96	5.01	0.37	1.77	3.9	1.06	0.37	0.48	0.19
		Shoots	3.92	352	2.2	0.68	0.93	0.41	0.85	3.56	0.3	0.47	2.93	0	0.29	0.35	0.12
55km West	With ash	Roots	4.36	180	2.1	1.39	0.91	0.16	1.08	3.5	0.34	1.35	1.74	0.99	0.15	0.38	0.26
		Shoots	3.06	610	4.66	1.95	0.68	0.12	0.88	2.18	0.24	0.29	1.29	0	0.07	0.31	0.04
	Without ash	Roots	7.19	680	5.1	2.78	1.43	0.9	1.21	4.18	0.89	2.17	3.42	1.85	0.59	0.49	0.18
		Shoots	6.06	478	4.42	1.95	1.3	0.83	1.18	4.17	0.74	0.71	3.11	0	0.51	0.48	0.04
20km West	With ash	Roots	1.84	135	1.59	0.72	1.22	0.05	0.29	1.93	0.14	0.94	2.11	0.4	0.11	0.16	0.69
		Shoots	0.97	340	2.28	1.3	0.63	0.03	0.17	0.97	0.05	0.08	1.26	0	0.05	0.1	0.09
2.5km West	With ash	Roots	1.4	76	0.92	0.39	1.1	0.05	0.38	2.1	0.09	0.51	1.62	0.16	0.38	0.07	0.72
		Shoots	0.51	166	1.09	0.5	0.38	0.01	0.03	0.56	0.04	0.05	0.91	0	0.02	0.03	0.23

The current study seeks to identify plants which can be used for phytoremediation of land east and west of mine smelter in Selebi-Phikwe Cu/Ni mine and so plants which are capable of producing a high biomass in the presence of heavy metal and soil acidity stress will be most suitable for use in phytoremediation of the area (Baker *et al.*, 1994; Ebbs and Kochian 1997; 1998). Biomass production by plants with phytoextraction (removal of metals by plants) abilities is therefore a significant factor contributing to the success of phytoremediation.

Table 3. Translocation factor (TF) from root to shoot of *M. oleifera* grown on heavy metal contaminated soil amended with coal fly ash. The % RSD for all metals was < 6.07 % (n=5).

Distance from Mine smelter		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5km East	With ash	0.52	2.52	2.34	1.29	0.64	0.44	0.64	0.57	0.6	0.21	0.62	0	0.46	0.46	0.57
	Without Ash	0.71	0.63	0.72	0.47	0.71	0.7	0.76	0.69	0.84	0.22	0.74	0	0.66	0.65	0.7
55km West	With ash	0.63	2.92	1.85	1.43	0.73	0.57	0.73	0.7	0.7	0.21	0.69	0	0.53	0.72	0.65
	Without ash	0.82	0.81	0.95	0.54	0.92	0.92	0.9	0.83	0.8	0.35	0.94	0	0.78	0.81	0.84
20km West	With ash	0.4	1.86	2	1.09	0.67	0.5	0.46	0.44	0.43	0.09	0.53	0	0.25	0.4	0.3
2.5km West	With Ash	0.32	1.28	1.33	1.38	0.49	0.25	0.11	0.24	0.22	0.07	0.43	0	0	0.22	0.2

Heavy metal and acidity stress in the soils collected 2.5 km east, 2.5, and 20 km west of the mine smelter significantly reduced total dry weight of all the plant species as compared to the control soil collected from the 55 km west ($p<0.05$) (Fig. 5). As compared to the dry weights of plants grown in the control soil, 36 and 27% reduction in total dry weight was observed for *M. oleifera* and *M. stenopetala*, respectively, when grown in soil collected from 2.5 km east of mine smelter. Therefore a degree of reduction followed the order *M. stenopetala* < *M. oleifera* in this case. Reduction in total dry weight can be ascribed to reduction in vegetative growth (leaf number, stem diameter and plant height) (Figs 6, 7, and 8).

Table 4. Translocation factor (TF) from root to shoot of *M. stenopetala* grown on heavy metal contaminated soil amended with coal fly. The % RSD for all metals was < 4.65 % (n=5).

Distance from mine smelter		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5km East	With ash	0.67	2.64	2.03	1.28	0.69	0.67	0.65	0.6	0.53	0.15	0.63	0	0.5	0.71	0.25
	Without Ash	0.74	0.6	0.47	0.56	0.72	0.77	0.89	0.71	0.81	0.27	0.75	0	0.78	0.73	0.63
55km West	With ash	0.7	3.39	2.22	1.4	0.75	0.75	0.81	0.62	0.71	0.21	0.74	0	0.47	0.82	0.15
	Without ash	0.84	0.7	0.87	0.7	0.91	0.92	0.98	0.87	0.83	0.33	0.91	0	0.87	0.98	0.22
20km West	With ash	0.53	2.52	1.44	1.81	0.52	0.6	0.59	0.5	0.36	0.09	0.6	0	0.45	0.63	0.13
2.5km West	With Ash	0.36	2.18	1.18	1.28	0.35	0.2	0.08	0.27	0.44	0.1	0.56	0	0.05	0.43	0.32

Leaf number, stem diameter and plant height of both *M. oleifera* and *M. stenopetala* were significantly reduced when grown in soil collected 2.5 km east as compared to the control soil from 55 km west ($p<0.05$). Leaf number decreased by 28 % in *M. oleifera* and 33 % in *M. stenopetala* (Fig. 6). Stem diameter decreased by 35 % in *M. stenopetala* and 55 % in *M. oleifera* while plant height decreased by 22 % in *M. stenopetala* and 25 % in *M. oleifera* (Figs 7 and 6).These observations suggest that vegetative growth of *M. oleifera* and *M. stenopetala* was reduced due to soil acidity and high heavy metal content. Prolonged exposure of roots to low pH leads to suppression of lateral root development and in extreme cases this leads to death of the root tips (Khan *et al.*, 2013). Availability of most plant essential elements depends on soil pH and so the contents of mineral nutrients in plants decrease with the decrease of pH (Pessarakli, 1999). Heavy metal also affect biological and physiological functions of plants which results in a decrease in vegetative growth or biomass production as shown in (Figs 6–8).

Table 5. Bioaccumulation factor (BAF) of *M. oleifera*. The %RSD for all metals was < 7.14 % (n=5).

Distance from mine smelter		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5km East	With ash	0.09	0.39	0.22	0.44	0.22	0.13	0.18	0.13	0.05	0.2	0.12	0	0.07	0.06	0.18
	Without ash	0.3	0.34	0.19	0.32	0.28	1.18	0.32	0.28	0.18	2.28	0.22	0	0.31	0.35	0.48
55km West	With ash	2.4	0.85	0.24	0.74	0.42	0.23	0.36	0.19	0.12	0.28	0.72	0	0.38	0.82	0.46
	Without ash	5.92	0.74	0.25	0.55	0.63	2.11	0.69	0.37	0.56	3.43	1.39	0	2.14	1.34	0.92
20km West	With ash	0.05	0.12	0.17	0.24	0.14	0.07	0.05	0.08	0.03	0.64	0.08	0	0.1	0.09	0.06
2.5km West	With ash	0.004	0.03	0.03	0.18	0.14	0.02	0.04	0.02	0.01	0.18	0.01	0	0	0.02	0.05

Addition of alkaline coal fly ash increased soil pH to above neutral 7 (Vurayai *et al.*, 2017), significantly increased vegetative growth which culminated into increase in total dry weight in *M. oleifera* and *M. stenopetala* grown in soil collected 2.5 km east and 55 km west (Figs 5–8). In soil collected 2.5 km east, *M. oleifera* had the greater enhancement in total dry weight as compared to *M. stenopetala* (Fig. 5). Enhanced growth after addition of fly ash has been reported in many plants (Jala and Goyal 2006; Agrawal *et al.* 2006). Mittra *et al* (2005) demonstrated increased growth of rice plants on soil amended with fly ash, and Yunusa *et al* (2009) observed increases in dry weight in canola with application of coal fly ash on acidic soil. Incorporation of alkaline coal fly ash increases soil pH and immobilizes heavy metals thus reducing their availability to plants (Polat *et al.*, 2002; Su and Wong, 2004; Tsang *et al.*, 2014), leading to improvement in plant growth. Incorporation of coal fly ash also enabled *M. oleifera* and *M. stenopetala* to survive in soil collected 2.5 km and 20 km west of the mine smelter (Figs 1-4). This shows that coal fly ash has potential for use as an aid in phytostabilisation.

Heavy metal uptake

Subsequently, the ability of plants in accumulating heavy metals under heavy metal and low pH stress was evaluated. *M. oleifera* and *M. stenopetala* accumulated heavy metals with varying degree

(Tables 1 and 2). Similar studies have shown that *M. oleifera* (Amadi and Tanee, 2014; Offor *et al*., 2014) and *M. stenopetala* (Yimer and Khan 2016) accumulated heavy metals. For plants to be successfully used for phytoextraction of heavy metals they have to accumulate metals in the shoots for easiness of harvest. *M. oleifera* and *M. stenopetala* accumulated most metals in the roots as compared to shoots (Tables 1 and 2), showing that both plants are not favorable for phytoextraction, since successful phytoextraction depends on plants which can accumulate metals in harvestable shoots (Cardwell *et al*., 2002; Chatterjee and Chatterjee, 2000).

Table 6. Bioaccumulation factor (BAF) of *M. stenopetala*. The %RSD for all metals was < 7. 45 % (n=5).

Distancefrom Mine smelter		Cu	Fe	Mn	Zn	As	Cd	Co	Cr	Li	Mo	Ni	Pb	Pt	Se	Sn
2.5km East	With ash	0.13	0.42	0.21	0.52	0.2	0.2	0.22	0.15	0.05	0.16	0.11	0	0.06	0.1	0.09
	Without ash	0.31	0.35	0.15	0.25	0.33	1.46	0.3	0.31	0.17	2.61	0.28	0	0.43	0.41	0.19
55km West	With ash	3.29	0.88	0.27	0.78	0.36	0.34	0.43	0.18	0.15	0.34	0.48	0	0.29	1.1	0.07
	Without ash	6.66	0.72	0.27	0.77	0.74	2.3	0.61	0.38	0.6	3.38	1.38	0	2.43	1.66	0.08
20km West	With ash	0.07	0.19	0.17	0.3	0.15	0.11	0.08	0.08	0.03	0.73	0.1	0	0.24	0.11	0.06
2.5km West	With ash	0.004	0.04	0.03	0.16	0.12	0.02	0.04	0.03	0.03	0.29	0.01	0	0.02	0.03	0.29

Translocation factors of heavy metals from root to shoot in both *M. oleifera* and *M. stenopetala* were less than 1 for most metals, except Fe, Mn and Zn where they were above 1 on soils where coal fly ash was added at all distances (Tables 3 and 4). Translocation factor for both *M. oleifera* and *M. stenopetala* was however highest on soils without ash except for Fe, Zn and Mn. Comparison of species has shown that both *M. oleifera* and *M. stenopetala* were able to translocate Fe, Zn and Mn to a TF> 1 in soils with ash (Table 3 and 4).

Fig. 1. *Moringa oleifera* plants growing in soil collected from 2.5 km west of mine smelter with and without coal fly ash.

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). These results therefore indicate that *M. oleifera* and *M. stenopetala* do not fulfil the requirements of a good phytoremediator for land east and west of the BCL mine smelter.

Fig. 2. *Moringa stenopetala* plants growing in soil collected from 2.5 km west of mine smelter with and without coal fly ash.

M. oleifera was an excluder for most metals especially on soils with coal fly ash except for Cu at 55 km west (Table 5). It is however an accumulator of Cu (55 km west with and without ash), Cd (2.5 km east without ash; 55 km west without ash), Mo (2.5 km east without ash; 55 km west without ash), Ni (55 km west without ash), Pt (55 km west without ash), and Se (55 km west without ash). *M. stenopetala* was also an excluder of most metals, except for Cu (55 km west with and without ash), Cd (2.5 km without ash), Mo (2.5 km east without ash; 55 km west without ash), Ni (55 km west without ash), Pt (55 km west without ash), and Se (55 km west with and without ash) (Table 6). Under ash condition, *M. stenopetala* was an excluder for all metals except Cu and Se at 55 km west. *M. oleifera* and *M. stenopetala* however failed to hyperaccumulate any metal and performance of species according to number of distances with BAF>1 (accumulators) follows the order *Moringa oleifera* (9) > *Moringa stenopetala* (7). Failure of *M. oleifera* and *M. stenopetala* to hyperaccumulate metals further indicates that both species are not favourable for phytoextraction of heavy metals of polluted soil east and west of the BCL Cu/Ni mine since the success of phytoextraction depends on use of plant species which are tolerant to pollution and have hyperaccumulation capacities (Gonzalez, 2012). *M. oleifera* and *M. stenopetala* failed to survive in soil collected 2.5 km west and 20 km west of the mine smelter (Fig. 1-4) thus showing that they are both not tolerant to pollution. *M. oleifera* and *M. stenopetala* accumulated more metals in soil without coal fly ash as compared to with fly ash and heavy metal levels in control plants (55 km west) was generally higher than the other distances (Table 1; Table 2). Coal fly ash immobilizes heavy metals in soil reducing their availability to plants (Polat *et al.*, 2002; Su and Wong, 2004; Tsang *et al.*, 2014).

Fig. 3. *Moringa oleifera* plants growing in soil collected from 20 km west of mine smelter with and without coal fly ash.

The reduction of heavy metal accumulation in plants growing in soil with ash therefore reduce the impact of heavy metals on plant growth which resulted in the increase in vegetative growth (leaf number, plant height and stem diameter) which increased biomass production (total dry weight) (Fig. 5; 6; 7; 8).

Fig. 4. *Moringa stenopetala* plants growing in soil collected from 20 km west of mine smelter with and without coal fly ash.

The indicators of heavy metal accumulation, TF and BAF generally followed the order 55 km west > 2.5 km east for plants grown in soil without ash, and 55 km west > 2.5 km east > 20 km west for plants grown in soil with ash (Table 3-6). This is attributed to soil pH which followed the same trend (55 km west > 2.5 km east > 20 km west > 2.5 km east) in both soil with and without coal fly ash (Vurayai *et al.*, 2015; Vurayai *et al.*, 2017). Heavy metal bioavailability increases at low soil pH (Mench *et al.*, 2003) and so a combination of heavy metal and low pH stress may have impacted the efficiency of plants accumulate metals.

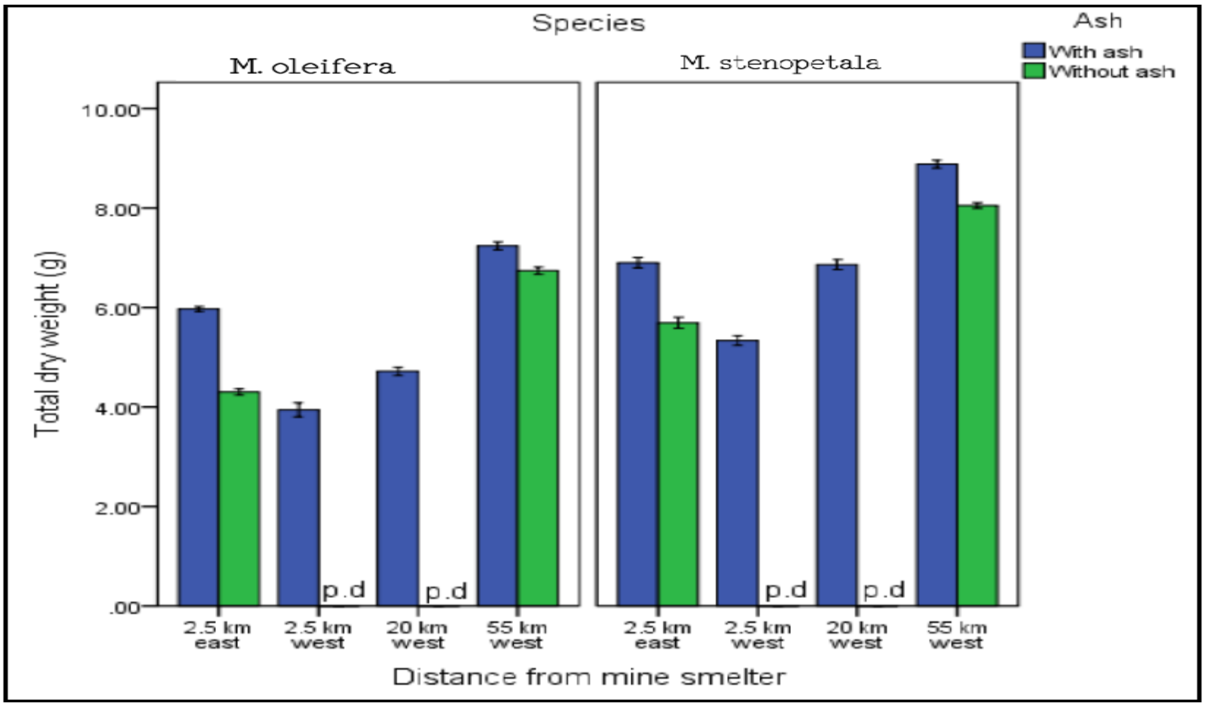

Fig. 5. Effect of coal fly ash treatment (7.5%) of polluted soil on total dry weight of *Moringa oleifera* (Mo O) and *Moringa stenopetala* (Mo S). Error bars indicate ± standard error (n=5) and p.d indicates that plants died.

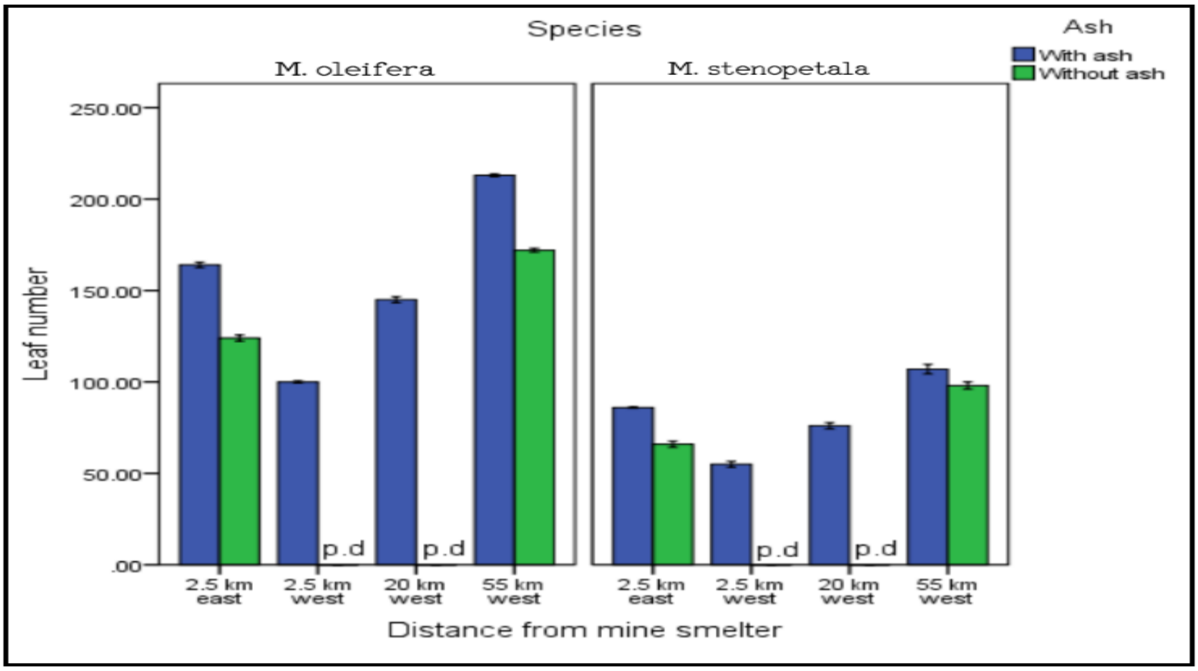

Fig. 6. Effect of coal fly ash treatment (7.5%) of polluted soil on leaf number *Moringa oleifera* (Mo O) and *Moringa stenopetala* (Mo S). Error bars indicate ± standard error (n=5) and p.d indicates that plants died.

The current study has indicated that *M. oleifera* and *M. stenopetala* are not good candidates for phytoextraction but this does not mean that they cannot be used for phytoremediation of the area east and west of the BCL mine smelter.

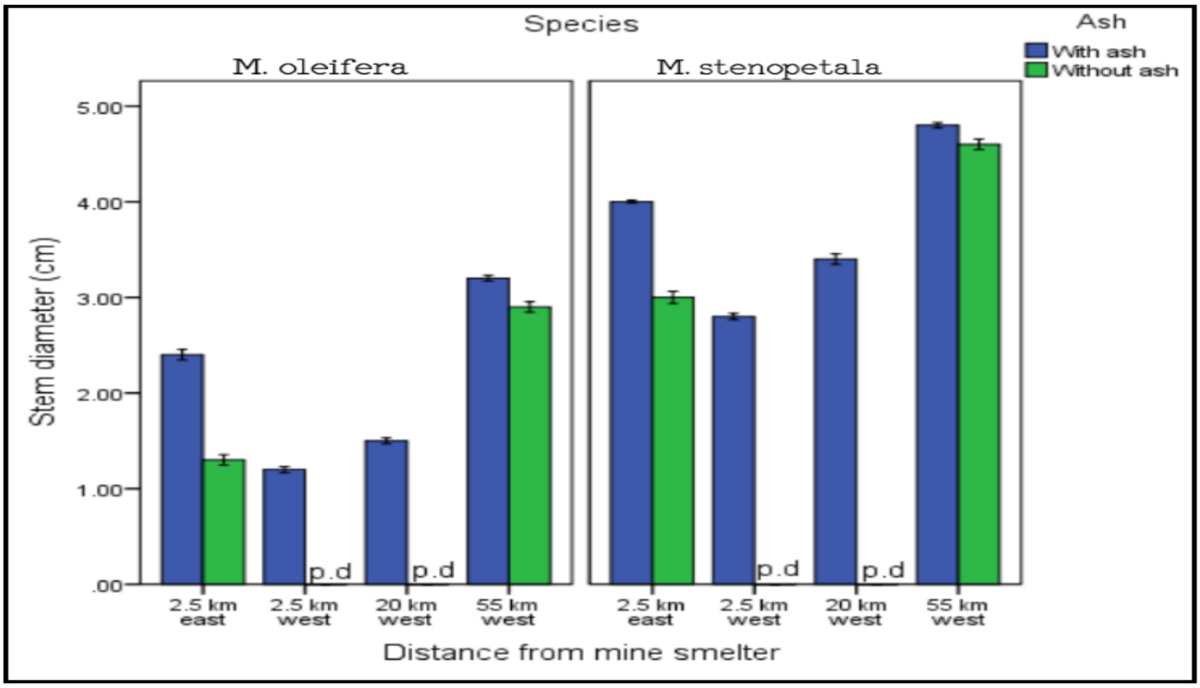

Fig. 7. Effect of coal fly ash treatment (7.5%) of polluted soil on stem diameter of *Moringa oleifera* (Mo O) and *Moringa stenopetala* (Mo S). Error bars indicate ± standard error (n=5) and p.d indicates that plants died.

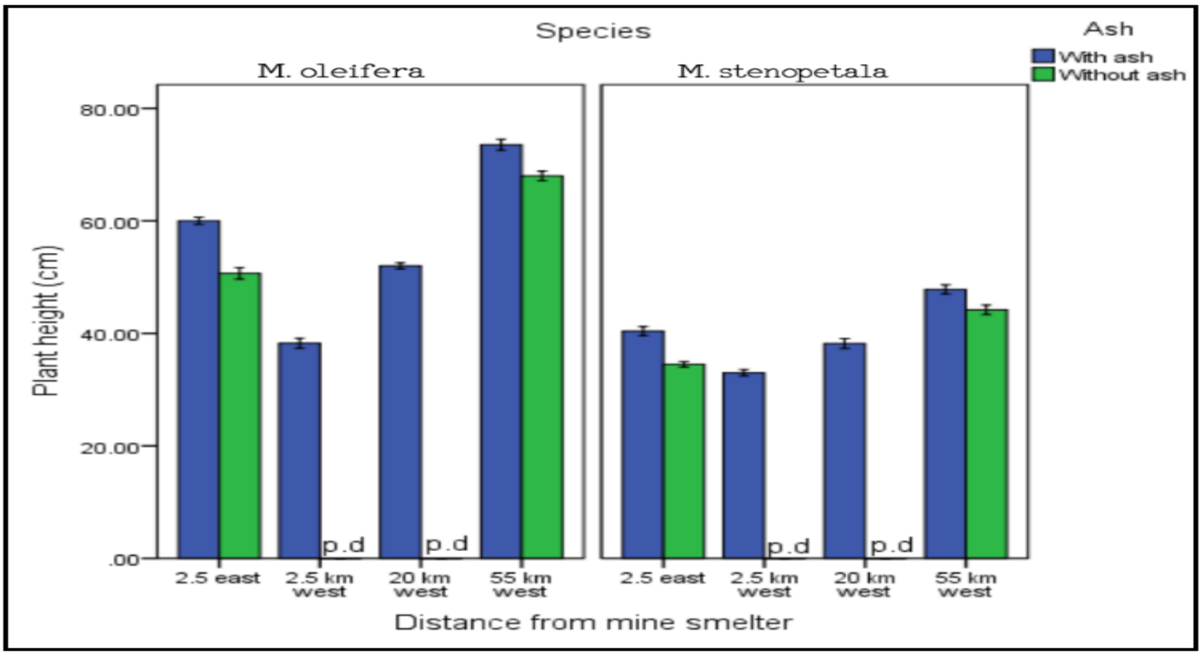

Fig. 8. Effect of coal fly ash treatment (7.5%) of polluted soil on plant height of *Moringa oleifera* (Mo O) and *Moringa stenopetala* (Mo S). Error bars indicate ± standard error (n=5) and p.d indicates that plant.

Coal fly ash has been shown to impede accumulation of heavy metals in plant biomass and so can be effective in situ remediation or aiding phytostabilisation since it enabled *M. oleifera* and *M. stenopetala* to survive polluted soils. Addition of coal fly ash enables plants to survive and grow, and immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing their migration in soil, as well as their movement by erosion and deflation (Erdei, 2005; Erakhrumen and Agbontalor, 2007).

Conclusion

M. oleifera and *M. stenopetala* failed to survive in polluted soils collected 2.5 and 20 km west of the mine smelter, showing that both species are not good candidates for phytoremediation of the area. Soil acidity and heavy metal stress reduced vegetative growth (leaf number, stem diameter, plant height) of both species, which resulted in decrease in total dry weight. Both species also failed to effectively translocate metals to shoots as they accumulated more metals in roots than in shoots.

They also both failed to hyperaccumulate any metal in all polluted soils examined, further confirming that both species are not good candidates for phytoremediation of the area. Application of coal fly ash enabled *M. oleifera* and *M. stenopetala* to survive in soil collected 2.5 and 20 km west of mine smelter. Coal fly ash reduced heavy metal accumulation in both species at all distances, which led to reduction in translocation and bioaccumulation factors.

Heavy metal accumulation generally followed the order 55 km west> 2.5 km east> 20 km west> 2.5 km east which is the same order for soil pH from highest to lowest. Coal fly ash can therefore be used for aiding phytostabilisation as it enables plants to survive in polluted soil. *M. oleifera* and *M. stenopetala* are not good candidates for phytoextraction of heavy metals east and west of the BCL Cu/Ni mine smelter, but can be used for phytostabilisation with the help of coal fly ash.

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