Science Park Journals

Vol. 4(3), pp. 031-042, June 2018 ISSN 2315-5396 DOI: 10.14412/SRI2018.042 Copyright© 2018 Author(s) retain the copyright of this article Available online at http://www.scienceparkjournals.com/SRI	Scientific Research and Impact

Full Length Research Paper

Effect of soil pollution and addition of coal fly ash on germination of *Jatropha curcas* and *Pennisetum clandestinum*: An initial approach to phytoremediation of Bamangwato Concessions Ltd (BCL) Cu/Ni mine, Botswana.

Baleseng Moseki[1], RaviroVurayai, Bonang Nkoane, PadmajaChaturvedi.

University of Botswana, P/Bag UB00704, Gaborone, Botswana

Abstract: Mining and smelting at BCL-Cu/Ni mine in Selebi-Phikwe, Botswana has been reported to have resulted in soil acidity and heavy metal contamination. Phytoremediation can be used to rehabilitate such soils. Research has shown *Jatropha curcas* and *Pennisetum clandestinum*as promising phytoremediators. The present study was conducted on soil collected east and west of mine smelter to analyze effect of heavy metals and soil acidity on germination of *Pennisetum clandestinum* (Kikuyu) andtwo *Jatropha* accessions from Tsamaya (J09) and Mosetse (J05), Botswana. Coal fly ash from Morupule power station, Palapye, Botswana, was added to soil to also assess its effect on seed germination in contaminated soils. Germination percentage, coefficient rate of germination, shoot length, root length, shoot dry weight, root dry weight, root: shootratio, vigour index and tolerance index of both species grown in soil without coal fly ash were reduced ($p<0.05$).Kikuyu failed to germinate in soil collected closest to the mine smelter (2.5 km west). Coal fly ash increased ($p<0.05$) the above mentioned parameters in both species and Kikuyu managed to germinate in soil with coal fly ash. The ability of *Jatropha carcas* species to germinate in polluted soil indicates tolerance to heavy metal stress and soil acidity unlike Kikuyu.

Keywords: Heavy metals; soil acidity; phytoremediation; coal fly ash; germination

Introduction

Mining and smelting in Selebi-Phikwe, Botswana by the Bamangwato Concessions Ltd (BCL) Cu/Ni mine has been reported to have resulted in heavy metal soil pollution (Ekosse *et al*. 2002, 2003; Vurayai et al. 2015) and soil acidity (Vurayai et al. 2015) and this has affected the atmosphere, soils, flora and fauna (Ekosse *et al*. 2003,2004, 2005). Vegetation around Selebi-Phikwemine smelter was depleted due to the formation of dead zones especially on the western side of the mine smelter (Ekosseet al. 2005) and this is shown inFigure 1. Heavy metals cannot be chemically degraded or biodegraded by microorganisms (Kramer, 2005) and bare soil is susceptible to wind erosion which consequently spreads contamination by airborne dust. Immediate remediation is thus required and establishment of a vegetative cover will also minimize soil erosion and spread of metal pollution.Onealternative biological approach to deal with this problem is phytoremediation. Phytoremediation is the use of plants to remove, destroy or sequester hazardous substances from the environment. It has become a topical research field in the last decades because it offers advantages of being safe, *in situ*, nondestructive and potentially cheap compared with traditional remediation techniques (Cunningham and Ow, 1996; Chaney *et al.* 1997; Pulford and Watson, 2003).

(a)

(b)

Figure 1. The effect of mining activity (signs of land degradation) on the western side (a) (2.5 km of mine smelter) compared to the eastern side (b) (2.5 km east of the mine smelter) of the BCL Cu-Ni mine smelter (Picture taken by RaviroVurayai, 2013).

The main initial steps in phytoremediation are the identification of species which are able to grow and develop in heavy metal contaminated soil. *Jatropha curcas* has been reported to have the capability to grow in dry and poor soils and to also have the ability to scavenge heavy metals from wastelands (Majid *et al*. 2012;Chang *et al.* 2014)and *Pennisetum clandestinum* has been shown to accumulate heavy metals (Bech *et al.* 2002; Söğüt *et al*. 2004). This therefore warrants their study for use in phytoremediation of heavy metal pollutedland around the BCL Cu/Ni mine in Selebi-Phikwe, Botswana.

The early growth status of plant seeds in the contaminated environment directly affects phytoremediation.Seed germination is the first physiological process affected by toxic elements and the ability of a seed to germinate and survive in a medium containing any metal element is a direct indicative of its level of tolerance to these metals (Peralta *et al*. 2001). Germination tests are therefore generally used for assessing heavy metal phytotoxicity andserve as an important indicator in determining the toxicity effects of heavy metals on plants (Kuriakose and Prasad, 2008; Salvatore *et al*., 2008) and so the possibility of plant growththus need to be evaluated.

In order to reduce pollution around mining areas, improving soil physical and chemical properties is required in many cases for successful phytoremediation of these polluted soils. Various amendments can be used to increase the phytoremediation potential of field crops. Recent studies have shown that coal fly ash from Morupule power station, Palapye, Botswanaincreased soil pH in soil collected east and west of the BCL Cu/Ni mine smelter (Vurayai *et al*.,2017). Coal fly ash also immobilizes heavy metals in soil reducing their availability to plants (Polat *et al*., 2002;Tsang *et al*., 2014). This effect is attributed to the alkaline nature of coal fly ash which raises the soil pH making heavy metals unavailable and to the coexistence of constituents potentially capable of absorbing heavy metals(Su and Wong, 2004; Tsang *et al*., 2014).

The aim of this study was therefore to investigate theimpact of soil acidity, heavy metal pollution and addition of coal fly ash on germination of *Jatropha curcas* and *Pennisetum clandestinum* for use in pollution reduction through phytoremediation.

Materials and methods

Seed source

Pennisetum clandestinum (Kikuyu) seeds and*Jatropha curcas* seeds [two accessions (J05 and J09] used in the study were collected from the seed stocks of Department of Agricultural Research, Ministry of Agriculture, Botswana. The *Jatrophacurcas*accessions were collected from different locations in Botswana namely Tsamaya (J09) and Mosetse (J05).

Soil and coal fly ash Samples

Soil samples were collected from four areas [2.5 km east, 2.5 km west, 20 km west and 55 km west (control)] of the BCL Cu/Ni mine smelter in Selebi-Phikwe, Botswana (Vurayai *et al*., 2015)]. Coal fly ash samples were collected from Morupule power station, Palapye, Botswana (Vurayai et al. 2017).

Experimental design

The experiment was arranged in a 2 x 3 x 4 factorial experiment in a completely randomized block design with 5 replications. The treatments were as follows: factor A (Fly ash) which had two treatments (with fly ash and without fly ash), factor B (Plant species) which had three treatments (J09, J05, and Kikuyu)and factor C (Distance where soil was collected) which had four treatments [2.5 km east, 2.5 km west, 20 km west and 55 km west (control) of mine smelter]

Procedure

This study was undertaken in the greenhouse at University of Botswana, Department of Biological sciences in October 2013 and 2014. Coal fly ash was added to soil from each of the 4 sites mentioned above to a final concentration of 7.5% per kg dry weight. 13cm round and 10.8 cm deep (1 litre) plastic pots were filled with 11 kg soil and 1 seed was planted per pot. The pots were watered to reach 100% plant available water (Rosenthal et al. 1987) everyday and the experiment ran for 14 days.

Measurements

Germinated seeds were counted daily for 14 days and seeds were considered germinated after the length of radicals had reached 2mm. Germination percentage was calculated according to Iqbal and Rahmati (1992).

Germination percent = (number of germinated seeds/ total number of planted seeds) x 100

Coefficient rate of germination (CRG) was calculated according to Mamo et al.(2006).

Coefficient rate of germination = [Σ n / Σ (n x d)] x100 (1)

Where

n = number of seeds completing germination on day

d = the time in days starting from day 0, the day of starting germination test

At the end of the 14 days of the experiment, root and shoot lengths of germinated seeds were measured with a millimeter ruler. Tolerance index was then calculated using the root and shoot lengths according to Iqbal and Rahmati (1992).

Tolerance index = (mean root length of polluted area seedlings/ mean root length of control area seedlings) x 100

(2)

Seedling vigour index was also calculated following the method by Moradi et al. (2008).

Vigour index = seedling length [root length + shoot length (cm)] x germination percentage (3)

Roots were separated from shoots and root and shoot fresh weight was measured with ahypersensitive electric balance. Seedlings were then placed in an oven and dried to constant weight at 60°c and dry weight was measured. Mean weight was then calculated and expressed in milligrams and root: shoot ratio was calculated.

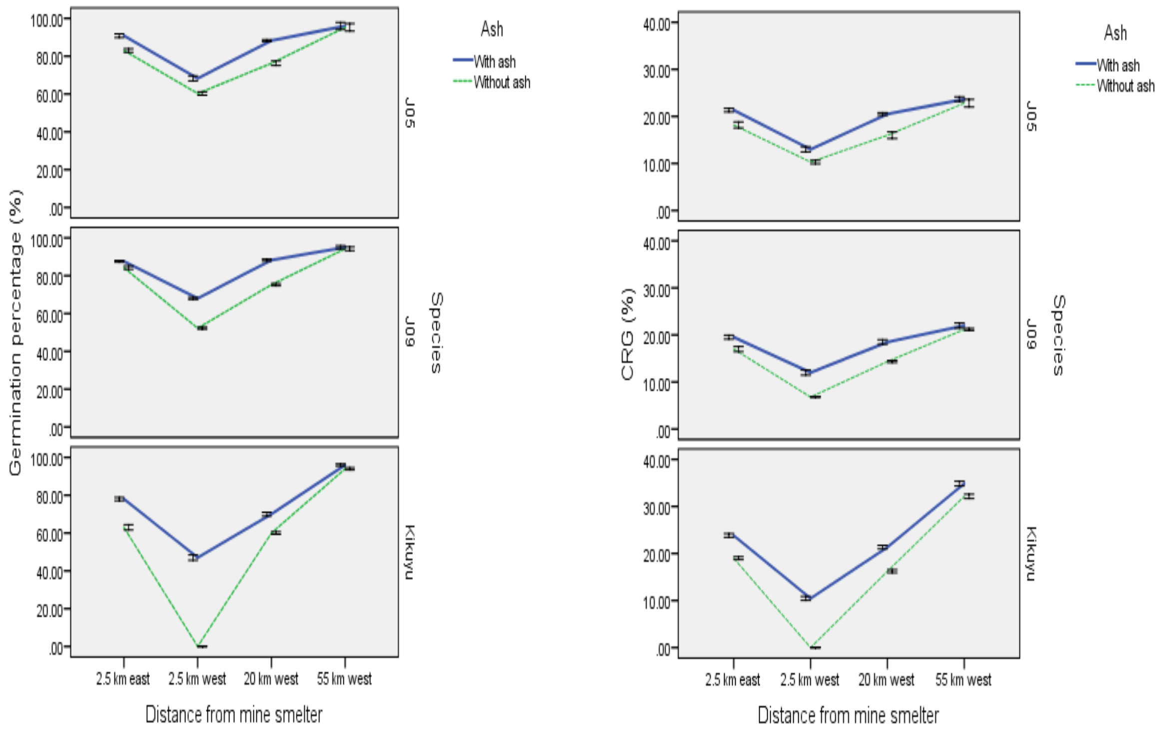
Statistical analysis

Data collected was subjected to analysis of variance (ANOVA) (IBM SPSS Statistics 22) and treatment means were compared using LSD at 0.05 probability levels.

Results and discussion

All parameters measured in this study (germination percentage, coefficient rate of germination, root and shoot length, fresh weight and dry weight, root: shoot ration, seedling vigour and tolerance index) for J05, J09 and Kikuyu were significantly lowest ($p< 0.05$) closest to the mine smelter (2.5 km west) followed by 20 km west, 2.5 km east and lastly 55 km west of mine smelter (control) (Figure 2; 3 and Table 1). This might be because soil pH is considered to be lowest closer to the mine smelter (2.5 km west). It increases exponentially with distance from the mine smelter towards the western side and follows the order 2.5 km west (3.86) < 20km west (4.31) < 2.5 km east (5.36) < 55 km west (6.28) (Vurayai et al. 2015).

Mining and smelting in Selebi-Phikwe, Botswana is said to have led to heavy metal contamination (Ekosse *et al*. 2004; Vurayai *et al*., 2015) and soil acidity (Vurayai et al. 2015) of the soil around the BCL Cu-Ni mine (study site). According to Vurayai *et al*. (2015)concentrations of Cu, Ni and Fe is higher in soils collected from 2.5 km west of the mine smelter and decreases exponentially with distance from the smelter towards the western side. Nevertheless dissolution and bio-availability of heavy metals is increased at very low pH thus heavy metal availability is bound to be higher at low soil pH and decrease with increase in soil pH (2.5 km west> 20 km west> 2.5 km east> 55 km west).



(a) (b)

Figure 2.Germination percentage (a) and coefficient rate of germination (CRG) (b) of *Jatropha curcas* (J05), *Jatrophacurcas* (J09) and *Pennisetumclandestinum* (Kikuyu) grown on polluted soil treated with 7.5 % coal fly ash. Error bars indicate ± standard error of the mean (n=5).

High concentrations of heavy metals and soil acidity can hinder seed germination,root elongation and seedling development. Soil acidity is a major growth-limiting factor for plants in many parts of the world (Foy, 1984). Soil pH is a measure of the concentration of hydrogen ions in the soil solution and the lower the pH of soil, the greater the concentration of H^+ ions. It has been suggested that excess H^+ competes with other cations for root absorption sites, interfering with ion transport and uptake, and causes root membranes to become leaky (Foy, 1992). This reduces root growth which culminates into reduction of germination percentage and rate, shoot growth, seedling vigour index and tolerance index as shown in this study. Reduction in root and shoot growth results in reduction in root and shoot dry weight and root: shoot as shown in Table 1.Excess heavy metals also inhibit germination.

J05 and J09 were able to germinate in soil collected from all four sites even at 2.5 km west (Figure 2) where soil pH was extremely acidic and some metals were above permissible limits (Vurayai *et al*., 2015). This might be because the supermidermal layers of their seed coats have thickened walls. Seed germination is the most resistant process to heavy metals (Seregin and Kozhevnikova, 2005). This resistance is due to weak penetration of heavy metals through the seed coat. The seed coat can be a barrier between the embryo and the environment in the immediate vicinity as it protects the embryo against the heavy metals toxicity (Araùjo and Monteiro, 2005).However Kikuyu did no manage to germinate in soil collected 2.5 km west of mine smelter(Figure 2). This might be attributed to seed coat having thin walls which allowed penetration of heavy metals. Heavy metals inhibit alpha amylase thus preventing hydrolysis of endosperm starch thus preventing germination.Previous studies have shown that seeds of some plants have highly permeable seed coats (Wierzbicka and Obidzinska 1998) something which may be the case with Kikuyu seeds.

The ability of both *Jatropha curcas* accessions (J09 and J05) to germinate in polluted soil(Figure 2) indicates tolerance to heavy metal stress and soil acidity. Therefore *Jatropha curcas* is a candidate for use in phytoremediation of land around the BCL Cu/Ni mine smelter.Kikuyu which was not able to germinate in soil collected 2.5 km west of mine smelter (Figure2) fails to be a candidate for phytoremediation of that area but has the potential to be used for phytoremediation of soils at 20 km west and 2.5 km east of mine smelter where it was able to germinate.

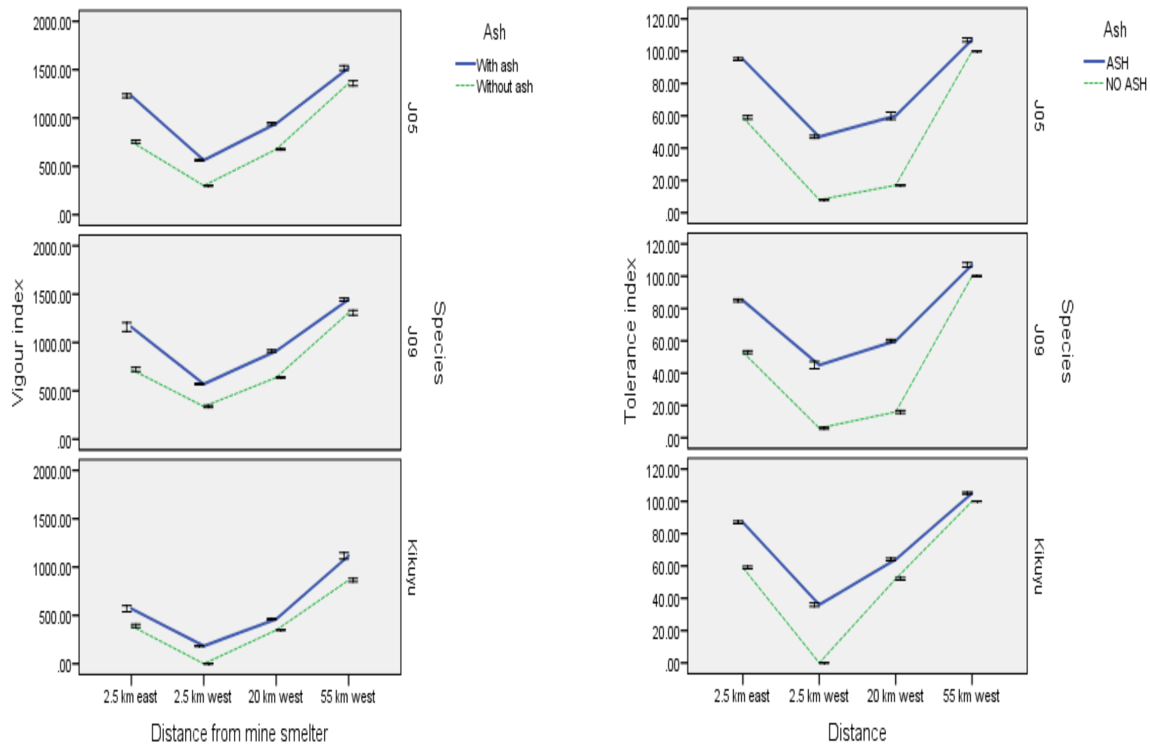
Germination percentages, coefficient rate of germination, root and shoot length, fresh weight and dry weight, root: shoot ration, seedling vigour and tolerance index varied ($p<0.05$) amongst species used in the experiment (Figure 2; Figure3; Table 1).However there was no significant difference ($p>0.05$) between J05 and J09.Variations observed between the species may be ascribed to their tolerance capacity and the role of genetic variation establishing individuality of species.

Table 1. Root and shoot length, root and shoot dry weight and root: shoot of *Jatropha curcas*(J05), *Jatrophacurcas* (J09) and *Pennisetumclandestinum*(Kikuyu) grown in coal fly ash (7.5%) amended soil. Error bars indicate ± standard error of the mean (n=5) and n.g indicates no seed germination.

	2.5 km east		2.5 km west		20 km west		55 km west	
	Ash	No Ash	Ash	No Ash	Ash	No Ash	Ash	No Ash

Root length	J05	12.8 ± 0.81	8 ± 0.59	6.43 ± 0.48	1.1 ± 0.25	8.2 ± 0.53	2.3 ± 0.19	14.4 ± 0.767	13.5 ± 0.725
	J09	11 ± 1.98	6.9 ± 0.8	5.8 ± 0.30	0.8 ± 0.11	7.8 ± 0.19	2.03 ± 0.19	13.8 ± 0.769	12.9 ± 0.802
	Kikuyu	9.3 ± 1.11	6.3 ± 0.65	3.9 ± 1.97	n.g	6.9 ± 0.36	5.6 ± 0.31	11.2 ± 1.566	10.7 ± 0.998
Shoot length	J05	13.5±0.69	9.1±0.78	8.325±1.2	5±0.83	10.675±1.1	8.925±1.13	15.825±0.71	14.33±0.66
	J09	13.2±0.64	8.6±0.98	8.4±0.9	6.5±0.47	10.6±0.89	8.5±0.52	15.175±1.16	13.85±1.04
	Kikuyu	7.30±0.31	6.2±0.57	3.9±0.22	n.g	6.63±0.45	5.8±0.38	11.6±0.97	9.2±0.71
Root dry weight	J05	0.148±0.01	0.073±0	0.072±0	0.019±0	0.099±0	0.0368±0.04	0.194±0.01	0.1±0.01
	J09	0.13±0.02	0.048±0	0.068±0	0.153±0	0.09±0	0.026±0	0.174±0	0.092±0
	Kikuyu	0.029±0	0.017±0	0.010±0	n.g	0.022±0	0.010±0	0.040±0	0.033±0
Shoot	J05	0.647±	0.481±	0.397±	0.284±	0.513±0.	0.43±0.	0.763±0.0	0.58±0.0

dry weight		0.02	0.02	0.01	0.02	02	01	3	2
	J09	0.644±0.02	0.434±0.07	0.4±0.01	0.327±0.03	0.514±0.03	0.417±0.02	0.72±0.01	0.571±0.25
	Kikuyu	.0448±0	.0280±0	.0200±0	n.g	.0363±0	.0250±0	.0593±0	.0500±0
Root: shoot	J05	0.233±0.02	0.15±0.01	0.182±0.01	0.067±0	0.193±0.01	0.087±0.01	0.24±0.03	0.236±0.03
	J09	0.2±0.03	0.111±0	0.169±0.03	0.046±0	0.175±0.01	0.063±0.01	0.227±0.01	0.223±0.02
	Kikuyu	0.641±0.03	0.604±0.02	0.500±0.02	n.g	0.613±0.01	0.40±0.02	0.67±0.03	0.66±0



(a) (b)

Figure 3. Mean vigour index and tolerance index of *Jatropha curcas*(J05), *Jatrophacurcas* (J09) and *Pennisetumclandestinum* (Kikuyu) grown on polluted soil treated with 7.5 % coal fly ash. Error bars indicate ± standard error of the mean (n=5).

Addition of coal fly ash significantly increased ($p<0.05$) germination percentage, coefficient rate of germination, root and shoot length, fresh weight and dry weight, root: shoot ration, seedling vigour and tolerance index in all species but more prominently in seedlings growing in soil collected from 2.5 km west followed by 20 km west, 2.5 km east and lastly 55 km west of mine smelter (control)(Figure 2; Figure3; Table 1). Similar results were also observed by other scientists where application of coal fly ash resulted in higher root length of lettuce (*Lactuca sativa*) (Lau and Wong, 2001), increased root number, root length, seedling height and germination percentage in *Allium cepa* (Swamy et al. 2010).

As mentioned earlier the soil pH of areas closest to the mine smelter is very low and contaminated with heavy metals. In order to reduce the effects of pollution addition of 7.5 % alkaline coal fly ash (pH 10.3) increased soil pH and reduced acidity. There was an increase in pH in soil collected 2.5 km westfrom 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) (Vurayai etal. 2017). The increase of soil pH in all soils can be attributed to the neutralization of H^+ by alkali salts and also due to solubilization of basic metallic oxides of fly ash in soil (Khan and Khan, 1996).The increase in soil pH thus reduces the effects of acidity on germination and also reduces the bio-availability of plant toxic heavymetals(Polat et al. 2002) resulting in an increase in germination percentage, coefficient rate of germination, root and shoot length, fresh weight and dry weight, root: shoot ration, seedling vigour and tolerance index in all species.The increase of germination performance in soil with coal fly ash followed the order 20 km west> 2.5 km east > 55 km east (Figure 2; Table 1; Figure 3). This is attributed to percentage increases in soil pH after coal fly ash addition which followed the same trend (Vurayai *et al*., 2017).While the addition of coal fly ash improves soil pH, it also simultaneously adds essential plant nutrients to the soil (Rai *et al*. 2004). Coal fly ash contains elements like Ca, Fe, Mg, and K, essential to plant growth, but also other elements such as B, Se, and Mo. Lime in fly ash readily reacts with acidic components in soil leading to release of nutrients such as S, B and Mo in the form and amount favourable to crop plants (Jaya and Goyal, 2006) thus results in increased growth and in this case increased root and shoot growth.

Both species (*Jatropha curcas* and *Pennisetum clandestinum*)were able to germinate in all soils collected east and west of mine smelter with coal fly ash showing that they both have the potential of germinating and establishing on fly ash amended soil.Coal fly ash thus increased the capability of growing plants in the polluted soils around the mine smelter. This increases phytoremediation potential of plants which can grow and either phytoextract heavy metals from the soil or revegetate the area. The plants together with coal fly ash stabilize heavy metals and prevent their spread. Growing of plants around the mine smelter especially on bare soils (dead zones) will reduce wind and soil erosion thus reducing transfer of pollutants to other areas.

Conclusion

Soil acidity and heavy metal pollution reduced germination parameters of *Pennisetum clandestinum* and two accessions of *Jatropha curcas* (J05 and J09). Reduction of germination was highest closest to the mine smelter and followed the order 2.5 km west < 20 km west < 2.5 km east < 55 km west (control) and followed the level of contamination. *Jatropha curcas*germinated in all soils but *Pennisetum clandestinum*failed to germinate in soil collected 2.5 km west of mine smelter. Application of coal fly ash increased germination parameters at all soil collection sites and also followed the order 2.5 km west < 20 km west < 2.5 km east < 55 km west.*Pennisetum clandestinumwas* able to germinate in soil collected 2.5 km west of mine smelter with coal fly ash. These results therefore suggest that coal fly ash enhances germination of *Jatropha curcas*and *Pennisetum clandestinum*in acidic and heavy metal polluted soils and can be used to amend polluted soil around the mine for phytoremediation purposes.

References

Araújo ASF,Monteiro RTR. 2005. Plant bioassays to assess toxicity of textile sludge compost. Sci. Agric.62: 286-290.

Bech J, Poschenrieder C, Barceló J, Lansac, A. 2002. Plants from mine spoils in the South American area as potential sources of germplasm for phytoremediation technologies. Eng. Life Sci.22(1-2): 5-11.

Chang FC, Ko CH, Tsai MJ, Wang YN, Chung CY. 2014. Phytoremediation of heavy metal contaminated soil by *Jatropha curcas*. Ecotoxicology.23(10): 1969-1978.

Cunningham SD, Ow DW. 1996. Promises and prospects of phytoremediation. Plant Physiol.110(3): 715.

Ekosse GIE, Ngila CJ, Forcheh N.2005. Multivariate analyses of heavy metals in soils and Colophospermum mopane leaves around the Selebi-Phikwe nickel-copper mine and smelter/concentrator plant area, Botswana.J. Appl. Sci. Environ. Mgt. 9 (1): 177-185.

Ekosse G, Van den Heever DJ, De Jager L and ToloIoO. 2004. Environmental chemistry and mineralogy of particulate air matter around Selebi Phikwe nickel-copper plant, Botswana. Miner Eng. 17: 349-353.

Ekosse G, Van Den Heever D, De Jager L, Totolo O. 2003. Environmental mineralogy of soils around Selebi Phikwe nickel-copper plant, Botswana. Int J Environ Studies.60(3): 251-262.

Foy CD. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. In: Adams F, editor. Soil Acidity and Liming. Madison(WI): American Society of Agronomy Inc; 57-97.

Foy CD. 1992. Soil chemical factors limiting plant root growth. In: Hatfield JL,Stewart BA, editors. Limitations to plant root growth. New York (NY): Springer New York; 97-149.

Iqbal MZ, Rahmati K.1992. Tolerance of *Albizia lebbeck* to Cu and Fe application. EKOL CSFR. 11: 427-430.

Jala S, Goyal D. 2006. Fly ash as a soil ameliorant for improving crop production-a review. Bioresour. Technol.97(9): 1136-1147.

Khan MR, Khan MW.1996. The effect of fly ash on plant growth and yield of tomato. Environ Pollut.92(2): 105-111.

KrämerU .2005. Phytoremediation: novel approaches to cleaning up polluted soils.Curr Opin Biotechnol.16(2): 133-141.

Kuriakose SV, Prasad MNV.2008. Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* L. Moench by changing the activities of hydrolyzing enzymes. Plant Growth Regul.54:143-156.

Lau SSS, Wong JWC. 2001. Toxicity evaluation of weathered coal flyash amended manure compost. Water Air Soil Pollut.128: 243-254.

Majid NM, Islam MM, Nap ME, Ghafoori M, Abdu A. 2012. Heavy metal uptake and translocation by *Justicia gendarussa* Burm F. from textile sludge contaminated soil. Acta Agric. Scand., Sect. B.62(2): 101-108.

Mamo N, Mihtetu M, Fekadu M, Tigabu M, Teketay D. 2006. Variation in seed and germination characteristics among *Juniperus procera*populations in Ethiopia. Forest Ecol Manag.225: 320-327.

Moradi Dezfuli P, Sharifzadeh F, Janmohammadi M. 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.).J. Agric. Biol. Sci.3(3):22-25.

Pulford, ID, Watson C. 2003. Phytoremediation of heavy metal-contaminated land by trees-a review. Environ Int.29(4): 529-540.

Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, Rascon E, Parsons JG.2001. Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). Bull Environ Contam Toxicol.66(6): 727-734.

Polat M, Lederman E, Pelly I, Cohen H. 2002. Chemical neutralization of acidic wastes using fly ash in Israel. J. Chem. Technol. Biotechnol.77(3): 377-381.

Rai V, Vajpayee P, Singh SN, Mehrotra S.2004. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. Plant Sci.167(5): 1159-1169.

Rosenthal W.D, Arkin GF, Shouse PE, Jordan WR.1987. Water deficit effects on transpiration and leaf growth. Agron J.79(6): 1019-1026.

Seregin IV, Kozhevnikova AD. 2005. Distribution of cadmium, lead, nickel, and strontium in imbibing maize caryopses. Russ J Plant Physl,52(4): 565-569.

Söğüt Z, Zaimoğlu BZ, Erdoğan R, Sucu MY. 2005. Phytoremediation of landfill leachate using *Pennisetum clandestinum*.J. Environ. Biol. 26: 13-20.

Swamy TN, Dash N, Nahak G, Deo B, Sahu RK. 2010. Effect of coal fly ash on growth, biochemistry, cytology and heavy metal content of *Allium cepa* L. N Y Sci J.3(5): 10-16.

Tsang DC, Yip AC, Olds WE, Weber PA. 2014. Arsenic and copper stabilisation in a contaminated soil by coal fly ash and green waste compost. Environ Sci Pollut Res Int.21(17): 10194-10204.

Vurayai R, Nkoane B, Moseki B, Chartuvedi P. 2015. Assessment of heavy metal pollution/contamination in soils east and west of the Bamangwato Concessions

Ltd (BCL) Cu/Ni mine smelter in Selebi-Phikwe, Botswana. J Biodivers Environ Sci.7(6): 111-120.

Vurayai R, Nkoane B, Moseki B, Chartuvedi P**.** 2017. 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. J Biodivers Environ Sci. 10(5): 193-206.

Wierzbicka M, Obidzińska J. 1998. The effect of lead on seed imbibition and germination in different plant species. Plant Sci.137(2): 155-171.