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Tolerance evaluation of *Jatropha curcas* and *Acacia burkei* to acidic and copper/nickel-contaminated soil

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Abstract

Aim : To evaluate tolerance of two tree species, *Jatropha curcas* and *Acacia burkei*, to an acidic and highly Cu/Ni-contaminated soil.

Methodology : Above-ground growth of *Jatropha* and *Acacia* were monitored for six months in two different soil types; a typical field soil (FS) and acidic and Cu/Ni-rich soil (ACNS), the latter containing 58- and 14-fold higher levels of Cu and Ni, respectively, than FS.

Results : Growth of *Acacia* was markedly inhibited in ACNS, as evidenced by low number of petioles and branches, thinner stem diameter, and low chlorophyll content compared with plants grown in FS. In contrast, tree height, stem diameter and length of emerged branches of *Jatropha* showed no significant differences between growth in FS and ACNS. In *Jatropha*, foliar Cu content grown in ACNS was not statistically different from that in FS whereas a small increase in foliar Ni content was observed in ACNS.

Interpretation : These observations suggested that *Jatropha* possess strong resistance to acidic and Cu/Ni-enriched soil. This trait might be advantageous for dual-purpose, utilization of *Jatropha* for renewable energy production and afforestation of Cu/Ni-polluted wastelands.

Key words: *Acacia burkei*, Acidic soil, Arid region, *Jatropha curcas*

Jatropha and Acacia growth were comparatively analyzed in two types of soils; control field soil versus acidic and Cu/Ni-rich soil



Parameters evaluated: Above-ground growth (plant height, stem diameter, branch/leaf formation), chlorophyll content, contents of absorbed mineral.

Jatropha



Not statistically different in both soils

Acacia



Markedly inhibited in acidic and Cu/Ni-rich soils

Resistance of *Jatropha* to highly acidic and Cu/Ni-rich soils can be useful for afforestation of wastelands

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Introduction

Jatropha curcas (*Jatropha*) belongs to family Euphorbiaceae and contains 25%–38% (w/w) oil in their seeds (Wani et al., 2012b) which can be converted to biodiesel. *Jatropha* has gained attention as a source of renewable energy (Parawira, 2010; Montes and Melchinger, 2016). The potential of mitigation of greenhouse gas emissions has been discussed (van Eijck et al., 2014; Ishimoto et al., 2018). In addition, the use of *Jatropha* non-oil biomass as a feedstock for organic fertilizer, soil conditioner (Abdul Khalil et al., 2013; Ogura et al., 2016), and renewable energy has also been reported (Jingura et al., 2010). Although several issues, such as oil production cost and the need for improved yield, have been highlighted for *Jatropha*-based bioenergy production (Borman et al., 2013), *Jatropha* is considered a suitable plant for sustainable energy production, especially for rural communities (Wicke et al., 2011). *Jatropha* is a drought tolerant plant (Quinn et al., 2015), that can thrive in semi-arid conditions and can survive even on degraded lands (Pandey et al., 2012). Moreover, in a previous study it has been reported that *Jatropha* deposits significant amount of carbon in soil (Wani et al., 2012a). These characteristics suggest that *Jatropha* can be an attractive tree for afforestation of otherwise unused wastelands.

The possibility of phytoremediation of potential toxic element (PTE)-contaminated lands using *Jatropha* has been suggested in previous studies (Agamuthu et al., 2010; Ghavri and Singh, 2012; Chang et al., 2014). Majid et al. (2012) studied the bio accumulation of copper, lead and zinc in *Jatropha* grown in a sawdust-contaminated soil. Abdullahi et al. (2017) demonstrated the potential of *Jatropha* for reclamation of soils polluted by zinc, copper and cadmium. Using stem cuttings, Ghavri and Singh (2012) reported copper accumulation in the roots of *Jatropha* grown in a contaminated soil. No decrease in shoot and root dry weight was observed in *Jatropha* grown in PTE-containing magnesite and bauxite mine soils as compared to control soil (Mathiyazhagan and Natarajan, 2012). These reports suggested that *Jatropha* might be tolerant to a wide range of PTEs. In Botswana, an area with high acidic and Cu/Ni-enriched soil has been reported in the vicinity of a Cu/Ni mining industrial site (Vurayai et al., 2015), which has adversely affected the vegetation of that area. It is hypothesized that *Jatropha* might be more resistant to Cu/Ni-rich soil as compared to ordinary tree species such as *Acacia burkei* (*Acacia*), which is one of the dominant tree species in African savanna (Masia et al., 2018) and adapts well to the climatological conditions of Botswana (van Wyk and van Wyk, 2013). In view of the above, this study was conducted to investigate the growth performance of *Jatropha* and *Acacia* in high acidic and Cu/Ni-rich soil.

Materials and Methods

Experimental soils: Two different soils were used in this experiment. One was collected from a field located approximately 4 km north-east the Department of Agricultural Research (DAR),

Ministry of Agriculture in Sebele, Gaborone, Botswana, that was used for *Jatropha* cultivation (Inafuku-Teramoto et al., 2013). This soil was designated as field soil (FS). The other soil was collected from the area around Cu/Ni mining site in Selebi-Phikwe, in the central district of Botswana. This soil was designated as acidic and Cu/Ni-rich soil (ACNS). The soil pH values were analyzed using an inoLab pH 720 meter (WTW GmbH, Weiheim, Germany). Organic carbon content in the soils were measured by potassium dichromate method with a spectrophotometer (UVD2950, Labomed, Los Angeles, CA, USA). For mineral analyses, soils were digested by the following method. Soil samples were dried at 70°C for two days. Dried soil samples (0.2 g) were ground using a mortar and pestle, and then 10 ml of concentrated HNO₃ was added and the mixtures were boiled until a final volume of 3 ml was reached. Subsequently, 10 ml of concentrated HCl was added, and the mixtures were evaporated to a volume of 3 ml. After filtration, the solutions were diluted to 40 ml with distilled water. The resultant decomposed materials were dissolved in 1% HNO₃, and their mineral contents were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES; SPECTRO CIROS CCD, SPECTRO Analytical Instruments GmbH, Nordrhein-Westfalen, Germany).

Plant materials and monitoring of growth: The branches of *Jatropha curcas* were collected from the trees at the Department of Water Affairs of the Ministry of Mineral Resources, Green Technology and Energy Security in Gaborone, Botswana. The branches were cut to approximately 15 cm long, and planted into a commercially-available horticultural organic soil for potting culture (Potting Soil, New Frontiers, Lobatse, Botswana) in 5 l polyethylene bags in a greenhouse at DAR. Approximately, 1 month after planting, sprouted *Jatropha* cuttings with a stem diameter of approximately 5 mm, 5 cm above ground level were used in this study. Seeds of *Acacia burkei* were collected from a mother tree on Block 8, Gaborone, Botswana, and germinated in the horticulture soil in a greenhouse at DAR. *Acacia* seedlings of approximately 10 cm long and 3 mm stem diameter, 5 cm from ground level were used in this study.

Three replicates of juvenile plants of *Jatropha* and *Acacia* were carefully transplanted into pots filled with 10 kg of experimental soils of FS or ACNS, and grown in a greenhouse at DAR. Approximately, one litre of water per pot was applied once a week; no fertilizers were applied during the study. Six months after transplantation, growth parameters such as plant height and stem diameter at a height of 5 cm above ground level were measured for all plants. For *Acacia* trees, the total numbers of first-and second-order branches (Ickert-Bond et al., 2015), and the total number of petioles per tree were counted. For *Jatropha*, the length of newly sprouted primary branch and total number of leaves on each tree were measured. Chlorophyll contents in the fully-expanded upper leaves was estimated following the method of (Han et al., 2018), with minor modification. The optical absorbance of the supernatant was measured at wavelengths of 645, 663, and 720 nm using a Ce2041 spectrophotometer (Cecil Instruments, Peterborough, UK), and used for the calculation of

the chlorophyll content (Porra *et al.*, 1989). For the estimation of nutrient minerals and PTEs in *Jatropha* leaves, approximately 200 mg dried leaves were dissolved in 10 ml concentrated HNO_3 in a flask, and digested on a hot plate at 240°C until the volume was reduced to 1 ml. The solutions were diluted to 25 ml with 1% HNO_3 and the mineral contents were measured using ICP-AES as described above. The enrichment factor of minerals and PTEs in the leaves was calculated by the following equation: $\text{EF} = [\text{element}]_{\text{leaf}} / [\text{element}]_{\text{soil}}$ (Zu *et al.*, 2005). Statistical significance of difference was evaluated by t-test using Microsoft Excel.

Results and Discussion

The major chemical components of two soils used in this study, FS and ACNS, are shown in Table 1. The pH of field soil was 4.92, and its organic carbon content was 0.31%. The five major mineral elements found in the FS were aluminum, iron, calcium, manganese and titanium. In contrast, the pH of ACNS was 3.39, revealing highly acidic nature of this soil. The organic carbon content of ACNS was 0.12%, which was lower than that of FS. Mineral contents in ACNS were mostly within the range of 5-fold differences when compared with those in FS, except for Cu, Ni, and Ti, which were 58-, 14-, and 8.9-fold higher, than those in FS. Notably, Cu (604 mg kg^{-1}) and Ni (365 mg kg^{-1}) levels in the ACNS were markedly higher than those in a previous report (Chang *et*

al., 2014), where *Jatropha* was used for phytoremediation of a PTE-contaminated field (Cu: 23.07 mg kg^{-1} and Ni: 20.99 mg kg^{-1}).

Growth of *Acacia* was adversely affected in ACNS as compared to FS (Table 2). The number of petioles on ACNS-grown plants was 11.7 per tree, which was only 14% of FS (82.7). The total number of first-and second-order branches was also significantly reduced in ACNS. In ACNS, plants had two branches per tree, as compared to five branches per tree in field soil. Although tree height was not significantly different between the two conditions, stem diameter 5 cm above ground level in ACNS was 10.3 mm, which was 26% thinner than FS (14.0 mm). In ACNS, the chlorophyll content of fully expanded petiolate leaves on the upper part of tree was $2.17 \mu\text{g chl cm}^{-2}$, which was significantly lower than that in FS ($7.21 \mu\text{g chl cm}^{-2}$). These results suggested that growth of *Acacia* was inhibited by acidic and Cu/Ni-rich soils. The detailed inhibitory mechanisms of *Acacia* trees in such soils should be examined in future studies.

In contrast, *Jatropha* trees grown in ACNS and FS showed no remarkable morphological differences. The height of *Jatropha* trees in ACNS was 25 cm, which was statistically insignificant from that in FS (24.3 cm; Table 2). Stem diameter 5 cm above ground level was 16.0 mm in ACNS, which was similar to that in FS (14.7 mm). In both conditions, only a single new

Table 1 : The pH, organic carbon and mineral contents in the experimental soils used in this study

	Soil		p-value ³	Fold difference ⁴
	FS ¹	ACNS ²		
pH	4.92	3.39		
Organic carbon (%)	0.31	0.12		
Mineral content (mg kg^{-1} soil) ⁵				
Al	$40,100 \pm 300$	$52,700 \pm 1,100$	2.9×10^{-4}	1.3
Ca	$5,880 \pm 40$	$19,300 \pm 100$	2.1×10^{-9}	3.3
Cd	3.42 ± 0.05	5.21 ± 0.04	3.2×10^{-5}	1.5
Co	78.6 ± 2.2	151 ± 1	2.8×10^{-5}	1.9
Cr	67.5 ± 0.5	52.4 ± 0.6	7.9×10^{-5}	0.78
Cu	10.5 ± 0.2	604 ± 3	9.1×10^{-6}	58
Fe	$13,100 \pm 100$	$28,900 \pm 200$	8.3×10^{-7}	2.2
K	299 ± 2	578 ± 2	8.1×10^{-7}	1.9
Mg	25.4 ± 0.3	90.4 ± 0.1	1.3×10^{-7}	3.6
Mn	704 ± 7	125 ± 1	1.7×10^{-6}	0.18
Ni	25.2 ± 0.3	365 ± 5	3.4×10^{-6}	14
P	136 ± 2	241 ± 2	1.1×10^{-5}	1.8
Pb	61.3 ± 0.8	61.0 ± 0.5	0.57	n.a. ⁶
Sn	19.6 ± 0.8	23.6 ± 0.9	1.3×10^{-2}	1.2
Ti	334 ± 3	$2,960 \pm 50$	1.8×10^{-6}	8.9
Zn	19.6 ± 0.1	60.9 ± 0.6	2.5×10^{-6}	3.1

¹ FS: field soil; ² ACNS: acidic and Cu/Ni-rich soil; ³ The probability values for the significant difference between the two soils were calculated by t-test ($p < 0.05$); ⁴ Fold difference of the value for ACNS in comparison to that of FS; ⁵ Values are the average and standard deviation ($n = 2-3$) and ⁶ n.a.: not applicable because the difference was not statistically significant

Table 2 : Growth data on *Acacia* and *Jatropha* plants grown in FS and ACNS after six months

Growth parameters	Acacia ^{*1}				Jatropha ^{*1}			
	FS ^{*2}	ACNS ^{*3}	p-value ^{*4}	Fold ^{*5}	FS ^{*2}	ACNS ^{*3}	p-value ^{*4}	Fold ^{*5}
Height (cm)	28.3 ± 6.0	31.0 ± 10.4	0.72	n.a. ^{*6}	24.3 ± 8.7	25.0 ± 7.0	0.92	n.a. ^{*6}
Diameter (mm)	14.0 ± 1.0	10.3 ± 0.6	5.3 × 10 ⁻³	0.74	14.7 ± 0.6	16.0 ± 1.0	0.12	n.a. ^{*6}
Number of petioles per tree	82.7 ± 4.7	11.7 ± 5.0	5.8 × 10 ⁻⁵	0.14	n.d. ^{*7}	n.d. ^{*7}	n.d. ^{*7}	n.d. ^{*7}
Number of branches per tree	5.0 ± 0.0	2.0 ± 1.0	6.5 × 10 ⁻³	0.40	1.0 ± 0.0	1.0 ± 0.0	1.0	1.0
Total length of emerged branch	n.d. ^{*7}	n.d. ^{*7}	n.d. ^{*7}	n.d. ^{*7}	45.0 ± 21.8	56.7 ± 55.1	0.75	n.a.
Number of leaves per tree	n.d. ^{*7}	n.d. ^{*7}	n.d. ^{*7}	n.d. ^{*7}	5.3 ± 2.9	10.3 ± 1.2	4.9 × 10 ⁻²	1.9

*¹ Values are mean ±SD (n = 3); *² FS: Field soil; *³ ACNS: Acidic and Cu/Ni-rich soil; *⁴ Probability values for significant difference between the two soils were calculated by t-test (p < 0.05); *⁵ Fold difference of the value for ACNS as compared to FS; *⁶ n.a.: not applicable as the difference was not statistically significant and *⁷ n.d.: not determined

branch emerged from the original cutting for a given plant. The length of the emerged branch was 56.7 and 45.0 mm in ACNS and FS, respectively, showing that the values were highly variable and statistically indifferent between the two conditions. Of note, the number of leaves on *Jatropha* grown on ACNS (10.3) were more than those grown on FS (5.3). The reason for higher number of leaves in ACNS is currently unknown. The chlorophyll content of *Jatropha* leaves in ACNS was 10.2 µg chl cm⁻², which was not

significantly different from those of FS (11.4 µg chl cm⁻²). Although the growth characteristics of *Jatropha* in the acidic and Cu/Ni-enriched soils awaits further evaluation in long term natural field conditions, the present results demonstrate that vegetative growth of *Jatropha* might not be negatively influenced by ACNS. The observed tolerance of *Jatropha* to Cu/Ni-enriched soils suggest that afforestation of *Jatropha* may serve as a measure for remediating Cu/Ni-polluted wastelands.

Table 3 : Contents and enrichment factors for the nutrient minerals and potentially toxic elements in *Jatropha* leaves

Mineral	FS ^{*1}		ACNS ^{*2}		p-value ^{*5}	Fold difference ^{*6}
	Content (mg kg ⁻¹) ^{*3}	EF ^{*4}	Content (mg kg ⁻¹) ^{*3}	EF ^{*4}		
Al	341 ± 200	8.5 × 10 ⁻³	503 ± 372	9.5 × 10 ⁻³	0.51	n.a. ^{*7}
Ca	5,120 ± 1,780	0.87	8,110 ± 7,670	0.42	0.54	n.a.
Cd	< 0.1	n.a.	< 0.1	n.a.	n.a.	n.a.
Co	3.52 ± 0.18	4.5 × 10 ⁻²	4.27 ± 0.36	2.8 × 10 ⁻²	7.7 × 10 ⁻²	n.a.
Cr	2.31 ± 0.60	3.4 × 10 ⁻²	1.93 ± 1.46	3.7 × 10 ⁻²	0.69	n.a.
Cu	3.90 ± 0.17	0.37	3.67 ± 1.16	6.1 × 10 ⁻³	0.81	n.a.
Fe	57.8 ± 19.1	4.4 × 10 ⁻³	79.1 ± 63.7	2.7 × 10 ⁻³	0.60	n.a.
K	9,180 ± 6,860	31	4,600 ± 3,530	8.0	0.21	n.a.
Mg	1,870 ± 2,430	74	1,860 ± 960	21	0.99	n.a.
Mn	48.0 ± 5.3	6.8 × 10 ⁻²	64.7 ± 37.1	0.52	0.48	n.a.
Ni	1.84 ± 1.28	7.3 × 10 ⁻²	5.55 ± 2.34	1.5 × 10 ⁻²	4.1 × 10 ⁻²	3.0
P	6,270 ± 1,500	46	5,190 ± 610	21	0.15	n.a.
Pb	2.54 ± 1.45	4.1 × 10 ⁻²	2.85 ± 2.03	4.7 × 10 ⁻²	0.83	n.a.
Sn	4.95 ± 1.02	0.25	4.79 ± 1.48	0.20	0.87	n.a.
Ti	1.10 ± 0.10	3.3 × 10 ⁻³	2.76 ± 0.05	9.3 × 10 ⁻⁴	1.1 × 10 ⁻⁴	2.5
Zn	35.5 ± 11.0	1.8	37.3 ± 10.5	0.61	0.82	n.a.

*¹ FS: field soil; *² ACNS: acidic and Cu/Ni-rich soil; *³ Values are mean ±SD (n = 2~6); *⁴ EF: Enrichment factor, [Elements]leaf / [Elements]soil; *⁵ Probability values for significant difference between the content of two soils were calculated by t-test (p < 0.05); *⁶ Fold difference of the value of content for ACNS as compared to FS and *⁷ n.a.: not applicable as the difference was statistically significant

The observed tolerance of *Jatropha* to ACNS, which was rich in Cu, Ni and Ti, prompted us to examine the foliar accumulation of these PTEs in *Jatropha*. The nutritive minerals and PTEs in *Jatropha* leaves revealed that the foliar contents of most elements were not statistically different between the plants grown in FS and ACNS, except for Ni and Ti (Table 3). The foliar Ni and Ti contents were 3- and 2.5-fold larger in ACNS (5.55 and 2.76 mg kg⁻¹ for Ni and Ti as compared to FS (1.84 and 1.10 mg kg⁻¹ for Ni and Ti). The observed fold differences were smaller than fold differences of soil Ni/Ti contents between ACNS and FS (14- and 8.9-fold higher Ni and Ti contents, Table 1). The enrichment factors (EFs) of Ni and Ti in ACNS, which reflect the fold-increase in foliar mineral content in comparison with that of soil, was only 1.5×10^{-2} and 9.3×10^{-4} for Ni and Ti, respectively (Table 3). This observation suggests that excess Ni and Ti in ACNS may be inefficiently absorbed by *Jatropha* roots, or may be inefficiently translocated to leaf tissues. Ni is an essential mineral nutrient for plant growth and plays a crucial role in certain enzymes such as urease, however, excess Ni causes cellular injuries and plant death (Yusuf et al., 2011). Whereas Ti is non-essential for plant growth, however, positive effects of adequate concentration of soil Ti on plant growth and phytotoxicity due to excess Ti has been reported (Lyu et al., 2017). The results of this study revealed that *Jatropha* did not hyperaccumulate these toxic metals in the leaf tissue. Rather, resistance in *Jatropha* may be an excluder strategy (Baker, 1981), which limits the accumulation of excess amount of toxic metals in leaves.

Notably, foliar Cu content in *Jatropha* grown in ACNS (3.67 mg kg⁻¹) was not statistically different from plants grown in FS (3.90 mg kg⁻¹; Table 3), showing that the foliar Cu contents were maintained at similar levels regardless of a large difference in the soil Cu content (58-fold higher in ACNS than in FS; Table 1). This observation suggested that foliar Cu homeostasis in *Jatropha* might be more strictly controlled than those of Ni and Ti in this study. Cu is a cofactor of many enzymes and plays essential roles in various biological processes, such as iron mobilization for normal growth and development (Festa and Thiele, 2011). However, high level of Cu in soils can be phytotoxic (Nagajyoti et al., 2010). Being one of the essential transition metals, Cu has unique redox-active properties to potentially enhance formation of reactive oxygen species (Shahid et al., 2014). Observed stability of Cu content in *Jatropha* leaves in this study may therefore indicate a mechanism of strict control for Cu homeostasis to avoid Cu-induced damage.

Previous studies have reported that *Jatropha* concentrated more Cu in their roots than leaves (Ghavri and Singh, 2012; Majid et al., 2012; Chang et al., 2014). In another study, *Jatropha* leaves and stems accumulated more Cu than their roots (Abdullahi et al., 2017). These previous reports, together with the present observations, suggested that the response of *Jatropha* to Cu-enriched soils might be variable, possibly influenced by multiple factors such as soil physico-chemical properties, *Jatropha* physiology and its underlying genetic varieties, and a combination thereof.

Of note, the EF of essential minerals such as potassium, magnesium and phosphorus, showed high values in ACNS (Table 3), which were in striking contrast to the behavior of PTEs. This observation suggested that *Jatropha* selectively translocated nutrient minerals for growth whereas translocation of PTEs from soil to foliar tissues was effectively suppressed.

In summary, this study showed that *Jatropha* can grow in highly acidic and Cu/Ni-rich soils, and its growth was similar to that in control soil. This trait was different from the growth characteristics of *Acacia*, which showed severe growth retardation in the acidic and Cu/Ni-rich soil. Analysis of mineral composition in *Jatropha* leaves showed a slight increase in the foliar Ni content in Cu/Ni-rich soil whereas foliar Cu contents were not statistically different between Cu/Ni-rich and control soil, suggesting that translocation of these PTEs to leaves, particularly for Cu, might be effectively suppressed in *Jatropha*. These observations highlighted potent resistance of *Jatropha* to acidic and Cu/Ni-enriched soil, which may be advantageous for the utilization of *Jatropha* for afforestation of Cu/Ni-polluted wastelands.

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