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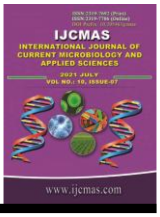

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Soil Ecology and Bioaccumulation of Heavy Metals by *Calotropis procera* (Ait) in Drylands of South Eastern Kenya

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ABSTRACT

Calotropis procera is a wild species that is drought-resistant and plays a number of economic and ecological uses. The species is important in soil fertility improvement, pollution control by monitoring sulphur dioxide emissions in the air and suitable indicator of exhausted soil. This study was undertaken in drylands of South Eastern Kenyato evaluate the species' soil ecology and ability to bioaccumulate heavy metalsThe objectives of the research were to determine the soil chemical and physical properties for survival of *C. procera* and to determine the ability of *C. procera* in phytoextraction of heavy metals from the soil. To understand the soil chemical and physical properties for growth of *C. procera*, two sites namely a farmland and a natural stand of *C. procera* were used. In the two sites, soil samples were collected and analysed for soil pH, total N, P, K and bulk density. *C. procera* tissues (leaves) were harvested and analyzed for total N, P, K, OC, Zn, Mn, Cu and Fe. One-way ANOVA was applied to assess the significance of variations in the soil chemical properties (pH, total N, P and K), Plant tissue data (total N, P, K, OC, Zn, Cu and Fe), *C. procera* provenances and spacing variables in relation to the field plots. Pearson simple linear correlation coefficient (r) was calculated for assessing the type of relationship between the study site and the natural stand of *C. procera* in relation to the soil chemical properties. There were no significant differences in subplots soil chemical properties. Correlation analysis showed a strong positive spatial relationship (Pearson, $P<0.01$, $r_s = 0.734$) in soil chemical properties between the artificial and the natural stand of *C. procera*. Further, there were no significant differences ($p<0.05$) in soil pH as well as in bioaccumulation of heavy metals in both the artificial and natural stands of *C. procera*. However, Pearson correlation analysis showed a very strong relationship (Pearson, $P<0.01$, $r_s = 0.966$) in terms of pytoextraction/phytoaccumulation of heavy metals (Zn, Mn, Cu and Fe) between the artificial and natural stands of *C. procera*. The study concluded that *C. procera* can grow in a wide range of soil pH levels and the species has the ability to bioaccumulate heavy metals in its leafy tissues. The study recommends use of *C. procera* as a remediator of soils contaminated with heavy metals (Zn, Mn, Cu and Fe).

Keywords

Calotropis procera, Heavy metals, Bioaccumulation, Phytoremediation, Phytoextraction

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Introduction

Calotropis procera (Aiton) W.T (Asclepiadaceae) is a xerophytic perennial shrub or small tree that grows in many arid and semi-arid countries (Hassan *et al.,* 2015). According to Farahat *et al.*, (2015), the species is a perennial xerophytic medicinal shrub or small tree that grows as a wasteland plant and reproduces by seeds. Frosi *et al.,* (2013) describes the species as a perennial Asian shrub that primarily reproduces via seeds. According to Galal *et al.*, (2015), the species is hardy xerophytic plant which is distributed globally in many countries and has important economic and ecological functions. The species is found in most parts of the world with a warm climate in dry, sandy and alkaline soils. It can tolerate adverse climatic conditions and poor soils (Parsons & Cuthbertson 1992; Lottermoser 2011; Kumar *et al.,* 2013). It has an evergreen behavior under field conditions, from young plant to the reproductive stage (Frosi *et al.*, 2012). It often grows in saline or slightly saline soils with low soil moisture, forming mono-specific stands (El-Midany, 2014). The species is drought-resistant and salt-tolerant xerophytic species, which is capable of surviving in a range of soil types including alkaline and saline soil, and prefers free-draining sandy soils. It grows in open habitats and is particularly common in overgrazed pastures and on poor soils, where there is a little competition from grasses (Kumar *et al.*, 2013; Galal, 2015).

In India, it is found at up to 1000 m altitude (Parrotta, 2001) in a wide variety of soil types and will survive on alkaline and saline soils though prefers free-draining sandy soils. It is found at a range of altitudes from exposed coastal sites to medium elevations up to 1300m. According to Kumar *et al.,* (2013) the species prefers disturbed sandy soils. It thrives on poor soils, particularly where overgrazing has removed competition from native grasses, and forms dense thickets which compete with native plant species and transform the appearance of savannah plant communities.

It has a preference for and is often dominant in areas of abandoned cultivation especially sandy soils in areas of low rainfall and is assumed to be an indicator of over-cultivation (Orwa *et al.*, 2009). The species is also found along roadsides, watercourses, river flats and coastal dunes, and is often prevalent in disturbed areas. It is deep-rooted, and so rarely grows in shallow soils over unfractured rock. Soils of all textures and derived from most parent materials are tolerated. It quickly becomes established as a weed along degraded roadsides, lagoon edges and in overgrazed native pastures.

The species is native to India, Pakistan, Nepal, Afghanistan, Algeria, Iran, Iraq, Israel, Kenya, Kuwait, Niger, Nigeria, Oman, Saudi Arabia, United Arab Emirates, Vietnam, Yemen and Zimbabwe (Kumar, *et al.*, 2013).

In Kenya, *C. procera* wildly grows in Kitui, Machakos, Makueni, Tharaka, Baringo, Kibwezi, Turkana, Wajir, Isiolo, Mandera among other arid and semi arid areas (ASALs). It is native to tropical and subtropical Africa and Asia and common in the Middle East (Parsons & Cuthbertson 1992; Lottermose 2011) and in Latin America. According to Orwa *et al.*, (2009) and Galal *et al.*, (2015), it has a wide range of uses such as medicinal, bark and latex are used in brewing and to curdle milk. Young pods used for fodder, stems produce good charcoal, fibre from stem and white silky wool, latex or rubber for tannin or dyestuff, poison for arrows and spears, soil fertility, pollution control by monitoring sulphur dioxide emissions in the air, suitable indicator of exhausted soil and plays a critical role in removal of heavy metals from the soil.

A study was undertaken to evaluate the soil physical and chemical properties for growth of *C. procera* in a typical farm setting as well as in a natural environment.

Its ability to phytoextract heavy metals from the soil and concentration of the same in its leafy tissues was evaluated.

Materials and Methods

Study Site

Geography

The study was carried out at South Eastern Kenya University (SEKU) situated in Kitui County, Kenya. The research site is located 15 Kilometers off Kwa Vonza Market, along the Kitui-Machakos main road, Kwa Vonza/Yatta ward, Lower Yatta, Kitui County. Geographically, the research plot lies at 01.31358°S, 037.75546°E and 01.31422°S, 037.75576°E at a general elevation of 1173m a.s.l (Figure 1).

Climate of the study site

The climate of the study areas is semi-arid (Agroecological zone IV) with very erratic and unreliable rainfall. The rainfall pattern is bimodal with the short rainy season occurring between November and December and the long one between April and May. The short rains are more reliable than the long rains (Pauw *et al.*, 2008).

The mean annual rainfall ranges between 500-1050 mm with 40 per cent reliability. The site experiences high average temperatures throughout the year, which range from 16^{0} C to 34^{0} C (Pauw *et al.*, 2008). The hot months are between January and February and June and September characterized by mean minimum and maximum temperatures of 28^{0} C and 32^{0} C, respectively.

Hydrology and Water Resources

Few water sources exist in the research site. The major sources of water are Mikuyuni and Mwita Syano seasonal streams. Virtually all of the seasonal rivers in the research area drain into the Tana River drainage basin, Kenya's largest river that drains the Eastern flank of the Aberdares and the Southern slopes of Mount Kenya. The river flows in the research area are characterized by very low flows (base flows) in dry season and high flows during rainy seasons, April-May and November-December respectively. Most of the ephemeral streams generally become dry within one month after the rainy season (Borst and De Haas, 2006). The flows are usually fast and turbid due to high sediment concentration associated with soil erosion in the catchment area.

Soils and Geology

Soils are predominantly sandy to loamy sand texture, hence they are susceptible to erosion and are limited in their capacity to retain water and nutrients. The major soil type of the area is lixisols (red soils). Alluvial deposits (fluvisols) occur in isolated patches along rivers and on hill slopes. The soils are generally poorly drained and easily eroded by runoff (Borst and De Haas, 2006). Some patches of the research area are overlain by red well drained sandy loam soils which have quartz and feldspar grains and felsic gravel rock fragments. Soil depths (thickness) vary from between 1.2m (upslope) to nearly 2.0m at the down slope side of Mwitasyano stream. The soils reduce in thickness upslope where rock outcrops are found jutting above the surface of the soils. The study site has a similar geology composed of high grade regional metamorphic granitoid granulites which are composed of quartz and feldspars (over 90%) and mafic hornblende and pyroxenes (about 10% or less).

Selection of the Study Site

The study site was selected purposively based on the following criteria. First, *C. procera* grows in drylands and the study site represents typical arid and semi-arid conditions. Secondly, the study site had a farmland where *C. procera* could be planted as well as existing natural stand of *C. procera.*

Selection of *C. Procera* Provenances

To capture the dry land conditions in the country, seeds were collected from three areas in Kenya: Baringo, Kibwezi and Tharaka Nithi.

Field Experimental Design

A natural stand of *C. procera* was identified and demarcated within the study site for evaluation of soil physical and chemical properties under natural conditions. For artificial conditions, a 60m by 80m plot was cleared and leveled. Within the main plot, 27 subplots were demarcated. The subplots were laid out in a randomized complete block design within the main plot. In each of the subplot, 12 planting pits (1ft by 1ft) were dug but at different spacing. For each provenance, three spacing types were used: 1.5m by 1.5m, 2m by 2m and 3m by 3m. These were replicated three times to give a total of 9 treatments. The spacing between subplots was 4m. The seedlings were transplanted into the pits. The planted *C. procera* were maintained in the field and monitored for four seasons (2 years).

Soil sampling and analysis

Soil samples were taken in the natural stand of *C. procera* as well as from the artificial plot before it was prepared for planting. In the natural stand of *C. procera*, sampling was done according to Kimiti *et al.*, (2016) whereby a 100m by 100m sampling plot was established. A diagonal transect was ran across the sampling plot. Three soil sampling points were identified and marked; one at the centre and two at the edges of the diagonal transect. At each point, three plants of *C. procera* were identified and from each plant, soil samples were collected by auguring at 30cm deep and 1m away from the plant base. The soil cores were pooled, mixed and subsamples were taken for laboratory analysis. In each of the 27 subplots, the zigzag sampling method was used to collect four soil samples which were pooled, mixed and subsamples were taken for laboratory analysis. Soil extracts were prepared to meet the requirements for the different parameters, 1:5 (w/v) soils (g): distilled water (ml). This extract was used to determine pH values using a glass electrode pH meter (Model 9107 BN, ORION type) and electrical conductivity (EC) with (conductivity meter 60 Sensor Operating Instruction Corning). Carbonates and bicarbonates were determined by titration against 0.1 N HCl using phenol phthalein and methyl orange as indicators. Total N was estimated using Micro-Kjeldahl method, while total P using a spectrophotometer (CECIL CE 1021) by applying Indo-Phenol blue and molybdenum blue methods, respectively. Sodium and potassium were determined using flame photometer. Zinc, copper, iron and manganese were determined using Atomic Absorption ectrophotometer (Shimadzu AA-6200).

Plant tissue sampling and analysis

At the end of the fourth season when the plants were 2 years old, four plants in each subplot were tagged and plant tissue samples were collected by harvesting the leaves of the plants. For each subplot, the harvested leaves were thoroughly mixed to form a bulk sample. For each subplot, a 500g sample (wet weight) was measured using a digital weighing balance. The samples were taken to the laboratory for complete chemical properties analysis (nitrogen, phosphorus, potassium, organic carbon, zinc, copper, manganese and iron).

Data analysis

One-way ANOVA was applied to assess the significance of variations in the soil chemical properties (pH, total N, P and K), Plant tissue data (total N, P, K, OC, Zn, Cu, Mn and Fe), *C. procera* provenances and spacing variables in relation to the artificial *C. procera* stand. A comparative soil chemical properties (pH (water), EC (ms/c), C(%), $NH^{3-}N$ (ppm), N (%),P (ppm), K (ppm), Mn (ppm), Zn (ppm), Fe (ppm) and Cu (ppm) between the artificial and the natural stand of *C. procera* was done. Pearson simple linear correlation coefficient (r) was calculated to assess the type of relationship between the artificial and the natural stand of *C. procera*. Bioaccumulation levels of heavy metals (Zn, Cu, Mn and Fe) in the plant tissues of *C. procera* was determined and compared with the benchmark concentrations required for plant growth. Toxicity levels of the heavy metals were determined. Duncan Multiple Range Test (DMRT) was done to detect existence of statistically significant differences in bioaccumulation of heavy metals in the artificial and natural stands of *C. procera.*

Results and Discussion

Soil properties at the trial plot and the natural stand of *C. procera*

The chemical properties of soil samples from the study site and the natural stand of *C. procera* were analyzed. The pH averaged at 6.3 in the *C. procera* field trial plot. When pH data for subplots was separated by both space and provenance and subjected to Duncan Multiple Range Test (DMTR), no statistically significant differences ($P<0.05$) were captured (Table 3.1). Similarly, no significant differences were obtained when the data was separated by space (Table 3.2). Analysis of soil pH in the naturally growing stand of *C. procera* gave an average of 6.23, which was slightly below the field trial plot average. Other soil chemical properties analyzed included soil total N (%), total P (ppm) and total K (ppm).

A comparative soil chemical properties (pH (water), EC (ms/c), C (%), $NH^{3-}N$ (ppm), N (%),P (ppm), K (ppm), Mn (ppm), Zn (ppm), Fe (ppm) and Cu (ppm) between the study site and the natural stand of *C. procera* was done. The comparative soil chemical properties (rhizosphere) only captured significant differences ($P< 0.05$) for K and Fe with the study site capturing relatively higher concentrations of the two nutrients compared to the *C. procera* natural stand. A comparative analysis of soil physical properties (rhizosphere) between the study site and the *C. procera* natural stand showed higher moisture content (2.03) in the natural stand compared to 1.58 in the study site. The two sites recorded almost similar bulk densities (g/cm^3) with the natural stand recording a mean of 1.08 compared to 1.1 for the study site. Correlation analysis showed a strong positive spatial relationship (Pearson, $P<0.01$, $r_s = 0.734$) in soil chemical properties between the study site and the natural stand of *C. procera.*

Analysis of *C. procera* leafy tissues showed a tendency by *C. procera* to bioaccumulate heavy metals mainly micronutrients such as Zn, Mn and Fe. Pearson correlation analysis showed a very strong relationship (Pearson, $P<0.01$, $r_s = 0.966$) in terms of pytoextraction/phytoaccumulation of heavy metals (Zn, Mn, Cu and Fe) between the artificial and natural stands of *C. procera*. For instance, mean bioaccumulation of Zn was 216.04 (ppm) which was slightly higher than the recommended concentrations (Table 3.3). Mn had a mean bioaccumulation of 151.62 (ppm) and 119.29 (ppm) in the artificial stand and in the natural stand respectively. Fe had a mean bioaccumulation of 502.86(ppm) which was within the toxic levels for plants. However, Cu had mean concentrations of 2.25 (ppm) which was within the safe levels for plant concentrations.

Lack of significant differences in soil chemical properties across the study site sub-plots is a clear indication that the microsites were homogenous and that the observed differences in performance of *C. procera* provenances cannot be attributed to the soil properties. With respect to pH, the study can authoritatively ascertain that the study site had a pH commonly found in natural habitats of *C. procera*. Comparison of soil pH in the study site and that of the natural stand of *C. procera* showed that the soils were slightly acidic (pH of 6.3 in the study site and 6.23 in the natural stand). The findings of this study are in agreement with Frosi *et al.*, (2012) who in a study of ecophysiological performance of *C. procera* in Brazil recorded a pH of 6.4 in a naturally growing stand of *C. procera*. Other studies have shown that *C. procera* can do well in soils that are acidic, neutral and basic. This implies that the species has a wide pH range and generally, the level of pH may not have played a critical role in determining the observed growth performance of the three provenances of *C. procera* in the current study.

Survival of the species in a wide range of pH levels has also been demonstrated in different studies. For instance, Galal *et al.*, (2015), in a study of *C. procera* growing in different habitats, noted that the residential habitat had a pH of 7.7 while the roadside habitat had 7.8. Similarly, Farahat *et al.,* (2015) analyzed soil pH in three *C. procera* growing habitats: residential areas, roadside and railway areas. The result showed good growth in railway habitats which are not only characterized by high soil pH (8.3) but also higher concentrations of heavy metals such as Zn, Cu, Pb, Cd, and Mn. Further, Oliveira *et al.*, (2009) documented that the species can grow in soils that are shallow, acidic, infertile as well as soils with toxic levels of aluminium and heavy metals.

The more or less homogenous soil bulk densities in the study site and in the *C. procera* natural stand can be attributed to the location of the two sites. The natural stand is located at the outskirts of an urban area hence subject to occasional anthropogenic disturbances while the current study site had been previously subjected to slash and burn agriculture. Based on these anthropogenic disturbances, the sites can be categorized as relatively disturbed hence more or less homogenous in terms of soil bulk densities.

C. procera is not selective in nutrients requirement and as such the insignificant differences in soil macronutrients in different sub-plots at the study site may not have influenced growth of the provenances. Generally, *C. procera* is well adapted to survive in a wide range of soil types ranging from poorly drained black cotton soils to well-drained but infertile sandy soils. The adaptive capacity of the species to a wide range of soil types was demonstrated by De Oliveira *et al.*, (2009) who, in a study in Brazil, found out that the species performed well in ironstone-rich areas in Canga and seasonally dry forest in Caatinga. *C. procera* survives in soils of all textures as well as soils with high concentration of sodium. According to Kumar *et al.*, (2013), *C. procera* has a relatively high degree of tolerance to salinity, prefers sandy soils and is capable of surviving in degraded areas. Adaptation to a wide range of edaphic factors by *C. procera* is further documented by Ara *et al.*, (2017) who found out that the species can tolerate poor soils with limited fertility and moisture level.

According to Francis (2002) and Orwa *et al.*, (2009), *C. procera* is drought and salt tolerant surviving in different types of soils as well as alkaline and saline soils. Similar tolerance to soil salinity is documented by Galal *et al.*, (2015). Similarly, El-Midany (2014) found out that *C. procera* is drought and salt tolerant surviving in a wide range of soils and prefers free draining sandy soils. Elsewhere, Lottermoser (2011), in a study on colonization of abandoned mines by *C. procera*, noted that the species can tolerate adverse climatic conditions, poor and polluted soils. According to Kumar *et al.*, (2013), one of the key features of *C. procera* is its ability to survive in different soil types.

The observed high concentration of heavy metals (ZN, Cu, Mn and Fe) in plant tissues of *C. procera* in the domestication site and the natural stand is an indicator of the species' ability to undertake phytoremediation. Pyhtoextraction/pytoremediation is the ability of plant species to remove contaminants such as heavy metals from the soil and air and accumulate them in their tissues such as the leaves, stems and roots. This aids in the removal of pollutants in the environment. Several studies have demonstrated the ability of *C. procera* to bioaccumulate heavy metals as observed in this study. For instance, Alyemeni *et al.*, (2011), detected higher mean Cd and Cu values in above ground tissues of *C. procera* though the level were below the phytotoxic ranges of 5–700 mg kg-1 and 25–40 mg kg-1 for Cd and Cu respectively. D'Souza *et al.*, (2010), in a study of soil contaminated with Pb and Cd in India, identified *C. procera* as an effective phyto-remediator of soils contaminated with heavy metals. In this study, though *C. procera* accumulated copper within its foliage, the concentrations (2.25) were within safe levels for plant growth. However, Galal *et al.*, (2015) obtained mean concentration values of Cu of 24.2 mg kg-1 in *C. procera* growing in railways habitat and the levels were considered to be within the toxic concentrations. It is important to note that though Cu is a heavy metal, it is vital a vital plant nutrient and plays a critical role in various enzymatic activities. Further, Cu tends to bio-accumulate in roots and is rarely concentrated into aboveground plant tissues such as the leaves and stems. Copper can cause toxicity when aboveground tissues pyto-accumulate levels beyond 20 mg kg-1 (Galal *et al.*, 2015). Plants affected by heavy metal toxicities show a wide range of leaf symptoms. For instance, Cu toxicity often causes foliar interveinal chlorosis with increasing exposure (Reichman, 2002).

This current study noted Zn bioaccumulation mean values of 216.04 mg kg-1 that were almost reaching toxic levels. According to Ghaderian and Ravandi (2012), Zn is a vital nutrient for plants and occurs in concentrations of between 10-200 mg kg-1 with toxic concentrations starting from 230 mg kg-1. Zinc toxicity is manifested by symptoms such as chlorosis and reddening of younger leaves (Reichman, 2002). In a different study, Galal *et al.*, (2015) obtained mean values of Zn pytoremediation of 58.5 mg kg-1 and 122.4 mg kg-1 in *C. procera* growing in railway and roadside habitats respectively. In a different study using *Sorghum bicolor*, Mirshekali *et al.*, (2012) reported that the plants tolerated <900 mg kg-1 of zinc concentrations and as such recommended the plant as a phyto-remediator of zinc contaminated soils.

Table.1 Means separation by both space and provenance

pH water			**Total P ppm**		
Baringo	6.54	a	2 Baringo	716.3	a
3 Tharaka	6.44	ab	3 Baringo	658.8	a
1.5 Kibwezi	6.41	ab	1.5 Tharaka	596	a
1.5 Baringo	6.41	ab	3 Kibwezi	563.9	a
3 Kibwezi	6.39	ab	1.5 Baringo	558.6	a
2 Baringo	6.38	ab	2 Kibwezi	498.4	a
1.5 Tharaka	6.34	ab	3 Tharaka	455.7	a
2 Kibwezi	6.21	ab	2 Tharaka	427.6	a
2 Tharaka	6.18	b	1.5 Kibwezi	422.3	a
p value	***0.542***		***p value***	***0.68***	

Table.1 Continued…..

Total N%			**Total K ppm**		
2 Baringo	0.2667	a	1.5 Baringo	1624	a
3 Kibwezi	0.2267	ab	3 Tharaka	1547	ab
3 Baringo	0.2033	ab	2 Baringo	1508	ab
1.5 Kibwezi	0.1867	ab	1.5 Kibwezi	1466	ab
2 Tharaka	0.1867	ab	1.5 Tharaka	1446	ab
2 Kibwezi	0.1867	ab	3 Kibwezi	1444	ab
3 Tharaka	0.1667	ab	3 Baringo	1437	ab
1.5 Tharaka	0.1633	ab	2 Tharaka	1426	ab
1.5 Baringo	0.1433	b	2 Kibwezi	1181	b
p value	***0.426***		***p value***	***0.646***	

*Means bearing the same letter in a column are not significantly different

Table.2 Means separation by space

1_5Total_K_ppm			**1_5Total_N%**			**1_5Total_p_ppm**			**1_5pH_water**		
Baringo	1624	a	Kibwezi	0.1867	a	Tharaka	596	a	Kibwezi	6.413	a
Kibwezi	1466	a	Tharaka	0.1633	a	Baringo	558.6	a	Baringo	6.407	a
Tharaka	1446	a	Baringo	0.1433	a	Kibwezi	422.3	a	Tharaka	6.34	a
p value	***0.232***		***p value***	***0.526***		***p value***	***0.723***		***p value***	***0.896***	

Table.2 Continued…..

2 Total_K_ppm			**2 Total_N%**			**2 Total_p_ppm**			**2 pH (water)**		
Baringo	1508	a	Baringo	0.2667	a	Baringo	716.3	a	Baringo	6.383	a
Tharaka	1426	a	Kibwezi	0.1867	a	Kibwezi	498.4	a	Kibwezi	6.213	a
Kibwezi	1181	a	Tharaka	0.1867	a	Tharaka	427.6	a	Tharaka	6.18	a
p value	***0.488***		***p value***	***0.394***		***p value***	***0.212***		***p value***	***0.214***	

Table.2 Continued…..

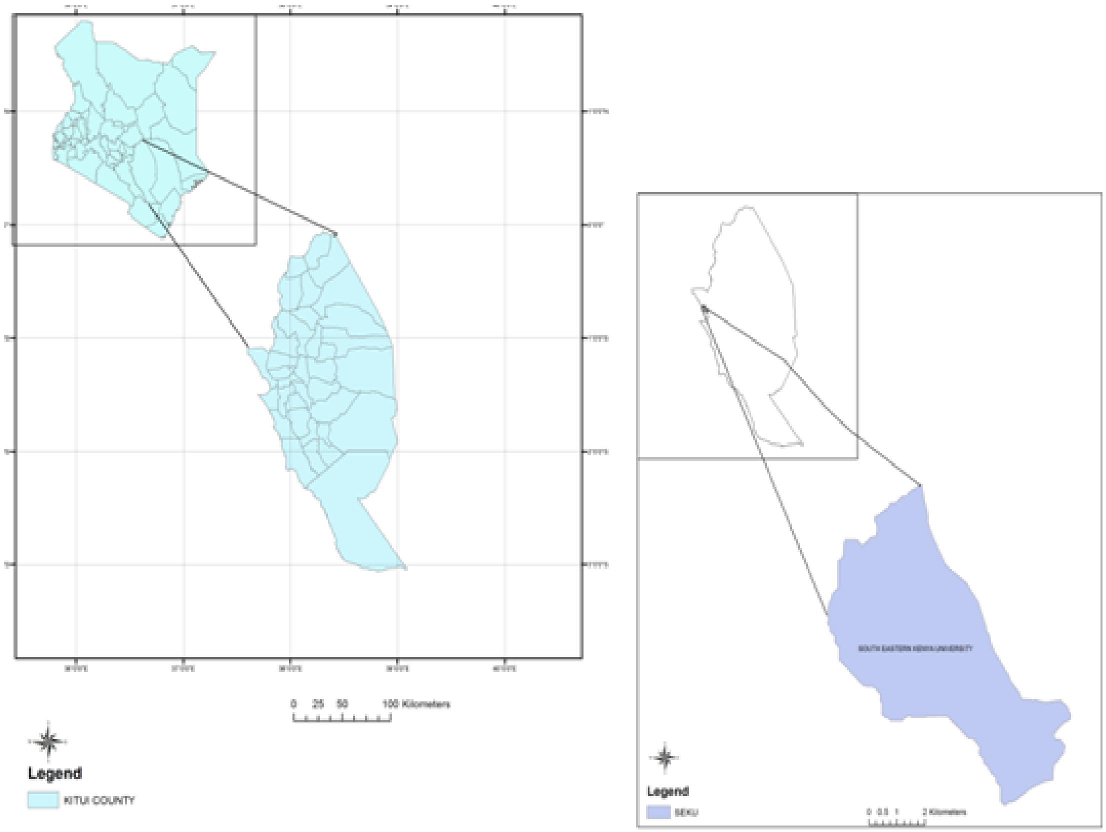
3 Total_K_ppm			**3 Total_N%**			**3 Total_p_ppm**			**3 pH_water**		
Tharaka	1547	a	Kibwezi	0.2267	a	Baringo	658.8	a	Baringo	6.537	a
Kibwezi	1444	a	Baringo	0.2033	a	Kibwezi	563.9	a	Tharaka	6.443	a
Baringo	1437	a	Tharaka	0.1667	a	Tharaka	455.7	a	Kibwezi	6.39	a
p value	***0.807***		***p value***	***0.504***		***p value***	***0.379***		***p value***	***0.776***	

**Means bearing the same letter in a column are not significantly different*

Table.3 Mean concentration of different heavy metals by *C. procera*

	Mn (ppm)	**Zn (ppm)**	**Fe (ppm)**	**Cu (ppm)**
Natural stand of *C. procera*	119.29	202.12	524.24	1.31
Artificial stand	151.62	216.04	502.86	2.25
Normal (safe) concentrations for plants	50-500	10-200	50-250	<20

Fig.1 Location of research site in SEKU, Kitui County, Kenya



This study reported Mn bioaccumulation in leafy tissues of *C. procera* both in the artificial stand (151.62 mg kg-1) and the natural stand (119.29 mg kg-1). According to Galal *et al.*, (2015), manganese plays a critical role in photosynthetic reaction and is important in a number of enzymes that catalyze redox, decarboxylation, and hydrolytic reactions.

However, concentrations of Mn above 50–500 mg kg-1 are considered toxic to plants. As such, the mean concentration values obtained from the domestication site (151.62 mg kg-1) and the natural stand of *C. procera* (119.29 mg kg-1) were within the normal range for plant growth.

Toxicity symptoms of Mn include chlorosis especially on older leaves and necrotic lessions on young foliage (Reichman, 2002). Elsewhere, Prajapati *et al.,* (2013) found out that *C. procera* is a phyto-remediator of soils contaminated with Mn owing to the species ability to bio-concentrate Mn in its above ground tissues. In a different study in Jeddah City-Kingdom of Saudi Arabia, Abdullatif *et al.,* (2016)estimated the amount of heavy metals such as Aluminum, Chromium, Boron, Barium, Copper, Manganese, Iron, Lead and Zinc in the soil and plant tissues of *C. procera*. Results revealed that *C. procera* is capable of phytoextraction of the heavy metals and as such the species can be used as a phyto-remediator by absorbing the pollutants from the soil as well as atmosphere and concentrating the same in the above ground tissues.

Out of the heavy metals analyzed, only Fe had bioaccumulation levels (502.86mg kg-1) that were within toxic concentrations for most plants. Similar accumulation of Fe to toxic concentrations were obtained by Galal *et al.,* (2015) who reported that Fe concentration in *C. procera* from residential and roadside habitats exceeded the normal concentration for plant survival. Out of the micronutrients required by plants, Fe is needed in high amounts with normal concentration for plant growth ranging between 50-250 mg kg-1. Iron is an essential micronutrient and plays critical role in DNA synthesis, enzymatically mediated processes, respiration and photosynthesis (Rout & Sahoo, 2015).Fe toxicity symptoms include yellowing and dieback of oldest leaves, necrotic lessions, growth retardation among others. It is important to note that the growth of *C. procera* in the artificial stand and the natural stand was characterized by occasional leaf yellowing followed by complete defoliation and dieback of aerial shoots. Given the observed levels of Fe and the effects of its excess bioaccumulation, it is likely that it played a critical role in the observed occasional loss of chlorophyll, defoliation and dieback of shoots.

Comparing the Fe normal ranges of 50 and 250 mg kg-1 and the mean values of 502.86mg kg-1 obtained in this study, it is evident that *C. procera* can tolerate exceptionally high Fe levels. This observation is further supported by De Oliveira1 *et al.,* (2009) who, in a study in Canga and Caatinga in Brazil, found out that *C. procera* had the capacity to tolerate high levels of iron which is characteristic of Canga soils. In Riyadh and Gazan, Saudi Arabia, Al-Yemni *et al.,* (2011) noted significant concentrations of nutrient and heavy metals such as N, Ca, Cd and Fe in above ground tissues of C. *procera* and *Citrullus colocynthis* indicating that the two species have the potential for pollution monitoring of soils contaminated with such heavy metals. Elsewhere, in a study to monitor distribution of Nickel and Vanadium in Jeddah City, Saudi Arabia, Al-Dhaibani *et al.,* (2018) noted concentrations of the two elements in the soil and *C. procera* tissues and recommended use of *C. procera* as a biological technique for monitoring the concentration of the two airbone heavy metals.

Recommendations

There were no significant differences in soil chemical properties in the field subplots and as such, chemical soil properties did not influence the observed growth and phenological characteristics in the three provenances of *C. procera*.

C. procera prefers a slightly acidic soil and has the ability to concentrate heavy metals (Zn, Cu, Mn and Fe) in the plant tissues indicating that the species has the ability to undertake phytoremediation/phytoextraction of soil contaminated with heavy metals.

The study recommends use of *C. Procera* as a heavy metal remediator especially in soils contaminated with ZN, Cu, Mn and Fe. There is need for further studies to establish if the observed periodic chlorosis followed by complete defoliation of *C. procera* stands is linked to bioaccumulation of heavy metals in the plant's leafy tissues. There is need for further research on the extent of bioaccumulation of heavy metals in other above-ground tissues of *C. procera* such as the stem.

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