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Full Length Research

An assessment of copper-contaminated soil using Kenaf (*Hibiscus cannabinus* l.) as a phytoaccumulator

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ABSTRACT: A pot experiment was conducted to study the potentials of Kenaf (*Hibiscus cannabinus* L.) as a Copper (Cu) phytoaccumulator at different levels of artificial contamination. Copper was applied as cupric sulphate ($CuSO_4.5H_2O$) and the treatments were 0, 50, 100 and 150 mg kg^{-1} of Cu. Kenaf was grown in each of the treated pots for 10 weeks, following which, leaf, stem and root samples were collected and analysed for Cu contents. The soil was analysed for physicochemical properties [viz. pH, electrical conductivity (EC), organic carbon (OC), exchangeable acidity (EA), exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and extractable and total Cu contents) before treatments/contamination and after harvesting. Kenaf showed symptoms of toxicity at 100 and 150 mg kg^{-1} treatments of Cu. It was observed that, compared with the levels of contamination of Cu, the concentration in kenaf was generally insignificant; thus, the concentration reduction in the soil at one cycle of cropping may not berealistic. Copper treatments at different levels significantly changed soil pH and EC. Therefore, more cycles of growth are needed to effectively remediate Cu-contaminated soils using Kenaf as Cu phytoaccumulator.

Key words: Copper, heavy metals, Kenaf, phytoaccumulator, phytoremediation, soil contamination.

INTRODUCTION

The concept of soil protection has recently received considerable attention around the globe due to the need to realise food security for the growing population. Contamination of soil by heavy metals was described by Logan (1990) as soil chemical degradation. Soil chemical degradation is caused by the build-up of some toxic chemicals and an elemental imbalance that is injurious to plant growth (Abrol et al., 2012).

Heavy metal contamination in the soil–water–plant ecosystem is of great concern because of its possible influence on the food chain (Mmolawa et al., 2010; Zakir et al., 2012). In the soil system, pollution by toxic metals is due to both natural processes, such as weathering of minerals, and anthropogenic activities related to industry, agriculture, burning of fossil fuels, vehicular emissions, mining and metallurgical processes and their waste disposal, as is the case in Nigeria (Human Rights Watch Staff, 2013). Mainly due to their harmful effects on plants, heavy metals have received enormous attention all over the world.

Pollution of the natural environment by heavy metals has become a global phenomenon, as these metals are indestructible, and most of them have toxic effects on living organisms when they exceed a certain concentration (Dalman et al., 2006; Ghrefat and Yusuf, 2006). Furthermore, heavy metals present a serious environmental risk when they accumulate in soils, especially for regions undergoing fast industrialisation and urbanisation (Zakir et al., 2017; Begum et al., 2014; Bakali et al., 2014; Hu et al., 2013). This implies a health risk, linked with the spread of pollution to agricultural areas, that poses a serious environmental threat and concern (Haque et al., 2018; Aysha et al., 2017; Tahar and Keltoum, 2011; Tahar et al., 2014).

Copper (Cu) is among the most frequently reported heavy metals concerning its potential hazards and occurrence in soils. It has a high affinity for soluble organic ligands, and the formation of these complexes may greatly increase Cu's mobility in soil. Its accumulation in topsoil is greatly influenced by traffic volume (Al-Kashman and Shawabkeh, 2009). In Maiduguri, Nigeria, the recent practice by many companies and industries of discharging untreated sewage, refuse and industrial wastewaters into nearby agricultural fields, as well as the indiscriminate dumping of refuse in neighbourhoods by inhabitants, indicate little concern related to environmental conservation in this area. These activities help in causing the accumulation of heavy metals like Cu in agricultural fields.

Phytoremediation, the use of living green plants specialized in cleaning up polluted soil (Singh et al., 2015), has become indispensable for addressing the problem of heavy metals in soils. Risk reduction may involve a process of removal, degradation or containment of a contaminant, or it may comprise a combination of any of these factors; hence, the understanding of different forms of phytoremediation will help in determining the different processes that occur due to vegetation, what happens to a contaminant, where the contaminant occurs and what should be done for effective phytoremediation (Pivetz, 2001). Phytoremediation is extremely competitive in relation to other treatment alternatives, as it is simple to use and has high public acceptability (Kaushik, 2015).

Several plant species have been used, assessed and ultimately reported to exhibit good performance in cleaning up soil and water and a natural propensity to take up metals, such as Cu and Pb (Singh et al., 2015). The potential of kenaf (*Hibiscus cannabinus L*.) and corn (*Zea mays* L.) for accumulating some heavy metals has been tested elsewhere for the remediation of dredging sludge contaminated with trace metals (Arbaoui et al., 2013). However, there has been no report on the use of kenaf as a phytoaccumulator in Borno State's (Nigeria) sandy loam. Therefore, the objectives of the study were to determine: the effects of Cu treatments on some physicochemical characteristics of soil along with the growth performance of Kenaf (*Hibiscus cannabinus* L.) and to assess its potentiality as Cu phytoaccumulator.

MATERIALS AND METHODS

Soil

To examine the potentials of Kenaf (*Hibiscus cannabinus*L.) as a phytoaccumulator of Cu, composite soil samples were used and the texture of the soil was sandy loam. The soil samples were collected from the teaching and research farm of the Faculty of Agriculture, University of Maiduguri at random at 25 different locations across the field at 0 to 30 cm depths. Large soil lumps were crushed, and the samples were passed through a 2-mm sieve to remove plant remains and materials larger than 2 mm. The sieved samples were kept in polythene bags. Tables 1 and 2 present the characteristics of the collected soil samples.

Treatments and experimental design

The study was conducted in a pot experiment which was laid in a completely randomized design (CRD). Copper (Cu) was used as treatments at four (4) different levels with three (3) replications. Cu was applied as cupric sulphate ($CuSO_4.5H_2O$) by wet contamination method at levels of treatments based on common ranges of metal contamination as given by Lindsay (1979) for contaminated soils. The treatments includes: 0, 50, 100 and 150 mg kg^{-1} Cu. Basal nutrients of N, P and K were prepared and applied to meet the N, P, K requirements of the potted plants. One hundred mg kg^{-1} of N was applied as ammonium nitrate (NH_4NO_3), One hundred (100) g Kg^{-1} P was applied as calcium hydrogen phosphate and 50 mg kg^{-1} K was applied as potassium chloride (KCl). After treatment, soils were kept for one week for incubation, and then seven certified seeds of Kenaf were planted per pot and watered at 10 KPa with distilled water and three days after planting, seedlings were thinned from 7 to 5 stands per pot, based on vigour to avoid overcrowding. Data collected includes- date of planting, weekly plant height (tape measurement), general physical appearance, blooming and harvest dates. During the growth period, soil water content was kept at 90% of field capacity and corrected daily by weight. Weeds were immediately handpicked once emerged; growth period of experimental plant was 10 weeks after which plants were harvested.

Laboratory analysis

Soil pH and electrical conductivity (EC) were measured using 1:2.5 soil weight to water volume ratio extract according to Thomas (1996). Soil Organic Carbon was determined using the wet oxidation method of Walkley and Black (1934) as reported by Sparks et al. (1996). Soil exchangeable acidity was determined by extracting the soil with Kcl using the extraction/titration method (McLean, 1965). The exchangeable bases in the soil were extracted with 1N neutral ammonium acetate (NH_4OAc) buffer according to Sparks et al. (1996). The concentrations of Na and K were determined with the flame photometer while that of Ca and Mg by Atomic Absorption Spectrophotometer (AAS) model AA-6800 (Shimadzu) (Stover et al., 1976). Determination of extractable and total Pb and plant Pb content were determined according to standard procedure using Atomic Absorption Spectrophotometer (AAS) model AA-6800 (Shimadzu) (Stover et al., 1976).

Table 1. Characteristics of the sampling site.

Sampling site	Characteristics
Coordinates	University of Maidduguri (11^{O} 54′ N and 13^{O} 15′ E) 354m ASL (Climate Chart, 2010)
Texture	Sandy loam (Soil Survey Staff, 2014)
Taxonomic classification	Typic Ustipsamment (Rayar, 1984)
Average annual rainfall	440-600mm (Climate Chart, 2010)
Temperature	15- 20°C(Min) 37- 45 °C(Max) (Climate Chart, 2010)
Steady infiltration rate	135mm/hr (Grema and Hess, 1994)
Average bulk density	1.5g/cm^3 (Grema and Hess, 1994)
Topography & Vegetation	Generally a low and plain topography with short grasses and thorny shrubs

Table 2. Initial (before kenaf grown) soil characteristics.

Soil characteristics	Values
pH	6.10
EC	0.09 dsm^{-1}
OC	0.24 (%)
EA	0.40
Ca^{2+}	13.20 cmol$_c$ kg^{-1}
Mg^{2+}	6.0 cmol$_c$ kg^{-1}
Na^+	0.29 cmol$_c$ kg^{-1}
K^+	0.28 cmol$_c$ kg^{-1}
Extr. Cu	0.04 mgkg^{-1}
Total Cu	0.64 mgkg^{-1}

EA - Exchangeable Acidity, Extr. – Extractable.

Statistical analysis

All data collected were subjected to statistical analysis using analysis of variance (ANOVA) with the help of a statistical package, statistix 10.0. Difference between treatments was separated using LSD at 5% probability level.

RESULTS

Characteristics of soil used

Physicochemical characteristics of soil used for the pot experiment before contamination with Cu at various levels and after harvesting of Kenaf are described as follows:

Effects of Cu treatments on soil characteristics after harvesting of Kenaf

Soil pH, Electrical Conductivity (EC), Exchangeable Acidity (EA) and percent Organic Carbon content (%OC) of soils treated with Cu at various levels are presented in Table 3.

pH values for soils treated with Cu ranged from 5.6 to 6.37, the differences amongst the treatments were significant ($P < 0.05$). However, the highest treatment level of Cu (150 mg kg^{-1}) had significant ($P < 0.01$) change in pH (5.46) compared to the control treatment. EC values ranged from 0.13 to 0.33 dsm^{-1}, with significant ($P < 0.05$) difference among the treatments.

Soil exchangeable bases (cations) at different levels of Cu treatments after harvesting of Kenaf is presented in Table 4. Treatments of Cu at the different levels had no significant ($P < 0.05$) effects on exchangeable Ca^{2+}, Mg^+, Na^+ and K^+. Values of exchangeable Ca^{2+} ranged between 1.20 and 1.30 cmol kg^{-1}, values of Mg^{2+} ranged from 0.53 to 1.50 cmol kg^{-1}. That of exchangeable Na^+ and K^+ had values from 0.39 to 0.41 and 0.22 to 0.33 cmol kg^{-1}, respectively.

Effect of Cu treatments on soil Cu contents after harvesting of Kenaf

The extractable and total Cu contents after harvesting of Kenaf are presented in Table 5. Extractable Cu of the soil treated with Cu at the different levels (0 to 150 mgkg^{-1} Cu) had no significant ($P < 0.05$) changes; values ranged between 0.00 mg kg^{-1} Cu to 1.71 mgkg^{-1} Cu. On the other hand, soil total Cu content significantly ($P < 0.01$) increased with the increasing levels of Cu treatments (Table 5).

Effects of Cu treatments on Kenaf growth and Cu content

The weekly heights of Kenaf plant grown in Cu treated soils at different levels of treatment is presented in Table 6. Values of Kenaf height grown in Cu treated soils were insignificant ($P < 0.05$). Growth generally was rapid within first three weeks in all the treatments, however, at third and fourth (treatments levels of 100 and 150 mg kg^{-1} Cu), plant growth kept being stunted. At these levels of treatment, plant showed signs of toxicity; plants were become yellow and stunted in growth and in extreme cases some plants

Table 3. Effects of different treatments of Cu on soil pH, EC, exchangeable acidity and organic carbon after harvesting of kenaf.

Treatments Cu($mgkg^{-1}$)	pH	Electrical Conductivity (dms^{-1})	Exchangeable Acidity ($cmol_cKg^{-1}$)	Organic Carbon (%)
0	6.33	0.13	0.67	0.39
50	6.37	0.13	0.53	0.39
100	6.07	0.19	0.60	0.37
150	5.60	0.33	0.73	0.28
LSD (P< 0.05)	0.37	0.12	NS	NS

NS=Not significant.

Table 4. Effect of different treatments of Cu on exchangeable bases (Cations) after harvesting of kenaf.

Treatments [Cu ($mgkg^{-1}$)]	Ca^{2+} ($cmol_c\ kg^{-1}$)	Mg^{2+} ($cmol_c\ kg^{-1}$)	Na^{+} ($cmol_c\ kg^{-1}$)	K^{+} ($cmol_c\ kg^{-1}$)
0	1.20	0.53	0.40	0.22
50	1.20	0.61	0.41	0.22
100	1.30	0.50	0.39	0.27
150	1.20	1.00	0.39	0.33
LSD (P< 0.05)	NS	NS	NS	NS

NS=Not significant.

Table 5. Effect of different treatments of Cu on soil extractable and total Cu content after harvesting of kenaf.

Treatments Cu ($mgkg^{-1}$)	Extractable Metal ($mgkg^{-1}$)	Total Metal ($mgkg^{-1}$)
0	0.00	0.27
50	1.64	16.81
100	1.71	34.36
150	1.28	42.90
LSD (P< 0.05)	NS	0.26

NS=Not significant.

Table 6. Effects of different treatments of Cu on weekly height of Kenaf plant.

Treatments	Weekly Plant Height of Kenaf Plant (cm)									
Cu ($mgkg^{-1}$)	WK 1	WK 2	WK 3	WK 4	WK 5	WK 6	WK 7	WK 8	WK 9	WK 10
0	5.90	11.00	16.33	21.83	28.33	34.00	39.67	45.33	50.67	56.00
50	5.73	10.17	14.73	18.60	23.50	28.67	33.33	38.33	43.33	48.00
100	5.63	9.17	13.67	17.67	22.67	27.17	31.50	36.50	43.00	44.67
150	5.67	9.60	12.33	17.17	21.50	25.67	30.67	34.33	38.33	42.33
LSD (P< .05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS=Not significant.

died (4 out of the 5 stands of Kenaf plants died at 150 mgkg-1 Cu in the third replicate). Fresh and dry weight of Kenaf at various treatment levels of Cu showed significantly (P < 0.05) differences (Table 7). It is aparant from Table 7 that both fresh and dry weights of plants decreased with increased levels of Cu treatments.

Total Cu contents in the different parts of Kenaf plant grown at various treatments showed significantly (P < 0.05) differences (Table 8). At all Cu treatment levels, leaf Cu content ranged from 0.46 to 2.08 mgkg-1; stem Cu contents varied from 2.22 to 7.13 mgkg-1 and root Cu contents ranged from 0.64 to 10.45 mgkg-1. The total plant

Table 7. Effect of different treatments of Cu on fresh and dry weights of Kenaf after harvesting.

TreatmentsCu (mgkg^{-1})	Plant Fresh Weight (g)	Plant Dry Weight (g)
0	118.33	22.07
50	101.67	20.67
100	91.67	16.87
150	23.33	3.40
LSD (P < 0.05)	14.88	3.74

NS=Not significant.

Table 8. Effect of different treatments on Cu content in different parts of Kenaf plant.

Heavy Meatal	Levels of Treatments (mgkg^{-1})	Leaf Metal Content (mgkg^{-1})	Stems Metal Content (mgkg^{-1})	Roots Metal Content (mgkg^{-1})	Plants Total Metal Content (mgkg^{-1})
	0	0.46	2.22	0.86	4.53
	50	0.27	7.13	5.28	12.83
Cu	100	1.93	3.46	0.64	4.75
	150	2.08	3.21	10.45	16.21
	LSD (P < 0.05)	NS	NS	4.74	5.29

NS=Not significant.

Cu contents of Kenaf grown in the different levels of Cu treatments also showed significant differences at P < 0.01 and the amount ranged from 4.53 to 16.21 mgkg^{-1} (Table 8). However, the sequence of Cu accumulation in Kenaf plant was: root > stem > leaf.

DISCUSSIONS

Soil physicochemical characteristics

The significant decrease of soil pH with increasing levels of treatment, especially at the treatment level of 150 mg kg^{-1} Cu indicates that, where there are higher levels of toxicity or contamination, soil pH will reduce making soil more acidic. Similar observation was also reported by Akan et al. (2010) and Babatunde and Kamar (2010) where they observed that pH decreased significantly (increased soil acidity with higher concentration of heavy metals). Soil electrical conductivity (EC), though varied significantly, no treatment recorded EC values that are less than two (<2), in other words soil still remained non-saline according to the rating scale of Boulding (1994). Percent OC content of the soil was generally low before and after the laboratory contamination. Extractable Cu contents of soils treated with different levels of Cu was insignificant although the content is increased. This is expected because, with increase in the sources of contamination of pollution in the environment, the levels or concentration of pollutants (in this case heavy metals) will increase in the soil. Similar circumstance was reported by Babatunde and Suleiman (2010) where the concentration/contents of residual metal in the soil after harvest of *Hibiscus cannabinus* increased with the increasing levels of metal concentration.

Total Cu contents at all levels of treatments increased significantly with the increasing levels of treatments. Meaning, the higher the level of contamination, the more the concentration expected in the soil as earlier suggested by the study of Babatunde and Suleiman (2010). Not only that, heavy metals do not degrade in the environment or soil quickly which can result in accumulation as supported by the studies of van Herk (2012) and Akan et al. (2010).

Agronomic performance and contents of Cu in Kenaf

Growth rate of Kenaf in Cu treated soils were generally slow. Though plant heights were reduced insignificantly (P < 0.05) with increase in levels of Cu treatment. Kenaf showed toxicity symptoms which included yellowing of plant and stunted growth and even recorded death at levels of 100 mgkg^{-1} and 150 mgkg^{-1} Cu treatments. The toxicity at this level was reported earlier in the study of van Herk (2012). Consequently, the growth rates of the plant reflected in the fresh and dry weights of Kenaf grown in Cu treated soil at all levels significantly (P < 0.05). Kenaf at the highest levels of Cu treated soil (150 mgkg^{-1}) weighed the least due to poor growth, toxicity and even death. Furthermore, the concentration of Cu reducing budding and growth rate of Kenaf as shown by Ihekeronye and Ngoddy (1985) influenced the overall biomass of the plant.

Total plant Cu content of Kenaf grown in Cu treated soil was significantly (P < 0.05) increased with the increasing levels of treatment. Leaf, stem and root Cu contents also increased with the increasing levels of Cu treatments. This phenomenon was due to the increased exchangeable form of metal concentrations in soil, resulting in increased plant uptake. The studies of van Herk (2012) and Amusan et al. (1999) supported this finding, They reported that plants (Okra, Kenaf, Waterleaf) grown on dump sites with high concentrations of heavy metals had high uptake and concentrations in the plant tissues than those grown on non-dump sites. Another factor for increased uptake and accumulation of Cu in tissue of plant in this study might be the pH levels of the soil which generally were moderately acidic. van Herk (2012) and Zornoza et al*.* (2010) in their study indicated that acidity range of soil is known to increase the mobilization of heavy metals, thus increasing their uptake. An earlier study of Smith and Giller (1992) also showed that soil pH is one of the factors that influence bioavailability and transport of heavy metals in the soil which influence plant uptake.

Conclusion

Kenaf (*Hibiscus cannabinus)* was found as a potential hyperaccumulator or phytoextractant of Cu for the remediation of contaminated soil. However, the plant grown showed severe symptoms of toxicity from the level of 100 mgkg^{-1} Cu and even death at 150 mgkg^{-1} Cu. Concentrations in plants of Cu was generally lower than anticipated. Therefore, more cycles of growth are needed to effectively remediate this metal from contaminated soils by Kenaf plant. At all treatment levels, soil physicochemical properties were generally not changed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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