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Research Paper

Influence of the application of biochar on the growth of groundnut (*Arachis hypogaea* L.) grown on lead contaminated soil

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This experiment was conducted at the screen house of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria, to determine the influence of the application of biochar on the growth of groundnut (*Arachis hypogaea* **L.**) grown on lead contaminated soil. SAMNUT-24 was used as the test crop for this research. Lead (Pb) was inoculated at the rate of 515 $mgkg^{-1}$ in the buckets containing 5 kg of top soil (thoroughly mixed) a week prior to biochar application. The biochar was applied two weeks before planting at the rates of 0, 2.5, 5, 7.5, 10 and 12.5 t/ha. The experiment was laid out in a completely randomized design and replicated three times. Data were collected on growth parameters (number of leaves and plant height), nodulation (effective and non-effective nodules) and yield (number of pods and number of kernels). Data collected were subjected to Analysis of Variance (ANOVA) and means were separated using Duncan's Multiple Range Test at 5% level of significance. The results showed that 12.5 and 10 t/ha of biochar significantly ($p<0.05$) increased number of leaves, plant height, shoot dry weight and number of effective nodules. 12.5 and 10 t/ha of biochar rates gave the best results. It is therefore recommended that these rates should be tried in field experiment to validate the results obtained from this study. Further studies should also be conducted in order to analyze and determine the shoot N, P, K, and Na as well as Lead concentration in shoot, root and yield.

Keywords: Biochar application, Lead inoculation, contaminated soil, agronomic parameters.

INTRODUCTION

Heavy metals constitute a very heterogeneous group of elements which are widely varied in their chemical properties and biological functions. The term "heavy metal" can be defined as metals which have specific weights more than 5 gcm^{-3} (Raikwar et al., 2008). When concentrations of metals within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly. Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Jadia and Fulekar, 2009). However, the indirect toxic effect is the replacement of essential nutrients at cation exchange sites of plants (Taiz and Zeiger, 2002). Plants are the target of a wide range of pollutants that vary in concentration, evolution, and toxicity. Such pollutants mainly enter the plant system through the soil (Arshad et al., 2008) or via the atmosphere (Uzu et al., 2010). Among common pollutants that affect plants, lead is one of the most toxic and frequently encountered (Cecchi et al., 2008; Grover et al., 2010; Shahid et al., 2011).

Lead occurs naturally in the earth's crust (Arias et al.,

2010) and its natural levels remain below 50 $mgkg^{-1}$ (Pais and Jones 2000). But, anthropogenic activities often modify the amount and nature of lead present in soil. Anthropogenic source of lead generally accumulates primarily in the surface layer of soil, and its concentration decreases with depth (Cecchi et al., 2008). Because of its strong binding with organic and/or colloidal materials, it is believed that only small amounts of the lead in soil are soluble, and thereby available for plant uptake (Kopittke et al., 2008; Punamiya et al., 2010). This metal impairs plant growth, root elongation, seed germination, seedling development, transpiration, chlorophyll production, lamellar organization in the chloroplast, and cell division (Sharma and Dubey, 2005; Krzesłowska et al., 2009; Gupta et al., 2009, 2010; Maestri et al., 2010). Plant biomass can also be restricted by high doses of lead exposure (Gopal and Rizvi, 2008; Gichner et al., 2008; Islam et al., 2008; Piotrowska et al., 2009; Sing et al., 2010). Under severe lead toxicity stress, plants displayed obvious symptoms of growth inhibition, with fewer, smaller, and more brittle leaves having dark purplish abaxial surfaces (Islam et al., 2007). Plant growth retardation from lead exposure may be attributed to nutrient metabolic disturbances (Kopittke et al., 2007; Gopal and Rizvi 2008) and disturbed photosynthesis (Islam et al., 2008). In most cases, the toxic effect of lead on plant growth is time and dose dependent (Dey et al., 2007; Gupta et al., 2009, 2010). In maize plant (*Zea mays*), reduction in germination percentage, suppression of growth, reduction in plant biomass, decrease in plant protein content has been reported by Hussain et al. (2013). Whereas in Portia tree (*Thespesia populnea*), reduction in number of leaves, leaf area and plant height were observed by Kabir et al. (2009). Recently, studies have paid considerable attention to in situ remediation of heavy metals by addition of soil amendments i.e. biochar addition (Xu et al., 2014).

Biochar is a porous carbonaceous solid produce by thermo chemical conversion of organic materials in an oxygen depleted atmosphere (Steinbeiss et al., 2009). One of the characteristics of biochar is possession of large surface areas, which implies a high capacity to complex heavy metals on their surface (Beesley and Marmiroli, 2011; Lu et al., 2012). This sorption can be due to complexation of the heavy metals with different functional groups by exchange of heavy metals with cations associated with biochar, such as Ca^{+2} and Mg^{+2} (Lu et al., 2012), K^{+}, Na^{+2} and S (Uchimiya et al., 2011), or due to physical adsorption (Lu et al., 2012). Some other compounds present in the ash, such as carbonates, phosphates or sulphates, can also help to stabilise heavy metals by precipitation of these compounds with the pollutants (Cao et al., 2009). Alkalinity (higher pH) of biochar can also be partially responsible for the lower concentrations of heavy metals by precipitation. The aim of the study was to determine the influence of biochar on the growth and yield of groundnut grown in a Lead (Pb) contaminated soil.

MATERIALS AND METHODS

Description of the study area and biochar production

The experiment was conducted at the biological science screen house of Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto State is located in Northwestern Nigeria, near the confluence of the Sokoto River and the Rima River, situated between longitudes 11°13′ to 13°50′E and latitudes 4° and 6°N having altitude of 350 m above sea level (Sokoto State Tourist Guide, 2010). Rice straw was used as the feedstock for biochar production (Table 1). The biochar was produced using gasification method. The feedstock (rice straw) was burned for 1 h 30 min at a temperature range of about 330°C–350°C.

Lead inoculation and biochar application

Lead (Pb) was inoculated at the rate of 515 $mgkg^{-1}$ in the buckets containing 5 kg of top soil (thoroughly mixed) a week prior to biochar application. The biochar was applied two weeks before planting at the rates of 0, 2.5, 5, 7.5, 10 and 12.5 t/ha, which is equivalent to 6.25, 12.5, 18.75, 25, and 31.25 g/5kg respectively.

Experimental design and layout

The experiment was conducted in the screen house and laid out in a completely randomized design and was replicated three times. The factor was the rates of biochar application (0, 2.5, 5, 7.5, 10, and 12.5 t/ha). SAMNUT-24 was used as test crop.

Agronomic data collection

Number of leaves and plant height were determined at 2, 4, 6 and 8 weeks after sowing (WAS). Other agronomic data collected include: nodulation count**,** root and shoot dry weights taken at 7 WAS and yield parameters viz pod yield, total kernel yield taken at harvest.

Data analysis

Data obtained was subjected to analysis of variance (ANOVA) and significant differences among means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Chemical properties of soil and biochar used for the study

The pre-sowing analyses of the soil and biochar used for this experiment are presented in (Table 1). The soil and

Table 1. Chemical properties of soil and biochar used for the study.

Properties	Soil	Biochar
pH	5.14	7.41
P (mg/kg)	0.38	1.20
OC (%)	0.66	4.59
N (%)	0.028	0.266
Exchangeable bases (cmol/kg)		
Ca	0.50	0.65
Mg	0.40	0.85
K	0.13	24.62
Na	0.13	2.96
CEC	2.26	46.3

O.C = Organic carbon, N = Nitrogen, P = Phosphorous, Ca = Calcium, Mg = Magnesium, Na = Sodium, CEC= Cation exchange capacity.

Table 2. Number of leaves as affected by biochar rates.

	Number of leaves			
Biochar rates (t/ha)	2 WAS	4 WAS	6 WAS	8 WAS
0	28.00^{a}	50.67^{c}	99.67^{a}	135.00^{a}
2.5	27.00^{a}	48.33^{c}	115.00^{a}	155.33^{a}
5	29.33^{a}	58.33^{bc}	123.33^{a}	166.33^{a}
7.5	28.67^{a}	48.33^{c}	98.00^{a}	138.67^{a}
10	27.67^{a}	73.33^{ab}	134.67^{a}	183.33^{a}
12.5	29.33^{a}	78.00^{a}	144.67^{a}	176.00^{a}
SE ±	0.642	3.496	6.484	7.058
Level of significance	ns	*	ns	ns

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at (p< 0.05), WAS = weeks after sowing, SE = Standard error, ns = Not significant, * = Significant at p<0.05.

biochar pH were 6.7 and 7.41 and are rated as neutral (6.6-7.3) and slightly alkaline respectively (Enwezor et al., 1989). The neutral condition of the soil might be due to the coarse nature of the soil and low rainfall of the area. The available P of the soil is 0.38 mg/kg, while that of the biochar is 1.20 mg/kg and are both rated as low according to Enwezor et al. (1989). The percentage organic carbon of the soil and biochar were 0.66% and 4.59% are rated as low (<1%) and high (>1.5%) respectively (Enwezor et al., 1989). The percentage nitrogen of the soil was 0.028% and is rated as low (<0.15%), while that of the biochar is 0.266% and is rated as medium (0.15 – 2.0%) (Enwezor et al.,1989). The cation exchange capacity (CEC) of the soil was 2.26 cmol/kg and is rated as low (<6 cmol/kg) while that of the biochar is 46.3 cmol/kg and is rated as high (>12 cmol/kg) (Enwezor et al., 1989). The exchangeable bases of the soil viz.; Ca was 0.50 cmol/kg and is rated as low (0-2 cmol/kg), Mg was 0.40 cmol/kg and is rated as medium (0.3-1.0ccmol/kg), K was 0.13 cmol/kg and is rated as low (0-0.15 cmol/kg) and Na was 0.13 cmol/kg and is rated as medium (0.1-0.3 cmol/kg) (Enwezor et al., 1989). Similarly, the exchangeable bases of the biochar viz.; Ca was 0.65 cmol/kg and is rated as low (0-2 cmol/kg), Mg was 0.85 cmol/kg and is rated as medium (0.3-1.0 cmol/kg), K was 24.62 cmol/kg and is rated as high (>0.3 cmol/kg) and Na was 2.96 cmol/kg and is rated as high (>0.3 cmol/kg) (Enwezor et al., 1989).

Effect of biochar on number of leaves

Table 2 presents the effects of biocahr on number of leaves. Biochar application at the rates assayed had no significant difference on number of leaves of groundnut at 2, 6 and 8 weeks after sowing (WAS) but had significant (p<0.05) effect at 4 WAS. However, the insignificant difference among biochar rates as observed in this study could be attributed to the slow nature of biochar in releasing nutrients. At 4 WAS the number of leaves per plant increased with increasing rates of biochar with 12.5 t/ha given the highest number of leaves (29.33) when compared with 0, 2.5, 5 and 7.5 t/ha but statistically comparable with 10 t/ha. The number of leaves per plant

Table 3. Plant Height as Affected by Biochar Rates.

	Plant height			
Biochar rates (t/ha)	**2 WAS (cm)**	**4 WAS (cm)**	**6 WAS (cm)**	**8 WAS (cm)**
0	9.92^{c}	15.17^{b}	27.50^{c}	48.67^{c}
2.5	11.50^{bc}	15.17^{b}	30.67^{c}	50.67^{c}
5	10.92^{bc}	16.17^{b}	34.67^{bc}	55.50^{bc}
7.5	9.83^{c}	15.17^{b}	27.17^{c}	48.00^{c}
10	12.75^{ab}	29.83^{a}	43.50^{ab}	50.00^{ab}
12.5	14.00^{a}	31.50^{a}	45.50^{a}	68.33^{a}
SE ±	0.454	1.793	2.048	2.269
Level of Significance	*	*	*	*

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at ($p<0.05$), WAS = weeks after sowing, SE = Standard error, * = Significant at $p<0.05$.

Table 4. Plant shoot and root dry weight as affected by biochar rates.

Biochar rates (t/ha)	**Shoot dry weight (kg/ha)**	**Root dry weight (kg/ha)**
0	8000^{b}	532^{a}
2.5	10400^{ab}	852^{a}
5	15200^{ab}	1028^{a}
7.5	8532^{b}	932^{a}
10	16132^{ab}	960^{a}
12.5	18132^{a}	1000^{a}
SE ±	1351.6	110
Level of Significance	*	ns

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at ($p <0.05$), SE = Standard error, ns = Not significant, * = Significant at $p<0.05$.

increased with increasing rates of biochar. This is in agreement with Carter et al. (2013) who stated that biochar treatments were found to have increased the number of leaves of lettuce plant in comparison to no biochar treatments. Increase in the number of leaves under the influence of biochar application as observed in this research, is likely due to biochar having a role in creating growing environment for plant growth by improving the physical, chemical and biological soil properties. Biochar potential as land reform media can improve the physical, chemical and biological properties of the soil and plant growth by supplying a number of useful nutrients (Glauser et al., 2002).

Effect of biochar on plant height

The effect of biochar on height per plant is shown in (Table 3). Biochar addition at the rate of 12.5 t/ha was significantly higher than 0, 2.5, 5 and 7.5 t/ha across the weeks though statistically similar with biochar rate of 10 t/ha across the weeks. Hence, the plant height also increased with increasing rates of biochar. The increase in plant height as observed in this research may be as a result of increase in nutrient release with increasing levels of biochar. This is because the higher the level of biochar the more effective it is in adsorbing Lead ions and neutralizing its effects thereby reducing its bioavailability and making beneficial nutrients for plant to be available. This finding is in agreement with Beesley and Marmiroli, (2011) and Lu et al. (2012) who stated that one of the characteristics of biochar is possession of large surface areas, which implies a high capacity to complex heavy metals on their surface. Carter et al. (2013) stated that biochar treatments were found to increase plant height of lettuce plant in comparison to no biochar treatments. Bishwoyog et al. (2015) reported a contrary opinion which stated that biochar did not influence plant height significantly.

Effect of biochar on shoot and root dry matter yields

Table 4 presents the effects of biochar on shoot and root dry matter yields of groundnut plant. Biochar rates differed significantly ($p<0.05$) in their effects on the shoot dry weight. Biochar rates of 12.5 t/ha was significantly higher than 0 and 7.5 t/ha with a weight of 18132 kg/ha soil, followed by 10, 5, and 2.5 t/ha giving weights of 16132, 15200 and 10400 kg/ha respectively. However,

Table 5. Number of Nodules as Affected by Biochar Rates.

Biochar rates (t/ha)	Effective nodules	Non effective nodules
0	25.3[b]	29.67[a]
2.5	41.00[b]	28.00[a]
5	46.33[a]	25.00[a]
7.5	47.00[a]	17.67[a]
10	56.67[a]	22.00[a]
12.5	56.67[a]	19.33[a]
SE ±	3.140	1.669
Level of Significance	*	ns

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at (p<0.05), SE = Standard error, ns = Not significant, * = Significant at p<0.05.

10, 5, and 2.5 t/ha were statistically comparable with 12.5 t/ha. One of the reasons for higher shoot dry matter yield with 12.5 t/ha in this study was the production of higher number of leaves and taller plants when compared with other biochar rates, this translated into a high vigorous growth (18132 kg/ha). Similar findings was reported by Biederman et al. (2013), that addition of biochar to soils resulted on average increase of the above ground biomass compared with control conditions. This is also similar to the findings of Shamshuddin et al. (2004), where it was reported that the highest shoot biomass yield was obtained under plants supplied with high rate of biochar.

No significant difference was observed in the root dry weight but biochar rate of 5 t/ha recorded the highest mean value (1028 kg/ha), followed by 12.5, 10, 7.5, 2.5 and the least was found with the control (0 t/ha). The non-significant difference recorded could be attributed to the fact that the roots of the plants are in direct contact with the Lead in the soil and its toxicity could affect the root growth in almost the same range across the biochar rates.

This is in agreement with what has been reported by Park et al. (2011) that biochar amendment has been investigated as a potential remediation technology for heavy metal-contaminated soil based on its properties, such as having a highly porous structure, the presence of various functional groups, as well as a high pH and cation exchange capacity. Another reason for the increased root dry biomass following biochar application could be attributed to its organic nature.

Effect of biochar on number of nodules

Table 5 presents biochar effect on plant nodules. The Table revealed that there was significant (p<0.05) difference among the biochar rates in number of nodules where 5, 7.5,10 and 12.5 t/ha recorded higher number of effective nodules when compared with 0 and 2.5 t/ha. Meanwhile, there was no significant (p<0.05) difference observed in biochar rates of application on non-effective nodules although treatment 0 t/ha topped the list with 29.67%, thereafter, the number of non-effective nodules decreases with increase in biochar rate. From the results above, it could be seen that the number of effective nodules increases with increase in biochar rate while the number of non-effective nodules decreases with increase in biochar rate. This implies that biochar has the capacity of reducing the number of non-effective nodules and this in turn leads to higher number of effective nodules and subsequently higher yield. This also proved that it can be a good medium for soil microbes growth and development. Similar findings was reported by Biederman et al. (2013), that addition of biochar to soils resulted on average increased in rhizobial nodulation compared with control conditions.

Effect of biochar on number of pod yield and total kernel yield

The effect of biochar on pod yield and total kernel yield per plant is shown in (Table 6). Although, there was no significant (p<0.05) difference observed among the biochar rates of application however, 12.5 t/ha showed the highest mean value (15.67) in both pod yield and total kernel yield (27.67) when compared with other biochar rates. However, the pod yield and total kernel yield increases with increasing biochar rates. Abewa et al. (2014) noted significant increase in grain yield with increasing rates of biochar. This might be due to the fact that soil amelioration and crops supplied with adequate nutrients have more vegetative growth, longer linear growth rate, and more dry matter accumulation which is directly related to an increment in pod number and total kernel yield. This result is in agreement with that of Agboola and Moses, (2015) who reported enhancement

Table 6. Pod yield and total kernel count as affected by biochar rates.

Biochar rates (t/ha)	Pod yield	Total kernel count
0	6.00[a]	8.33[a]
2.5	10.67[a]	12.00[a]
5	10.00[a]	9.33[a]
7.5	9.67[a]	11.00[a]
10	11.67[a]	18.00[a]
12.5	15.67[a]	27.67[a]
SE ±	2.268	4.002
Level of Significance	ns	ns

Means within the same column with the same letters are not significantly different according to Duncan's Multiple Range Test at ($p<0.05$), SE = Standard error, ns = Not significant at $p<0.05$.

of pod number of pea in response to applying of rice husk biochar. The insignificant difference recorded in pod number and total kernel yield could be due to the fact that the duration for the completion of the experiment was short that the biochar supply of nutrient could not support the plants full development of pods and kernels owing to its slow nature in nutrient release and as such differences among the rates could not be seen. This verified the findings of Major et al. (2010) who reported that application of biochar on maize grain yield had no significant effect in the first year; however, in subsequent years, maize yield increased with increasing biochar rate, and the positive effect of biochar was most prominent in the third year after application.

Conclusion

The rice straw biochar examined in this study showed potential for increasing the growth and yield of groundnut. Although, yield increases were not significant, the biochar rates of application examined did not compromise groundnut productivity.

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