

Efficacy of biochar on the phytoremediation potential of *Tithonia diversifolia* on spent oil-contaminated soil

Olamide Omolafe Ogunremi

folarinmide@gmail.com

First Technical University

Omolara Faith Amubieya

University of Ilorin

Clement Oluseye Ogunkunle

University of Ilorin

Paul Ojo Fatoba

University of Ilorin

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Abstract

The application of biochar has gained attention as a simple, affordable and sustainable strategy for the remediation of contaminated soils. However, the phytoremediating potential of certain plants and interactions with biochar are necessary to achieve effective environmental clean-up. This study evaluated the influence of biochar on the remediation potential of *Tithonia diversifolia* grown in spent oil-contaminated soil. *T. diversifolia* was grown in spent oil-contaminated soil amended with rice biochar and sorghum biochar at four different application rates (1, 2 and 3% w/w) for 6 weeks. The heavy metals studied included copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd) and chromium (Cr). The results of this study showed that compared with the other treatments, rice biochar strongly enhanced the growth of *T. diversifolia*. RB3% influenced the phytostabilization potential of *T. diversifolia* for Cu. However, StB3% and SB3% enhanced *T. diversifolia*'s potential capacity for the phytoextraction of Cr and Pb, respectively. Generally, the response of *T. diversifolia* to phytoextraction and phytostabilization is dependent on the biochar and application rate used. Hence, there is a need for further research on the use of *T. diversifolia* for remediation purposes under the influence of specific biochar types and application rates.

INTRODUCTION

Tithonia diversifolia is a shrub in the Asteraceae family that is widely spread throughout the world but is presently distributed in 64 countries [1], and it has been reported to be a native plant of Mexico. This plant was introduced as ornamental/green manure in the humid and sub-humid tropics of Africa [2, 3] and is regarded as a dicot weed found on farms, waste areas and roads in Nigeria [4].

T. diversifolia (TD) is widely valued in several cultures for its medicinal properties [5] due to the presence of valuable sources of bioactive compounds with significant therapeutic implications and favorable safety indices [2]. Comprehensive research on the phytochemical composition, therapeutic efficiency, soil fertility capacity, antioxidant capability, toxicity and allergenic potential of these plants has been reported [2, 6–11]. However, there are few studies on the phytoremediation potential of *T. diversifolia* in heavy metal-polluted soils [12–13].

Reports on the responses of *T. diversifolia* at polluted sites can serve as indicators for soil recovery, which is a cheap, sustainable strategy for cleaning the environment [14]. Ayesa et al. [15] and Al.-Jobori and Kadhim [16] identified *T. diversifolia* as a promising species for the phytoextraction of heavy metals from contaminated sites without being affected. The invasive nature of *T. diversifolia* and its capacity to produce massive biomass and a well-developed expanded root system [2], particularly in soils with low nutrient levels, suggest the need to investigate this plant for its metal accumulation potential. In addition, plants found growing naturally in polluted environments are suggestive of their potential for remediation purposes in such areas [17]. Additionally, the focus can shift to noncrops such as *T. diversifolia*, which are not consumed to avoid threats to human wellbeing. Studies have shown that plants that produce specific secondary metabolites (medicinal plants) can create an adaptive mechanism of tolerance by forming complexes with heavy metals against divalent metals [18–19]. This mechanism is interrelated

with the chelation of metal ions by specific secondary metabolites in medicinal plants [20–21]. *T. diversifolia*, as a medicinal plant, possesses specific secondary metabolites known as targitinins [2] that could be responsible for its capacity to tolerate heavy metals.

Few studies have been conducted on the synergistic effects of biochar and plants on the remediation of heavy metal-polluted soils [22–23]. However, no studies on the potential of *T. diversifolia* grown on spent oil-contaminated soil amended with biochar for soil remediation have investigated the responses of *T. diversifolia* to environmental pollution in terms of its growth and potential accumulation of pollutants. The objective of this research was to evaluate the influence of biochar on the potential of *T. diversifolia* for phytoremediation of spent oil-contaminated soil.

MATERIALS AND METHODS

Study area

The study was carried out at the Green House of Botanical Garden at the University of Ilorin, Ilorin, Kwara State, Nigeria. The University of Ilorin Botanical Garden lies between latitude 08° 28'N and longitude 04° 40' E and latitude 8.500 °N and longitude 4.550 °E, with an annual rainfall of approximately 1200 mm and a temperature varying between 33°C and 34°C.

Collection of substrates

Seeds of *T. diversifolia* were collected from the Botanical Garden, University of Ilorin, Ilorin, Kwara State. The soil samples were collected from the surroundings of the Botanical Garden, University of Ilorin, Ilorin, Kwara State. The spent engine oil was collected from the Toyota Automobile Company, Ilorin, Kwara State. Commercial grade biochar (wheat straw, 700°C) was obtained from the UK Biochar Research Centre (UKBRC). Rice and sorghum straw were collected from Rice Farm, Patigi, Kwara State, and Sorghum Farm, Ilorin, Kwara State, respectively.

Preparation of substrate

Sandy loam soil was bulked and homogenized to give one representative sample of approximately 300 kg. The soil was sieved through a 2 mm mesh to ensure that the soil was free of unwanted materials, which could disturb the proper functioning of the soil microorganisms, proper stretching of the roots and proper organization of the soil. The soil was spiked with 50 ml/kg (5% v/w) spent engine oil, thoroughly mixed manually with hand-protected gloves and left for two weeks in a moist, cool garden environment [24] to ensure the uniformity of the oil, moisture content, air content, temperature, and effective activities of the soil microorganisms. Biochar was introduced to the spiked soil at different rates of 1%, 2% and 3% w/w with thorough mixing under 60% moisture, after which it was allowed to incubate for one month. The soil was sampled for laboratory analysis.

Pot experiment

The experiment was conducted in 30 pots. Polythene pots (19 cm surface diameter and 23 cm depth) were filled with 3 kg of topsoil, and ten *T. diversifolia* seeds were planted in each polythene pot. The germinated plants were thinned to two plants per pot. Watering was performed daily with 180 ml of tap water, and regular weeding was performed by hand picking. The treatments were replicated three times with a complete randomized design (CRD).

Plant growth measurement

Plant height was measured using a meter ruler from the soil level to the collar of the uppermost leaf [25]. Stem girth was measured with thread, which was later extrapolated on a ruler. Leaf area was measured using the method of Watts [26]. The leaves were counted and harvested after 6 weeks. After harvesting, the shoot biomass was collected from the base of the plant stalks. The root biomass was manually separated from the potted soil, and the soil and biochar particles were removed. The root lengths were measured, and the fresh biomass was weighed immediately, while the dry biomass was weighed after oven drying to a constant weight.

Heavy metal determination: The method of Thompson and Wood [27] was adopted. One gram of each plant sample was digested in a 4:1 ratio of concentrated 69% HNO₃ (analyzer grade) and concentrated 60% HClO₄ (Merck, Germany). The mixture was heated to dryness. The residue was then leached with 5 M 36% HCl (analyzer grade) and filtered through Whatman No. 41 filter paper. The filtrate was finally diluted with distilled water to 1 M HCl. The samples were analyzed for multiple elements, including Cd, Cu, Pb, Ni and Zn, with an atomic absorption spectrophotometer (AAS model 210VGP, Buck Scientific Incorporated USA) with an air-acetylene flame. Procedural blanks were carried out, and samples were tested in triplicate to validate the accuracy of the procedure.

Soil and Plant Analyses

Heavy metal determination was performed using the method of Thompson and Wood [27]. One gram of each sample was digested in a 4:1 ratio of concentrated 69% HNO₃ (Analar grade) and concentrated 60% HClO₄ (Merck, Germany). The mixture was heated to dryness. The residue was then leached with 5 M 36% HCl (analyzer grade) and filtered through Whatman No. 41 filter paper. The filtrate was finally diluted with distilled water to 1 M HCl. The samples were analyzed for multiple elements, including Cd, Cu, Pb, Ni and Zn, with an atomic absorption spectrophotometer (AAS model 210VGP, Buck Scientific Incorporated USA) with an air-acetylene flame. Procedural blanks were carried out, and samples were tested in triplicate to validate the accuracy of the procedure.

Plant–soil relationship

The movement of heavy metals from polluted soil into the roots of plants and their ability to translocate metals from roots to aerial parts were assessed by means of bioconcentration factor (BCF) and translocation factor (TF) models [28]. Eq. 1 represent the bioconcentration factor (BCF) calculated as the ratio of the concentration of heavy metals in plant roots to that in soil: $BCF = \frac{C_{mr}}{C_{ms}} (1)$

where C_{mr} is the concentration of heavy metals in roots, C_{ms} is the concentration of heavy metals in soil. BCF values > 2 are regarded as high values, which implies better phytoaccumulation capabilities of the plant [29]. The higher the BCF is, the more suitable the plant is for phytoextraction.

Equation 2 represent the translocation factor (TF) calculated as the ratio of the heavy metal concentration in the plant shoot to that in the plant root: $TF = \frac{C_{msh}}{C_{mr}}$ (2)

where C_{msh} is the metal concentration in the shoot, and C_{mr} is the concentration of heavy metals in the root.

A $TF < 1$ indicates that the plant is an excluder, while a $TF > 1$ indicates that the plant is an accumulator. $TF = 1$ indicates that the plant is an indicator [30].

Statistical analyses

One-way analysis of variance (ANOVA) was used to assess the significant differences among the biochar treatments, and Duncan's multiple range test (DMRT) was used to determine the significance of the differences among the biochar treatments based on the soil properties, growth parameters and heavy metal contents ($p < 0.05$).

RESULTS

Table 1 shows the growth parameters of *T. diversifolia*. The height of *T. diversifolia* decreased with increasing standard biochar (StB) and increased with increasing sorghum and rice biochar. Rice biochar (RB 3%) had the greatest percentage (13.36 cm) of *T. diversifolia*. The number of leaves of *T. diversifolia* in the 1% (8.00) and 3% (7.00) RB treatment groups was significantly greater than that in the untreated group. Only the leaf area of *T. diversifolia* plants treated with rice biochar was significantly greater than that of the control plants. In addition, the 1% RB treatment had the greatest number of leaves and greatest leaf area. Rice biochar (3%)-treated *T. diversifolia* had the greatest percentage of stem girth. Stem girth decreased with increasing standard biochar concentration, while rice biochar tended to increase. However, the sorghum biochar (1%) value was not determined as a result of plant death before measurement. In general, compared with the other treatments, the rice biochar treatment improved the growth of *T. diversifolia*.

Table 1

Effect of different concentrations of standard, sorghum and rice biochars on the growth of *T. diversifolia*

Biochar	rate (%)	Height (cm)	No of leaves	Leaf area (cm ²)	Stem girth (cm)
Control	0	9.60 ± 0.95 ^c	6.00 ± 0.00 ^b	2.45 ± 1.75 ^{bc}	1.46 ± 0.09 ^d
StB	1	9.40 ± 1.61 ^c	4.00 ± 0.00 ^c	1.70 ± 0.32 ^c	1.66 ± 0.08 ^c
	2	8.27 ± 0.72 ^d	6.00 ± 0.00 ^b	1.58 ± 0.29 ^c	1.62 ± 0.08 ^c
	3	5.50 ± 0.20 ^e	4.00 ± 0.00 ^c	0.40 ± 0.26 ^d	1.25 ± 0.00 ^e
SB	1	N	N	N	N
	2	8.93 ± 0.85 ^d	4.00 ± 0.00 ^c	1.06 ± 0.26 ^c	1.52 ± 0.09 ^{cd}
	3	10.33 ± 0.25 ^b	5.00 ± 0.00 ^c	1.74 ± 0.48 ^c	1.33 ± 0.09 ^d
RB	1	10.70 ± 0.46 ^b	8.00 ± 0.00 ^a	5.08 ± 3.95 ^a	1.57 ± 0.00 ^{cd}
	2	12.90 ± 0.72 ^a	6.00 ± 0.00 ^b	3.42 ± 2.59 ^{bc}	1.98 ± 0.18 ^b
	3	13.37 ± 1.08 ^a	7.00 ± 0.00 ^a	4.40 ± 4.25 ^a	2.30 ± 0.48 ^a
Values are presented as means ± S.D of triplicate values. Values with different superscripts in the same column differ significantly at $p \leq 0.05$. StB – Standard Biochar; SB – Sorghum Biochar; RB - Rice Biochar, N- Necrosis					

Table 2 shows the biomass of *T. diversifolia* under the different biochar treatments. At 2%, sorghum biochar had the greatest effect on the length (9.73 ± 2.46 cm) of the roots of *T. diversifolia*. *T. diversifolia* plants treated with rice biochar had significantly greater shoot and root fresh weights ($p < 0.05$) than did the control plants. The sorghum and rice biochar treatments significantly ($p < 0.05$) increased the shoot dry weight of *T. diversifolia*, while the root dry weight of untreated *T. diversifolia* was significantly greater than that of the treated plants, except for SB2%.

Table 2

Effect of different concentrations of standard, sorghum and rice biochars on biomass of *T. diversifolia*

Biochar	rate (%)	RL (cm)	SFW (g/kg)	RFW (g/kg)	SDW (g/kg)	RDW (g/kg)
Control	0	8.20 ± 1.90 ^a	0.32 ± 0.05 ^{abc}	0.06 ± 0.02 ^{ab}	0.02 ± 0.01 ^{bc}	0.02 ± 0.03 ^a
StB	1	6.97 ± 1.21 ^a	0.20 ± 0.07 ^{bc}	0.10 ± 0.02 ^a	0.02 ± 0.01 ^{bc}	0.001 ± 0.00 ^a
	2	5.50 ± 0.40 ^a	0.20 ± 0.01 ^{bc}	0.06 ± 0.04 ^{ab}	0.01 ± 0.001 ^c	0.002 ± 0.001 ^a
	3	8.17 ± 0.55 ^a	0.09 ± 0.01 ^{bc}	0.05 ± 0.02 ^{ab}	0.002 ± 0.001 ^c	0.001 ± 0.001 ^a
SB	1	N	N	N	N	N
	2	9.73 ± 2.46 ^a	0.17 ± 0.04 ^{bc}	0.07 ± 0.05 ^a	0.05 ± 0.01 ^{bc}	0.02 ± 0.03 ^a
	3	6.23 ± 0.45 ^a	0.25 ± 0.07 ^{bc}	0.05 ± 0.03 ^{ab}	0.12 ± 0.08 ^a	0.001 ± 0.001 ^a
RB	1	8.40 ± 6.02 ^a	0.47 ± 0.31 ^{ab}	0.08 ± 0.04 ^a	0.08 ± 0.07 ^{ab}	0.01 ± 0.01 ^a
	2	8.83 ± 1.17 ^a	0.47 ± 0.15 ^{ab}	0.08 ± 0.01 ^a	0.08 ± 0.03 ^{ab}	0.001 ± 0.001 ^a
	3	8.00 ± 2.54 ^a	0.65 ± 0.55 ^a	0.07 ± 0.06 ^a	0.09 ± 0.08 ^{ab}	0.01 ± 0.01 ^a

Values are presented as means ± S.D of triplicate values. Values with different superscripts on the same column differ significantly at $p \leq 0.05$. **StB** – Standard Biochar; **SB** – Sorghum Biochar; **RB** - Rice Biochar; **RL** – root length, **SFW** – shoot fresh weight; **RFW** – Root fresh weight; **SDW** – shoot dry weight; **RDW** – root dry weight, N- Necrosis.

Table 3 shows that the control *T. diversifolia* and StB3%-treated *T. diversifolia* phytoextracted Cr, while SB3%-treated *T. diversifolia* phytoextracted Pb. However, in the RB3%-treated group, *T. diversifolia* phytostabilized Cu.

Table 3
Bioconcentration factors and translocation factors of heavy metals in *T. diversifolia*

		Cu		Zn		Pb		Cd		Cr	
Biochar rate (%)		BCF	TF	BCF	TF	BCF	TF	BCF	TF	BCF	TF
Control	0	1.00	Null	0.00	Null	1.00	7.60	Null	2.17	1.33**	1.50**
StB	1	0.66	1.09	1.23*	0.00*	0.60	1.67	0.62	1.30	0.00	Null
	2	Null	0.06	1.30*	0.00*	2.30*	0.00*	1.76*	0.00*	1.57*	0.64*
	3	0.00	Null	0.00	Null	0.46	2.67	0.00	Null	1.25**	1.60**
SB	1	0.24	1.25	0.00	Null	0.38	2.17	0.00	Null	1.10*	0.36*
	2	Null	1.38	0.00	Null	0.00	Null	0.00	Null	0.57	0.75
	3	1.00	5.51	0.46	1.00	1.66**	1.30**	Null	2.00	1.00	1.25
RB	1	0.00	Null	1.00	0.63	1.00	0.50	1.00	1.54	0.64	0.86
	2	0.63	2.03	1.00	1.00	0.77	1.30	1.00	2.00	0.86	0.67
	3	2.00*	0.78*	0.50	0.00	1.00	0.77	Null	0.00	0.71	1.00

StB – Standard Biochar, **SB** – Sorghum Biochar, **RB** –Rice Biochar, **BCF**- Bioconcentration factor, **TF**- Translocation factor, Phytoextractor** (BCF &TF > 1), Phytostabilizer * ($BCF > 1$ and $TF < 1$), Indicator, $TF = 1$.

Figure 1-5 revealed higher concentrations of all heavy metals in the roots of *T. diversifolia* treated with StB2% than in the shoots. In addition, the roots of *T. diversifolia* treated with RB3% contained higher concentrations of Cd and Cu than the shoots.

DISCUSSION

Research carried out by Ayesa et al. [15] revealed a decrease in the growth biomass of *T. diversifolia* in simulated polluted soil. They noted that water uptake and heavy metal toxicity could be the causes of reduced growth. In relation to this study, the presence of biochar, especially rice biochar, has been able to address the problems highlighted by Ayesa et al. [15]. This implies that the addition of biochar in this study improved *T. diversifolia* growth and biomass in polluted soil. This could result from the porous structure of biochar, which is responsible for its high adsorption and water retention capacity [31–32]. According to Genesio et al. [33], physical conditions change with biochar; its dark color alters thermal dynamics and alleviates rapid plant germination, allowing more time for growth compared with controls.

Additionally, the ability of rice biochar to restrict the movement of heavy metals to *T. diversifolia* could be responsible for its improved growth and biomass.

Phytoextraction is a phytoremediation technique in which heavy metals are removed from polluted soils and then translocated to aboveground tissues [34].

Plants that are recognized as hyperaccumulators must be able to accumulate high levels of heavy metals and still retain their health status, such as high biomass, tolerance to metal toxicity, and resistance to pathogens and pests [35]. For plants to be efficient tools for the phytoextraction of heavy metals in polluted soils, both the bioconcentration factor and translocation factor must be greater than one [36–37]. With respect to the current research, *T. diversifolia* was able to phytoextract Pb in the presence of sorghum biochar (3%), with a BCF of 1.66 and a TF of -1.30, while untreated *T. diversifolia* had BCF and TF values of 1.33 and 1.50, respectively. In addition, standard biochar (3%) was used to treat *T. diversifolia* with BCF and TF values of 1.25 and 1.60, respectively, of potentially phytoextracted Cr. A report by Ayesa et al. [15] revealed that *T. diversifolia* phytoextracted Cd, Zn and Cu but not Pb, which was dissimilar to the results of this study. This could be a result of the incorporation of biochar into the soil, which supported *T. diversifolia* in the phytoextraction of Pb and phytostabilization of Cu, Zn, and Cd, as confirmed in this study.

Phytostabilization is another phytoremediation technique that involves immobilization of heavy metals and accumulation of these metals in underground biomass [38–39] and primarily focuses on heavy metal sequestration in roots. Plants with BCF values greater than 1 and TF values less than one are appropriate for phytostabilization [40–41]. The current study showed that rice biochar application at 3% restricted the movement of Cu from *T. diversifolia* to the roots. The mechanisms that could result in the restriction of heavy metal mobility include water erosion and leaching prevented by plant roots, precipitation [42–43], root adsorption or absorption and a reduction in metal valency [44]. Moreover, plants with BCF values that lack the ability to translocate heavy metals to aerial parts are suitable for phytostabilization [45–46]. Therefore, the current study confirmed the influence of biochar treatments on the phytostabilization potential of *T. diversifolia* for all heavy metals except StB3% and SB3%.

CONCLUSION

This study revealed that rice biochar promoted the growth of *T. diversifolia* and supported the phytostabilization ability of *T. diversifolia* for the studied heavy metals. However, StB3% and SB3% were the only treatments that enhanced the phytoextraction potential of *T. diversifolia* for Cr and Pb, respectively. In this view, the response of *T. diversifolia* to phytoextraction and phytostabilization is dependent on the biochar and dose used in this study. Hence, further research on the use of *T. diversifolia* for remediation purposes in relation to specific biochar types and application rates is needed.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not Applicable

Availability of data and materials: The authors declare that all data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Figures

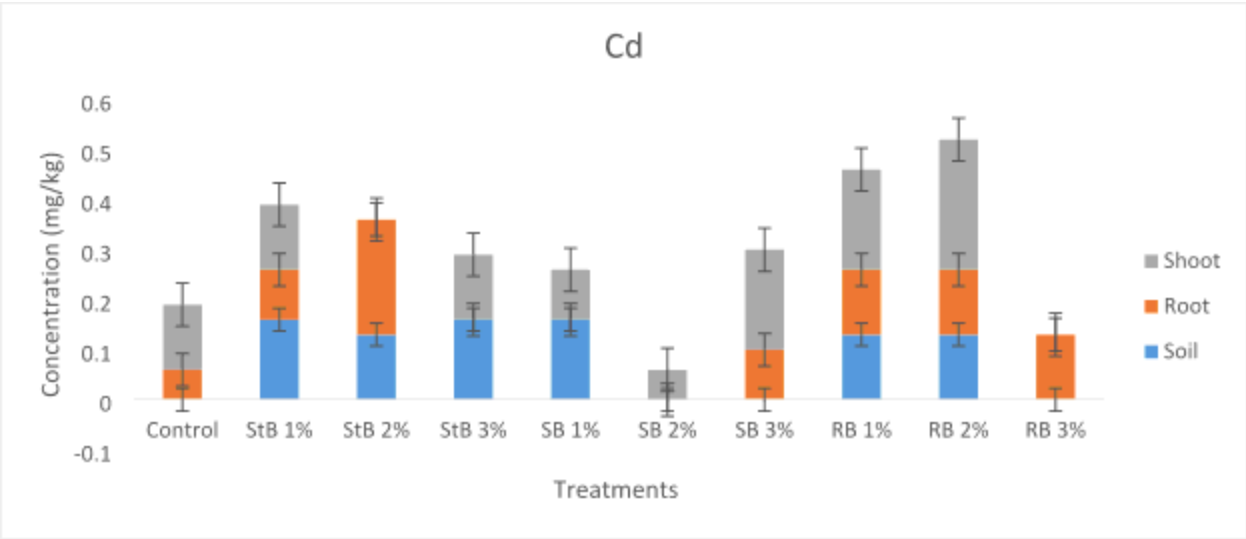


Figure 1

The effect of different concentrations of standard, sorghum and rice biochars on Cu content in soil, root and shoot of *T. diversifolia*

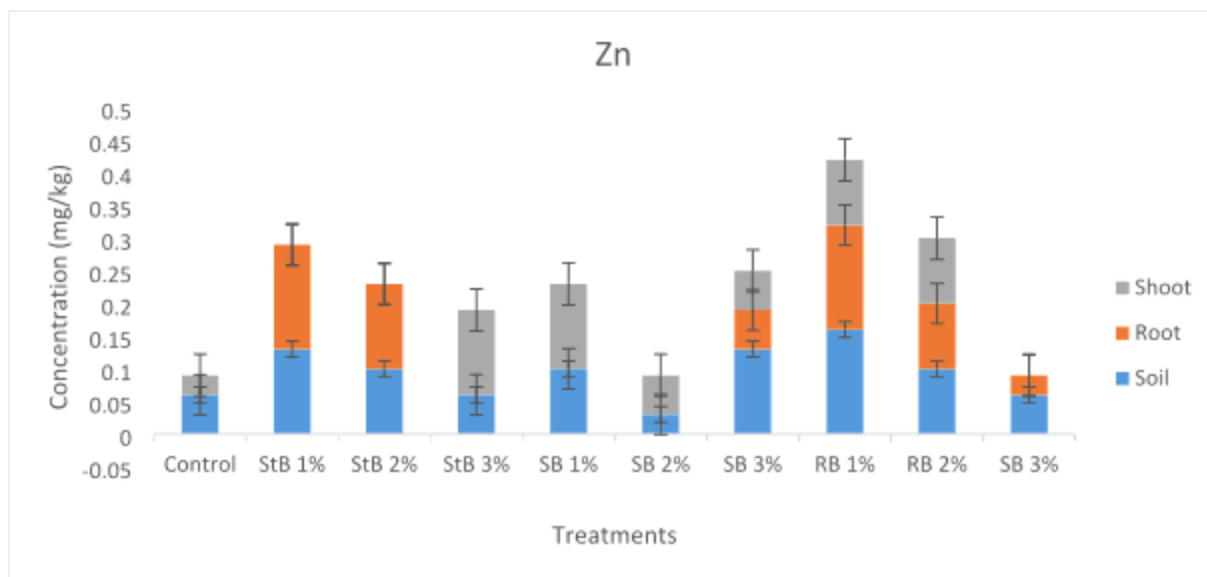


Figure 2

The effect of different concentrations of standard, sorghum and rice biochars on Zn content in soil, root and shoot of *T. diversifolia*

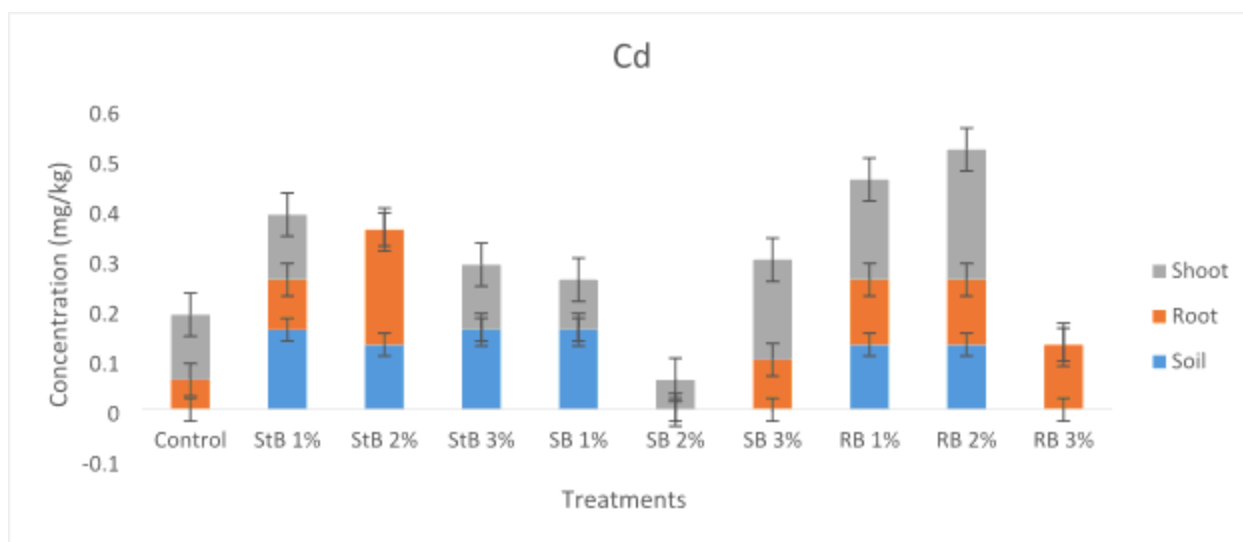


Figure 3

The effect of different concentrations of standard, sorghum and rice biochars on Cu content in soil, root and shoot of *T. diversifolia*

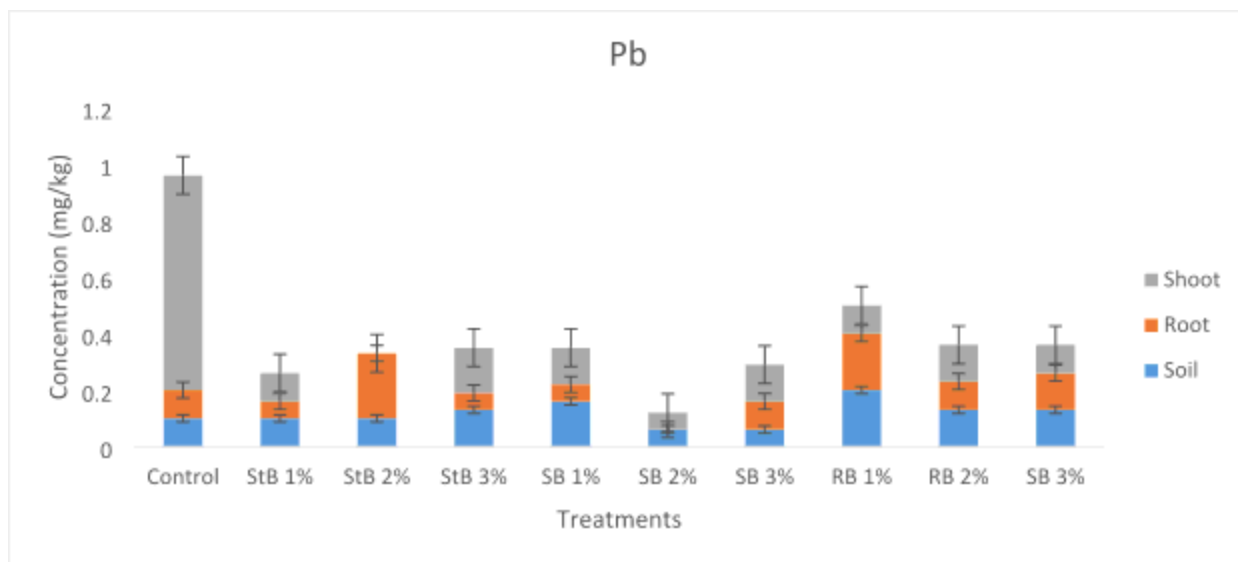


Figure 4

The effect of different concentrations of standard, sorghum and rice biochars on Pb content in soil, root and shoot of *T. diversifolia*

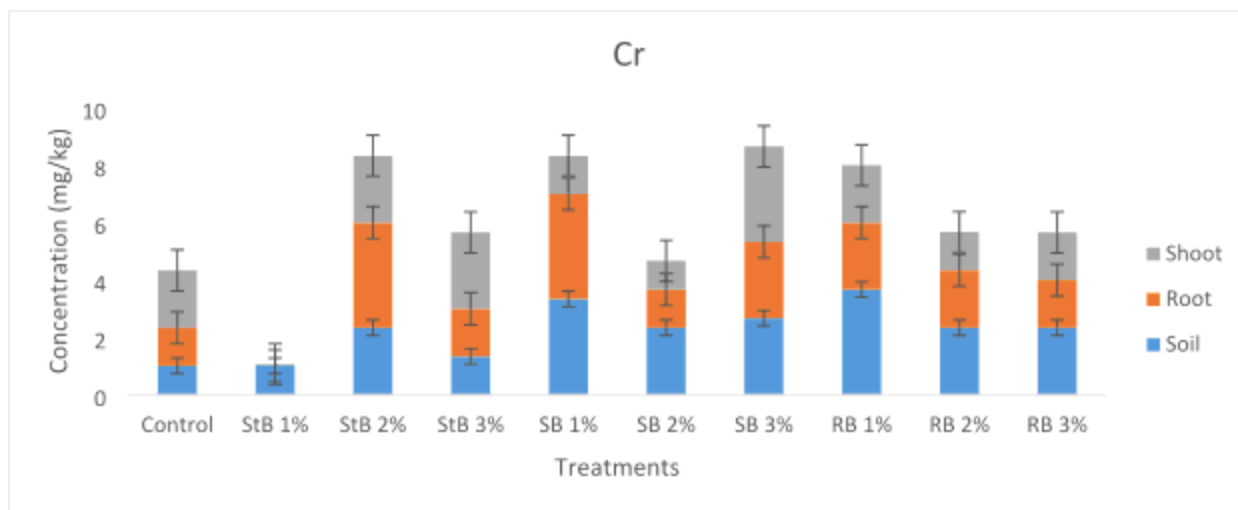


Figure 5

The effect of different concentrations of standard, sorghum and rice biochars on Cr content in soil, root and shoot of *T. diversifolia*