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Effects of ZnO Nanoparticles on the Heavy Metal Concentration of Cement Contaminated Soil and Tissues of *Jatropha curcas* as Test Plant in Phytoremediation Experiment

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ABSTRACT

This study determined the effects of ZnO nanoparticles (ZNP) on the heavy metal concentration of cement contaminated soil and tissues of *Jatropha curcas* test plant. The was carried out to determine the suitability of the test plant assisted with ZNP in the phytoremediation of cement contaminated soil. Pre-soil analysis of heavy metals was done. Cement contaminated soil preliminarily contained 10.0 mg/kg Mn, 10.0 mg/kg Pb and 10.0mg/kg Pb. Significant reduction in heavy metals were recorded in *J. curcas* phytoremediated soils as the concentrations of ZNP treatments increased. Significant accumulation in heavy metals were recorded in shoot and root of test plant as the concentrations of ZNP treatments increased from 20-100ppm. Results showed that phytoremediated soils treated with ZnO nanoparticles at 40-100ppm had PLI <1. There were clear evidences that *J. curcas* was a good candidate for phytoremediation of cement pollutant in by reducing the amount of heavy metals in soil. As these heavy metals are accumulated in the shoot and root of the plant, the pollution index is drastically reduced. The test plant is thus recommended for cultivation around cement factories where wastes are disposed.

Key words: Cement**,** Heavy metals, *Jatropha curcas,* Nanoparticle, Phytoremediation

INTRODUCTION

The heavy metals are among the most insidious soil pollutants because of their non-degradable nature and so affect all forms of ecological systems (Kim *et al.,* 2015). Consequently, they are one of the most pressing problems of the present world (Sharma *et al*., 2019). Heavy metals are long term contaminants with ability to accumulate in the soil and in plants for over a long period of time, and there is no natural way to remove them. Even low concentrations of heavy metals are toxic because there is no good mechanism for their elimination from the body. Mobilization of heavy metals in the biosphere, caused by human activity, significantly increased the circulation of toxic metals in soil, water and air. Many studies have shown that heavy metals are extremely persistent in the environment, non-biodegradable and readily accumulate to toxic levels (Kim *et al.,* 2015; Sharma *et al*., 2019). Among the very toxic and bioavailable ones are Beryllium, Chromium, Manganese, Iron, Copper, Zinc, Cadmium and Lead. Potentially toxic metals are Lead, Zinc, Copper, Arsenic, Mercury, Barium, Cadmium, Chromium, Nickel and Selenium. Others include Cobalt and Manganese (Kim *et al.,* 2015; Sharma *et al*., 2019).

Cement manufacturing is a complex process that begins with mining and then grinding of raw materials that include limestone and clay, to a fine powder called raw meal, which is then heated to a very high temperature of about 1450ºC in a Kiln (Gundu *et al.,* 2020). Chemical analysis of cement dust shows that they contain some appreciable amounts of basic oxides, sulfides and heavy metals in various proportions and when they dissolve in water, they react to form various products (Gundu *et al.,* 2020). The negative consequence of cement industries in urban cities has become a major concern (Laniyan *et al*., 2014; Gundu *et al.,* 2020).

Phytoremediation consists of four different plant-based technologies, each having a different mechanism of action for the remediation of metal polluted soil, sediment or water. These include phytoextraction, where plants absorb metals from soil and translocate them to harvestable shoots where they accumulate (Akomolafe *et al.,* 2018; Onwusiri *et al.,* 2021). Currently, sustainable environment is needed. The development of nanochemicals has appeared as promising agents for assisted phytoremediation (Azeez *et al.,* 2021). Metal nanoparticles present more surface area for valance electron exchange with bio-molecules, due to their higher surface area to volume ratio. Therefore, use of metal nanoparticles can alter the antioxidant status of treated plants, by virtue of their ability to participate in cellular redox reactions (Awwad and Ahmad, 2014; Ghidan *et al.,* 2018). Jatropha has been extensively researched for phytoremediation The plant can be used in remediation without losing their economic values while nanotechnology offers a new dimension as augmented and accelerated method of heavy metals uptake from contaminated soil. This study determined the effects of ZnO nanoparticles (ZNP) on the heavy metal concentration of cement contaminated soil and tissues of *Jatropha curcas* test plant.

MATERIALS AND METHODS

Study Area

This study was carried out in Makurdi metropolis of Benue State (Longitude 8º30'E, 8º30'E and Latitude 7º30'N, 7º43'N). Climatically, Makurdi falls within the tropical, sub humid, wet and dry climate which has two distinct seasons, namely wet season and dry season. The wet season starts from April and lasts till October; while the dry season starts in November and lasts till March. Rainfall ranges from 775 millimeters to 1792 millimeters, with a mean annual value of 1190 millimeters. Mean Monthly Relative Humidity in Makurdi LGA varies between 43% in January to 81% in July-August perio. Makurdi L.G.A. falls within the Guinea Savannah belt of Nigeria. The Guinea Savannah belt is a transitional vegetation zone separating the forested belt of southern Nigeria from the true savannah of the north. It is characterized by a mixture of tall grasses and trees of average height. Most of the trees are deciduous and shed their leaves during dry season (Tyowua *et al*., 2013).

Collection of Cement Kiln Waste and Spent Engine Oil as Contaminants

Cement kiln waste (50kg) was collected from Benue Cement Industry, Gboko, Benue State. These materials were transported to the Advanced Biology Laboratory of the Joseph Sarwuan Tarka University Makurdi. They were stored inside the cupboard for safe keeping.

Collection and Preparation of Soil Samples

Soil samples were collected from an undisturbed area within the University farm land using the soil auger. Two depths were covered: 0-15cm and 15-30cm. Samples were collected in sterilized polythene bags and taken to the laboratory. Soil samples were air-dried and disaggregated where 1.0 g of was weighed into a 250.00cm^3 beaker followed by digestion and evaporation to dryness over a water bath. The residue was re-dissolved with 10ml HCl, and transferred to a 50.00ml volumetric flask. The content was made up to 50.00ml mark with de-ionized water. Heavy metal analyses were carried out in a pre-experimental stage following the procedures used by Paliza *et al.* (2019).

Heavy metal analysis using Atomic Absorption Spectrophotometer (AAS)

Heavy metal analysis was carried out at the Chemistry Advanced Research Center, SHETSCO, Abuja. The amount of Phosphate (mg/kg), Lead (mg/kg), Chromium (mg/kg) and Manganese (mg/kg) were determined using the Atomic Absorption Emission Spectrophotometer (Model Perkin Elmer) (Benavides *et al.,* 2018).

Preliminary Heavy Metal Analysis of Soil Samples Prior to Contamination

The following heavy metals were determined from freshly prepared soil samples: Lead (mg/kg), Chromium (mg/kg) and Manganese (mg/kg), using standard methods (Gundu *et al.,* 2020; Onwusiri *et al.,* 2021) using AAS (atomic absorption spectrophotometer).

Heavy Metal Analysis of Contaminated Soil Samples

Fresh soil sample was thoroughly mixed with cement kiln waste (6:1) in a clean bucket (9kg of soil mixed with 1.5kg of cement waste) (Jones and Smith, 2017) (Agamuthu *et al.,* 2015). Each mixture was tightly covered and allowed to stand for 7 days (Agamuthu *et al.,* 2015). Soil samples were analyzed for Lead (mg/kg), Chromium (mg/kg) and Manganese (mg/kg) (Gundu *et al.,* 2020; Onwusiri *et al.,* 2021) using AAS (atomic absorption spectrophotometer).

Collection of Test Plants

Jatropha curcas seeds were collected from mature plants within Makurdi metropolis in Benue State. Authentication was done by Taxonomists aided by relevant monographs/albums in the Department of Botany, Joseph Sarwuan Tarka University Makurdi. Seedlings were raised in the nursery for 30 days before use in phytoremediation experiments. Duly (Arora *et al.,* 2012).

Green Synthesis of Zinc Oxide Nanoparticles (ZnONP)

Freshly synthesized ZnO nanoparticles were obtained from Chemistry Department, JOSTUM, Nigeria. It consisted of mixtures of Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), sodium hydroxide (NaOH) and ethanol 99.9% of analytical grade. The green method was employed in the synthesis using *Jatropha tajonensis* leaves. Fine powdered *J. tanjorensis* leaves of 6g were added into 100 mL deionized water which was heated at 80°C for 20 to 30 minutes. The obtained extract was filtered using filter paper (Whatman no. 1) and stored at 4°C for further use (Attah *et al*., 2020). The solution of (Zn(NO3)2·6H2O) was prepared in deionized water. For the preparation of zinc oxide NPs, the flask containing 17.85g zinc nitrate hexahydrate (5.95g, 0.2M) in 240 mL of deionized water reacted with 60 mL of the aqueous leaf extract and stirred using a magnetic stirrer heated at 70°C with the addition of 2M NaOH solution and stirring is non-stop until a homogenous mixture of the solution is attained. The uniform solution was desiccated in a warm air oven at the temperature of 100-110°C for 90 minutes (until dried), washed with ethanol and water and annealed at 300-350°C for 1 h in a muffle furnace. The particles with yellow colour obtained are wrinkled in a metallic mortar and pestle to get zinc oxide nanoparticles (Al-Khaial *et al*., 2021).

Experimental Design

The Completely Randomized Experimental Design was used at 5 levels of nanoparticles (20, 40, 60, 80 and 100ppm) and one test plant. Zinc salt and NPK inorganic fertilizers also served as positive control treatment while test plant with contaminated soil but without treatment served as the negative control (Cumba *et al.,* 2019). Altogether, eight (8) treatments were used, each replicated 5 times. In total, there were 40 experimental units (pots).

Planting and Treatment Application

Three (3) stands of test plant (30 day old) were transplanted in each pot (sized 18 by 9cm) filled with 25kg of contaminated soil. All pots were tagged. They were irrigated with 50 mL de-ionized water twice a week for the first two weeks, and then every other day as the plants grew taller. The pots containing experimental plants were kept in the screen house for 12 weeks (Paliza *et al*., 2019). After establishment at day 20 after planting (DAP), treatments were applied with five different concentrations of ZnO nanoparticles (20, 40, 60, 80, 100ppm) using micropipette. Exactly 5g of Zinc salt and inorganic fertilizer (NPK) were applied in powdered form (Arora *et al.*, 2012).

Re-Analysis of Contaminated Soil Samples for Heavy Metals (Post phytoremediation)

After the experiment at week 12, contaminated soil samples undergoing phytoremediation experiments were re-analyzed for heavy metals including Pb (mg/kg), Cr (mg/kg) and Mn (mg/kg) (Gundu *et al.,* 2020; Onwusiri *et al.,* 2021).

Determination of Heavy Metal Concentration in Shoots and Roots of Test Plants

Wet digester method was applied for plant shoot and root analysis using perchloric acid, nitric acid and sulphuric acid. The digested plant samples were analyzed for the heavy metals using atomic absorption spectrophotometer.as described by Arora *et al.* (2012).

Determination of Pollution Load Indices (PLI) in Contaminated Soil Samples

Contamination factor (CF) was estimated for each heavy metal using standard baseline permissible level of the heavy metal in soil to divide the concentration in polluted soil. Pollution Load Indices (PLI) was estimated by taking the average values of the CFs. This was done for contaminated soil, control soil with only test plants and ZNP treated soils at varying concentrations (20-100ppm) (Joanna *et al.*, 2018).

Data Analysis

Soil and plant data were computed and analysed on the Genstat application package, version 17.1 for mean, standard deviation and one-way ANOVA test statistics. Mean separation (Post-hoc analysis) was performed using the LSD method at 95% confidence limit.

RESULTS

Table 1 gives the outcome of preliminary analysis of soil samples. Lead and chromium were not detected (<0.01 mg/kg) while manganese level was 2.18 mg/kg. Lead levels were 15.0 mg/kg in cement contaminated soils. Chromium level was 10.0 mg/kg while Manganese was 10.0 mg/kg.

Table 2 shows the effects of ZnO nanoparticle (at different concentrations) on heavy metal concentration of cement contaminated soil undergoing phytoremediation by *J. curcas*. Levels of Pb, Cr and Mn were found highest in the control soil with values of 8.11, 5.55 and 3.23 mg/kg respectively. Significant reduction in heavy metals were recorded in phytoremediated soils as the concentrations of ZNP treatments increased (P<0.05). At 100ppm of treatments, the levels of the three heavy metals analyzed were found to 5.01, 3.33 and 1.73 mg/kg respectively.

Table 3 shows the effects of ZnO nanoparticle (at different concentrations) on heavy metal concentration in shoot of *J. curcas* test plant. Levels of Pb, Cr and Mn were found lowest in the control shoot (without ZNP treatment) with values of 0.13, 0.09 and 0.04 mg/kg respectively. Significant accumulation in heavy metals were recorded in shoot of test plant as the concentrations of ZNP treatments increased from 20-100ppm (P<0.05). At 100ppm of treatments, the levels of the three heavy metals analysed in the shoot were found to 0.37, 0.27 and 0.11 mg/kg respectively.

Table 4 shows the effects of ZnO nanoparticle (at different concentrations) on heavy metal concentration in root of *J. curcas* test plant. Levels of Pb, Cr and Mn were found lowest in the control root (without nano treatment) with values of 0.54, 0.49 and 0.14 mg/kg respectively. Significant accumulation in heavy metals were recorded in root of test plant as the concentrations of ZnO nano treatments increased from 20-100ppm (P<0.05). At 100ppm of treatments, the levels of the three heavy metals analysed in the shoot were found to 1.78, 1.22 and 0.93 mg/kg respectively.

Table 5 gives the effects of ZnO nano treated *J. curcas* on contamination factor (CF) and pollution load indices (PL1) of cement contaminated soil. Results showed that phytoremediated soils treated with ZnO nanoparticles at 40-100ppm had PLI <1. Phytoremediated soil using *J.curcas* alone or augmented with 20ppm ZnO nano particle had PLI >1.0 in cement contaminated soil

Table 1: Preliminary Soil Heavy Metal Analysis Pre and Post Contamination with Cement

Soil Properties	Pre-contamination	Post-contamination with cement @ 7 days
Lead (mg/kg)	<0.01	15.0
Chromium (mg/kg)	<0.01	10.0
Manganese (mg/kg)	2.18	10.0

Table 2: Effects of ZnO Nanoparticles on Heavy Metals Concentration in Soil Undergoing Phytoremediation by *J. curcas*

Treatment	Pb (mg/kg)	Cr (mg/kg)	Mn (mg/kg)
Control	8.11 ±0.00[a]	5.55 ±0.00[a]	3.23 ±0.00[a]
Nano@20ppm	6.60 ±0.01[b]	4.68 ±0.03[b]	2.94 ±0.00[b]
Nano@40ppm	6.00 ±0.00[c]	3.99 ±0.00[c]	2.72 ±0.00[c]
Nano@60ppm	5.77 ±0.00[d]	3.72 ±0.00[d]	2.27 ±0.00[d]
Nano@80ppm	5.32 ±0.01[e]	3.50 ±0.00[e]	1.99 ±0.00[e]
Nano@100ppm	5.01 ±0.00[f]	3.33 ±0.01[f]	1.73 ±0.00[f]
P-value	0.000	0.000	0.000
LSD	0.29	0.10	0.22

Control = Test plant in contaminated soil only (without nano-treatment)

Means with different superscripts along the column are significantly different at P≤0.05 level of significance

LSD = Least Significant Difference; P = probability value at 5% level of significance; ppm = part per million; Pb= Lead; Cr = Chromium; Mn = Manganese

Table 3: Effects of ZnO Nanoparticles on Heavy Metals Concentration in Shoot of *J. curcas* Test Plant

Treatment	Pb (mg/kg)	Cr (mg/kg)	Mn (mg/kg)
Control	0.13 $\pm 0.00^{f}$	0.09 $\pm 0.00^{f}$	0.04 $\pm 0.00^{d}$
Nano@20ppm	0.25 $\pm 0.00^{e}$	0.13 $\pm 0.00^{e}$	0.05 $\pm 0.00^{c}$
Nano@40ppm	0.28 $\pm 0.00^{d}$	0.16 $\pm 0.00^{d}$	0.06 $\pm 0.00^{c}$
Nano@60ppm	0.31 $\pm 0.00^{c}$	0.18 $\pm 0.00^{c}$	0.08 $\pm 0.00^{b}$
Nano@80ppm	0.36 $\pm 0.00^{b}$	0.22 $\pm 0.00^{b}$	0.11 $\pm 0.00^{a}$
Nano@100ppm	0.37 $\pm 0.00^{a}$	0.27 $\pm 0.00^{a}$	0.11 $\pm 0.00^{a}$
P-value	0.000	0.000	0.000
LSD	0,01	0.02	0.01

Control = Test plant in contaminated soil only (without nano-treatment)

Means with different superscripts along the column are significantly different at P≤0.05 level of significance

LSD = Least Significant Difference; P = probability value at 5% level of significance; ppm = part per million; Pb= Lead; Cr = Chromium; Mn = Manganese

Table 4: Effects of ZnO Nanoparticles on Heavy Metals Concentration in Root of *J. curcas* Test Plant

Treatment	Pb (mg/kg)	Cr (mg/kg)	Mn (mg/kg)
Control	0.54 $\pm 0.00^{f}$	0.49 $\pm 0.00^{f}$	0.14 $\pm 0.00^{f}$
Nano@20ppm	1.01 $\pm 0.00^{e}$	0.59 $\pm 0.00^{e}$	0.40 $\pm 0.00^{e}$
Nano@40ppm	1.11 $\pm 0.00^{d}$	0.72 $\pm 0.00^{d}$	0.54 $\pm 0.00^{d}$
Nano@60ppm	1.26 $\pm 0.00^{c}$	0.87 $\pm 0.00^{c}$	0.66 $\pm 0.00^{c}$
Nano@80ppm	1.45 $\pm 0.00^{b}$	1.06 $\pm 0.00^{b}$	0.74 $\pm 0.00^{b}$
Nano@100ppm	1.78 $\pm 0.00^{a}$	1.22 $\pm 0.00^{a}$	0.93 $\pm 0.00^{a}$
P-value	0.000	0.000	0.000
LSD	0.10	0.15	0.11

Control = Test plant in contaminated soil only (without nano-treatment)

Means with different superscripts along the column are significantly different at P≤0.05 level of significance

LSD = Least Significant Difference; P = probability value at 5% level of significance; ppm = part per million; Pb= Lead; Cr = Chromium; Mn = Manganese

Table 5: Effects of ZnO Nano Treated *J. curcas* on Contamination Factor (CF) and Pollution Load Indices (PLI) of Cement Contaminated Soil

Treatment	Pb CF	Cr CF	Mn CF	PLI
Contaminated soil only	6.000	1.250	0.040	2.43
Control	3.243	0.694	0.012	1.32
Nano@20ppm	2.639	0.585	0.012	1.08
Nano@40ppm	2.400	0.499	0.011	0.97
Nano@60ppm	2.308	0.465	0.009	0.93
Nano@80ppm	2.128	0.438	0.008	0.86
Nano@100ppm	2.003	0.417	0.009	0.81

Control = Test plant in contaminated soil only (without nano-treatment)

Means with different superscripts along the column are significantly different at $P \leq 0.05$ level of significance; ppm = part per million; Pb= Lead; Cr = Chromium; Mn = Manganese; CF = Contamination factor; PLI = Pollution load index

DISCUSSION

Soil samples contaminated with cement increased in heavy metal concentrations when compared with non-contaminated soil. Similar findings were reported by Subhashini and Swamy (2013). Nanoparticles play important roles in enzyme activation to reduce the level of toxic elements in soil. Heavy metals are generally more mobile, therefore easily bioavailable to plants, and subsequently have the possibility of being transferred into the food chain (Xiao *et al*. 2017; Alengebawy *et al*., 2021). Studies have reported the potential of ZnO nanoparticles to mitigate the toxic effects of heavy metals on plants, making them a promising solution for crops grown in contaminated soils (Kim *et al*., 2021). Therefore, nanoparticle-assisted phytoremediation increase tolerance to stress from contaminants, and increase the absorption of nutrients and water. It makes phytoremediation more efficient by eliminating, immobilizing, or reducing HMs from the soil (Alaboudi *et al*., 2018).

The finding of this research has provided valuable information on the combination of phytoremediation with *Jatropha* or *Nicotiana* and the application of nanoparticles to remove heavy metals from contaminated soil. Performances of the test plant in cement contaminated soil showed that phytoremediated soils augmented with ZNP at 40-100ppm had PLI <1 whereas soil treated with test plant alone or augmented with 20ppm ZnO nano particle had PLI >1.0.

CONCLUSION

Results showed that phytoremediated soils treated with ZnO nanoparticles at 40-100ppm had pollution load index (PLI) of <1. Therefore, *J. curcas* plant is a good candidate for phytoremediation of cement pollutant by reducing the amount of heavy metals in soil. As these heavy metals are accumulated in the shoot and root of the plant, the pollution index is drastically reduced. The test plant is thus recommended for cultivation around cement factories where wastes are disposed.

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