Pb UPTAKE BY QUAIL GRASS (*CELOSIA ARGENTEA)* GROWN ON Pb - ACID BATTERY SOIL TREATED WITH STARCH STABILIZED ZERO-VALENT IRON NANOPARTICLES.

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ABSTRACT

The effect of zero-valent iron nanoparticles (nZVI) on the physiology and uptake of heavy metals and nutrients in plants is crucial in the development of nanotechnology for the reclamation of contaminated soils. Zerovalent iron nanoparticles are able to immobilize heavy metals thereby reducing its bioavailability. Plant uptake experiment was thus used to evaluate the immobilizing potentials of starch stabilized zerovalent iron nanoparticles (S-nZVI) in the uptake of Pb by *Celosia argentea* grown on Pb-acid battery contaminated soil. Application of 1000 mg/kg of S-nZVI to the parent soil reduced bioavailable Pb in *Celosia argentea* (Quail grass). *Celosia argentea* grown on the starch stabilized zerovalent iron nanoparticles soil showed better tolerance to Pb than that grown on the parent soil. This was ascertained by the higher shoot and root biomass (dry weight matter). The concentration of iron was found to be higher in the shoots of *Celosia argentea* grown on the parent soil than for the treated soil. The higher translocation factor (TF) for iron in *Celosia argentea* (Quail grass) grown on the untreated soil might be one of the factors responsible for the observed trend. The TF for Pb was greater than one, an indication that *Celosia argentea* could be used in phytoremediation. The result from the pot experiment showed that S-nZVI significantly reduced phytoavavilable Pb.

Key words: Heavy metals; starch stabilized zero-valent iron nanoparticles; *Celosia argentea*; Phytoremediation

INTRODUCTION

Lead is one of the most widespread heavy metals in soils and groundwater. (Tangahu *et al.,* 2011 and Zhao *et al.*, 2009). Minute quantity of Pb can be very toxic to biotas (Zimmerman, 2010). The U.S. Environmental Protection Agency (USEPA) and Agency for Toxic Substances and Disease Registry (ATSDR) have ranked lead as the second most hazardous substances in the U.S. (ATSDR, 2005). Accumulated levels of these heavy metals could have a negative influence on the physiological procedures of the plant (Ioan *et al.*, 2008; Moosavi and Seghatoleslami, 2013). Plants are able to uptake, translocate, and store toxic elements, whose chemical properties simulate those of essential elements. The roots are able to immobilize these heavy metals in the soil and groundwater through adsorption onto the roots or precipitation within the root zone (Tangahu *et al.*, 2011). Plants therefore have the ability to absorb the bioavailable fractions of these heavy metals from the surrounding soil through their roots and translocating these heavy metals so absorbed into its edible tissues thus bioaccumulating these heavy metals in its tissue (Rahman and Zaim, 2015; Tangahu *et al.*, 2011).

The thermodynamic solubility of a contaminant containing mineral in the environment can directly influence the chemical reactivity, transport and ecotoxicology of the heavy metal ions since contaminant uptake and contaminant mobility of these heavy metals in natural systems occur typically through the solution phase (Kalembkiewicz and Soèo, 2005; Traina and Laperche, 1999). That is to say, for a heavy metal to be available for uptake by plants, it has to be present in the soil solution. This therefore means that ideally there exist a relationship between the concentration of the free metal ion activity in the soil solution and the level of heavy metal uptake by plant. The concentration of heavy metals is thus controlled by the dissolution and precipitation of discreet mineral phase (Traina and Laperche, 1999). Natural and synthetic amendments/additives such as phosphates, biochar, lignite, Fe and Mn oxides, Fe(II)sulphate, aluminosilicates, coal, flyash, clay minerals, microbes, organic compost, sulphate salts, compounds of sulphur, micro and nanoscale iron

have been added to soils to alter soil chemistry. (Anegbe *et al.*, 2014; Gaast *et al.,* 2003; Gatma *et al.,* 2005; Mench *et al.,* 1998; Uzinger and Anton 2008; Weigand *et al.,* 2003; Zubillaga *et al.,* 2008). These additives are able to immobilize these heavy metals through adsorption or change in the redox state of these heavy metals thus effectively reducing the mobility, bioavailability and toxicity of the heavy metals in soils/sediments (Galdames *et al.,* 2017; Wang *et al.,* 2009; Wang *et al.,* 2015).

In recent times, the use of zerovalent iron nanoparticles (especially the stabilized zerovalent iron nanoparticles) have received considerable attention and is preferred to other methods of immobilization because of their extremely large surface area, high reactivity and ability to diffuse and disperse in water and soils. These attributes enables the stabilized zerovalent iron nanoparticles to get to target contaminant for effective immobilization thus eliminating excessive input of the immobilizing agent and thus reducing cost (Prabu and Parthiban, 2013; Zhao *et al.,* 2009; Zhang, 2003). Several literatures have reported huge success in the use of zerovalent iron nanoparticles in the immobilization of heavy metals (Gil- Díaz *et al.,* 2013; Gueye *et al.,* 2015; Xu *et al.,* 2007; Zhao *et al.,* 2009). Studies on its effect on plants is however still unclear and has not been given considerable attention (Li *et al.,* 2015).

Chemical sequential extraction procedure has often been used to evaluate phytoavailable metal in nZVI treated soils (Gil - Díaz *et al.,* 2014). The uptake of heavy metals by plants is however dependent on plant species and the physicochemical factors governing soil chemistry (Tangahu *et al.,* 2011) and as such a chemical extraction though an important tool in estimating phytoavailable heavy metal may not be sufficient in evaluating the immobilizing effect of zerovalent nanoparticles in plants. This work therefore is an attempt at prognosis and prophylaxis on the reclamation of arable soils and would hence provide an insight into the immobilizing effects of zerovalent iron nanoparticles on Pb uptake and plant physiology using *Celosia argentea* as an indicator of the phytoavailable Pb in starch stabilized zerovalent iron nanoparticles treated soils.

MATERIALS AND METHODS
Preparation of S-nZVI

All reagents used in this study were all of analytical grade. The reduction method proposed by (Wang and Zhang, 1997) was used in preparing S-nZVI. Aqueous solutions were purged with nitrogen gas prior to synthesis and all the reactions were done in a 250 mL conical flask under nitrogen purge at ambient temperature. 1.62 g of iron (III) chloride hexahydrate was dissolved in 24 mL deionized water the ferric solution was then added drop wise through a syringe to a 6 mL solution of starch under simultaneous stirring to yield a 0.2 % w/v of starch concentration. 0.80 g of sodium borohydride was dissolved in 50 mL nitrogen purged deionized water and was thereafter added drop wise through a syringe to the iron (III) chloride/starch complex under vigorous stirring. Slowly the solution turned black and was stirred for five minutes after all the sodium borohydride had been added. The black particles were collected using a magnet, washed via centrifugation with 50 mL of nitrogen saturated deionized water followed with washing in three portions of 25 mL absolute ethanol and then oven dried at a temperature of $60^{\circ}C$ for 12 hours. This procedure was repeated several times to obtain about 1.5 g of S-nZVI. Bare zerovalent iron nanoparticles (ZVI) were synthesized likewise but in the absence of starch ($FeCl_3$ solution was prepared in a mixture of 24 mL ethanol and 6 ml deionized water to prevent rapid oxidation). (Fig. 1)

Treatment of Soil with Starch Stabilized Zerovalent Iron Nanoparticles

Soil from a Pb battery manufacturing site was treated with S-nZVI. A gram of zerovalent iron nanoparticles was added to 999 g of the parent soil (a dosage level of 0.1 % S-nZVI). The soil and nanoparticles were thoroughly mixed followed by the addition of 100 mL of deionized water. The soil was thoroughly mixed again to ensure uniformity and proper dispersion of the nanoparticles. The soil was then incubated for 3weeks. The parent soil and treated soils were then used to evaluate the plant uptake of heavy metals.

Planting

Two weeks old uniform seedlings of *Celosia argentea* from Obasanjo Farms Ota were transplanted into 200 g of parent and 0.1% starch stabilized zerovalent iron nanoparticles treated soils. The experiment was arranged in completely randomized design with five replications for each soil. The plants were harvested after two weeks. They were first washed in tap water to remove sand particles and then rinsed in distilled water. The shoots were thereafter separated from the roots.

Digestion of Plant Samples

The plant samples were air dried to a constant weight after which they were ground into powder. Plant samples were first pre-digested in concentrated HNO_3 followed by digestion in a 3:2 diacid mixture of HNO_3 and $HClO_4$. Deionized water was added followed by filtration with Whatmann No 1 filter paper. The digestate was then diluted appropriately and analyzed for mineral uptake using AAS Buck scientific VGP 210 model (Deo *et al.,* 2011).

RESULTS AND DISCUSSION
Characterization of Starch Stabilized Zerovalent Iron Nanoparticles

The crystallographic analysis of S-nZVI used in this study was performed by powder x-ray diffraction using X- ray diffractometer (Philip's X'pert Pro, UK) with Cu Kα radiation (λ = 0.154). The diffraction pattern obtained over a 2θ range of 25-70^0 is shown in Fig. 1. The diffraction pattern showed that of an amorphous phase of Fe and the characteristic broad peak at 2θ of about 45 showed that of zerovalent iron (Rashmi *et al.,* 2013; Selvarani and Prema, 2012; Sun *et al.,* 2006). Iron oxide peaks as seen in the spectra of bare ZVI (1) and (2) were not noticed in XRD spectra

of S-nZVI. Same results were obtained by Selvarani and Prema, (2010), using starch stabilized formation of iron oxide (FeO). Other related works have however reported that there was no appearance of oxide / hydroxide shell formation in the outer surfaces of the bare zerovalent iron nanoparticles (Rashmi *et al.,* 2013; Yuvakkumar, 2011). Since both zerovalent iron nanoparticles were synthesized in the same manner, it is most likely that the starch used in the synthesis of the modified zerovalent iron nanoparticles protected the zerovalent iron nanoparticles from rapid oxidation and the synthesized S-nZVI was predominantly in its zero-valent state. The average particle size of S-nZVI was 9.58 nm as calculated from Debye Scherer formula in equation 1 (Huang and Tang, 2005).

$$d = \frac{K\lambda}{\beta \cos \theta} \qquad (1)$$

Where d is the particle size of the crystal, k is Scherer constant (0.9), λ is the X- ray wavelength (0.15406 nm), β is the line broadening in radian obtained from width at half maximum, and θ is the Bragg diffraction angle of the XRD diffraction pattern.

The magnetic properties as obtained using physical property measuring system PPMS is shown in Fig. 2. The hyteresis loop shows that the particles were superparamagnetic (Faraji *et al.,* 2010; Krajewski *et al.,* 2015). As extrapolated from the magnetization curve, the synthesized starch stabilized zerovalent iron nanoparticles had a coercivity value of 1628 Oe and a saturation magnetization (M_s) of 27 emu/g which is lower than that of bulk iron which has a M_s of 92 emu/g (Alagiri *et al.,* 2015; Vecchia *et al.,* 2008).
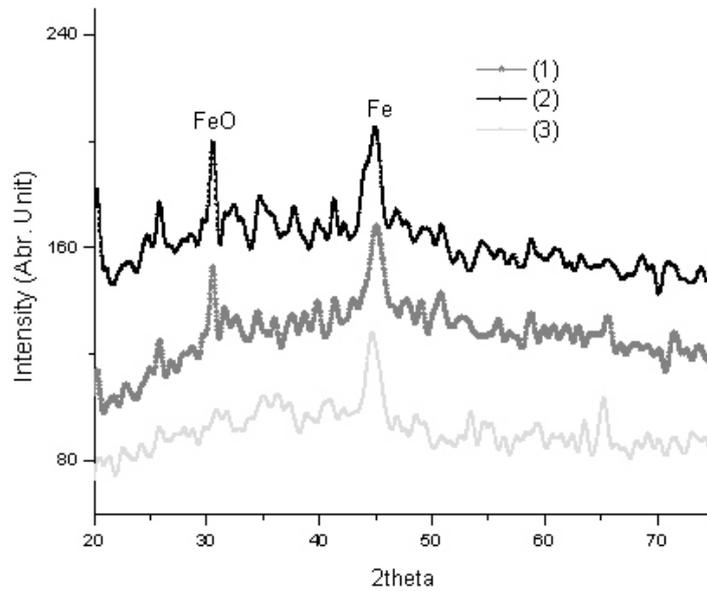
Fig. 1: X-Ray Diffraction Pattern of nZVI and Starch Stabilized Zerovalent Iron Nanoparticles (1&2 = nZVI, 3= S-nZVI) .
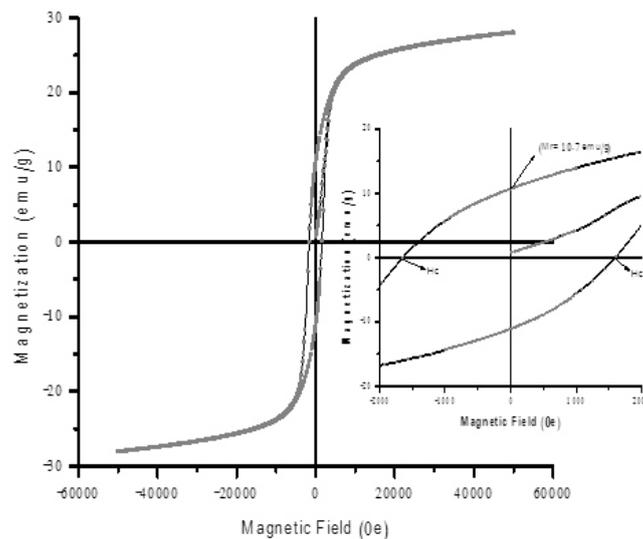

Fig. 2: Magnetic Hysteresis Loop of S-nZVI under Magnetic Field upto 50 K Oe at 300 K. Hyteresis Parameters such as Saturation Magnetization, Magnetization Coercivity and Remenance Magnetization are shown.

Table 1: Selected Physicochemical Properties of Pb Acid Battery Soil.

pH	Organic carbon (%)	Organic matter (%)	Sand (%)	Clay (%)	Silt (%)	CEC (meq/100g)	Pb (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
4.20±0.10	2.7332±0.28	4.725±0.55	73.40±0.98	23.60±0.45	3.00±0.33	5.23±0.04	4980±20.50	100±2.00	15249.50±72.56

Effect of Starch Stabilized Zerovalent Iron Nanoparticles on the Physiology of *Celosia Argentea*

Celosia argentea grown in parent and treated soils both showed the toxic effect of Pb as there was withering of leaves. However, *Celosia argentea* grown in the starch stabilized zero iron nanoparticles treated soil showed better tolerance to the Pb contaminated soil than that grown on the untreated soil. This can be ascertained by the lower rate of withering and higher plant biomass (dry weight matter) as observed in Tables 2 and 3. The little withering observed in the treated soil is as a result of the extremely high concentration of Pb in the soil and therefore a higher dose of zerovalent iron nanoparticles would be needed to reduce the phytoavailable Pb to concentrations that are not phytotoxic.

The tolerance index, TI based on the dry weight of plant is an indicator of the toxic effect of metals on plants growing in heavy metal contaminated soil. The equation for the calculation TI for heavy metals is shown in equation 2 (Ebrahimi, 2013).

$$TI = \frac{dry\ weight\ of\ the\ plant\ grown\ in\ heavy\ metal\ soil}{dry\ weight\ of\ plant\ in\ control} \quad (2)$$

Consequently, in this study the TI indicator for toxic effect of zerovalent iron nanoparticles in heavy metal soil is given by

$$TI = \frac{dry\ weight\ of\ the\ plant\ grown\ S-nZVI\ soil}{dry\ weight\ of\ plant\ in\ control} \quad (3)$$

Where the control is the heavy metal soil without starch stabilized zerovalent iron nanoparticles.

While TI for Pb in the root increased from 0.28 to 0.34 in treated soil that of the shoots increased from 0.27 to 0.32. TI for starch stabilized zerovalent iron nanoparticles in heavy metal soil for root and shoot were 1.24 and 1.15 respectively. These values are greater than 1, an indication that the application of starch stabilized zerovalent iron nanoparticles did not have a negative effect on the plant physiology but rather a positive effect. Plant turgidity is a term that is used to refer to the ability of plants to remain upright in the soil. *Celosia argentea* grown on the treated soil also showed greater turgid strength as shown Fig. 3.

Table 2: Effect of S-nZVI on Leaf Withering

	Number of withered leaves (dropped leaves)									
Day	Parent soil					0.1% treated soil				
	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	1	2	1	2	1	0	0	0	0	0
4	0	0	0	0	1	0	1	1	1	0
5	0	0	0	0	0	0	0	0	0	1
6	1	1	1	0	1	1	0	0	0	0
7	1	1	0	1	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	1	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
Total	3	4	2	3	3	1	2	1	1	1

Table 3: Dry Weight (g) of Plants (Mean dry weight of seedling in distilled water (control): root 0.25 g, shoot 0.75 g)

Treatment levels of S-nZVI (%)	Dry weight of Root	Dry weight of Shoot	TI (Root)	TI (Shoot)	TI_{S-nZVI} (Root)	TI_{S-nZVI} (Shoot)
0	0.070±0.008	0.207±0.050	0.28	0.27	-	-
0.1	0.087±0.006	0.240±0.072	0.34	0.32	1.24	1.15
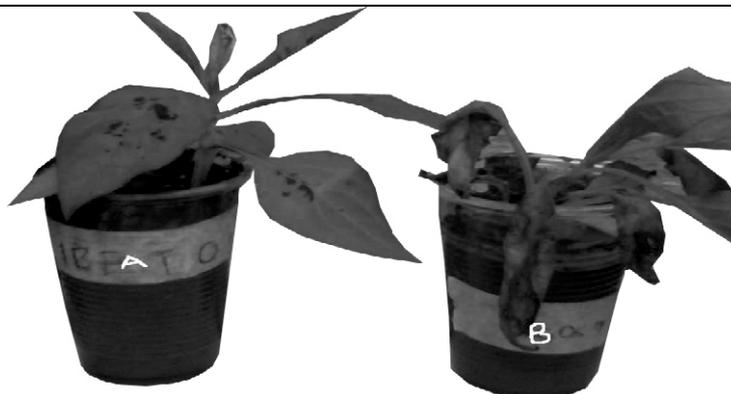
Fig. 3: *Celosia Argentea* Grown on Treated Soil (A) and Parent Soil (B)

From the results, the phytoxic symptoms observed in the plants is as a result of the extremely high concentration of the Pb in the soils used rather than the application of zerovalent iron nanoparticles.

Heavy Metal Uptake

The uptake of Pb is quite high in *Celosia argentea* grown on both the parent and treated soil. However, the application of zerovalent iron nanoparticles to the soil significantly reduced the uptake of Pb grown on the treated soil. In the shoots, Pb uptake was reduced from 147.78 mg/kg to 89.84 mg/kg after the application of 1000 mg/kg starch modified zerovalent iron nanoparticles. In the roots, Pb uptake was reduced from 146.31 mg/kg to 88.07 mg/kg. The ability of roots to transfer heavy metals to the shoots can be explained by a translocation factor or transfer factor (TF). It is given as the ratio of metal concentration in plant shoot to that in the root. The translocation factor for Pb in both parent and treated soils were 1.01 and 1.02 respectively. A translocation factor that is equal to 1 is an indication that the plant is a potential phytoaccumulator (Ebrahimi *et al.*, 2007; Aiyesanmi, 2012). These values therefore indicate that *Celosia argentea* may be a good hyperaccumulator of Pb in phytoremediation.

The application of zerovalent iron nanoparticles to the soil significantly reduced the uptake of Zn in *Celosia argentea* grown on the treated soil in both shoots and roots. The translocation factor for Zn in plants grown on parent and treated soils were 1.35 ± 0.33 and 1.14 ± 0.31 respectively. These values again indicate that C*elosia argentea* may be a good hyperaccumulator of Zn in phytoremediation. However, the application of zerovalent iron nanoparticles may lead to Zn deficiency since it is an essential nutrient. Though Zn deficiency was not established in this report as

can be seen from the high concentration of Zn in *Celosia argentea.*

Nanoparticles can be taken up by the root and transported to the above parts of the plant or may adhere to the roots depending on the anatomy of the plant, composition, size and shape of nanoparticles (Nedecky *et al.*, 2017). Although total heavy metal may not always be used in predicting bioavailable metals (Anarwar, 2008; Zimmerman and Weindorf, 2010), most research works have however reported an increase in uptake of metals with increase in metal concentration (Intawongse and Dean, 2006; Nouri *et a.,* 2009; Orhue and Ekhomun, 2010). The addition of S-nZVI to parent soil would increase the total Fe concentration and thus likely increase uptake in plant. The level of Fe uptake in the roots was slightly higher in *Celosia argentea* grown on the untreated soil. Conversely in the shoots, the level of Fe was reduced from 953.64 mg/kg in the parent soil to 602.33 mg/kg in the treated soil despite the addition of Fe. This is probably due to higher translocation factor (TF) for iron in *Celosia argentea* grown on untreated soil. Since uniform seedlings were collected from the same source, the obviously lower concentration of Fe in the shoots and hence lower TF of plants grown on treated soil can be attributed to change in the surface chemistry of the soil and root zone owing to the presence S-nZVI (Nedecky *et al.*, 2017). The lower TF is an indication that *Celosia argentea* may not have the internal detoxification mechanism to tolerate an eventual phytotoxicity of Fe nanoparticles (Nouri *et al.*, 2009). The above observations show that iron nanoparticles are more likely to adsorb to the root zone and may not be easily translocated to the shoots. It is also an indication that the addition of S-nZVI may not have significantly increased leachable Fe (phytoavailable Fe).

Table 4: Heavy Metal Uptake (mg/kg)

	Parent soil			Treated **soil**		
	Root	Shoot	TF	Root	Shoot	TF
Pb	146.31± 2.67	147.78 ± 13.10	1.01	88.04 ± 1.99	89.84 ±1.14	1.02
Zn	605.44±172.50	726.41± 49.02	1.31	534.76 ± 33.79	538.24 ± 20.67	1.14
Fe	1773 ± 20.50	953.64± 67.54	0.46	1800 ± 44.11	602.33 ± 77.32	0.33

The bioconcentration factor shown in equations 4 and 5 is used in classifying plants as either bioaccumulators or excluders. A bioaccumulator has BCF values > 1 while an excluder plant has BCF values < 1 (Ebrahimi, 2013). The results show that *Celosia argentea* is an excluder of Pb and iron and a bioaccumulator of Zn. The application of S-nZVI reduced the ability of *Celosia argentea* to function as either an excluder or a bioaccumulator.

$$BCF_{root} = \frac{(C_{root})}{(C_{soil})} \qquad (4)$$

$$BCF_{shoot} = \frac{(C_{shoot})}{(C_{soil})} \qquad (5)$$

Where C_{root} and C_{shoot} are the metal concentration in the roots and shoots respectively and C_{soil} is the metal concentration in the soil.

Table 5: Bioconcentration Factor of *Celosia argentea*

Metal	Parent soil		Treated Soil	
	BCF $_{root}$	BCF $_{shoot}$	BCF $_{root}$	BCF $_{shoot}$
Pb	0.029	0.030	0.018	0.018
Zn	6.054	7.264	5.3476	5.382
Fe	0.110	0.062	0.118	0.039

CONCLUSION

Starch stabilized zerovalent iron nanoparticles was able to immobilize phytoavailable Pb and Zn in lead-acid battery soil. It was ecofriendly and had a positive effect on the physiology of *Celosia argentea*. The use of zerovalent iron nanoparticles may create a means of reclaiming arable soil if the immobilization of essential nutrients can be controlled.

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