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Enhanced Phytoremediation Efficiency of Lead Contaminated Soil by Zero Valent Nano Iron

Harikumar P S[1], Lamya T V[2], Shalna T[3]
[1,2,3]*Water Quality Division,*
Centre for Water Resources Development and Management, Kozhikode, Kerala,India

Abstract-Nano-phytoremediation is an advanced technology using nanotechnology and phytotechnology for remediation of contaminated environments. This research aimed to investigate the capability of combination between phytoremediation and nanoscale zero valent iron (nZVI) for removal of lead (Pb)from contaminated soil. For the present study five hyper accumulating plants were selected according to their accumulation efficiency namely,Alternathera dentate, Wediliacheninsis,Tradescantiaspathacea,Plectranthusamboinicus&Heliconiapsittacorum. The selected plants were transferred to the pots containingPbcontaminated soilwith or without nZVIparticles and allowed to grow for 60 days. Subsequently they were harvested and then analyzedby using Atomic Absorption Spectroscopy (AAS).Among the studied plants, Wediliacheninsisexhibited highest removal efficiency(97.82%) followed by Alter nantheradentate (97.00%), Plectranthusamboinicus (87.12%), Tradescantiaspathacea (84.92%) & Heliconiapsittacorum (52.82%) during nano-phytoremediation method.Translocation factors (TF) and bioconcentration factor (BCF) of the plants are <1 which suggests that, translocation of Pb from root to shoot was comparatively less and both phytoremediation &nanophytoremediation resulted in phytostabilization.Thereby plants couldminimizethe availability of heavy metal (Pb) through immobilization in contaminated soils.
Keywords-Hyper accumulating plants, Phytoremediation, Nano-phytoremediation,Bioconcentration factor, Translocation factor

I. INTRODUCTION

Heavy metal contamination is a major environmental problem generated by human activities due to the uncontrolled disposal of industrial and urban wastes, unscientific agricultural activities, etc. The non- biodegradable nature of heavy metals can adverselyaffect the ecosystem. Heavy metal contamination in soil represents one of the most severe threats to soil and water resources as well as human health [32] andmany of the heavy metals are toxic even at low concentrations.They can accumulate in the environment and can subsequently find their way into the food chains [11].Several technologies can beapplied to remediate heavy metal contaminated soils, but most of them are expensive[6-22].

However; phytoremediation can be potentially used to remediate metal-contaminated site using hyper accumulating plants, which is an emerging cleanup technology for contaminated soils that employs a cost effective, eco-friendly, long-lasting and creativesolution for remediation of contaminated sites [20].Nano-phytoremediation is a combined technology between nanotechnology and phytotechnology for remediation of contaminated environments. Nano materials have significant surface area, high number of active surface sites, and high adsorption capacities make them very promising as cost-effective amendments for the remediation of contaminated soils.In a recent study,Harikumar et al. [10] observed maximum phytoremediation efficiency for Pb and copper in TiO_2 nanoparticle exposed plants. In recent year nanoparticles of Fe (0) also have become a strategic material with great application potential in the broad range of modern nanotechnologies, due to the extraordinary reduction capabilities, small size in the range of several tens of nanometers and high reactivity with a broad spectrum of toxic substances.Zerovalent iron nanoparticles (nZVI) are a promising material which plays a very important rolein environmental remediation [33]. nZVI, as a powerful, inexpensively and environmental friendly agent, has been used for heavy metal soil remediation, and satisfactory results have been achieved not only in the laboratory but also in the field. Experiments were conducted in the fields using nZVI for the reduction of Cr (VI) and got satisfactory results [25].In this perspective, new studies are still necessary to find new accumulator plants growing in a local area. The selection of plant species is an important decision for phytoremediation study. While the application of locally available plant species for phytoremediation is often favoured as it requires less management and acclimatizes successfully in our native climate and seasonal conditions.With this idea, we did experiments using locally available hyper accumulating plants species namely, *Alternatheradentate,Wediliacheninsis,Tradescantiaspathacea, Plectranthusamboinicus&Heliconiapsittacorum*to investigate the ability of their phytoremediation efficiency and also to study the effect of zerovalent iron nano particle on heavy metal uptake and translocation in different parts of the plants.

II. MATERIALS AND METHODS

2.1 Soil characteristics and preparation

The soil used for this study was collectedlocally from the surface layer of 0-90 cm. The collected soil was dried in air and twigs and stones were removed manually by sieving using a 2 mm sieve. The physico-chemical properties of soil such aspH, electrical conductivity (EC), organic carbon, texture and exchangeable cationswere determined and tabulated (Table 1).

Table1.Physico-chemical characteristics of soil

Parameters	
pH	4.85 ±0.05
Electrical conducticity, µS/cm	71.1 ±0.05
Organic carbon, %	1.2±1.24
Sand, %	83.75
Clay, %	13.75
Silt, %	2.50
Cation exchange capacity,milli equivalents/100g soil	2.10

2.2 Experimental Design

Locally available non-edible terrestrial plant species such as *Alternathera dentate, Scoparisdulcis,Clerodendronviscosum,Abutilonindicum,Wediliacheninsis,Tradescantiaspathacea,Catharanthusroseus,Plectranthusamboinicus,Heliconiapsittacorum&Ervalaneta jus* were selected and screened for the phytoremediation study. The experiment was continued after the screening period and 5 different species of hyper accumulated plants were selected according to their removalefficiency.The selected plants are:*Alternantheradentata,Tradescantiaspathacea,Plectranthusamboinicus,Heliconiapsittacorum&Wediliacheninsis.* The cultivation of plants were carried out in plastic pots (with an inner diameter 20 cm and depth of 17 cm) filled with 7 kg of dry soil. Plants were placed in a temperature controlled greenhouse. Water was added periodically to maintain the soil moisture contentat the ratio of 1:10.The soil in each pot was artificially spiked with Pbin the form of $Pb(NO_3)_2$, to a level of 40 ppm. The soil was equilibrated for 48 hrs. Five hundred milligrams of finely powdered nZVIparticle was added to one kilogram of soil and mixed uniformly. Allchemicals used were of the highest purity available and of analytical grade procured from Merck.The plant samples were collected from the pots after a period of 60 days of experiment. Root and shoot of the plants were separated, dried and analyzed using Atomic Absorption Spectroscopy (AAS).

2.3 Synthesis of nanozerovalent iron particles

The nZVI particles were synthesized by the reductive precipitation process using sodium borohydride ($NaBH_4$) and iron (III) chloride ($FeCl_3$) [30,8,27 and 12]. $NaBH_4$ (0.25 M) aqueous solution was added drop wise to $FeCl_3.6H_2O$ (0.045 M) aqueoussolution at 1:1 volume ratio.The formed blacksolidiron nano particles were filtered through whatman No 41. The synthesized iron nanoparticles were then washed several times with distilled water and absolute ethanol and were dried in an oven at 50^0C overnight. For storage, ethanol was added to preserve the nano iron particles from oxidation.

2.4 Extraction and analysis of heavy metals in plants

After 60 days, the plants were harvested and heavy metal concentrations in plants were determined. The plants were separated into root and shoot and thoroughly washed with distilled water to remove soil particles adhered to the plants. After washing, plant samples were dried in an oven at 80°C for 48 hours and then ground to powders and sieved by 0.2mm sieve. Samples (0.2g) of finely ground plant tissue were digested with 10 ml mixtures of $HNO_3/HClO_4$ (4:1) at 150°C for 200 min [31]. After complete digestion, the digested samples were diluted to 50 ml with deionized water, and analyzed for total heavy metals by flame atomic absorption spectrometry (Thermo seriesUSA) using acetylene/air as gas mixture.

2.5 Bio concentration factor and Translocation factor

Bio concentration factor (BCF) indicates the efficiency of a plant in up-taking heavy metals from soil and accumulating them into its tissues. It is a ratio of the heavy metal concentration in the plant tissue (root, stem or leaves) to that in soil [34]. The high BCF value,indicates more suitability for phytoextraction (BCF Values>2 is regarded as highvalues) [5 and 21].

$$BCF = \frac{\text{Metal concentration in the plant tissue}}{\text{Metal concentration in the soil}}$$

Translocation factor (TF) indicates the potency of the plant in translocating the accumulated heavy metals from roots to shoots. It is the ratio of the concentration of the heavy metal in shoots (stem or leaves) to that in its roots.Metals that are accumulated largely in the roots of plants are indicated by TF values<1, with values greater indicating translocation to the aerial part of the plant [21].TF was calculated using the following method [23].

$$TF = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in roots}}$$

III. RESULTS AND DISCUSSION

3.1 Characterization ofnZVI particles

nZVI particles used for the study were synthesized by chemical precipitation method and characterization were done by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The SEM image of synthesized nZVI particles is shown in the figure 1.

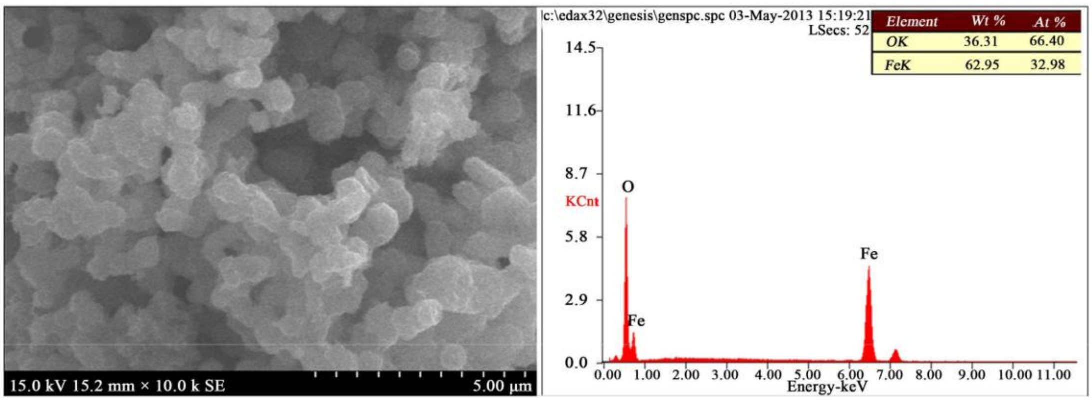

Figure1.Scanning electron microscopy-energy dispersive X-ray spectroscopy image of the zerovalent iron nanoparticles

Results indicated that the synthesized nZVI particles are in nano scale (can be seen on the scale bar at the bottom of the SEM image) and the nanoparticles are spherical in shape and exist as chain like aggregates. Aggregation of the nanoparticles is reported to be caused by the large surface area and magnetic dipole–dipole interactions of the individual particles [29].

TEM images (figure 2) ofnZVI nanoparticles indicated that iron nanoparticles possess a core–shell structure, in which the shell represents the oxidisedregion that surrounds the Fe^0core and preserves it against further oxidation. It appears that many primary nano particles are interconnected with one another to form larger nano clusters, and single nano particle sizes are around 5–40 nm. The size distribution survey from TEM images of over 400 nanoparticles suggests that over 80% of the nanoparticles had diameters of less than 100 nm whereas 50% were less than 60 nm. The synthesized nanoparticles are formed chain-like as aggregated structures because of nano material have a natural tendency to remain in amore thermodynamically stable state. The absence of high-resolution fringes in the shell indicates that the shell is amorphous. Sun et al.[26], synthesized nZVI nanoparticles and got similar results. The laboratory prepared iron particles were largely spherical, characteristic of particles formed in solution. A representative single particle size isapproximatly 60–70 nm. A few particles had size as large as 200–250 nm, whereas 92% particles were less than 100 nm. TEM images also showed that most particles formed chain-like aggregates.

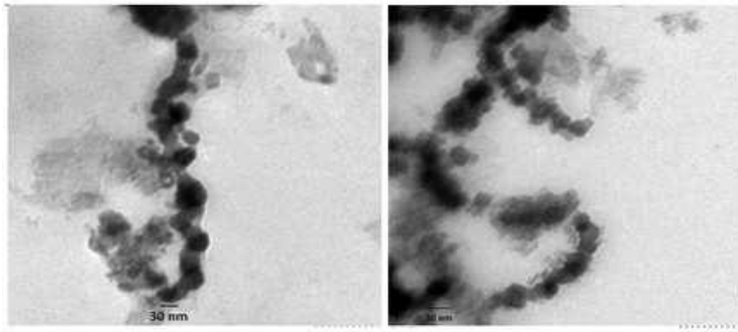

Figure 2.Transmission electron microscopy images of the zerovalent iron nanoparticles

3.2Phytoremediation of heavy metals

3.2.1 Metal concentrations in plants

In the present investigation, a total of 5 hyper accumulating plants were selected after continuous screening.Chaney [7] first suggested the concept of using hyperaccumulator plants to accumulate heavy metals in their biomass as to remove metals from soil environment.Under normal growing conditions, plants can potentially accumulate certain metal ions an order of magnitude greater than the surrounding medium [14].

The concentration of Pbin the plants after phytoremediation ranged from 17.88 to 35.00 mg kg−1, with the maximum being reported in the *Alternathera dentate.* In addition, *Wediliacheninsis* also contained significant amounts of Pb (27.41 mg kg−1). *Heliconiapsittacorum*accumulated least amount of Pb while comparing with other hyper accumulators.It may be because of *Heliconiapsittacorum* had less water logging capacity than other selected hyperaccumulators. The uniform sprinkling of water wasinsuffient for thegrowth& showed less biomass yield.Alloway et al. [1] suggested that metal concentrations in plants vary with plant species. Natural hyperaccumulator plants often grow slowly and have low biomass yield[24,28,16,17and18].Root characteristics and depth of root zone also plays an important role in metal uptake.In 95% of the plant samples, the Pb concentrations in the roots were much greater than those in the shoot.This indicates low mobility ofPb from the roots to the shoots and immobilization of heavy metals in roots. For instance, Chaney and Giordano classified Mn, Zn, Cd, B, Mo, and Se as elements, which were readily translocated to the plant shoots[7-9]. Ni, Co, and Cu, were intermediate, and Cr, Pb, and Hg were translocated to the lowest extent [2].Concentrations of Pb in plant samples are given in table 2.

Table 2.Concentration ofPbin various plant species (mg kg−1)

Plant species	Phytoremediation		Total (mg/kg)
	Pb (40 mg/kg)		
	Root (mg/kg)	Shoot (mg/kg)	
Alternathera dentate	29.68	5.32	35.00
Wediliacheninsis	23.17	4.24	27.41
Tradescantiaspathacea	17.76	1.51	19.27
Plectranthusamboinicus	16.29	2.34	18.63
Heliconiapsittacorum	15.07	2.81	17.88

3.2.2 Metal concentrations in nZVI exposed plants

nZVI has emerged as an effective option forthe treatment of contaminated soil. Thenanosize effect of nZVI played an important role in the degradation of pollutants[13]. nZVI has proved to have a good performance on decomposition of p-nitrophenol and was greatly superior to that of commercial iron power [19].The application ofnZVI particles enhance Pb uptake in all plant species. Among them the maximum uptake of Pb was observed in *Wediliacheninsis,* which is 39.13mg kg−1 followed by *Alternathera dentate* (38.80 mg kg−1).*Tradescantiaspathacea*&*Plectranthusamboinicus* also showed an increased concentration of Pb 33.97&34.85mg kg−1 respectively. The least concentration of Pb was observed in *Heliconiapsittacorum (*21.13 mg kg−1).Accumulation of Pb in the selected hyperaccumulators were higher in roots than that of shoots. Roots are important part of underground planting, which are also the first part of the contact with nZVI. A recent study by Kim showed that exposure of plants to 500 mg/L nZVI can enhance *Arabidospis thaliana* root elongation because nZVI induces cell wall loosening [15].The observation indicated thatcontaminants are immobilized through absorption by roots, adsorption onto root surface and precipitation within the area of plant roots-Phytostabilization(Table 3).

Table 3. Concentrations of Pb in plant biomass

Plant species	Nano- phytoremediation Pb (40 ppm)		Total (mg/kg)
	Root (mg/kg)	Shoot (mg/kg)	
Alternatheradentate	32.65	6.15	38.80
Wediliacheninsis	32.46	6.67	39.13
Tradescantiaspathacea	29.76	4.21	33.97
Plectranthusamboinicus	27.87	6.98	34.85
Heliconiapsittacorum	18.71	2.42	21.13

3.2.3 Percentage of accumulation of heavy metal in plants

The percentage removal of heavy metals by hyper accumulating plants with phytoremediation and nano-phytoremediation is shown in figure 3.The results indicated that the accumulation of heavy metal concentration in plant biomass was higher inexperiment pot with nano-phytoremediation compare to phytoremediation. In nano-phytoremediationexperiment the *Wediliacheninsis*&*Alternathera dentate*reported 97.82% & 97.00 % of uptake whereas accumulation of Pb inphytoremediation without nZVIreported68.52% & 87.50% of uptakerespectievely.The study indicatedthat, allselectedhyper accumulator plants except*Heliconiapsittacorum*reportedhigh accumulation of heavy metalwhenZVI particles were applied.

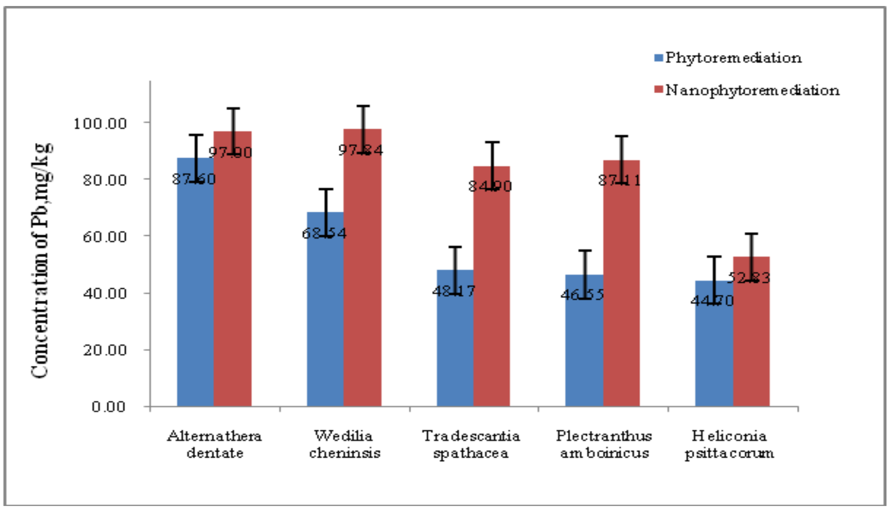

Figure 3. Percentage of accumulation of Pb in plants

3.2.4 Accumulation and translocation of metals in plants

Both bioconcentration factors (BCF) and translocation factors (TF) can be used toevaluvate a plant's potential for phytoremediation purpose. A plant's ability to accumulate metals from soils can be estimated using the BCF and TF, which is the measure of the translocation of metals from the roots to the shoots. Metal that is accumulated by plants largely stored in the root of the plants are indicated by TF < 1 and value > 1 indicate that, the metals are stored in the shoot.Yoon et al. [32] evaluated the potential of 36 plants (17 species) growing on a contaminated site and found that plants with a high BCF (BCF, metal concentration ratio of plant roots to soil) and low TF (TF, metal concentration ratio of plant shoots to roots) have the potential for phytostabilization.In the present study, TF value were calculated where TF <1 which suggests that, Pb concentration was more in root than shoot. This indicates that heavy metals are immobilized through adsorption by roots or precipitation within the area of plant roots. The TF and BCFfor Pb in phytoremediation and nano- phytoremediation using five hyper accumulators are shown in Table 4.In particular, the selected five hyper accumulators shows adventitious root system, they are underground roots which arise from the node of an horizontal stem.They give off small thread like branches with equal length of main root.Adventitious roots are important for tolerance to stresses. Each root type forms in different vertical positions, exposing them to different layers of the soil[3].Changes in root architecture can change the efficiency of metal uptake. In this respect, Bidar et al.[4] studied the suitability of *Loliumperenne* and *Trifoliumrepens* for the phytostabilization of a metal field located near a closed Pb smelter, discovering that metal was preferentially accumulated in roots rather than shoots, as follows: Cd>Zn>Pb.

Table 4. BioconcentrationFactors (BCF) and Translocation Factors (TF)

Plant species	BCF		TF	
	Phytoremediation	Nano-Phytoremediation	Phytoremediation	Nano-Phytoremediation
Alternatheradentate	0.87	0.97	0.18	0.19
Wediliacheninsis	0.68	0.98	0.18	0.20
Tradescantiaspathacea	0.48	0.85	0.08	0.14
Plectranthusamboinicus	0.46	0.87	0.14	0.25
Heliconiapsittacorum	0.44	0.52	0.19	0.13

IV. CONCLUSION

Phytoremediation technology is an efficient method to remediate heavy metal from polluted environment. However, phytoremediation with the addition of nZVI particlesresults in more efficiency.The application of nano-remediation using nZVI could be applied for the detoxification of heavy metal pollutants from soil.The present study demonstrated that all selected hyper accumulator plants except *Heliconiapsittacorum*reportedenhanced accumulation of heavy metal by the application ofnZVI particles than that of normal phytoremediation. Among the selected plants, the maximum uptake of Pb was observed in *Wediliacheninsis*followed by *Alternathera dentate*. Moreover phytostabilizationwas found in the plants, in which metals that are accumulated by plants were stored largely in the roots of plants (TF<1).

V. ACKNOWLEDGEMENT

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