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Heavy metal accumulation in *Pterisvittata* L. growing on Abandoned Lime Kiln and Abandoned Coal Stockpile of Meghalaya, India

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Abstract

The objective of the study was to assess the ability of P. vittata to accumulate Arsenic (As) from a mixed heavy metals soil contaminations in the Abandoned Lime Kiln (ALK) and Abandoned Coal Stockpile (ACS). The results showed that metal concentrations in the soil are of descending order Ca>Fe>Mg>Se>As>Na>Mn>Zn >Pb>Cu>Cr>Ni>Co in ALK and Fe>Mg>Zn>Mn>Se>Na> As>Ca>Pb>Cu>Ni=Cr>Co in ACS. As concentration in the soils of these two study sites have a relatively low As content and P. vittata could efficiently accumulates As to its roots and fronds parts from soils with low As concentration. An Enrichment Coefficient (EC) and Translocation Factor (TF) indicate a plant's ability for phytoremediation. The EF value of Co, Zn, Ni, Ca, Mg, Se, As, and Na was found to have an EF value greater than 1 which can be considered that P. vittata as a good accumulator of these metals in a mixed metals soil contamination. The TF values >1 was found for As, Pb, Mn, Fe, Se, Ni, Zn, Na, Co and Mg which indicates that P. vittata was efficient to accumulate more than one metal and translocate metals from soil to shoots. As-phytoremediation efficiency of P. vittata in a mixed metals soil contamination may be influence by the presence of other metals like Ca, Fe, Se, Mg, and Cu in the soil.

Keywords: Abandoned Lime Kiln (ALK), Abandoned Coal Stockpile (ACS), Enrichment Coefficient (EC), Translocation Factor (TF), Arsenic (As).

Introduction

The natural occurrence of metals maybe through the geological process such as modifications and weathering of rocks which have led to their formation in the soils, however, mining, industrial emission, vehicular traffic, etc are the anthropogenic activities which have led to the elevated increase of concentration in the soil by heavy metals which has become an environmental hazard. In recent past, heavy metal contamination in the soils have become a critical topic due to its effects on the soil environment and ultimately on human health and to control soil heavy metal pollution in a sustainable manner[1]. Meghalaya is gifted with great wealth of minerals and extensive mining of the two important minerals i.e., coal and limestone have been carried out. However, unscientific mining, lacking of post mining treatment and inefficient management have led to large scale degradation of land cover in these areas. The coal dumped in the vicinity of unmined site leads to the creation of large quantity of overburden such assoil, rocks, gravels, sand, etc and mine spoils which leads to severe soil and water pollution and deterioration of surrounding vegetation. Limekiln is one of the important small-scale sector industries in the state which contributes employment and uplift the economic development of the rural people and the state as whole. The proximity location to the limestone deposits, primitive and outdated technologies which are employed in these limekilns results to low yield and environmental degradation such runoff from these kilns leads to pollution of the streams, severe degradation of vegetation and its floor.

Phytoremediation is a process of using plants to remove, destroyed or sequester hazardous contaminants from contaminated water, soil, and air[2,3] and has derived a great deal significance in the last decade since it is cost effective, cheap and is a sustainable process[4]as compared to traditional remediation techniques[5]. The used of plants as an environmental remediation techniques has been carried long time ago. However, with the advent of many fascinating scientific discoveries in collaboration with many other interdisciplinary researches approaches have led this study into an environmentally friendly technology which is promising and cost-effective[6]. Hyper accumulators plants are those plants which uptake and accumulate heavy metals at levels 100 times greater into their tissues[7]. Therefore, the used of hyper accumulating plants in contaminate soils may decontaminate the toxic elements and restore fertility of the polluted soils[8]. The first known plant to hyper accumulate arsenic in its fronds was *Pterisvittata* L. (Chinese brake fern)[9] and their ability to hyper accumulates arsenic was because of their high biomass production and rapid growth[10]. There also a report that Chinese brake fern is also a Selenium accumulator[11]. *P.vittata* are often found growing in metal-rich regions[9,12] and they possessed high tolerance and accumulation properties which give them a competitive edge over the other species of plant.

During survey in abandoned lime kiln and abandoned coal stockpile sites it has been observed that *P. vittata* was growing abundantly in these two sites. Therefore, the objective of the study was to assess the ability of *P. vittata* to uptake heavy metals which is grown in these two sites, where it has been known that *P. vittata* is a potential arsenic hyper accumulator.

Material and Methods

Study site: For this study, soil samples and *P. vittata* which is found to grow in Cherrapunjee, India and were collected from the two sites, an abandoned coal stockpile (ACS) and abandoned lime kiln (ALK) during the month of May, 2010.

Sampling technique and Preparation: Soil analysis: A hand driven steel corers was used to randomly sample the surface soil up to the depth of 10cm and are transferred to the laboratory and stored in labeled polythene bags before pre-treatment and analysis. In laboratory, the soil samples were spread in trays and air dried for 15 days at room temperature. Before analysis, the air dried soil was homogenized by utilizing a mortar and pestle and stored in a labelled polyethylene bags after sieving by 2 mm mesh. Perkin Elmer Microwave digester 3000 were used for digestion of soil samples and were followed according to Marbaniang et al.[13]. Atomic Absorption Spectrometry (AAS 3110, Perkin Elmer) was utilized for analysis of the metals like As, Ca, Se, Fe, Pb, Mn, Ni, Zn, Mg, Cu, Co, Cr and Nain the soil samples.

Plant Analysis: The collected plants were wash properly with tap water and then with deionized water to removed soils and dust deposits adhering to the plant surface and then segregated into fronds and roots. An absorbent paper were used to dry the adherent water in plant samples. Plant samples were then dried in an oven at $70±5^0$C for 48h. To ensure homogeneity for organic matter digestion, the dried roots and fronds were separately chopped and grounded to fine size through a mortar and pestle. Kara and Zeytunluoglu[14] methods of digestion for the plant samples were carried out. Atomic Absorption Spectrophotometer (AAS 3110, Perkin-Elmer) was used to determine the heavy metals concentration in the plant samples.

Enrichment Coefficient (EC): EC indicates the ability of a plant species for phytoremediation[15]. EC is determined as the ratio of metal concentrations in shoots to soil metal concentrations.

$$\text{Enrichment Coefficient} = \frac{\text{Metal concentration in sHoot}}{\text{Metal concentration in soil}}$$

Translocation Factor: TF is the internal-metal transportation of the plant which indicates the translocation of metal from root to aerial part and it can be determined by a ratio of shoot metal accumulation to root metal accumulation[16].

$$\text{TF} = \frac{\text{Shoot metal accumulation}}{\text{Rootmetal accumulation}}$$

Where: TF>1 indicates root to shoot translocation of metals was effective.

Statistical analysis: Pearson correlation matrix of enrichment coefficients for different heavy metals in *P. vittata* plant samples were calculated and the data of three replicates were presented in mean ±standard error. The validity of the data were performed using ANOVA through SPSS 17.

Results and Discussion

Metals concentration in soils: Concentration of As, Ca, Se, Fe, Pb, Mn, Ni, Zn, Mg, Cu, Co, Cr, and Na in the soil sample of ALK and ACS sites are represented in Table-1.

Table-1
Metals concentration in the soil of ALK and ACS

Metals ↓	Mean metals concentration in the soil	
	ALK	ACS
As	1.28±0.1**	0.91±0.1**
Ca	32.54±1.06**	0.37±0.1**
Se	1.49±0.17*	1.22±0.6
Fe	23.67±0.05**	25.16±0.1**
Pb	0.32±0.1**	0.29±0.1*
Mn	0.79±0.01**	1.33±0.1**
Ni	0.12±0.1*	0.15±0.1
Zn	0.4±0.1**	1.61±0.1**
Mg	2.05±0.01**	2.08±0.1**
Cu	0.24±0.01**	0.209±0.1**
Co	0.11±0.01*	0.11±0.1**
Cr	0.16±0.1**	0.15±0.1**
Na	1.05±0.01**	1.2±0.1**

*p< 0.05, **p<0.01

The metal concentration in the soils of ALK and ACS are in the descending order Ca>Fe>Mg>Se>As>Na>Mn> Zn>Pb>Cu>> Cr> Ni>Co and Fe>Mg>Zn>Mn>Se>Na> As>Ca>Pb>Cu>Ni=

Cr>Co respectively. The concentrations of Fe, Mn, Zn and Mg in ACS site was found to higher as compared to that in ALK site, this higher in concentration of these metals in ACS site maybe due to the results of anthropogenic activities by dumping of coal and soils on the surface soils. Whereas, concentration of As, Ca and Se in the soil of ALK were comparatively high as to ACS, a high concentration of Ca in ALK site is due to the industrial Limekiln which may have a direct influence on increase Ca concentration in ALK. The natural abundance of Fe concentration maybe one of the reason for insignificant mean differences in both the study sites[13].

Accumulation of heavy metals by *P. vittata:* Accumulation of As, Ca, Se, Fe, Pb, Mn, Ni, Zn, Mg, Cu, Co, Cr, and Na in *P. vittata* roots and fronds from ALK and ACS are presented in Table-2.

The results indicates that metal accumulation in *P. vittata* roots from the two sites (Figure-2) are shown in descending order of Ca>Fe>As>Mg>Se>Na>Zn>Pb=Co>Ni>Cu>Cr>Pb in ALK and Ca> Fe> Mg> Se> Na> Zn> Mn> Cu> As> Pb> Cr> Co> Ni in ACS respectively. Maximum and minimum concentration of metals in *P. vittata* roots was found to be Ca (41.57) and Pb (0.02) in ALK and Ca (34.46) and Ni (0.02) in ACS. Metal accumulation in the fronds are in descending order of Ca>Fe>As>Mg>Zn>Na>Mn>Pb>Co>Ni>Cu>Cr in ALK and Ca>Fe>Mg>Se>Na>As>Zn>Mn>Pb>Ni>Ca>Co=Ni in ACS. The maximum and minimum concentration of metals in the fronds was Ca (31.41) and Cr (0.01) in ALK and Ca (13.02) and Co (0.02) and Ni (0.02) in ACS. In both the sites metals accumulation shows a significantly different (p<0.05) in *P. vittata* roots and fronds.

Table-2
Metals accumulation (mg/Kg) in *P. vittata* roots and fronds

Metals	ALK		ACS	
	Roots	Fronds	Roots	Fronds
Cu	0.15±0.01*	0.12±0.1*	0.16±0.1*	0.08±0.01*
Fe	7.33±0.05*	16.73±0.2*	7.22±7.2*	8.17±0.01*
Mn	0.12±0.01*	0.32±0.1*	0.17±0.1*	0.25±0.001*
Co	0.12±0.02*	0.14±0.03*	0.04±0.01*	0.02±0.01*
Pb	0.02±0.1*	0.165±0.01*	0.12±0.01*	0.17±0.001*
Cr	0.08±0.001*	0.014±0.1*	0.08±0.01*	0.02±0.001*
Ni	0.11±0.001*	0.109±0.01*	0.02±0.01*	0.15±0.001*
Se	2.21±0.08*	3.04±0.7*	1.95±0.01*	2.34±0.01*
Zn	0.51±0.001*	0.55±0.01*	0.57±0.1*	0.5±0.001*
Ca	41.57±0.08*	31.41±2.69*	34.46±0.01*	13.02±0.01*
Mg	2.25±0.008*	2.24±0.02*	2.53±0.01*	2.44±0.01*
Na	0.7±0.001*	0.49±0.02*	0.63±0.01*	0.99±0.001*
As	3.75±0.01*	5.02±0.9*	3.26±0.01*	6.54±0.1*

*p< 0.05

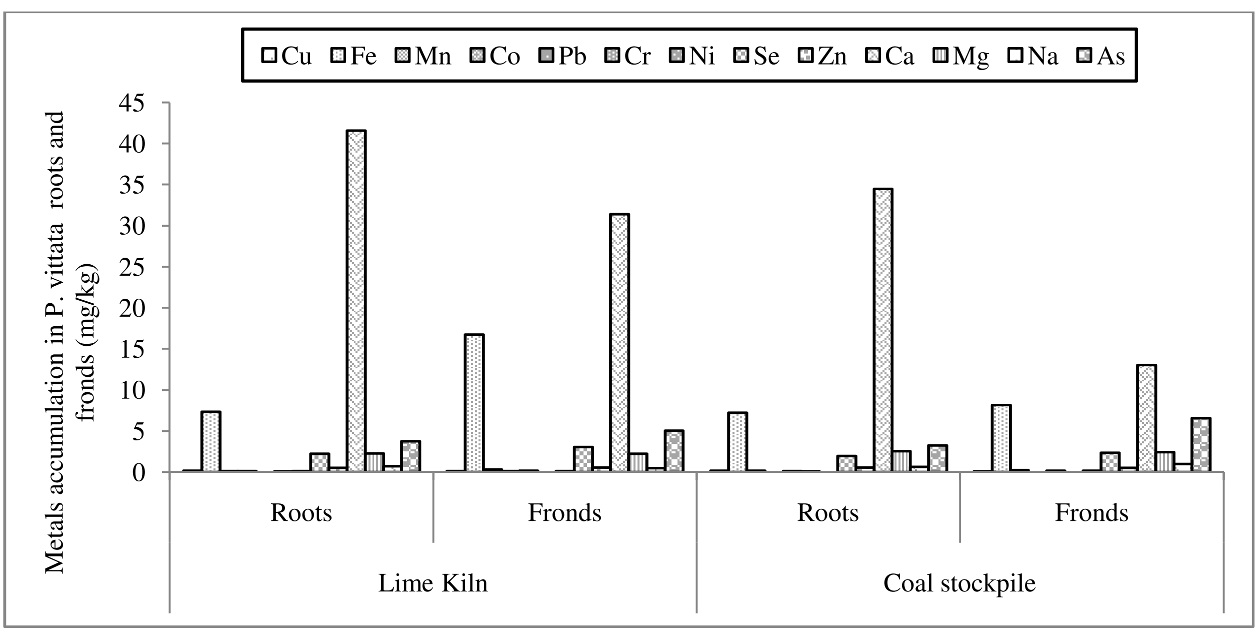

Figure-2
Metals accumulation (mg/kg) in *P. vittata* roots and fronds

The results indicate that As accumulation in *P. vittata* roots and fronds were significantly different between the two study sites. Our observations corresponds with the finding of Wei et al.[17] where it also shows that As concentration in the soils of these two study sites have a relatively low As content and *P. vittata* could efficiently accumulates As to its roots and fronds parts from soils with low As concentration. *P. vittata* selectively uptake certain metals like Ca, Fe, Se which are contrary with the studies by Caille et al.[18], Fayiga et al.[19], Tu and Ma[20] and Wei et al.[17]. Whereas, Pb, Mg, Ni, Mn, Cu, Cr, Zn, Na, and Co shows a similar pattern of accumulation in the roots and fronds, this maybe probably due to these metals translocation to the fronds which are consistency with the finding of Kumari et al.[21]. *P. vittata* showed a higher content of Fe, Se and As and lower Ca content in the fronds in both the study sites. The Fe content in *P. vittata* fronds were much higher as compared to the concentration in the roots which is in conformity with the studies by Wei et al.[22] which suggests that *P. vittata* translocation both As and Fe to fronds.

The high soil Fe concentrations in ACS and ALK sites are in accordance with Fitz et al.[23] and Wei et al.[22] where the roots of *P. vittata* played an important purpose in mobilizing Fe from the soil. *P. vittata* was reported that it is also a Se-accumulator[6], our results also shows that Se concentrations in the soils of these two study sites is much higher than the As concentration in the soils. Studies by Khattak et al.[24] and Feng et al.[25] shows that high concentrations of Se in the solution suppresses the uptake of As by plants which is also consistent with the present studies. It has been reported by Jones[26] that *P. vittata* favor to grow in calcium rich soils and studies by Tu and Ma[27] and Fagiya et al.[19] described that as concentration in fronds effectively increases in the presence of calcium which corroborates with the present study.

Enrichment Coefficient (EC) and metals Translocation factor (TF) in *P. vittata:* Enrichment Coefficient (EC): Enrichment factor of metals in *P. vittata* roots and fronds of ALK and ACS are presented in Table 3. The enrichment factor in both the study sites were found of the following sequence As>Na>Se>Zn>Co>Mg>Ca>Ni>Fe>Pb>Cu>Mn>Cr in ALK and Ca>Na>Se>Mg>Ni>As>Pb>Cu>Fe>Zn>Co>Mn>Cr in ACS. The results indicates that the EC value of Co (1.23), Se (2.03), Zn (1.3), Mg (1.1), Na (2.5) and As (3.9) in ALK and Ni (1.14), Se (1.89), Ca (34.67), Mg (1.17) and Na (5.44) in ACS have an EC value >1 which indicates that accumulation of metals in *P. vittata* found on these two sites increases which may be due to the higher availability and distribution of these metals in these two sites which corresponds with the study of Singh et al.[28]. Based on EC values, *P. vittata* could be considered as a good accumulator of Co, Se, Ni, Zn, Mg, Na and Ca in a mixed metals contamination apart it being an arsenic hyper accumulator.

Translocation factor (TF): TF i.e., the metals ratio mobilized to aerial part from roots has been presented in Table-3.

Table-3
Enrichment Coefficient and Translocation Factor from *P. vittata* root to fronds in the two sites

Metals ↓	ALK		ACS	
	EF	TF	EF	TF
Cu	0.5	0.81	0.38	0.5
Fe	0.7	2.27	0.32	1.13
Mn	0.4	2.5	0.18	1.42
Co	1.23	1.11	0.19	0.54
Pb	0.51	6.6	0.55	1.5
Cr	0.08	0.16	0.12	0.23
Ni	0.8	0.97	1.14	7.02
Se	2.03	1.37	1.89	1.18
Zn	1.3	1.07	0.3	0.88
Ca	0.96	0.75	34.67	0.37
Mg	1.1	1.0	1.17	0.97
Na	2.5	0.91	5.44	1.99
As	3.9	1.33	7.18	4.32

Metals translocation from roots to fronds of *P. vittata* were in the order of Pb>Mn>Fe>Se>As> Co>Zn>Mg>Ni>Na>Cu> Ca>Cr in ALK and Ni>As>Na>Pb>Mn>Se>Fe>Mg> Zn>Co>Cu>Ca>Cr in ACS (Table-3). The results for translocation factor (TF)ranges was 0.16 to 6.6with Pb (6.6), Fe (2.27), Mn (2.5), Co (1.11), Se (1.37), Zn (1.07), Mg (1.0) and As (1.33) have TF values >1 in the ALK while in the ACS TF values ranges from 0.23 to 7.02, with Fe (1.13), Mn (1.42), Pb (1.5), Ni (7.02), Se (1.18), Na (1.99) and As (4.32) recorded TF values >1 respectively. In the present study, translocation of Pb and Ni was greater than 1 (>1) in the ALK and ACS, the high translocation of Pb and Ni in *P. vittata* may be due to the high mobility within the plant which is in conformity with that of Singh et al.[28]. Among the metals screened for TF, it was found that *P. vittata* can take up and efficiently translocate more than one metals to the fronds from soil and roots which corroborates with the finding of Malik et al.[29].

Correlation: The correlation analysis (Table-4) were carried out between the As accumulation in *P. Vittata* fronds and soil metals concentrations in the study sites. In ALK site, there is a positive relationship between As and Pb, Co, Zn, As, Ni, Ca, and Na, but a negative relationship for Cu, Fe, Mn, Cr, Se and

Table-4
Correlation of As accumulation in the fronds and soil metals concentrations in ALK and ACS site

Metals concentrations in the soil	Ca	Fe	Pb	Se	Cr	As	Mn	Cu	Zn	Na	Ni	Co	Mg
ALK													
Fronds As→ accumulation	.824	-.923	.736	-.865	-.836	.660	-.494	-.981	.879	.911	.760	.918	-.920
ACS													
Fronds As accumulation	.998*	1.000**	-.988	-1.000*	-.992	-.990	.000	1.000**	-.980	.999	.928	.982	

*p< 0.05, **p<0.01

Mg at ALK site. In ACS site, a positive relation was found between As and Ni, Fe, Co, Na, Mn, Cu and Ca and negative relation between As and Pb, C, Se, Zn, Mg and As. In the ACS site, As have a significant positive correlation with Cu, Fe and Ca, whereas a significant negative correlation with Se and Mg. Ca is immobile and therefore Ca remained concentrated more in roots as compared in fronds. In this study, there is a significant positive correlation on As accumulation in *P. vittata* fronds and soil Ca concentration which is in consistent with the findings of Tu and Ma[27] and Faqiya and Ma[30] where the increased As concentration in *P. Vittata* fronds were effective in existence of high Ca concentration in the soil. Fe and Cu are micronutrients which are the structural components of the antioxidant enzymatic system of plant cells[31]. A correlation was also found among As in the fronds with soil Fe and Cu concentration which is significantly positive, which corroborates with the findings of Poter and Peterson[32]and Reed[33]. However, there are evidence that Se concentration may have an antagonistic effect on As uptake, thus a significant negative correlation between Se and Mg concentration and As accumulation in the fronds was observed in this study which corresponds with that of Khattak et al.[24], Marschner[34], Feng et al.[25] and Islam et al.[35].

Conclusion

This study shows that *P. vittata* from both the two study sites is a good accumulator and efficiently accumulate and translocate more than one metal from soil to fronds in a mixed metals soil contaminations. In the field, As-phytoremediation efficiency can be determined by other important factors like metals (Cu, Fe, Ca, Se and Mg) concentrations in the soil.

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