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Potential screening of phytoremediating crops and performance of maize in photoremediated coal mined acid soil with phosphorus application

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Abstract

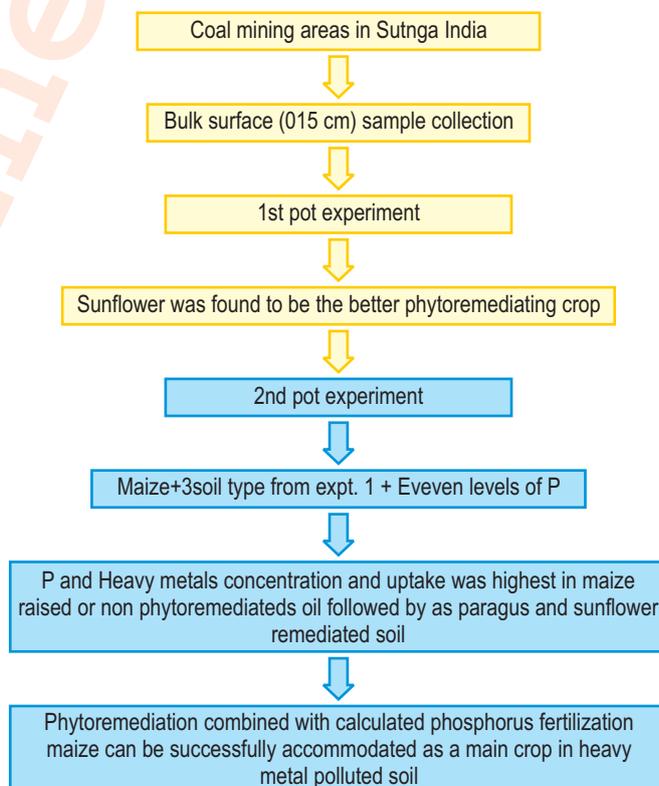
Aim: To assess the ability of phosphorus to counteract harmful effects of heavy metals by reducing their concentration and increasing the maize yield.

Methodology: Bulk surface soil sample (0-15 cm) was collected from heavy metal polluted soil of coal mine areas of Sutnga. Two pot experiments were conducted. Ten kg capacity pots were laid out for three phytoremediating crop and replicated 33 times. The processed soil was used for filling the pots. The first pot experiment was conducted to assess the phytoremediation efficiency of *Helianthus annuus* and *Vigna unguiculata* on heavy metal polluted soil. Thereafter in the second pot experiment *Zea mays* L. was planted in pots maintained under experiment 1 and subsequently eleven levels of phosphorus i.e. 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg P kg⁻¹ soil were super imposed in non-phytoremediated soil, phytoremediated soils through SSP. The elemental uptake and concentration of available phosphorus and heavy metal was determined.

Results: From the first pot culture experiment, it was observed that heavy metals content in soil after harvesting of *phytoremediating* crops reduced significantly in both the phytoremediated soils as compared to non-phytoremediated soil. From the second pot experiment it was observed that phosphorus as well as the heavy metals concentration and uptake in maize decreased significantly with increasing doses of phosphorus added in non phytoremediated soil.

Interpretation: This study clearly indicates that sunflower can be successfully grown as a phytoremediating crop in coal mine affected soils of Jaintia hills for phytoremediation of heavy metal polluted soils.

Key words: Acid soil, Heavy metal pollution, Maize, Phosphorus, Phytoremediation



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Introduction

Phosphorus is an essential nutrient for crop production due to its extensive physiological functions. Therefore, the performance of any crop is largely dependent on the phosphorus supplement. However, the majority of soils in north-east regions of India are acidic resulting from high rainfall (pH 5.0–6.0) which makes them deficient in available phosphorus (Sanjay-Swami and Lyngdoh, 2019). More than 95 per cent of the soils are acidic in reaction with 65 per cent being strongly acidic (pH<5.5) (Sanjay-Swami, 2019). Coal mining activities are highly prevalent in North-East India significantly contributing to heavy metal pollution of the soil and metal accumulation, especially with Co, Cu, Zn, Ni and Pb (Cui *et al.*, 2004) which further aggravates soil acidity. Heavy metal contamination affects the biosphere many places world-wide. Being primary producers, green plants accumulate contaminants from the soil and can contribute elements in toxic concentrations through food chain (Borah and Devi, 2012). Soil is considered major sink for trace elements released into the environment because of its high metal-scavenging potential (Banat *et al.*, 2005). Non-essential heavy metals like Cr, Cd, As, and Hg are highly toxic with no known function in plants (Fasani *et al.*, 2018) and severely affects variety of physiological and biochemical processes in crop plants, thereby disrupting absorption of essential plant nutrients required for proper growth and development, thus, reducing agricultural productivity. Heavy metal contamination coupled with strongly acidic soil further alleviates prevalent phosphorus deficiency which is detrimental to crop growth and productivity in this region.

Phytoremediation is a new encouraging and optimistic technology that uses plants to annihilate contaminated areas. This method uses specific plants and wild species known as accumulators which are highly effective in accumulating higher amount of toxic heavy metals (Ghosh and Singh, 2005; Brunet *et al.*, 2008). Plants extend their root system into the soil depth and accumulates heavy metals which in return modulates their bioavailability, thereby reclaiming the polluted soil and stabilizing soil fertility (Jacob *et al.*, 2018; DalCorso *et al.*, 2019). Besides studies have proved phytoremediation to be a successful tool in converting contaminated land into a cultivable land when coupled with phosphorus can positively affect the growth and nutrition absorbing capacity of crops grown in such soil (Yan *et al.*, 2020). Phosphorus application not only affects its own absorption and assimilation in plants but also influences number of other essential as well as non-essential elements present in the soil. Phosphorus is known to play an important role in several metabolic pathways, and its dominant position in its interaction with trace elements are bound to be an area of interest for further studies (Bolan *et al.*, 2005).

Phosphate fertilizers typically contain soluble forms of P that are immediately available to plants. The reaction of phosphate in the soil has an important contribution to crop growth and fertilizer use efficiency (Sushanta *et al.*, 2014). Upon P

fertilization several specific and non-specific nutrient interactions became apparent in soils and plant. Specific interactions occur between elements with similar physico-chemical properties as competition for adsorption and absorption sites (Wilkinson *et al.*, 2000). Non-specific interactions become apparent when the concentrations of elements in question are changed. Consequently, modified growth responses and mineral uptake and concentration may result in different crops grown in different environmental conditions. Therefore, it is the need of the hour to phytoremediate heavy metal polluted soils in coal mine areas and to also determine the effect of P fertilization so that farmers can apply adequate amount of phosphorus in maize growing areas in order to get definite economic response. To attend to the prevailing situation, this study was divided into two parts. Firstly, pot experiment was carried out to evaluate the phytoremediation potential of sunflower and Asparagus bean in heavy metal polluted soil, thereafter a second pot experiment was conducted to explore the effects of P fertilization on counteracting heavy metal toxicity and its role in improving maize performance.

Materials and Methods

In order to assess the phytoremediation effect of heavy metals, bulk surface soil sample (0-15 cm) was collected from heavy metal polluted soil of coal mine area of Sutnga in East Jaintia Hills. The physico-chemical and heavy metal analysis of soil sample was estimated using standard procedures. The physico-chemical properties of experimental soil is given in Table 1.

A pot culture experiment was conducted at Research Farm of School of Natural Resource Management, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam, Meghalaya using processed coal mined soil with two phytoremediating crops viz. PC1 Sunflower cv. EC-68913, and PC2 Asparagus bean cv. UPC-287 along with control C₃ without phytoremediating crop and replicated 33 times. To find out the variability in phytoremediation effect of both the crops on heavy metals in coal mined soil, 33 pots for each phytoremediating crop including no crop/control was considered as 33 replications and significant means were separated by One-way ANOVA. The crop plants were harvested at 60 DAS and heavy metal concentration in the plant and soil were estimated by Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) using standards made up in the DTPA solution as described by Lindsay and Norvell (1978).

To determine the elemental uptake and concentration of available phosphorus in phytoremediated heavy metals polluted soils for maize (*Zea mays*), another pot culture experiment was conducted. Eleven levels of phosphorus, *i.e.*, 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg P kg⁻¹ soil were super imposed in non-phytoremediated soil, Asparagus bean remediated and sunflower remediated soils through SSP and mixed thoroughly. These phosphorus treated soils were incubated in pots at field capacity for 28 days. The soils were mixed thoroughly at regular interval of time in order to maintain homogeneity. In order to assess the

Table 1 : Physico-chemical properties of experimental soil

Parameters	Value	References
pH	3.93 (Very or extremely acidic)	Jackson (1973)
Electrical conductivity (1:2:5) (dSm ⁻¹)	0.08	Jackson (1973)
Bulk Density (g cc ⁻¹)	1.2	Black (1965)
Organic carbon (%)	0.83 (High)	Walkley and Black (1934)
Available nitrogen (kg ha ⁻¹)	259.87 kg ha ⁻¹ soil (low)	Subbiah and Asija (1956)
Available phosphorus (kg ha ⁻¹)	9.18 kg ha ⁻¹ or 4.1 mg kg ⁻¹ soil (Low)	Bray and Kurtz (1945)
Available potassium (kg ha ⁻¹)	166 kg ha ⁻¹ soil (Medium)	Jackson (1973)
Soil texture	Sandy clay loam Sand: 55.6 % Silt: 13.8 % Clay: 30.6 %	Piper (1966)
Soil structure	Medium sub angular blocky	Piper (1966)
Heavy metal content (mg kg ⁻¹)		
Chromium	95.393 mg kg ⁻¹	Lindsay and Norvell (1978)
Cadmium	25.936 mg kg ⁻¹	
Lead	17.410 mg kg ⁻¹	
Nickel	51.127 mg kg ⁻¹	
Cobalt	6.462 mg kg ⁻¹	

status of available phosphorus, a 200 g representative sample of soil from each treatment was drawn after 28 days of incubation. After thorough mixing of nutrients in non-phytoremediated and phytoremediated soils, three healthy seeds of maize (cv. DA 61A) were sown in each pot at 2.5 cm depth. Thinning of plants was performed after one week of germination and only one healthy plant per pot was kept to grow further. Weeding was done frequently to avoid any loss of nutrients. The pots were irrigated with distilled water as and when required. The position of pots were changed randomly at different time intervals in order to minimize the effect of position of the pots. The crop was harvested from just above the soil surface 60 days after planting (DAP).

The dry matter yield of phytoremediating crops was weighed separately in digital electronic balance and finally converted in terms of grams per pot. The plants of phytoremediating crops were harvested at 60 DAS and heavy metals concentrations in the samples were estimated following standard procedures. The details of plant analysis are described in following section.

The heavy metals concentration in plant samples were determined by Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES). In this method, 0.5 g powdered plant sample was digested by di-acid mixture (HNO₃ + HClO₄) in 3:1 ratio until the clear solution appeared. The concentrations of heavy metals in the samples were determined by ICP-OES using standards made up in the DTPA solution

The uptake of heavy metals in plant samples of phytoremediating crops was calculated by multiplying per cent nutrient concentration with their respective plant yield as per the formula given below:

Heavy metals uptake (μ pot⁻¹) = Concentration (mg kg⁻¹) x Dry matter yield (g pot⁻¹)

Phosphorus concentration in plant samples was determined by Vanadomolybdate phosphoric yellow colour method proposed by Koenig and Johnson (1942).

Table 2 : Mean heavy metal concentration (mg kg⁻¹) and uptake (μ g pot⁻¹) by phytoremediating crops grown in heavy metals polluted soil of coal mine area of Jaintia Hills of Meghalaya

	Chromium		Cadmium		Lead		Nickel		Cobalt	
	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake
Asparagus (PC1)	3.67 ±0.36	42.98 ±2.37	1.22 ±0.16	14.30 ±1.60	0.78 ±0.08	9.20 ±1.07	1.83 ±0.18	21.43 ±1.25	0.35 ±0.08	4.06 ±0.85
Sunflower (PC2)	10.22 ±0.34	51.83 ±1.50	2.99 ±1.06	15.05 ±4.08	2.07 ±0.21	10.40 ±0.88	6.15 ±0.68	30.88 ±1.20	1.13 ±0.15	5.68 ±0.40

Values are mean of replicates ±SD.

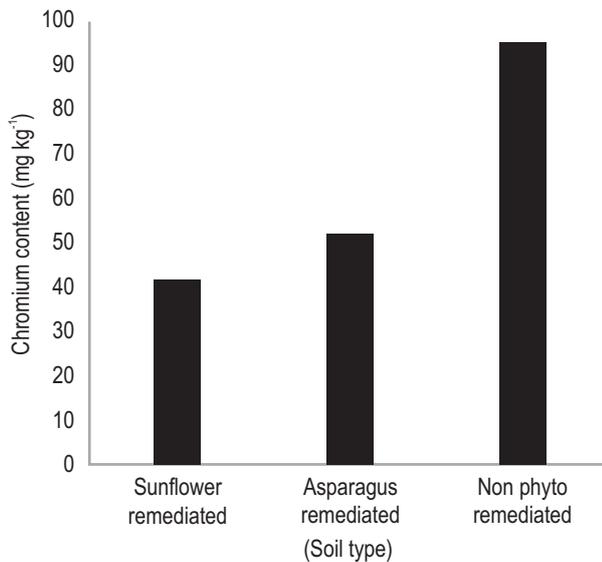


Fig. 1 : Chromium content in soil after harvesting of phyto-remediating crops.

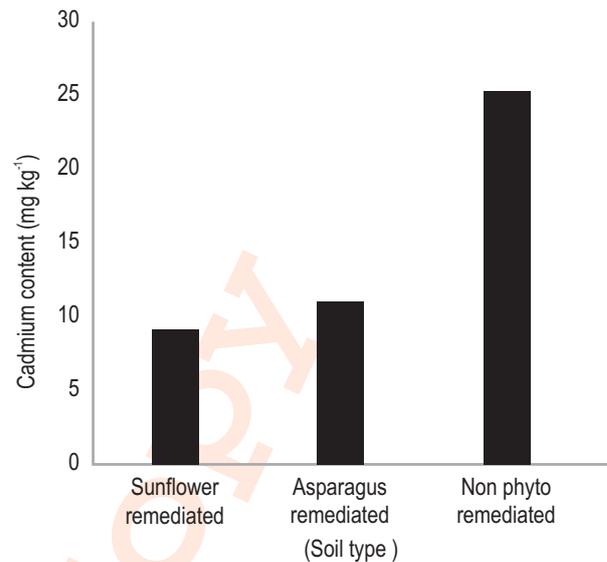


Fig. 2 : Cadmium content in soil after harvesting of phyto-remediating crops.

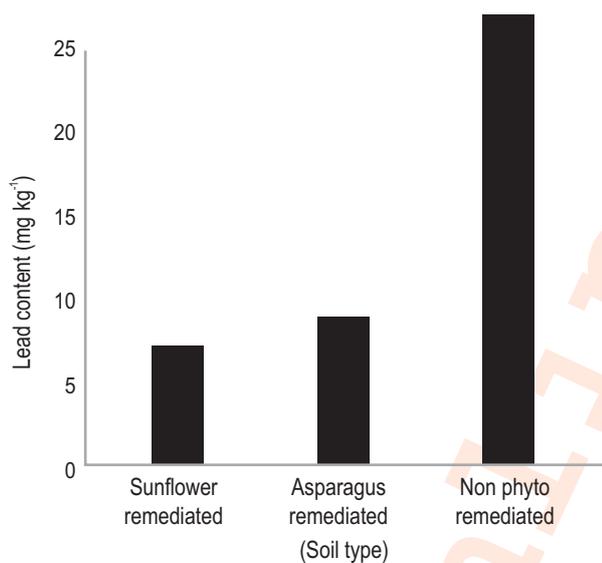


Fig. 3 : Lead content in soil after harvesting of phyto-remediating crops.

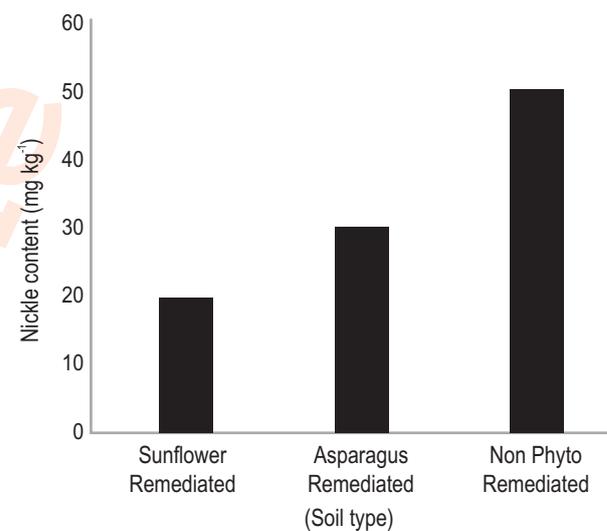


Fig. 4 : Nickel content in soil after harvesting of phyto-remediating crops.

In the second pot experiment, the dry matter yield of maize was recorded treatment-wise and uptake of phosphorus and heavy metals were estimated in plant samples following the same methods as described above.

Results and Discussion

Phytoremediation potential of sunflower and asparagus bean : The result of the first pot experiment revealed that dry matter yield of Asparagus bean (11.79 g pot⁻¹) was higher than the sunflower crop (5.08 g pot⁻¹). However, sunflower was found to be

superior phytoremediating crop in comparison to Asparagus bean as it accumulated more heavy metals (Table 2). The heavy metal content in soil after harvesting phytoremediating crops grown in polluted soil of coal mined area reduced significantly in both the soils phytoremediated by sunflower and asparagus bean as compared to non-phytoremediated soil (Fig 1-5). These results are similar to those described by other workers in different plant species (Tewari *et al.*, 2002; Zhou and Qiu, 2005; Gajewaska and Sklodowska, 2007; Mahardika *et al.*, 2018 Yazdanbakhsh *et al.*, 2020). However, the soil phytoremediated by sunflower recorded least heavy metals content indicating its superiority over

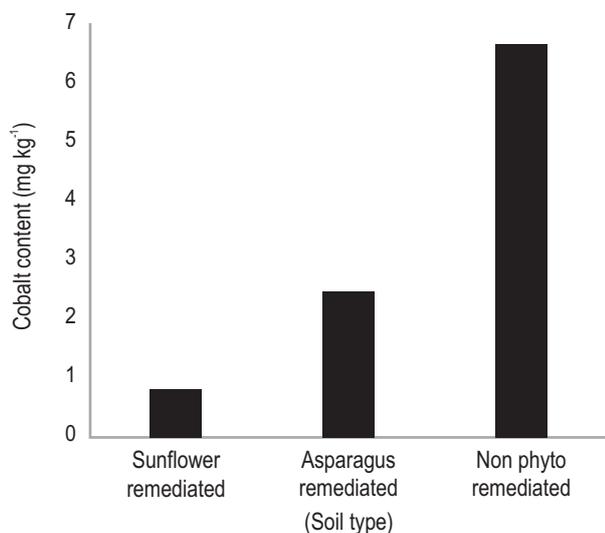


Fig. 5 : Cobalt content in soil after harvesting of phyto-remediating crops.

Asparagus bean. Similarly, Nehnevajova *et al.*, (2005); Rizwan *et al.* (2016); Chauhan and Mathur (2020) evaluated the potential use of sunflower as a phytoremediating plant and reported that sunflower's high tolerance to metal toxicity and high biomass makes it a good contender for phytoremediation.

Effect of phosphorus application on dry matter yield of maize: Graded application of phosphorus fertilizer significantly and markedly increased the mean dry matter yield of maize planted in sunflower and asparagus bean remediated soils as compared to maize grown in non-phytoremediated soil or control. Similar results was reported by Ahmed (2017) and Silveira *et al.*, (2018), Getnet and Dugasa, (2019) for Faba beans, sorghum and maize. The dry matter yield of maize grown in Asparagus bean and sunflower remediated soils, as evident in Table 4, increased significantly with increasing levels of phosphorus up to 90 mg P kg⁻¹ soil, respectively. The dry matter yield of maize plants differed significantly among non-phytoremediated and phytoremediated soils. The mean dry matter yield produced was highest in maize raised in sunflower remediated soil (19.67 g pot⁻¹) followed by Asparagus bean remediated (16.25 g pot⁻¹) and the least was observed in non-phytoremediated soils (13.50 g pot⁻¹). The results are in agreement with those obtained by Taalab *et al.*, (2007); Wortmann *et al.*, (2009); Taliman *et al.*, (2019) who reported that dry matter yield increased with increasing phosphorus application.

Effect of phosphorus application on elemental concentration and uptake by maize

Concentration and uptake of phosphorus: The perusal of data in Table 5 showed a significant increase in as concentration of phosphorus with the increasing levels of applied phosphorus in

non-phytoremediated and phytoremediated soils. This conforms to earlier findings of Hossain *et al.* (2007) who reported that concentration of phosphorus in leaf and stem of maize was influenced by applied fertilizer. Similarly, Wang *et al.* (2018), Turuko and Muhammed (2014) reported the same in soyabean and common bean, respectively. The highest phosphorus concentration was observed in maize raised in sunflower remediated soil (mean 0.14 per cent), which was significantly higher than maize grown in Asparagus bean soil (mean 0.12 per cent) and in non-phytoremediated soil (mean 0.10 per cent). A significant lower concentration of P was observed in plants grown in non-phytoremediated soils with respect to phytoremediated soils. The mean P concentration of maize produced in Asparagus bean and sunflower remediated soils significantly increased to 10 and 27 per cent, respectively over P concentration of non-phytoremediated maize which proves the positive effects of phytoremediation (Table 5). Similarly, a significant increase in uptake of phosphorus with the increasing levels of applied phosphorus in non-phytoremediated and phytoremediated soils was observed (Table 5). Several field and glasshouse studies have shown that the increased P concentration and uptake with increased P application was most likely due to plant establishment and proper root development induced by the water soluble P mineralized into the soil (Pantigoso *et al.*, 2020; Kacar and Katkat, 2011; Zhoa *et al.*, 2007). Plants grown in extremely P deficient soil exhibit higher P sorption at lower doses of P. This might be due to the availability of more phosphorus in soils with the application of P as well as due to low available P status of the experimental soils as reported by Gaur (1990) and Kuntuyastuti and Suryantini (2015) which supports the findings in this research. The highest value of 29.65 mg P pot⁻¹ was recorded in sunflower remediated soil followed by Asparagus bean remediated soil (21.45 mg P pot⁻¹) and the least was recorded in non-phytoremediated soil (17.03 mg P pot⁻¹).

Concentration and uptake of heavy metals : The mean concentration of heavy metals decreased significantly with increasing levels of applied phosphorus in non-phytoremediated and phytoremediated soils. The highest mean concentration of heavy metals was recorded in maize grown in non-phytoremediated soil which was significantly higher than that obtained in Asparagus bean remediated and sunflower remediated soils revealing the fact that phytoremediation helped in reducing the concentration and toxic effects of heavy metals. These results were similar to the studies conducted by Singh *et al.* (2010) where they found out that *Brassica campestris*, showed higher level of accumulation of Cd, Zn, Cr, Ni and Mn in contaminated soil as compared to uncontaminated soil. Reduced heavy metal concentration with P application was also reported by Ahmad (2017) in his experiment with Faba beans,

Effect of phosphorus application on Cr concentration and uptake by maize : The mean Cr concentration decreased significantly with the increasing levels of applied phosphorus in

Table 4 : Effect of phosphorus application on dry matter yield of maize (g pot⁻¹) grown in non-phytoremediated and phytoremediated soils

Phosphorus levels (mg kg ⁻¹ soil)	Non-phytoremediated soil	Asparagus remediated soil	Sunflower remediated soil	Mean
0	4.65	8.65	10.10	7.80
10	6.26	10.26	13.54	10.02
20	8.99	13.99	16.33	13.10
30	10.02	14.02	17.92	13.99
40	11.77	15.77	19.07	15.54
50	13.26	16.26	21.64	17.05
60	15.34	17.84	22.70	18.63
70	17.18	19.78	23.25	20.07
80	18.09	20.09	24.39	20.86
90	20.62	21.02	24.41	22.02
100	22.39	21.02	23.06	22.16
Mean	13.50	16.265	19.67	
C.D. (p ≤ 0.05)	P levels (P)	Soils (S)	P x S	
	0.21	0.12	0.62	

Table 5 : Effect of phosphorus application on phosphorus concentration (per cent) and uptake (mg pot⁻¹) of maize grown in non-phytoremediated and phytoremediated soils

Phosphorus levels (mg kg ⁻¹ soil)	Non-phytoremediated soil		Asparagus remediated soil		Sunflower remediated soil		Mean	
	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake
0	0.08	3.72	0.12	8.65	0.11	11.11	0.10	7.83
10	0.09	5.63	0.11	11.29	0.12	16.25	0.11	11.06
20	0.10	8.99	0.11	15.39	0.13	21.23	0.11	15.20
30	0.10	10.02	0.12	16.82	0.13	23.30	0.12	16.71
40	0.11	12.95	0.12	18.92	0.14	26.70	0.12	19.52
50	0.12	15.91	0.13	21.14	0.15	32.46	0.13	23.17
60	0.13	19.94	0.14	24.98	0.15	34.05	0.14	26.32
70	0.13	22.33	0.14	27.69	0.16	37.20	0.14	29.08
80	0.14	25.33	0.15	30.14	0.18	42.93	0.15	32.80
90	0.14	28.87	0.15	31.53	0.18	41.74	0.16	34.05
100	0.15	33.59	0.16	33.44	0.19	41.20	0.16	34.08
Mean	0.11	17.03	0.12	21.45	0.14	29.65		
C.D. (p ≤ 0.05)	P levels (P)		Soils (S)		P x S			
	Conc.	Uptake	Conc.	Uptake	Conc.	Uptake		
	0.003	0.22	0.02	0.12	0.009	0.56		

soils from 2.38 mg Cr kg⁻¹ in control to 1.34 mg Cr kg⁻¹ in 100 mg P kg⁻¹ soil application as observed in Table 6. The highest mean Cr concentration was recorded in maize grown in non-phytoremediated soil (2.91 mg Cr kg⁻¹), which was significantly higher than 1.45 mg Cr kg⁻¹ obtained in Asparagus bean remediated and 1.29 mg Cr kg⁻¹ obtained in sunflower remediated soils. Similarly, the mean Cr uptake observed in Table 7 was 16.55 µg pot⁻¹ in control, which increased significantly to 33.03 µg pot⁻¹ with the application of 70 mg P kg⁻¹ soil. Sayanthan and Shardendu (2013, 2015) reported decreased Cr concentration and uptake with increasing P in radish and spinach. However, at higher level of applied phosphorus, Cr uptake decreased significantly due to decrease in the concentration of heavy metals

in maize at higher levels of phosphorus in which phosphorus and chromium forms chromium complexes such as chromium phosphate. The highest mean Cr uptake was recorded 37.28 µg Cr pot⁻¹ in non-phytoremediated soil which was followed by sunflower remediated soil (24.87 µg Cr pot⁻¹) and Asparagus bean remediated soil (21.69 µg Cr pot⁻¹) indicating the effect of phytoremediation in reducing heavy metal concentration in soil.

Effect of phosphorus application on Cd concentration and uptake by maize : The perusal data presented in Table 6 showed a significant decrease with the increasing levels of applied P in soils from 0.40 mg Cd kg⁻¹ in control to 0.12 mg Cd kg⁻¹ in 100 mg P kg⁻¹ soil application. The lowest Cd concentration was observed in maize

Table 6 : Effect of phosphorus application on heavy metal concentration (mg kg^{-1}) of maize grown in non-phytoremediated and phytoremediated soils

P levels (mg kg^{-1} soil)	Non-phytoremediated soil					Asparagus remediated soil					Sunflower remediated soil					Mean				
	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co
0	3.59	0.62	1.06	3.36	0.30	2.07	0.35	0.38	1.73	0.15	1.49	0.24	0.32	0.69	0.05	2.38	0.40	0.59	1.93	0.17
10	3.39	0.58	1.03	3.3	0.29	2.00	0.35	0.34	1.72	0.12	1.48	0.24	0.31	0.68	0.05	2.29	0.39	0.56	1.90	0.15
20	3.35	0.53	1.00	3.16	0.28	1.97	0.33	0.34	1.53	0.11	1.42	0.21	0.28	0.66	0.05	2.25	0.36	0.54	1.78	0.15
30	3.04	0.47	1.00	2.89	0.27	1.67	0.32	0.34	1.44	0.11	1.39	0.19	0.25	0.62	0.05	2.03	0.33	0.53	1.65	0.14
40	2.90	0.4	0.99	2.85	0.27	1.65	0.29	0.32	1.34	0.11	1.33	0.14	0.21	0.6	0.05	1.96	0.28	0.51	1.60	0.14
50	2.83	0.35	0.96	2.75	0.23	1.53	0.23	0.25	1.22	0.10	1.31	0.13	0.19	0.58	0.05	1.89	0.24	0.47	1.52	0.13
60	2.72	0.3	0.88	2.67	0.23	1.44	0.19	0.22	1.17	0.10	1.24	0.11	0.14	0.53	0.04	1.80	0.20	0.41	1.46	0.12
70	2.68	0.26	0.80	2.49	0.22	1.26	0.17	0.18	1.14	0.09	1.21	0.1	0.08	0.49	0.04	1.72	0.18	0.35	1.37	0.12
80	2.68	0.24	0.76	2.35	0.19	0.96	0.17	0.17	0.95	0.08	1.18	0.09	0.06	0.49	0.03	1.61	0.17	0.33	1.26	0.10
90	2.50	0.21	0.74	2.21	0.18	0.7	0.13	0.13	0.83	0.07	1.14	0.08	0.06	0.42	0.02	1.45	0.14	0.31	1.15	0.09
100	2.33	0.19	0.59	2.11	0.12	0.65	0.13	0.10	0.7	0.04	1.03	0.03	0.05	0.33	0.02	1.34	0.12	0.25	1.05	0.06
Mean	2.91	0.38	0.89	2.74	0.23	1.45	0.24	0.25	1.25	0.10	1.29	0.14	0.18	0.55	0.04					
C.D. ($p \leq 0.05$)	P levels (P)					Soils (S)					P x S									
	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co					
	0.06	0.01	0.02	0.05	0.01	0.11	0.008	0.01	0.09	0.003	0.32	0.04	0.06	0.26	0.02					

Table 7 : Effect of phosphorus application on heavymetal uptake ($\mu\text{g pot}^{-1}$) of maize grown in non-phytoremediated and phytoremediated soils

P levels (mg kg^{-1} soil)	Non-phytoremediated soil					Asparagus remediated soil					Sunflower remediated soil					Mean				
	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co
0	16.69	2.88	4.93	15.62	1.40	17.91	3.54	3.29	14.96	1.30	15.05	2.08	3.23	6.97	0.51	16.55	2.83	3.82	12.52	1.07
10	21.22	3.63	6.45	20.66	1.82	20.52	4.74	3.49	17.65	1.23	20.04	2.46	4.20	9.21	0.68	20.59	3.61	4.71	15.84	1.24
20	30.12	4.76	8.99	28.41	2.52	27.56	5.39	4.76	21.40	1.54	23.19	2.94	4.57	10.78	0.82	26.96	4.36	6.11	20.20	1.63
30	30.46	4.71	10.02	28.96	2.71	23.41	5.73	4.77	20.19	1.54	24.91	2.66	4.48	11.11	0.90	26.26	4.37	6.42	20.09	1.72
40	34.13	4.71	11.65	33.54	3.18	26.02	5.53	5.05	21.13	1.73	25.36	2.21	4.00	11.44	0.95	28.50	4.15	6.90	22.04	1.95
50	37.53	4.64	12.73	36.47	3.05	24.88	4.98	4.07	19.84	1.63	28.35	2.11	4.11	12.55	1.08	30.25	3.91	6.97	22.95	1.92
60	41.72	4.60	13.50	40.96	3.53	25.69	4.31	3.92	20.87	1.78	28.15	1.96	3.18	12.03	0.91	31.85	3.62	6.87	24.62	2.07
70	46.04	4.47	13.74	42.78	3.78	24.92	3.95	3.56	22.55	1.78	28.13	1.98	1.86	11.39	0.93	33.03	3.47	6.39	25.57	2.16
80	48.48	4.34	13.75	42.51	3.44	19.29	4.15	3.42	19.09	1.61	28.78	1.81	1.46	11.95	0.73	32.18	3.43	6.21	24.52	1.93
90	51.55	4.33	15.26	45.57	3.71	14.71	3.17	2.73	17.45	1.47	27.83	1.68	1.46	10.25	0.49	31.36	3.06	6.48	24.42	1.89
100	52.17	4.25	13.21	47.24	2.69	13.67	3.00	2.10	14.72	0.84	23.75	0.63	1.15	7.61	0.46	29.86	2.63	5.49	23.19	1.33
Mean	37.28	5.96	11.29	34.79	2.89	21.69	4.41	3.74	19.08	1.50	24.87	2.05	3.07	10.48	0.77					
C.D. ($p \leq 0.05$)	P levels (P)					Soils (S)					P x S									
	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co	Cr	Cd	Pb	Ni	Co					
	0.20	0.14	0.05	0.23	0.09	0.11	0.08	0.15	0.12	0.04	0.57	0.41	0.41	0.65	0.26					

raised in sunflower remediated soil ($0.14 \text{ mg Cd kg}^{-1}$) followed by maize grown in Asparagus bean remediated soil ($0.24 \text{ mg Cd kg}^{-1}$) and non-phytoremediated soil ($0.38 \text{ mg Cd kg}^{-1}$). The mean Cd uptake was $2.83 \mu\text{g pot}^{-1}$ in control which increased significantly to $4.37 \mu\text{g pot}^{-1}$ with the application of 30 mg P kg^{-1} soil as shown in Table 7. The application of 40 mg P kg^{-1} soil and higher levels of P significantly and subsequently decreased Cd uptake of maize over lower levels of applied P. However, studies on Cd uptake with increased P application are contrasting in nature while some researchers observed a reduction in the availability of Cd eventually

decreasing Cd uptake by plants, as reported by Yan *et al.* (2015) either by interfering with Cd translocation from roots to above ground plant parts or by promoting the capacity of soil or its constituents to adsorb Cd (McGowan and Basta, 2001; Bolan and Duraisamy 2003; Seshadri *et al.*, 2016; Zhao *et al.*, 2020). Phosphate fertilization is known to increase adsorption which in turn increases negative charge of soil and Cd adsorption (Ruangcharus, 2020). Other studies, however, showed that application of phosphate fertilizer containing Cd increased the Cd concentration in wide range of crops (Grant *et al.* 1999; Jiao *et al.* 2004).

Effect of phosphorus application on Pb concentration and uptake by maize:

The results depicted in Table 6 indicated that the highest mean Pb concentration was recorded in maize grown in non-phytoremediated soil ($0.89 \text{ mg Pb kg}^{-1}$), which was significantly higher than $0.25 \text{ mg Pb kg}^{-1}$ in Asparagus bean remediated and $0.18 \text{ mg Pb kg}^{-1}$ in sunflower remediated soil as shown in Table 6. Pb uptake by maize crop at different levels of applied P indicated that the mean Pb uptake was $3.82 \text{ } \mu\text{g pot}^{-1}$ in control which increased significantly to $6.97 \text{ } \mu\text{g pot}^{-1}$ with the application of 50 mg P kg^{-1} soil. However, as depicted in Table 7, at higher levels of applied phosphorus, Pb uptake decreased significantly which might be due to decrease in the concentration of maize at higher levels of P. The highest mean Pb uptake was recorded $11.29 \text{ } \mu\text{g Pb pot}^{-1}$ in non-phytoremediated soil, followed by Asparagus bean remediated soil ($3.74 \text{ } \mu\text{g Pb pot}^{-1}$) and sunflower remediated soil ($3.07 \text{ } \mu\text{g Pb pot}^{-1}$). Netherway *et al.* (2020) reported the effective use of phosphorus rich biochar in reducing lead bioavailability in soil. Addition of TSP substantially decreased Pb and Cd availability through modification of metals partitioning from non-residual to residual forms (Valipour *et al.*, 2016). Reduction in Pb concentration and uptake in plants with higher application of P has been reported earlier (Brown *et al.*, 2004; Hettiarachchi *et al.*, 2001; Cao *et al.*, 2002). Reduced plant uptake of Pb was also observed upon Rock Phosphate (PR) or Single Super Phosphate (SSP) addition to lead contaminated soils (Hettiarachchi and Pierzynski, 2002; Chen and Zhu, 2004). Co-precipitation reaction between alkaline phosphate materials and calcium compounds can lead to metal oxide precipitation as described by Zhao and Song (2004). Similarly, phosphates and heavy metals can form insoluble phosphates to encapsitate migration of Cd and Pb in (Chen *et al.*, 2006).

Effect of phosphorus application on Ni concentration and uptake by maize :

The concentration of Ni in maize plants had significantly and subsequently decreased with the successive application of P. The lowest mean Ni concentration ($0.55 \text{ mg Ni kg}^{-1}$ soil) was recorded in sunflower remediated soil, which was significantly lower than Asparagus bean remediated soil and the highest value of Ni concentration was recorded in non-phytoremediated soil ($2.74 \text{ mg Ni kg}^{-1}$ soil). The mean Ni uptake was $12.52 \text{ } \mu\text{g pot}^{-1}$ in control, which increased significantly to $25.57 \text{ } \mu\text{g Ni pot}^{-1}$ with the application of 70 mg P kg^{-1} soil. Application of 80 mg P kg^{-1} soil and higher levels of P significantly and subsequently decreased Ni uptake in maize over lower levels of applied P. The highest mean Ni uptake was obtained in non-phytoremediated soil ($34.79 \text{ } \mu\text{g Ni pot}^{-1}$), followed by $19.08 \text{ } \mu\text{g Ni pot}^{-1}$ in Asparagus bean remediated soil and $10.48 \text{ } \mu\text{g Ni pot}^{-1}$ in sunflower remediated soil (Table 7). Several soil treatments such as addition of lime, phosphorus, organic matter, etc. are known to decrease Ni availability in plants (Chaney *et al.*, 1984). Ahmad and Esmail (2014) reported a reduced Ni concentration in roots, shoots and grains with increasing levels of P in wheat plants. Formation of complexes of heavy metals (Ni, As, Cd, Pb, etc.) on

the surface of phosphate grains and partial dissolution of amendments and precipitation of heavy metal-containing phosphates are the dominant immobilization mechanisms. Phosphate amendments like rock phosphate and hydroxyapatite immobilize Co and Ni in heavy metal contaminated soil (Mignarde *et al.*, 2013) thereby reducing uptake and concentration of heavy metals in crops.

Effect of phosphorus application on Co concentration and uptake by maize :

The mean Co concentration as observed in Table 6 decreased significantly with the increasing levels of applied P in soils from $0.17 \text{ mg Co kg}^{-1}$ in control to $0.06 \text{ mg Co kg}^{-1}$ in 100 mg P kg^{-1} soil application. The highest mean Co concentration was recorded in maize grown in non-phytoremediated soil ($0.23 \text{ mg Co kg}^{-1}$), which was significantly higher than $0.10 \text{ mg Co kg}^{-1}$ in Asparagus bean remediated and $0.04 \text{ mg Co kg}^{-1}$ in sunflower remediated soil. Co uptake by maize crop at different levels of applied P indicated that the mean Co uptake was $1.07 \text{ } \mu\text{g pot}^{-1}$ in control which increased significantly to $2.16 \text{ } \mu\text{g pot}^{-1}$ on applying 70 mg P kg^{-1} soil (Table 7). However, at higher levels of applied P, Co uptake decreased significantly which was due to decrease in concentration of maize at higher levels of P. The highest mean Co uptake was recorded $2.89 \text{ } \mu\text{g Co pot}^{-1}$ in non-phytoremediated soil which, followed by sunflower ($1.50 \text{ } \mu\text{g Co pot}^{-1}$) and Asparagus bean ($0.77 \text{ } \mu\text{g Co pot}^{-1}$) remediated soil. The results of present study contradict the results of Gad and Hassan (2011), who reported that cobalt addition caused enhancement in plant growth parameters with all type of phosphorus fertilizer, especially with Mono super phosphate form. Gad (2002) also found that cobalt uptake from soil by corn and soybean plant increased enhanced phosphorus levels phosphorus. Abde-Kader *et al.* (2013) reported that extractable Co and Cr showed consistent decrease (nearly 50 percent) of un-amended soils due to the effect of added rock phosphate amendments after one year.

Based on the results of the present investigation, it may be concluded that sunflower is a superior phytoremediating crop in comparison to Asparagus bean (*Vigna unguiculata*) extracting more amount of heavy metals from polluted soil of coal mine area of Jaintia Hills of Meghalaya. The results of this study throws light on the importance of P in amending heavy metal polluted soil, where incorporation of P as an amendment helps in the reduction of heavy metal toxicity thereby improving crop growth and productivity in heavy metal polluted soils. Further, when used in conjunction with phytoremediation in heavy metal polluted soils of coal mine areas, will help the farmers to apply judicious amount of phosphorus in these soils, which in turn will reduce the cost of maize crop cultivation in heavy metal polluted soils and also ensure an increase in crop growth and productivity.

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References

- Abdel-Kader, N.H., R.R. Shahin and H.A. Khater: Assessment of heavy metals immobilization in artificially contaminated soils using some local amendments. *Open J. Metal*, **3**, 68-76 (2013).
- Ahmad, I.: The Effect of Phosphorus Fertilizers on Faba Bean Plant Growth in Soils were Polluted by Heavy Metals. *Eur. J. Sci. Eng.*, **3**, 175-180 (2017).
- Ahmad, T. and A.O. Esmail: Effect of different levels of phosphorus fertilizer on 11 heavy metals concentration in different parts of wheat plant. *Mesopotamia J. Agric.*, **42**, 328-334 (2014).
- Balemi, T. and K. Negisho: Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. *J. Soil Sci. Pl. Nutri.*, **12**, 547-561 (2012).
- Banat, K.M., F.M. Howari and A.A. Al-Hamad: Heavy metals in urban soils of central Jordan: Should we worry about their environmental risks? *Environ. Res.*, **97**, 258-273 (2005).
- Black, G.R.: Bulk density. In: Methods of Soil Analysis, Part 1, Edn., Black, C.A. American Society of Agronomy (1965).
- Bolan, N.S. and V.P. Duraisamy: Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: A review involving specific case studies. *Australian J. Soil Res.*, **4**, 533-555 (2003).
- Bolan, N.S., D.C. Adriano, R. Naidu, M.D.L. Mora and M. Santiago: Phosphorus - Trace Element Interactions in Soil - Plant Systems. In: Phosphorus: Agriculture and the Environment. **Vol. 46**. Book series: Agronomy Monograph, pp. 317-352 (2005).
- Borah, M. and A. Devi: Effect of heavy metals on *Pisum sativum* Linn. *Int. J. Adv. Bio. Res.*, **2**, 314-321 (2012).
- Bray, R.H. and L.T. Kurtz: Determination of total organic and available phosphate in soils. *Soil Sci.*, **59**, 39-45 (1945).
- Brown, S.R., J. Hallfrisch, J. Ryan and W. Berti: *In-situ* soil treatments to reduce the phyto- and bioavailability of lead, zinc and cadmium. *J. Environ. Qual.*, **33**, 522-531 (2004).
- Brunet, J., A. Repellin., G. Varrault., N. Terryn and Y. Zuily-Fodil: Lead accumulation in the roots of grass pea (*Lathyrus sativus* L.): A novel plant for phytoremediation systems? *Com. Ren. Biolo.*, **331**, 859-864 (2008).
- Cao, R.X., L.Q. Ma., M. Chen., S. Singh and W. Harris: Impacts of phosphate amendments on lead biogeochemistry at a contaminated site. *Environ. Sci. Tech.*, **36**, 5296-5304 (2002).
- Chaney, L.R., S.B. Sterrett and H.W. Mielke: The potential for heavy metal exposure from urban gardens and soils, *USDA Agric. Res. Service. Biolo. Waste Manage. Org. Res. Lab. Study*, Beltsville, MD 20705 (1984).
- Chauhan, P. and J. Mathur: Phytoremediation efficiency of *Helianthus annuus* L. for reclamation of heavy metals-contaminated industrial soil. *Environ. Sci. Pollut. Res.*, **27**, 29954-29966 (2020).
- Chen, S., Y. Zhu and Y. Ma: The effect of grain size of rock phosphate amendment on metal immobilization in contaminated soils. *J. Hazard. Mater.*, **134**, 74-79 (2006).
- Chen, S.B. and Y.G. Zhu: Effect of different phosphorus-compounds on Pb uptake by *Brassica oleracea*. *Acta Sci. Circum.*, **24**, 707-712 (2004).
- Cui, L.P., J.F. Bai, Y.H. Shi, S.L. Yan, W.H. Wuang and X.Y. Tang: Heavy metals in soil contaminated by coal mining activity. *Acta Ped. Sini*, **41**, 896-904 (2004).
- DalCorso, G., E. Fasani, A. Manara, G. Visioli and A. Furini: Heavy metal pollutions: State of the art and innovation in phytoremediation. *Int. J. Mol. Sci.* **20**, 3412 (2019).
- Fasani, E., A. Manara, F. Martini, A. Furini and G. DalCorso: The potential of genetic engineering of plants for the remediation of soils contaminated with heavy metals. *Plant Cell Environ.*, **41**, 1201-1232 (2018).
- Getnet, B.E. and T. Dugasa: Response of maize yield and yield related components to different levels of nitrogen and phosphorus fertilizers. *Acta Sci. Agric.*, **3**, 03-08 (2019).
- Gad, N.: Uptake of cobalt and some other trace elements as affected by phosphorus levels and mycorrhizae inoculation. *Egypt J. Soil Sci.*, **42**, 609-623 (2002).
- Gad, N. and M.K. Hassan: Influence of cobalt and phosphorus on growth, yield and quality of sweet potato (*Ipomoea batatas* L.). *J. Appl. Sci. Res.*, **7**, 1501-1506 (2011).
- Gajewska, E. and M. Sklodowska: Relations between tocopherol, chlorophyll and lipid peroxides contents in shoots of Ni-treated wheat. *J. Plant Physiol.*, **164**, 364-366 (2007).
- Gaur, A.C.: *Phosphate Solubilizing Micro-organisms as Bio-fertilizers*. Omega Scientific Publications, New Delhi, pp 176 (1990).
- Ghosh, M. and S.P. Singh: A review on phytoremediation of heavy metals and utilization of it's by products. *Appl. Ecol. Environ. Res.*, **3**, 1-18 (2005).
- Grant, C.A., L.D. Bailey, M.J. McLaughlin and B.R. Singh: Management factors which influence cadmium concentration in crops. In: Cadmium in Soils and Plants (Eds.: B.R. Singh and M.J. McLaughlin). Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 151-198 (1999).
- Hettiarachchi, G.M. and G.M. Pierzynski: *In-situ* stabilization of soil lead using phosphorus and manganese oxide: Influence of plant growth. *J. Environ. Qual.*, **31**, 564-572 (2002).
- Hettiarachchi, G.M., G.M. Pierzynski and M.D. Ransom: *In-situ* stabilization of soil lead using phosphorus. *J. Environ. Qual.*, **30**, 1214-1221 (2001).
- Hossain, M.A., S. Hamid and S. Nasreen: Effect of nitrogen and phosphorus fertilizer on N/P uptake and yield performance of groundnut (*Arachis hypogaea* L.). *J. Agric. Res.*, **45**, 119-125 (2007).
- Jacob, J.M., C. Karthik., R.G. Saratale, S.S. Kumar, D. Prabakar., K. Kadirvelu and A. Pugazhendhi: Biological approaches to tackle heavy metal pollution: A survey of literature. *J. Environ. Manage.* **21**, 756-70 (2018).
- Jackson, M.L.: *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi (1973).
- Jiao, Y., C.A. Grant and L.D. Bailey: Effects of phosphorus and zinc fertilizers on cadmium uptake and distribution in flax and durum wheat. *J. Sci. Food Agric.*, **84**, 777-785 (2004).
- Kuntyastuti, H. and Suryantin: Effect of phosphorus fertilization on soil phosphorus level, growth and yield of soybean (*Glycin max* l.) in paddy soil, *J. Exp. Bio. Ag. Sci.*, **3**, 1-9 (2015).
- Kacar, B. and A.V. Katkat: BitkiBesleme. Nobel Yayinlari (5. Baski), Turkey, pp. 1-678 (2011).
- Lindsay, W.L. and W.A. Norvell: Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. America J.*, **42**, 421-428 (1978).

- Mahardika, G., A. Rinanti and M.F. Fachrul: Phytoremediation of heavy metal copper (Cu²⁺) by sunflower (*Helianthus annuus* L.). *IOP Conf. Ser.: Earth Environ. Sci.*, **106**, 012120 (2018).
- McGowen, S.L. and N.T. Basta: Heavy metal solubility and transport in soil contaminated by mining and smelting. In: Heavy Metal Release in Soils (Eds.: H.M. Selim and D.L. Sparks). CRC Press, Inc, Boca Raton, FL, pp. 89-107 (2001).
- Mignardi S., A. Corami and V. Ferrini: Immobilization of Co and Ni in mining-impacted soils using phosphate amendments. *Water, Air Soil Poll.*, **224**, 1-10 (2013).
- Nehnevajova, E., R. Herzig, G. Federer, K.H. Erismann and J.P. Schwitzguebel: Screening of sunflower cultivars for metal phytoextraction in a contaminated field prior to mutagenesis. *Int. J. Phytorem.*, **7**, 337-349 (2005).
- Netherway, P., G. Gascó, A. Méndez, A. Surapaneni, S. Reichman, K. Shah and J. Paz-Ferreiro: Using phosphorus-rich biochars to remediate lead-contaminated soil: Influence on soil enzymes and extractable P. *Agronomy*, **10**, 454 (2020).
- Pantigoso, H.A., J. Yuan, Y. He, Q. Guo, C. Vollmer and J.M. Vivanco: Role of root exudates on assimilation of phosphorus in young and old *Arabidopsis thaliana* plants. *PLoS ONE*, **15**, 112-23 (2020).
- Piper, C.S.: Soil and Plant Analysis. The University of Adelaide, Australia. (1966).
- Ruangcharus, C., S.U. Kim and C.O. Hong: Mechanism of cadmium immobilization in phosphate-amended arable soils. *Appl Biol Chem.*, **63**, 36 (2020).
- Rizwan, M., S. Ali, H. Rizvi, J. Rinklebe, D.C.W. Tsang, E. Meers, Y.S. Ok and W. Ishaque: Phytomanagement of heavy metals in contaminated soils using sunflower: A review. *Critical Rev. Environ. Sci. Tech.*, **46**, 1498-1528 (2016).
- Sanjay-Swami and E.A.S. Lyngdoh: Restoration of degraded land in coal mine areas of Jaintia Hills, Meghalaya through phytoremediation. *Soil & Water Conser. Bull.*, No. 4, Indian Association of Soil and Water Conservationists, Dehradun, UK, pp. 17-24 (2019).
- Sanjay-Swami: Managing fragile hill ecosystems of North Eastern Region. In: *Soil Water Conser. Today*, **14**, 02 (2019).
- Sayantan, D. and Shardendu: Amendment in phosphorus levels moderate the chromium toxicity in *Raphanus sativus* L. as assayed by antioxidant enzymes activities. *Ecotoxic. Environ. Safety*, **95**, 161-170 (2013).
- Seshadri, B., N.S. Bolan, H. Wijesekara and R. Naidu: Phosphorus-cadmium interactions in paddy soils. *Geoderma*, **270**, 43-59 (2016).
- Singh, R., D.P. Singh, N. Kumar, S.K. Bhargava and S.C. Barma: Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. *J. Environ. Biol.*, **31**, 421-430 (2010).
- Silveira, T.C., R.F. Pegoraro, M.K. Kondo, A.F. Portugal and Á.V. Resende: Sorghum yield after liming and combinations of phosphorus sources. *R. Brasil de Eng. Agrí. e Amb.*, **22**, 243-248 (2018).
- Subbiah, B.V. and G.L. Asija: A rapid procedure for the determination of available nitrogen in soils. *Curr. Sci.*, **25**, 259-260 (1956).
- Sushanta, S., S. Bholanath, M. Sidhu, P. Sajal and D.R. Partha: Grain yield and phosphorus uptake by wheat as influenced by long-term phosphorus fertilization. *African J. Agric. Res.*, **9**, 607-612 (2014).
- Taalab, A.S. and M.A. Badr: Phosphorous availability from compacted rock phosphate with nitrogen to sorghum inoculated with phosphor-bacterium. *J. Appl. Sci. Res.*, **3**, 195-201 (2007).
- Taliman, N.A., Q. Dong, K. Echigo, V. Raboy and H. Saneoka: Effect of phosphorus fertilization on the growth, photosynthesis, nitrogen fixation, mineral accumulation, seed yield, and seed quality of a soybean low-phytate line. *Plants*, **8**, 119 (2019).
- Turuko, M. and A. Mohammed: Effect of different phosphorus fertilizer rates on growth, dry matter yield and yield components of common bean (*Phaseolus vulgaris* L.). *World J. Agric. Res.*, **2**, 88-92 (2014).
- Tewari, R.K., P. Kumar, P.N. Sharma and S.S. Bisht: Modulation of oxidative stress responsive enzymes by excess cobalt. *Plant Sci.*, **162**, 381-388 (2002).
- Valipour, M., S. Karim and K. Ali: Chemical immobilization of lead, cadmium, copper, and nickel in contaminated soils by phosphate amendments. *CLEAN - Soil, Air, Water*, **44**, 572-578 (2016).
- Wang, J., Y. Chen and P. Wang, Y. S. Li, G. Wang, P. Liu and A. Khan: Leaf gas exchange, phosphorus uptake, growth and yield responses of cotton cultivars to different phosphorus rates. *Photosynthetica*, **56**, 1414-1421 (2018).
- Walkley, A. and L.R. Black: An examination of degtryeff method for determination of soil organic matter and a proposed modification of chromic acid titration method. *Soil Sci.*, **37**, 29-38 (1934).
- Wilkinson, S.R., D.L. Grunes and M.E. Sumner: Nutrient interactions in soil and plant nutrition. In: Handbook of Soil Science (Ed.: M.E. Sumner). CRC Press, Boca Raton, FL, pp. D-89-D-111 (2000).
- Wortmann, C.S., A.R. Dobermann, R.B. Ferguson, G.W. Hergert, C.A. Shapiro, D.D. Tarkalson and D.T. Walters: High-yielding corn response to applied phosphorus, potassium, and sulfur in Nebraska. *Agron. J.*, **101**, 546-555 (2009).
- Yan, Y., Y.Q. Zhou and C.H. Zhou: Evaluation of phosphate fertilizers for the immobilization of Cd in contaminated soils. *PLoS ONE*, **10**, (2015) e0124022.
- Yan, A., Y. Wang, S.N. Tan, M.L. MohdYusof, S. Ghosh and Z. Chen: Phytoremediation: A promising approach for revegetation of heavy Metal-polluted land. *Front. Plant Sci.*, **11**, 359 (2020). doi: 10.3389/fpls.2020.00359
- Yazdanbakhsh, A., S.N. Alavi, S.A. Valadabadi, F. Karimi and K. Zainab: Heavy metals uptake of salty soils by ornamental sunflower, using cow manure and bio-solids: A case study in Alborz city, Iran. *Air, Soil Water Res.*, **13**, 1-13 (2020).
- Zhao, Q.X. and Y.F. Song: Principles and methods of contaminated soil remediation. Beijing (China): *China Environ. Sci. Press*, (2004).
- Zhoa, R.F., C. Zou and F. Zhang: Effects of long-term P fertilization on P and Zn availability in winter wheat rhizosphere and their nutrition. *Pl. Nutr. Ferti. Sci.*, **13**, 368-372 (2007).
- Zhou, W. and B.W. Qiu: Effects of cadmium hyper-accumulation on physiological characteristics of *Sedum alfredii* Hance (Crassulaceae). *Plant Sci.*, **169**, 737-745 (2005).
- Zhou, J., C. Zhang, B. Du, H. Cui, X. Fan, D. Zhou and J. Zhou: Effects of zinc application on cadmium (Cd) accumulation and plant growth through modulation of the antioxidant system and translocation of Cd in low- and high-Cd wheat cultivars. *Environ. Poll.*, **95**, 115045, (2020).