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## Effect of lime, compost and microbial inoculants on removal of cadmium and lead by mustard and maize in trace metal contaminated soil of Jharkhand

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### Abstract

A Farmers' field trial was conducted at Patratu (Ramgarh) to study the effect of lime, compost, plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi for remediation of high trace metal levels in mustard-maize cropping system. Results reveal that microbial inoculants with or without vermicompost increased the trace metal removal, however, vermicompost alone decreased the removal. Vermicompost, lime and lime + vermicompost significantly reduced the total Cd uptake by mustard and maize. Inoculation with *Glomus mossae* resulted in elevated level of Cd in mustard and maize plants. Total trace metal content in soil was significantly reduced by microbial inoculation alone or that in combination with vermicompost. However, DTPA-extractable trace metals decreased with addition of amendments as well as inoculation of microbes. *Glomus mossae* was most effective in remediating the trace metals. Under this study, the total metal content reduced effectively by their inoculation alone while inoculation along with vermicompost resulted in reducing the DTPA-extractable fraction, more effectively. The extent of reduction in total Cd and Pb after harvest of both crops was 6 to 26 and 5 to 12 per cent, respectively over control. However, the corresponding values observed for DTPA extractable Cd and Pb was 53 to 65 and 20 to 32 per cent over control in microbial inoculation and 46 to 47 and 14 to 17 per cent in case of amendments.

**Keywords:** remediation of Cd, Pb, mustard, maize, lime, compost, plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi

### 1. Introduction

Contamination of trace metals refers to their anthropogenic accumulation, which may or may not inflict any harm to the system or organism. Pollution is the worst example of contamination where irreversible toxicity-damage has already occurred due to buildup of the toxic substances in the system. Heavy metal pollution, particularly after the reports of infamous *itai-itai* and *minamata* diseases from Japan, has created an environmental scare. In India, arsenic poisoning in human beings and selenium toxicity in the live stock are the two similar examples (Rattan *et al.*, 2005) [16]. Anthropogenic sources of trace elements are a consequence of industrial development and urbanization. These sources are related to human activities such as mining and smelter activities, fossil fuel combustion, waste incineration and disposal, agricultural practices like use of fertilizers and pesticides (Adriano *et al.*, 1995) [1]. Soil remediation is the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to condition prior to disturbance. One of the effective remediation technologies of metal contaminated soils has been the excavation of soil followed by soil washing and subsequent disposal of treated soils (US Environmental Protection Agency, 1991). Since soil removal is prohibitively expensive and

Impractical in the context of our country, there is a need to evolve cost-effective indigenous *in-situ* technology. Use of chemical amendments like lime, phosphates and organic matter for scavenging, precipitating and inactivating the heavy metals and plant species capable of hyper-accumulating these metals for alleviating the metal-toxicity seem probable options. Plant based bioremediation technologies have been collectively termed as phytoremediation, refers to the use of green plants and their associated micro biota for the *in-situ* treatment of contaminated soil and ground water (Sadovsky, 1999) [14]. The inoculation with plant growth promoting rhizo bacteria may facilitate plant growth and thus increase phytoextraction efficiency, although it did not greatly influence metal concentrations in plant tissues, but achieved a much larger above ground biomass harvest, thus resulting in a much higher metal removal (Wu *et al.*, 2006) [19]. Arbuscular mycorrhizal fungi are soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots (Barera and Jeffries, 1995). Jharkhand has several coal mines. The Damodar river basin is a repository of approximately 46 per cent of the Indian coal reserves. Due to extensive coal mining and rapid growth of industries, soil and water resources have been badly contaminated. Besides mining, coal-based industries like coal washeries, coke oven plant, coal fired thermal power plant, steel plants and other related industries in the region are responsible for degradation of environmental quality.

### Materials and Methods

A farmer's field experiment was conducted during *rabi* 2009-10 and *kharif* 2010 at Patratu (Ramgarh) to investigate the effect of lime, compost, plant growth promoting rhizo bacteria and arbuscular mycorrhizal fungi on remediation of trace metal contamination. The soil was sandy loam in texture with pH 6.08, EC 0.07 dS m<sup>-1</sup>, organic carbon 2.85 g kg<sup>-1</sup>, DTPA-extractable Cd 0.20 mg kg<sup>-1</sup>, DTPA-extractable Pb 3.71 mg kg<sup>-1</sup>, Total Cd 19.5 mg kg<sup>-1</sup> and Total Pb 58.2 mg kg<sup>-1</sup>. Mustard (cv. T 59) and maize (cv. PEHM 2) were grown in sequence at the same site with 10 treatments in randomized block design having three replications. Trace metals concentration including Zn, Cu, Mn, Fe, Cd, Pb, Ni and Co in soil and plant samples were analysed. The available trace metal was determined by extracting soil with DTPA (0.005M DTPA, 0.01M CaCl<sub>2</sub>, 0.1M TEA), pH adjusted to 7.3 with the help of dilute HCl, maintaining 1:2 soil to extractant ratio and shaking for 2 hrs at 120 rpm (Lindsay and Norvell, 1978). For estimation of total trace metal, soil sample was digested in perchloric-hydrofluoric mixture on platinum crucible near to dryness, residue was redissolved in hydrochloric acid (Hesse, 1994) and metal content was determined on Atomic Absorption Spectrophotometer (EICL AAS4139). Oven dried plant sample was digested in mixture of HNO<sub>3</sub>:HClO<sub>4</sub> in the ratio of 9:4 at 80°C until a transparent solution was obtained (Allen *et al.*, 1986). The transparent solution was diluted with double distilled water and filtered. The content of trace metal was determined on Atomic Absorption Spectrophotometer (EICL AAS4139) by employing the appropriate hollow cathode lamp.

### Results and Discussion

#### *Cd and Pb removal by mustard and maize*

The effect of lime, compost, PGPR and AMF on removal of Cd, Pb, Ni and Co is presented in table 1. Results indicate high Cd uptake by mustard grain (0.89 g ha<sup>-1</sup>), maize straw

(42.26 g ha<sup>-1</sup>), maize grain (4.75 g ha<sup>-1</sup>) as well as mustard-maize system (47.86 g ha<sup>-1</sup>) with *Glomus mossae* inoculation. But total Cd removal by mustard (11.92 g ha<sup>-1</sup>) was high with *Pseudomonas striata* inoculation. Results on total Cd removal by crops indicate that microbial inoculants with or without vermin compost showed similar influence, however, vermin compost alone tend to decrease the removal. Vermi compost, lime and lime + vermin compost significantly reduced the total Cd removal by mustard and maize. It was noticed that 19 to 33 per cent reduction in Cd removal was due to vermin compost, lime and their combination (Fig 1), while 10 to 38 per cent Cd removal was due to microbial inoculants either alone or in combination with vermin compost. Inoculation with *Glomus mossae* resulted in elevated level of Cd in mustard and maize plants (Fig 2).

Inoculation with *G. mossae* resulted in significantly high Pb removal by mustard grain (4.69 g ha<sup>-1</sup>), maize straw (55.56 g ha<sup>-1</sup>) and maize grain (11.02 g ha<sup>-1</sup>). Significantly high Pb uptake by mustard (47.97 g ha<sup>-1</sup>) was recorded with *P. striata* inoculation. However, *A. chroococcum* recorded the highest Pb removal by mustard-maize cropping system. Total Pb removal by maize and mustard-maize system was significantly reduced by vermin compost application either alone or in combination with lime and microbial inoculants. However, in case of mustard the effect of vermin compost was not significant. Increase in Pb removal up to 14 per cent was observed due to amendments (Fig 3), while vermin compost decreased the removal when applied with microbial inoculants. Thirteen to 15 per cent reduction in Pb removal was recorded in maize and mustard-maize system due to amendments. Application of vermin compost, lime and lime + vermin compost recorded reduction in Pb content of both crops (Fig 4). Microbial inoculants when applied with vermin compost also resulted in reduced Pb concentration.

Inoculation with *G. mossae*, *P. striata* and *A. chroococcum* resulted in increased content and removal of Cd and Pb by above ground parts of mustard and maize. The possible mechanisms for bioaccumulation of harmful trace metal in plant parts have been suggested by several workers. Maywald and Weigel (1997) [13] reported that phytochelatins like low molecular weight  $\alpha$ -Glu-Cyspeptides with high affinity for certain metals are assumed to be involved in accumulation, detoxification and metabolism of metal ions (Cd, Zn, Cu, Pb and Hg) in plant cells. Organic carboxylic acid like maleic, citric, oxalic, succinic acids etc. are commonly found in crop rhizo sphere as secretion or exudation products from plant roots or associated microbes, help in complexing metals to ease their entry to the plant cell. This bound form has less free energy and thus does not get involved easily in metabolic activities. Metal might be bound to the cell wall without actually entering the protoplasmic body thus saving the plant from any toxicity hazard (Tomsett and Thurman, 1988) [15]. The results obtained in present study are in agreement with Weng *et al.* (2004) [18], Citterio *et al.* (2005) [7], Madhaiyan *et al.* (2007) [12] and Arora and Sharma (2009) [3].

Microbial inoculation along with vermin compost application resulted in reduced Cd and Pb removal. Bolan and Duriasamy (2003) [5] suggested that the resulting metal complex with dissolved organic matter is not taken up by plants due to its inability to pass through the plasma lemma. The Pb has strong affinity to organic carbon which may reduce their removal (Assami *et al.*, 1995) [4]. Vermi compost, lime and lime + vermin compost significantly reduced Cd and Pb content and removal. This might be ascribed as reduction of trace metal mobility by promoting the formation of insoluble precipitates

or by enhancing the soils capacity to bind the trace element, directly through the addition of adsorbent material or indirectly by adjusting the soil's pH-Eh conditions to promote trace element adsorption onto the soil's matrix.

#### *Cd and Pb content in soil after harvest of mustard and maize*

The effect of lime, compost, PGPR and AMF on trace metal status of soil after harvest of mustard and maize is presented in table 2. It was observed that total trace metal content in soil was significantly reduced by microbial inoculation alone or that in combination with vermin compost. However, DTPA extractable trace metals decreased with addition of amendments as well as inoculation of microbes. *Glomus mossae* was most effective in remediating the trace metals, the total metal content reduced effectively by their inoculation alone while inoculation along with vermin compost resulted in reducing the DTPA extractable fraction, more effectively. The extent of reduction in total Cd and Pb after harvest of both crops was 6 to 26 and 5 to 12 per cent, respectively over control. However, the corresponding value observed for DTPA extractable was 53 to 65 and 20 to 32 per cent over control in microbial inoculation and 46 to 47 and 14 to 17 per cent in case of amendments.

Significant reduction in total and DTPA extractable Cd and Pb by microbial inoculation may be attributed to their increased uptake by mustard and maize. However, reduction in DTPA extractable trace metal due to application of vermin compost, lime and lime + vermin compost may be attributed to formation of insoluble precipitates, complexation or adsorption. Similar results are found by Chen and Liu (1999), Jha (2001)<sup>[9]</sup> and Lombi *et al.* (2002)<sup>[11]</sup> earlier.

Results thus indicate that microbial inoculation resulted in increased trace metal removal by mustard, maize and system. However, amendments (vermin compost, lime and their combination) reduced the trace metal removal by the crops. Reduction in total trace metal content in soil after harvest of crops was recorded with microbial inoculation. However, DTPA extractable trace metals decreased with addition of amendments as well as inoculation of microbes. *Glomus mossae* was most effective in remediating the trace metals under this study. This indicates that such bioremediation measures in the affected areas could be highly effective in abatement of trace metal contamination of soils and crops.

**Table 1:** Effect of lime, compost, PGPR and AMF on Cd and Pb removal (g ha<sup>-1</sup>) by mustard and maize

Treatments	Mustard			Maize		
	Stover	Grain	Total	Straw	Grain	Total
<b>Cd removal</b>						
Control (RDF)	7.91	0.72	8.63	34.66	3.73	38.39
Vermicompost (VC)	6.25	0.48	6.74	22.97	2.77	25.74
Lime	6.25	0.48	6.74	23.91	2.51	26.42
Vermicompost + Lime	6.52	0.46	6.98	22.89	2.74	25.62
<i>Pseudomonas striata</i>	11.05	0.86	11.91	39.74	4.22	43.96
<i>Pseudomonas striata</i> + VC	11.12	0.80	11.92	39.17	4.58	43.75
<i>Azotobacterchroococcum</i>	9.96	0.81	10.77	37.89	4.48	42.37
<i>A.chroococcum</i> + VC	10.73	0.78	11.51	38.46	4.62	43.08
<i>Glomus mossae</i>	10.75	0.89	11.65	42.26	4.44	46.69
<i>Glomus mossae</i> + VC	10.98	0.81	11.79	39.15	4.75	43.89
SEm±	0.56	0.02	0.57	1.43	0.13	1.56
CD (P = 0.05)	1.66	0.06	1.69	4.26	0.38	4.62
CV %	5.06	4.09	6.46	6.52	4.33	6.74
<b>Pb removal</b>						
Control (RDF)	29.08	2.91	31.99	44.25	7.48	51.73
Vermicompost (VC)	32.28	2.52	34.80	38.33	5.72	44.05
Lime	31.69	2.54	34.24	39.27	5.29	44.56
Vermicompost + Lime	34.03	2.40	36.43	38.57	5.47	44.03
<i>Pseudomonas strata</i>	43.64	4.33	47.97	53.41	10.63	64.04
<i>Pseudomonas strata</i> + VC	41.12	3.46	44.58	49.49	9.71	59.20
<i>Azotobacterchroococcum</i>	40.18	4.07	44.26	54.76	12.52	67.27
<i>A.chroococcum</i> + VC	39.60	3.36	42.96	50.19	10.39	60.58
<i>Glomus mosseae</i>	43.09	4.69	47.78	55.56	11.02	66.58
<i>Glomus mosseae</i> + VC	41.25	3.62	44.87	49.60	10.23	59.83
SEm±	1.82	0.06	1.81	0.51	0.24	0.68
CD (P = 0.05)	5.41	0.18	5.39	1.52	0.70	2.03
CV %	5.46	5.61	6.06	8.90	7.73	9.76

**Table 2:** Effect of lime, compost, PGPR and AMF on remediation of Cd and Pb (mg kg<sup>-1</sup>) contaminated soil in mustard-maize cropping system

Treatments	Total		DTPA-extractable	
	Mustard	Maize	Mustard	Maize
<b>Cd</b>				
Control (RDF)	18.75	17.17	0.19	0.17
Vermicompost (VC)	18.58	17.00	0.10	0.09
Lime	18.33	16.75	0.10	0.09
Vermicompost + Lime	18.33	16.67	0.10	0.09
<i>Pseudomonas strata</i>	16.50	14.67	0.08	0.06
<i>Pseudomonas strata</i> + VC	17.33	15.75	0.09	0.08
<i>Azotobacterchroococcum</i>	16.75	14.00	0.08	0.06

<i>A.chroococcum</i> + VC	17.58	15.67	0.09	0.07
<i>Glomus mosseae</i>	15.08	12.75	0.07	0.05
<i>Glomus mosseae</i> + VC	16.00	14.50	0.08	0.07
SEm±	0.27	0.36	0.01	0.01
CD (P = 0.05)	0.81	1.07	0.02	0.02
CV %	11.34	14.80	4.07	3.25
Pb				
Control (RDF)	56.92	52.75	3.63	3.28
Vermicompost (VC)	56.33	52.33	3.11	2.77
Lime	56.42	52.33	3.04	2.74
Vermicompost + Lime	56.08	52.00	3.08	2.73
<i>Pseudomonas strata</i>	53.00	48.83	2.75	2.59
<i>Pseudomonas strata</i> + VC	53.75	49.50	2.90	2.63
<i>Azotobacterchroococcum</i>	53.33	49.08	2.82	2.49
<i>A.chroococcum</i> + VC	54.17	50.17	2.90	2.59
<i>Glomus mosseae</i>	50.33	46.17	2.46	2.18
<i>Glomus mosseae</i> + VC	51.42	47.25	2.68	2.47
SEm±	0.29	0.34	0.05	0.06
CD (P = 0.05)	0.86	1.00	0.15	0.17
CV %	6.80	8.24	5.15	6.12

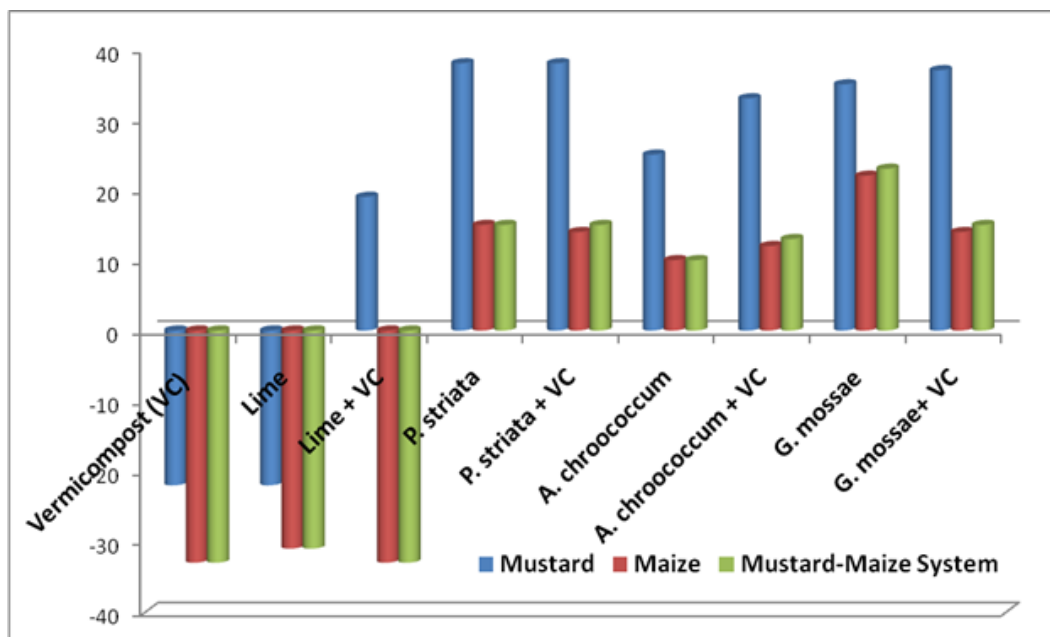


Fig 1: Effect of treatments on per cent increase or decrease in Cd removal

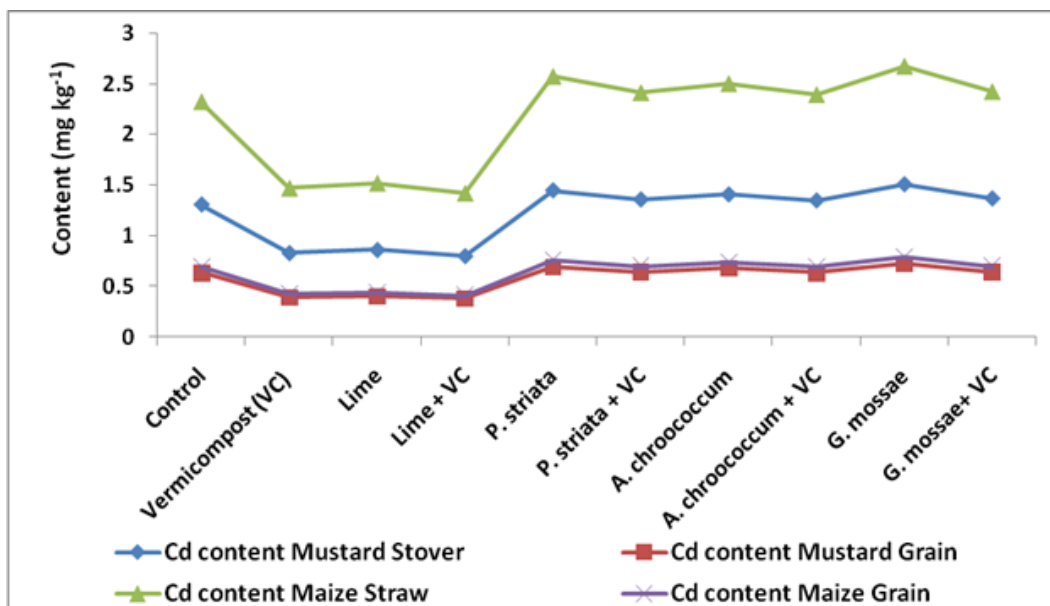


Fig 2: Effect of lime, compost, PGPR and AMF on Cd content (mg kg⁻¹) in plant parts

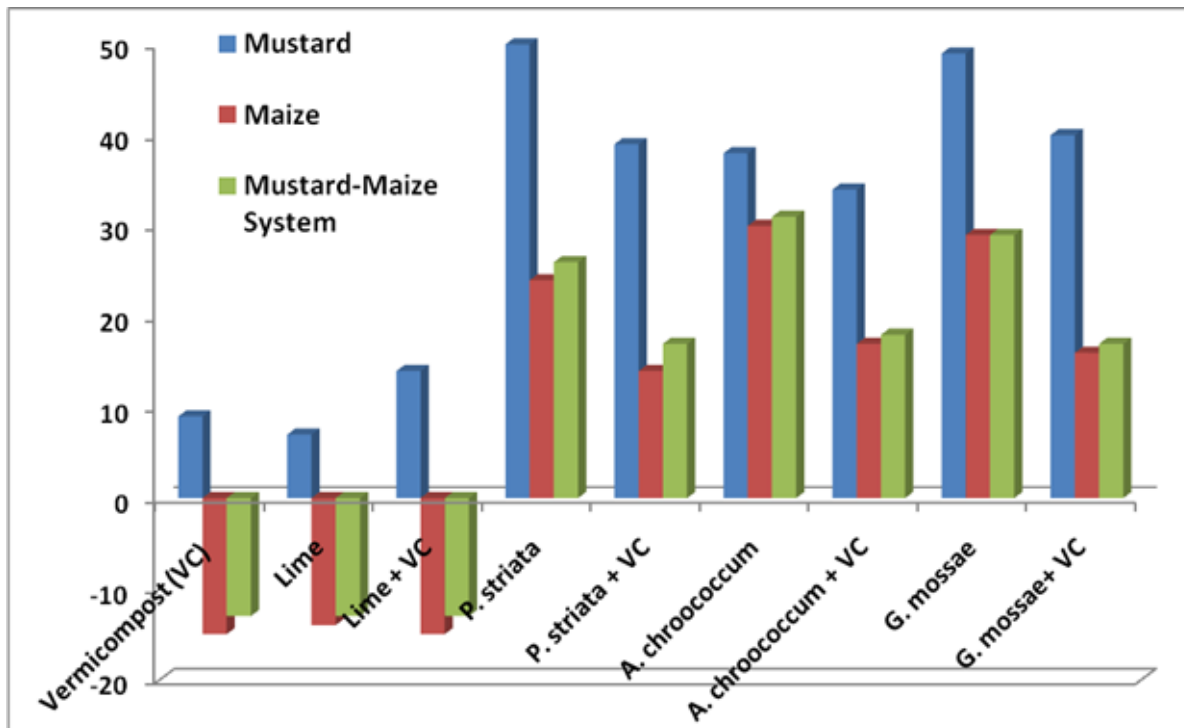


Fig 3: Effect of treatments on per cent increase or decrease in Pb uptake

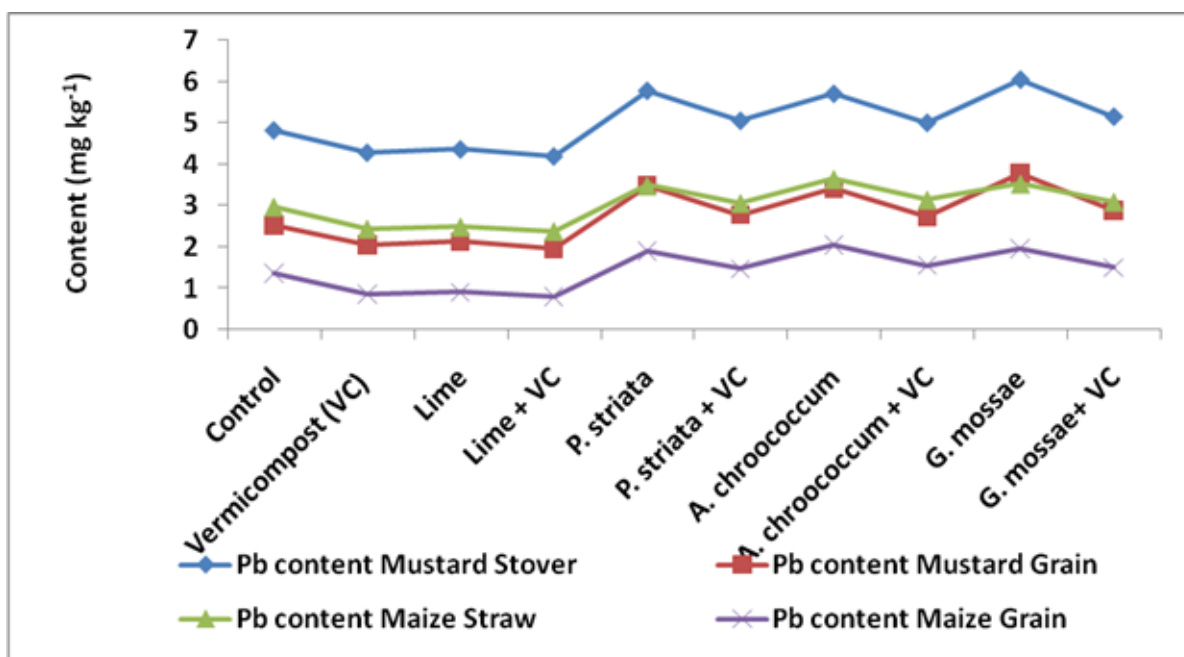


Fig 4: Effect of lime, compost, PGPR and AMF on Pb content (mg kg<sup>-1</sup>)

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