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Different sources of phosphorus fertilizers and soil amendments affected the phosphorus and cadmium content in soil, roots and seeds of maize (*Zea mays* L.)

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Research Article	Phosphorus (P) fertilizers contain cadmium (Cd) as a contaminant at levels varying from trace amounts to high levels and therefore, can be a major source of Cd to agricultural systems. This study was designed to assess the impact on application of Eppawala rock phosphate (ERP) and triple super
Received : 15/04/2020 Accepted : 01/02/2021	phosphate (TSP) as P fertilizers and different soil amendments on P and Cd uptake in maize (<i>Zea mays</i> L.). The field trial was carried out at Field Crop Research and Development Institute at Mahailluppallama, Sri Lanka. A randomized complete block design was employed with three replicates as ERP and TSP separately applied with arbuscular mycorrhizal fungi (AMF) and three
<i>Keywords:</i> Phosphorus Cadmium Triple Super Phosphate Eppawala Rock Phosphate <i>Zea mays</i> L.	types of amendments (biochar, compost and dolomite) and the control without adding P fertilizers. Phosphorus content and Cd content of soil, maize roots and seeds were quantified. Results revealed that available soil Cd and total accumulated root and seed Cd amounts were significantly higher in TSP added treatments with and without amendments compared with ERP added soil. Considering soil available P, root and seed P, there was no significant difference observed in different treatments of TSP and ERP added treatments. A similar phenomenon was also observed in growth and yield parameters with both fertilizers added and with the added amendments. There was no colonization of AMF in maize roots in TSP applied soil while 25-60% of colonization was recorded with ERP. Synthetic fertilizer (TSP) must have inhibited the AMF colonization and thereby increasing the Cd content in maize seeds. AMF colonization increased with comparatively low soil available P in ERP added treatments. The results revealed that TSP could be effectively substituted by ERP as a source of P for maize soils. The addition of AMF, compost and biochar further increased the effect.
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Introduction

Phosphorus (P) has acquired a significant role in plant growth and metabolism. It involves many important processes in plants such as energy transfer and storage, respiration, photosynthesis and enzyme regulation (Schachtman et al., 1998; Malhotra et al., 2018). Also P is part of structural component of DNA, RNA and membranes (Holford, 1997; Smith et al., 2011). Therefore, deficiency of P directly leads to loss of agricultural productivity in the world (Hinsinger, 2001; Leghari et al., 2016). It implies the necessity of adding P to agricultural field externally. As a result, about 30 million tons of P fertilizer is applied worldwide every year. Mainly P fertilizers that are applied to agricultural fields can be categorized as basic slag (12-18% P2O5), single super phosphate (17-20% P₂O₅), rock phosphate (26-37% P₂O₅), di-calcium phosphate (35-52% P₂O₅) and triple super phosphate (44-48% P₂O₅) (Dissanayake and Chandrajith,

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2009). However, higher percentage of P (up to 80%) is escaped by becoming immobile or unavailable to uptake by plants because of adsorption, precipitation, leaching and conversion to organic forms (López-Bucio et al., 2000; Saleque et al., 2004; Ayaga et al., 2006). Therefore, efficient management of P fertilizers in cropping fields is required.

However, there are many limitations in soil, which leads to phytoavailability of P from the fertilizers. Such factors can be categorized as plant factors as crop variety, root development and distribution, crop yield level and soil factors such as soil texture, aeration, compaction, soil moisture, soil pH, organic matter content and interaction with other nutrients (Beauchemin and Simard, 1999). Fertilizer based factors such as water solubility, chemical and physical forms of P (Armstrong, 1999; Alkhader, 2015). The effect of soil microorganisms such as arbuscular mycorrhizal fungi (AMF) and P solubilizing bacteria can be categorized as the soil biological factors. Therefore, manipulation of these factors that can enhance efficiency of P uptake by plants is a currently important concern (Miyasaka and Habte, 2001). However, excessive regular application of synthetic inorganic fertilizer to agricultural fields leads many environmental and health hazardous situations (Singh et al., 2017). Specially, accumulation of harmful residues such as heavy metals from fertilizers is more prominent (Grant et al., 2004; Singh et al., 2017). Therefore, implementation of the methods to increase the efficiency of P acquisition and usage by plants and to substitute synthetic P fertilizers with minimum hazardous impurities such as heavy metals is one of the research priorities. Both advantages and limitations of triple super phosphate have been reported as well (Edward et al., 2000; Grant and Sheppard, 2008). However, there is limited data in scientific literature about the usage of Eppawala rock phosphate (ERP) for crops such as maize, which is in short time period in field. This ERP normally contain low level of heavy metals than TSP because ERP originate from bed- rock, which is closer to the igneous type (Dissanayake and Chandrajith, 2009). Although there are limitations in the usage of ERP as a source of P due to its lower solubility than TSP, there are opportunities to make use of ERP when it applies with some soil amendments.

Here, the clear idea of the study is to manipulate the factors that can effect to absorption of P and Cd from fertilizers. In this case, selected amendments namely biochar, compost, dolomite and AMF use to manipulate soil conditions physically, chemically and biologically leading to increase P phytoavailability and reduce absorption of Cd in to plant. The phenomenon about the usage of compost, it can reduce the phosphorus fixation in soils by creating a competition between organic acids and orthophosphate for adsorption sites resulting release of phosphorus by this organic material in the case of decomposition. Also combine application of compost with commercial inorganic fertilizers can increase the efficiency of inorganic fertilizer and reduce the cost for frequent addition of synthetic fertilizers (Kwabiah et al., 2003). The idea for the selection of biochar for the study is that biochar application have resulted a higher grain yields at sites with low P availability and improved the response to nitrogen and phosphorus chemical fertilizer treatments according to the literature (Jha et al., 2010). Biochar appear as multischeme player by improving soil physical properties (Herath et al., 2013), reducing soil fertility constraints (Mia et al., 2014) and stimulating soil biological processes (Wang et al., 2015) leading to enhance crop performance. As well as, Physical amelioration of lime or dolomite occurs through flocculation of colloid particles which leads to changes in surface potential and charge densities (Bolan et al., 2003). Accordingly, it reduces P sorption and increase the concentration of HPO42- in the soil solution (Straaten, 2007) leading to increase the amount of available P for plant uptake (Kisinyo, 2013). When consider the reason to AMF selection for the study, this AMF colonization directly provides the advantages to host plants through contributing the nutrient uptake process especially to phosphorus uptake even in low nutrient conditions in rhizosphere using its very large surface area that is involving efficient nutrient uptake from different regions and volumes of soil while rapidly delivered to cortical cells within the root (Zhu et al., 2001). Therefore, the aim of the study was to assess P and cadmium (Cd) in soil, roots and seeds of maize (*Zea mays* L.) as affected by the application of ERP and TSP fertilizers with combine application of AMF and the other soil amendments such as biochar, compost and dolomite.

Materials and Methods

Experimental Sites

The field experiment was carried out at Field Crops Research and Development Institute, Mahailluppallama (08.60° N, 80.27° E, 137 m amsl), Sri Lanka, which is in Low country Dry Zone from January 2018 to June 2018. The annual average rainfall of this region is 900-1100 mm. The soil at the experimental site is Rhodustalfs, with a moderately well-drained sandy clay loam texture (Panabokke, 1996). The initial pH of the soil was 6.7.

Experimental Treatments and Design

The experimental design was randomized complete block design with three replicates. The plot size was 2.4 m x 1.2 m spaced at 30 cm apart from each other. ERP and TSP were separately applied with AMF and three types of amendments namely biochar, compost and dolomite as different treatments, T₁- no any P addition, T₂ - TSP alone (100 kg ha⁻¹), T₃ - ERP alone (153.3 kg ha⁻¹), T₄ - AMF inoculants only (4 t ha⁻¹), T₅- TSP (100 kg ha⁻¹) + AMF , T₆- ERP (153.3 kg ha⁻¹) + AMF (4 t ha⁻¹), T₇ - compost only (8 t ha⁻¹), T₈ - TSP (100 kg ha⁻¹) + compost (8 t ha⁻¹), T₉ - ERP (153.3 kg ha⁻¹) + compost (8 t ha⁻¹), T₁₀ - dolomite (2 t ha⁻¹) only, T₁₁- TSP (100 kg ha⁻¹) + dolomite (2 t ha⁻¹), T₁₂- ERP (153.3 kg ha⁻¹) + dolomite (2 t ha⁻¹), T₁₃ - biochar (4 t ha⁻¹) only, T₁₄ - TSP (100 kg ha⁻¹) + biochar (4 t ha⁻¹), T₁₅ - ERP (153.3 kg ha⁻¹) + biochar (4 t ha⁻¹).

Crop Establishment and Maintenance

Maize (variety MIMZ HY01), was established by direct after disc-plouhging and harrowing seeding the experimental field. Plots were prepared with shallow drains around them. The crop was planted at an inter-row spacing of 60 cm and intra-row spacing of 30 cm. Two seeds per hill were sown at the beginning and thinned out to have a plant density of 5.55m⁻². An equal amount of (3 kg) common organic nutrition supplement mixture (1:1, powdered Gliricidia sp. leaves: cow dung) was incorporated to each plot and mixed with soil by racking before the establishment of the crop. Compost was prepared using house yard manures while biochar was prepared using partial hydrolysis of wood chips. AMF inoculants were prepared using trap culture method using maize plant as the host and initial colonization percentage was verified following McGonigle method (McGonigle et al., 1990). Then compost, dolomite, AMF inoculants (with 75% initial colonization percentage) and biochar were added to plots according to the treatment plan. Pests and diseases were controlled by periodic spraving of liquid prepared with using neem (Azadiractha indica) plant parts and by cultural practices. Plots were kept weed-free and all other cultivation practices were followed as recommended by the Department of Agriculture, Sri Lanka.

Measurements and Data Analysis

Growth and yield parameters

Plant height, number of leaves per plant, total leaf area was measured at flowering and harvesting stages of maize plants taking randomly-selected plants per plot. A destructive sampling of randomly selected plants was used to measure the dry weight of roots, leaves and seeds at harvesting stage. Total grain yield with moisture percentage was measured in the net plot area and the moisture was adjusted to 14%.

Cadmium analysis of soil, roots and seeds of maize

The maize was harvested and seeds were separated from the cob at 105 days after establishment. The remaining plants were uprooted and separated into roots, shoot and leaves. Roots were washed with tap water to remove all the soil particles. Thereafter, seed, leaf and root samples were oven dried at 65° C. Soil samples collected from each plot separately was air dried for 2 days and sieved through the 2 mm mesh.

The digestion of sample was carried out using, 0.5 g of ground plant sample, 5 ml of acid mixture (HNO₃:HClO₄, 3:2) and acid digestion was carried out on block digester at 200 °C about 2 hours. Quantification of Cd was done using Atomic Absorption Spectrophotometer (GBC 902/903 AAS-Australia). The Cd in soil was also quantified following this procedure and 1 g of sieved soil was used.

Phosphorus analysis of soil, roots and seeds of maize

The Olsen method (Watanabe and Olsen, 1965) was followed to determine soil P content of the sample and ammonium vana-molybdate was used as coloring reagent. Quantification of P was done using Spectrophotometer (Model No. UV/1800 APC) at 882 nm wave length. Phosphorus content of plant samples were measured using dry-ashing method. Then samples were read for P using Spectrophotometer at 410 nm wave length.

Determination of final AMF colonization percentage in roots and pH of soil

Determination of AMF colonization percentage was done following the modified grid transects method (McGonigle et al., 1990) and soil pH was also estimated before and at the end of trial.

Statistical Analysis

Data were analyzed using Analysis of Variance in MINITAB version 16.2.1. Tukey's Method at P<0.05 was used to test the differences among treatment means. All statistical analysis was performed using MINITAB version 16.2.1.

Results

Initial Cd level of added TSP was 1.00 ppm and Cd level of ERP was below detection level. The statistical analysis of present study revealed that, the soil, maize root and seed Cd concentrations were significantly high (P<0.05) in TSP added treatments compared with the ERP added treatments. However, the soil Cd concentration of the other treatments with added AMF and the amendments with TSP was significantly lower than the treatment of only added TSP (Table 1). However, even with the addition of AMF, compost, biochar and dolomite has shown significantly high (P<0.05) Cd concentrations in soil, maize roots and seeds of TSP added treatments compared to the ERP added treatments of respective amendments. Further it was shown that AMF with TSP treatment was shown significantly higher Cd level in soil, maize roots and seeds (P<0.05) and lower level of Cd was recorded in ERP with AMF (Table 1). This can be explained by considering the estimated percentage of colonization of AMF in plant roots. Although initial AMF colonization of maize root samples taken from the trap culture was approximately 75%, there was no colonization observed in the maize roots harvested from soil of TSP added treatments. As well as, AMF colonization of maize roots in soil ERP added treatments was ranged between 25-60%. This can be explained by the adverse impact of synthetic TSP on soil AMF propagules which lower the AMF colonization of roots. Further the study was shown that ERP with AMF has significantly contributed to lower the seed Cd level retaining higher Cd amounts in roots. Further, the study has also revealed that the compost and biochar with both TSP and ERP have obtained significantly lower mean (P<0.05) of Cd levels in soil, roots and seeds, independent from the type of tested P fertilizers.

Table 1. Mean Cd concentrations of soil, roots and seeds at the harvesting stage of maize (ppm). BDL - Below detection level

Treatments	Cd concentration in soil	ncentration in soil Cd concentration in roots		
Treatments	(ppm) (ppm)		(ppm)	
no any P addition	BDL	BDL	BDL	
TSP alone	0.12	0.09	0.05	
ERP alone	0.08	0.05	0.03	
AMF inoculants only	BDL	BDL	BDL	
TSP+ AMF	0.09	0.06	0.06	
ERP+ AMF	0.03	0.05	0.01	
compost only	BDL	BDL	BDL	
TSP +compost	0.05	0.02	0.02	
ERP +compost	0.01	0.01	BDL	
Dolomite only	BDL	BDL	BDL	
TSP +dolomite	0.08	0.05	0.02	
ERP +dolomite	0.07	0.02	0.02	
Biochar only	BDL	BDL	BDL	
TSP +biochar	0.04	0.02	0.02	
ERP +biochar	0.02	0.01	BDL	

Table 2. Mean P conc	entrations of soil, i	roots and seeds	at the harvesting	g stage of maize	(ppm)
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Treatmonts	Concentration in soil	Concentration in roots	Concentration in seeds	
Treatments	(ppm)	(ppm)	(ppm)	
No any P addition	12.20	83.83	160.21	
TSP alone	21.59	189.52	239.04	
ERP alone	17.94	139.56	237.90	
AMF inoculants only	23.09	94.33	236.95	
TSP+ AMF	21.95	125.36	238.76	
ERP+ AMF	18.03	136.14	233.09	
compost only	23.16	171.83	225.61	
TSP +compost	37.46	161.16	237.62	
ERP +compost	23.88	198.86	239.14	
Dolomite only	20.59	165.68	171.17	
TSP +dolomite	26.81	120.17	236.33	
ERP +dolomite	22.81	219.74	182.61	
Biochar only	20.30	208.54	235.04	
TSP +biochar	32.38	179.56	238.09	
ERP +biochar	20.52	190.85	238.31	

Table 3. Growth and yield parameters of maize plants. Means that do not share a same letter are significantly different (P<0.05).

Traatmant No	Growth parameters of maize plants for different treatment (Mean)			Dry biomass of plant parts (Mean)		
Treatment No.	Height of plant	No of leaves	Leaf area	Seeds	Roots	Leaves
	(cm)		(cm^2)	(g plant ⁻¹)	(g)	(g)
T_1	31.2 ^a	10 ^a	226.7ª	94.5ª	4.2 ^a	41.8 ^a
T_2	59.3 ^b	11 ^a	518.9 ^b	97.2ª	6.0^{a}	73.2 ^b
T ₃	61.3 ^b	11 ^a	520.4 ^b	91.6 ^a	5.3ª	60.9 ^b
T_4	54.2 ^b	11 ^a	442.3 ^b	90.4 ^a	5.4 ^a	68.8 ^b
T ₅	53.0 ^b	11 ^a	490.6 ^b	88.0 ^a	5.7 ^a	73.6 ^b
T ₆	63.9 ^b	11 ^a	518.3 ^b	95.2ª	4.5 ^a	68.8 ^b
T ₇	61.4 ^b	10 ^a	473.9 ^b	94.4 ^a	6.6^{a}	80.9 ^b
T ₈	72.8 ^b	11 ^a	555.8 ^b	96.8ª	4.8^{a}	78.2 ^b
T9	71.0 ^b	11 ^a	504.3 ^b	94.0 ^a	6.0^{a}	68.9 ^b
T ₁₀	60.2 ^b	11 ^a	471.2 ^b	96.4ª	5.3ª	72.7 ^b
T ₁₁	61.7 ^b	11 ^a	537.8 ^b	94.0 ^a	5.4 ^a	77.1 ^b
T ₁₂	67.6 ^b	10 ^a	522.1 ^b	87.6 ^a	8.2ª	76.5 ^b
T ₁₃	62.5 ^b	10 ^a	400.9 ^b	91.2ª	3.1ª	60.5 ^b
T ₁₄	65.9 ^b	10 ^a	512.9 ^b	95.2ª	5.7ª	68.5 ^b
T ₁₅	78.6 ^b	12 ^a	575.4 ^b	98.4ª	8.1 ^a	71.5 ^b

 $\begin{bmatrix} T_1 - no \text{ any } P \text{ addition}, T_2 - TSP \text{ alone}, T_3 - ERP \text{ alone}, T_4 - AMF \text{ inoculants only}, T_5 - TSP + AMF \text{ inoculants}, T_6 - ERP + AMF \text{ inoculants}, T_7 - compost only, T_8 - TSP + compost, T_9 - ERP + compost, T_{10} - Dolomite only, T_{11} - TSP + dolomite, T_{12} - ERP + dolomite, T_{13} - Biochar only, T_{14} - TSP + biochar, T_{15} - ERP + biochar]$

The dolomite added with fertilizers was not recorded considerable performance to manipulate Cd level when compared with other amendments.

When consider the P analysis, it was revealed that there was no significant difference (P>0.05) between TSP added treatments and ERP added treatment for soil P concentration. However, mean soil P content was higher in compost added plots with TSP and ERP fertilizers. Also mean higher soil content next to compost amendment was shown in biochar added treatment with both ERP and TSP fertilizers. It was observed in the present study also, there were no considerable effect for soil P was recorded TSP and ERP with AMF.

Considering growth and yield parameters it was revealed that there was a significant difference (P<0.05) between control (without addition of any P fertilizers) and the other treatments (Table 3) and there was no significant difference (P>0.05) between any other treatments. Considering height, leaf area and dry mass of roots, biochar added treatment with ERP has acquired higher means than the others. Soil pH values of all control and experimental plots were reached 7.2 - 7.9 range and initial pH was 6.7 in the field. At the harvesting period there was no significant difference (P>0.05) between any treatments. But compost added treatments have shown higher pH of 7.9. Considering biochar added treatments with fertilizers in to soil, the pH has reached 7.8.

Discussion

Soil application of triple super phosphate increases the available soil Cd than the application of Eppawala rock phosphate (Wijewardhana, 1998). Further the TSP contributes to increase of accumulated Cd in maize root and seeds compared with ERP. Therefore, an application of sparingly soluble ERP as soil P fertilizer reduces available soil Cd and it is important in having a healthy soil. Considering Cd accumulation of roots and edible seeds, application of ERP increases safe consumption of maize. ERP with AMF significantly contribute to lower the seed Cd level retaining higher Cd amounts in roots because AMF can minimize the translocation of heavy metal (HM) from roots to shoot by compartmentalization HM in roots (Jiang et al., 2016). Biochar and compost are effective soil amendments for maize cultivation with the ameliorating effect of soil Cd absorption. When consider the compost and biochar, both these amendments have high binding capacity for cationic and organic contaminants which might lead to immobilization of heavy metals (Planquart et al., 1999; Murray et al., 2011). Also compost addition play another important role by enhancing soil organic matter content and their further decomposition released organic acids and CO₂ gas leading to reduce pH while raising P availability and decrease P fixation by soil (Kulasinghe et al., 2013). Compost might possess specific ability to adsorb the organic anions and the corresponding release of hydroxyl ions (Hue, 1992), may be the reason of slight increase of pH. Because of such ameliorating properties of compost can be effectively used in maize cultivation in slightly acidic soil. As well as, biochar addition increase soil P because, application of biochar to soil can dissolve phosphates bounds to free cations such as Ca²⁺, Mg²⁺, Fe³⁺ and Al³⁺ leading to release P for plant uptake (Zhang et al., 2016).In the case of pH manipulation by biochar, it starts to release cations in to the soil solution and these cations displace exchangeable acidity and contribute to neutralize soil pH and furthermore, the biochar with higher ash alkalinity has a stronger ability to adjust soil acidity (Gaskin et al., 2008; Keith et al., 2011).

Although many scientific researchers have proved that colonization of AMF contribute to raise P bioavailability leading to increase the growth and yield of maize plants (Higo et al., 2018), some other studies were shown that available soil P content has inversely affect the colonization level of AMF (Xu et al., 2014). According to Xu et al., (2014), host plants tend to increase their restriction to AMF colonization when plant has abundant soluble P for their usage. Therefore, soil application of TSP adversely affects soil AMF and also the potential of root colonization. Further, addition of ERP to soil reduces the potential of root AMF colonization, because of the available P in soil. AMF colonized maize roots are compartmentalized the Cd in roots of maize and hence reduce the accumulation of seed cadmium. Dolomite/lime need significantly long time period to manipulate soil pH to effect ion exchange process (Watt, 1991). Considering soil P availability and Cd accumulation in edible seeds of maize, TSP can be effectively substitute by ERP for maize crop P requirement and sustainable maize crop cultivation. Further, ERP addition with AMF, compost and biochar further enhance the effect.

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