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EFFECT OF BIOORGANIC ADDITIVES ON CALCIUM SOLUBILITY FROM ROCK PHOSPHATE UNDER SANDY AND CLAYEY SOILS CONDITIONS

I.A. Hammouda[(1)], M.A. El-Howeity[(2)] and Amira E. El-Meselawe[(2)]
[(1)] Soils, Water and Environment Res. Inst.(SWERI). Agric. Res. Center (ARC).
[(2)] Envir. Stud. Res. Inst. (ESRI), University of Sadat City.

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ABSTRACT: *The present work was planned to study the influence of bio – organic additives (i.e. compost (CM) and farmyard manure (FYM), as organic fertilizers and Bacillus megaterium (Bm) and Bacillus polymyxa (Bp), as biofertilizers) on calcium solubilization from rock phosphate in sandy and alluvial clayey soils. Pot experiment was conducted in a greenhouse of the Environmental Studies and Research Institute (ESRI), University of Sadat, Menoufia Governorate, Egypt during summer season of 2014.*

The experiment was laid out in a complete randomized design (CRD) arrangement with three replications. Each soil bulk was divided into portions, each was assigned to one treatment, and each treatment replicated 3 times. Rock phosphate, as insoluble calcium source was added at a rate of 4 gm kg^{-1}. Compost or FYM was added at a rate of 2% organic carbon (OC) to each soil. Each pot was contained 500 g of the soil mixed with rock phosphate and organic fertilizer. Bm or Bp was added to each potted soil at a level of 2 ml of culture suspension. Different parameters were determined along the periods of incubation (120 days).

Results showed that, all added amendments increased calcium solubilization in both soils with time of incubation. Soil pH values were decreased with incubation time up to 60 days. EC dSm^{-1} and soil CO_2 release rate significantly increased in both soil as an indicator of increasing the bio activity and calcium solubilization in the soil solution by increasing the incubation time. The highest increasing rate of soil EC and CO_2 release were obtained with added bio-organic fertilizer mixture than each one alone. Soluble calcium in the soil solution raised with the individual additions of organic or biological with prolonging the incubation time, according to the descending order CM > FYM, Bm > Bp. The order for the effect of the common treatments was followed: CM + Bm > CM + Bp, FYM + Bm > FYM + Bp. Overall total comparison, the descending order was followed: CM + Bm > CM + Bp > FYM + Bm > FYM + Bp > CM > FYM > Bm > BP. The single application of each bio-and organic fertilizers showed the following increases mean values of calcium solubilization compared to the control (W.B.O.): CM (88.89 & 98.26%), FYM (57.78 & 68.70%), Bm (20.00 & 26.09%) and Bp (4.44 & 9.57%), for the sandy and alluvial clayey soils, respectively. Incorporation of CM or FYM plus each of the biofertilizers greatly augmented calcium solubilization, as compared with the single additive of each of the bio-organics. The highest increases rates induced by using any of CM or FYM each with Bm, reached 166.67 & 128.89% and 197.39 & 147.83% above their control (W.B.O.) treatment in the sandy and alluvial clayey soils, respectively.

Key words : *Rock phosphate, Calcium solubilization, Soil properties, Compost, Farmyard manure, Bacillus megaterium, Bacillus polymyxa.*

INTRODUCTION

Calcium (Ca) is one of the major nutrients, its an essential nutrient for growth and productivity of plants. It plays an important role in plants in many physiological activities. So, calcium is a secondary nutrient that is critical to crop development. It is needed in large amounts by all plants for the formation of cell walls and cell membranes, and it plays a vital role in soil structure. Due to the immobility of calcium in the soil and plant tissues, a continuous supply must be present for plants to access (Marcherer, 1995). Calcium can also be added to soil fertility programs and applied in irrigation water to ensure adequate levels are spoon fed to the plant. Monitoring soil calcium levels and sampling plant tissue can help you make good management decisions for proper calcium fertilization (Marcherer, 1998).

The direct application of rock phosphate is generally successfully used where (i) local sources represent an economically viable option, a situation often found in developing countries, ; (ii) properties of soil-cropping systems offer conditions favorable for dissolution of phosphate rock (Nishanth and Biswas, 2008). Commercial rock phosphate occurs in nature as deposits of apatites (P bearing minerals) along with other accessory minerals such as quartz, silicates, carbonates, sulphates, sesquioxides etc. Four types of rock phosphate minerals are: Carbonate apatite [$3Ca_3(PO_4)_2.CaCO_3$], Fluoro apatite [$3Ca_3 (PO_4)_2.CaF_2$], Hydroxy apatite [$3Ca_3(PO_4)_2.Ca(OH)_2$], Sulpho apatite [$3Ca_3 (PO_4)_2.CaSO_4$]. The apatites of igneous and metamorphic origin are generally regarded as less reactive because of their well developed crystalline form. However, the apatites of sedimentary rock deposits are soft minerals possessing micro-crystalline structure and are of major commercial importance for direct application in the soil (Kiran and Phogat, 2008).

Himani and Sudhakara, (2011) found that to enhance the fertilizer value of rock phosphate (RP) in alkaline soils, the phosphate (P) solubilizing fungus *Penicillium oxalicum* was isolated from the rhizosphere soil of rock phosphate mine landfills and tested for its efficacy to solubilize rock phosphate, as well as promotion of the growth of wheat and maize plants grown in soil amended with RP. The results showed that P*. oxalicum* effectively solubilized RP in Pikovskaya's medium and released higher amount of phosphorus. The solubilization was increased with increase in concentration of RP. Field experiments showed that inoculation of *P. oxalicum* significantly increased the growth and yield of two consecutive crops i.e. wheat and maize compared to the control soil. The P content was significantly increased in the plants. The available P and organic carbon levels increased in RP amended soil inoculated with *P. oxalicum* compared to the control soil. It was found that the *P. oxalicum* along with RP can substitute the chemical fertilizer in alkaline soil and help in improving the crop production.

Several mechanisms have been proposed to explain the microbial solubilization of P compounds. The mechanisms consist of: (i) release of inorganic and organic acids produced during organic residue decomposition; (ii) excretion of protons due to NH/ assimilation by microorganisms; (iii) formation of complexes between organic acids/anions with cations (Al^{3+}, Fe^{3+}, Ca^{2+}) (Whitelaw, 2000 and Hameeda *et al.,* 2006)

Increase in soil P availability may be caused by several reactions involving microorganisms that produce organic acids and humic substances (Stevenson, 1986). Presumably, these substances can replace or compete with Pi ions for sorption sites. In addition, Lopez-Bucio et al. (2007) reported that *Bacillus megaterium* (a known PSM) promoted plant growth and stimulated root branching of *Arabidopsis thaliana.*

That may be ascribed to release Ca from the added fertilizers into the tested soil, also to the release of CO2 as a result of added organic materials decomposition, forming carbonic acid. This acid, in turn, might form a soluble calcium carbonate salt in soil (Barber, 1995 and Brady and Weil, 2008)

This investigation was carried out to study the effect of individual and combined applications of organic and bio fertilizers on soil pH, EC, calcium solubilization from rock phosphate and soil respiration under sandy and clayey soils conditions.

MATERIALS AND METHODS

Soils sampling

Soil samples were collected from the surface layer (0 - 30 cm) of two locations in Menoufia Governorate of Egypt:

1. Sandy soil, from the Experimental Farm of the Environmental Studies and Research Institute, Sadat University, Sadat City.
2. Alluvial clayey soil, from the Experimental Farm of Faculty of Agriculture, Menoufia University, Shibin El - Kom.

Soil samples were air - dried, separately grounded and sieved through a 2-mm sieve and well mixed. Routine analyses for some physical and chemical characteristics of the soils were performed, following the standard methods stated by Cottenie *et al.* (1982) and Klute (1986), and the data are presented in Table (1a and b).

Rock phosphate

Rock phosphate (Fluorapatite, $Ca_{10}F_2(PO_4)_6$, was used as a source of insoluble calcium and phosphate mineral fertilizer, having a pH value 7.9 (1 : 2.5 , water suspension), total calcium was 42.11 % and total phosphorus was10.5%.

Table (1a): Chemical properties of the tested soils (sandy and alluvial clayey soil).

Soils	pH Sus.1:2.5	TSS		Soluble ions meq / 100 g soil							
		EC dSm^{-1}	%	Cations				anions			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
Sandy	7.81	0.28	0.09	0.11	0.05	0.13	0.01	-	0.10	0.03	0.15
Clayey	7.58	0.62	0.20	0.28	0.36	2.45	0.15	-	0.58	0.48	2.09

Soils	CEC meq./100g Soil	Total $CaCO_3$%	organic matter %	Soil content of macronutrients							
				Total concentration (%)				Soluble concentration mg kg-1			
				N	P	K	Ca	N	P	K	Ca
Sandy	6.90	2.37	0.60	0.05	0.03	0.15	0.95	13.90	8.53	60.10	22.00
Clayey	29.8	2.10	1.42	0.15	0.10	0.31	0.84	58.11	11.04	270.00	56.00

Table (1b): Physical properties of the tested soils (sandy and alluvial clayey soil).

Soils	Soil moisture %		% Particle size distribution			Textural Class
	Water holding capacity	Field capacity	Sand	Silt	Clay	
Sandy	30	18	88.50	8.90	2.60	Sandy
Clayey	60	36	25.00	28.20	46.80	Clayey

Organic fertilizers

Compost (CM) and farmyard manure (FYM) were chosen as a sources of organic fertilizers. Chemical analysis of organic fertilizers were carried out according to the methods described by Cottenie *et al.* (1982), and presented in Table (2).

Bio-fertilizers

The microorganisms, Bacillus megaterium (Bm) and Bacillus polymyxa (Bp) were employed herein as phosphate solubilizing bacteria (PSB). Such agents were obtained from the Biofertilizers Production Unit of the Agric. Microbiol. Dept., Soils, Water and Environ. Res. Inst.(SWERI) Agric. Res. Center (ARC), Giza, Egypt.

Preparation of the bio-fertilizers

The strains of either Bacillus megaterium or Bacillus polymyxa were pre-cultured on nutrient agar media, then each was grown in a nutrient broth liquid medium for 2 days at 30°C. The suspended cultures were then centrifugated at 1000 rpm for 30 min., at 10°C. The sediment was re-suspended in 5 ml sterilized 0.8 % KCl solution (w/v). The bacterial suspension was again shaken for 5 min. (Collins and Lyne 1980). These suspensions were introduced as biofertilizer inoculants.

The experiment was conducted at the greenhouse of the Environmental Studies and Research Institute (ESRI), at the University of Sadat, Menoufia Governorate, Egypt during summer season of 2014 under greenhouse conditions. Rock phosphate was applied at a rate of 4 g Ca/kg soil (4.75 g from rock phosphate/pot), CM or FYM, as sources of organic fertilizers were applied at a rate of 2% organic carbon basis (57.9 g or 76.9 g/pot from CM or FYM respectively). Plastic pots were used, each contained 500 g of the treated soil. The potted soils then received each biofertilizer agent, at a rate of 2 ml/pot (containing 10^3-10^4 cell ml^{-1}per ml). Each experimental treatment was replicated three times. Moisture content of the potted soils was kept at the field capacity of each soil. Pertinent chemical and biological associating attributes of the treated soils had been determined at intervals along 120 day-incubation period at the ambient temperature. These determinations were carried out at 0, 15, 30, 45, 60, 75, 90, 105, and 120 days of incubations according to Cottenie *et al.* (1982).

Experimental treatments

So, this study included the following ten treatments :

1. Control (W.B.O): Soil + rock phosphate (RP) without bio-organics.
2. Soil + Rock phosphate (RP) + Compost (CM) .
3. Soil + Rock phosphate (RP) + Farmyard Manure (FYM).
4. Soil + Rock phosphate (RP) + Bacillus megaterium (Bm).
5. Soil + Rock phosphate (RP) + Bacillus polymyxa (Bp).
6. Soil + RP + CM + Bm.
7. Soil + RP + CM + Bp.
8. Soil + RP + FYM + Bm.
9. Soil + RP + FYM + BP.

Table (2): Chemical properties of the organic fertilizers used .

Organic fertilizers	pH *	E.C** dSm^{-1}	Organic C %	Total N %	C:N ratio	Total P %	Total Ca %
Compost (CM)	7.62	2.46	17.28	1.21	14.3	0.52	0.61
Farmyard manure (FYM)	8.41	5.10	13.01	0.72	18.1	0.32	1.12

* pH :In the 1 : 10 water suspension. **E.C, dSm^{-1} :In the 1 : 10 water extract.

Microbial activity in soil

To assess the microbial activity in soil, the daily evolution rate of CO_2 had been measured every 15-day intervals up to 120 day- experimental duration, according to the procedure of Paul and Clark (1996). 20 g of each potted soil, at each interval, was placed in a pored polyethylene bag. The filled bag was hung to a hook connected with the lower surface of a rubber stopper, which was fitted precisely to the neck of a 500-ml conical flask. Exactly 25 ml of 0.5 N NaOH solution were pipetted into each flask, to absorb the released CO_2. The flasks were immediately closed with the stoppers hanging the soil bags, to provide an air-tight condition. The flasks were incubated at 25° C for 24 hours. The sodium hydroxide solution was then back titrated with 0.5 HCl in presence of 5 ml $BaCl_2$ (40%) and phenolphthalein indicator. NaOH flasks without soil bag were used as reference controls.

Concentration of CO_2 (mg / g soil / time 24 h) was estimated according to the following formula:

$$CO_2\ (\text{mg/sw/t}) = \frac{[(v_0 - v) - (v_0 - v_c)] \times 1.1}{dsw}$$

Where: (sw) is the amount of soil used in grams, (t) is the incubation time in hours, (v_o) is the NaOH aliquot volume in milliliters, (v) is the HCl milliliters used for titration, (v_c) is the HCl volume used for titration of the control NaOH , (dsw) is the dry weight of 1.0 g soil, and (1.1) is the conversion factor (1 ml 0.5 N NaOH consumed equals 1.1 mg CO_2).

Calculations

Raw results (analytical data) of the various treatments were further calculated on the dry weight basis of the used substrates.

Rates of the relative changes of results (as percent RC %) ware calculated for the mean value listed for each treatment for all intervals, referring to the mean results of the control (W.B.O.) (Soil with RP and no bio-organics):

$$RC\,\% = \frac{\text{Mean result of the particular treatment} - \text{Mean result of the control "W.B.O"}}{\text{Mean result of the control}} \times 100$$

Statistical analysis

The experiment was laid out in a complete randomized design (CRD) arrangement with three replications. The data obtained were statistically analyzed (LSD, at 0.05&0.01), according to the method outlined by Sendecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of the bio-organic additives on soil reaction (pH)

Changes in the soil reaction (pH) of sandy and alluvial clayey soils as a resolute applications of the bio-organic amendments, i.e. compost (CM) and farmyard manure (FYM), *Bacillus megaterium* (Bm) and *Bacillus polymyxa* (Bp), within 120 days of experimental duration for both tested soils, are shown in Table (3). All soil treatments decreased the soil pH, especially at the first intervals up to 60 days of incubation time. Incorporation of CM or FYM diminished the pH values at first then modestly moved up towards the end of experimental period, The CM induced a lower values than the FYM. Introduction of the bio-fertilizers, *Bm* and *Bp,* revealed the same trend, but at somewhat higher values, whereas *Bm* was mostly more effective on lowering the pH value than *Bp*. Combinations of the organic and bio-fertilizers showed inconsistent changes of the pH values. However, the dual applications of CM + *Bm* (or *Bp*) were generally more active in reducing the pH value than that of FYM correspondents. This was the case in both soils tested. Generally, changes of the pH values of both soils detected throughout the experimental period were inconsiderable.

Table (3): Effect of the bio-organic additives on soil reaction (pH) in the rock phosphate treated sandy and alluvial clayey soils.

Treatments *			Incubation times (Days)									Mean
			0	15	30	45	60	75	90	105	120	
Soil pH value in treated sandy soil												
4 g Ca/kg soil as RP	W.B.O		7.83	7.87	7.95	8.05	8.10	8.14	8.34	8.36	8.39	8.11
	CM		7.77	7.75	7.63	7.51	7.56	7.61	7.81	7.78	7.80	7.69
	FYM		7.79	7.75	7.70	7.62	7.60	7.68	7.80	7.77	7.82	7.73
	Bm		7.80	7.80	7.78	7.68	7.72	7.18	7.90	7.98	8.12	7.77
	Bp		7.80	7.78	7.76	7.71	7.80	7.92	8.01	8.14	8.28	7.91
	CM	*Bm*	7.70	7.62	7.43	7.21	7.28	7.35	7.35	7.48	7.60	7.45
		Bp	7.72	7.70	7.61	7.50	7.41	7.44	7.60	7.63	7.60	7.58
	FYM	*Bm*	7.78	7.66	7.52	7.50	7.62	7.61	7.80	8.12	8.01	7.74
		Bp	7.83	7.76	7.60	7.52	7.40	7.53	7.92	8.10	8.22	7.76
General Mean			7.78	7.75	7.69	7.62	7.65	7.65	7.86	7.94	7.99	7.77
Soil pH value in treated alluvial clayey soil												
4 g Ca/kg soil as RP	W.B.O		7.60	7.64	7.72	7.82	7.86	7.90	8.10	8.12	8.15	7.88
	CM		7.54	7.52	7.41	7.22	7.20	7.33	7.60	7.71	7.82	7.48
	FYM		7.56	7.60	7.48	7.40	7.31	7.30	7.55	7.82	8.02	7.56
	Bm		7.57	7.63	7.60	7.55	7.53	7.60	7.66	7.82	8.10	7.67
	Bp		7.78	7.63	7.61	7.58	7.76	7.81	7.93	8.15	8.15	7.82
	CM	*Bm*	7.52	7.41	7.32	7.30	7.21	7.20	7.15	7.33	7.68	7.35
		Bp	7.55	7.42	7.38	7.32	7.30	7.60	7.68	7.66	7.72	7.51
	FYM	*Bm*	7.56	7.48	7.40	7.43	7.50	7.66	7.95	7.98	8.10	7.67
		Bp	7.56	7.51	7.48	7.45	7.48	7.56	7.66	8.00	8.11	7.65
General Mean			7.58	7.55	7.51	7.48	7.49	7.57	7.71	7.84	7.97	7.63

* Control (Soil pH at without any additions), RP (Rock Phosphate Fluorapatite), W.B.O. (without bio-organic addition), CM (compost), FYM (farmyard manure), Bm (Bacillus megaterium), Bp (Bacillus polymyx).

The initial pH values of the experimental substrates, soils and organic fertilizers (Tables 1 and 2) contributed to the alterations of pH values recorded within the duration of study. Moreover, amount added and composition of the used organics, i.e. compost and farmyard manure determined their rate of decomposition (particularly the C/N ratios) and contents of salts, which ware reflected on the release of carbonic and organic acids at the first intervals of incubation time, then liberation of mineral elements latter on.

This explains the decrease of pH value at first, and the recovery occurring later on, as the rate of breakdown had declined by elapse of time. It was also reported that phosphate dissolving bacteria produce a number of organic acids that could participate in decreasing the pH value in soil (Khan *et al.,* 2007). In this regard, it is assumed that extent of phosphate solubilization extent and pH change in soil, as influenced by the bio-organic treatments, are governed by the net difference between the rates of organic acids formation and their decay in soil medium.

Effect of bio-organic additives on soil EC (dS m^{-1})

Soil EC values (dS m^{-1}), as effected by applications of using bio-organic additives (CM, FYM, Bm and Bp) during 120 day-incubation period of both treated soils, are presented in Table (4). The gained data revealed that, all the employed amendments resulted in progressive mostly significant increases of soil EC at all soils with incubation time. Incorporation of CM or FYM raised the levels of EC in the sandy soil by RC values of 20.00 and 32.50% compared to the control, respectively. On the other hand, introduction of the biofertilizer *Bm* slightly increased the EC concentration (RC, 7.50%), but the *Bp* showed mostly no alterations. The highest values of EC were obtained by the dual addition of both organic and bio-fertilizers. Combination of CM + *Bm* gave higher values than the double treatment of CM + *Bp,* as they gave RC values 45.00 and 40.00%, in order. Likewise, combination of FYM + each of *Bm* or *Bp* raised the contents of EC, but at somewhat lower values than those of the CM. Extents of increases, referring to the control treatment were RC 37.50 and 40.00%, for FYM + *Bm* and FYM + *Bp*, respectively. The mean RC value of the bio-organic additions collectively was 20.00% (Table 4).

Values of EC (dS m^{-1}) in the alluvial clayey soil exhibited a similar trend to that of the sandy soil, when increased with the time of incubation (Table 4). Effect of CM and FYM, as organic fertilizers, and Bm and Bp, as bio-fertilizers, augmented the level of EC in soil solution. Such increases reached RC values of 31.18 and 40.86 and 10.75 and 4.30 %, respectively. Incorporation of the combined additives of the bio-organic fertilizers, significantly raised the soil EC (dSm^{-1}) in the alluvial clayey soil (Table, 4), as they gave RC values of 59.14, 51.61, 79.57, and 65.59%, referring to the control treatment, for CM + Bm, CM + Bp, FYM + Bm and FYM + Bp, respectively. The mean RC value for the bio-organic additions recorded 32.26% collectively.

Composition of each of the submitted organic fertilizers determined their rate of mineralization in the tested soils. For instance, the narrower C:N ratio of compost (CM) hastened the degradation rate of its organic compounds, as compared with the farmyard manure (FYM) Table (2). Presence of cattle dung and urine, as well as a soil portion (clay and silt) (as a bedding material) within the makeup of farmyard manure, contributed to some extent to the relatively higher contents of salts in the experimental components. Data appearing in Table (2) confirm this interpretation. Breakdown of the organic compounds composing the added organic fertilizers eventually results in accumulation of mineral elements and thus the contents of EC are elevated in the soil medium (Barber, 1995 and Brady and Weil , 2008).

Participation of the bio-fertilizers in such concern was confined to their moderately activation of the decomposition of organic fertilizers added. Rates of EC relative changes (RC) principally depended on soil properties, whereas the clayey soil exhibited higher values of EC-RC than the sandy soils (Tables 1 and 4).

Table (4): Effect of the bio-organic additives on soil EC (dS m^{-1}) in the rock phosphate treated sandy and alluvial clayey soils.

Treatments *			Incubation times (Days)									Mean	RC ** (%)
			0	15	30	45	60	75	90	105	120		
Soil EC (dSm-1) in treated sandy soil													
4 g Ca/kg soil as RP	W.B.O		0.30	0.31	0.33	0.35	0.36	0.40	0.46	0.51	0.55	0.40	0
	CM		0.34	0.35	0.38	0.38	0.46	0.48	0.61	0.65	0.70	0.48	20.00
	FYM		0.34	0.38	0.40	0.41	0.56	0.54	0.65	0.73	0.77	0.53	32.50
	Bm		0.31	0.33	0.35	0.35	0.39	0.44	0.53	0.56	0.59	0.43	7.50
	Bp		0.31	0.32	0.33	0.35	0.37	0.41	0.48	0.52	0.56	0.41	2.50
	CM	Bm	0.37	0.38	0.49	0.48	0.51	0.60	0.73	0.81	0.89	0.58	45.00
		Bp	0.38	0.38	0.46	0.45	0.50	0.57	0.70	0.76	0.83	0.56	40.00
	FYM	Bm	0.44	0.44	0.52	0.54	0.54	0.66	0.42	0.43	1.00	0.55	37.50
		Bp	0.42	0.41	0.50	0.42	0.57	0.62	0.79	0.89	0.43	0.56	40.00
General Mean			0.35	0.36	0.41	0.40	0.46	0.51	0.58	0.63	0.68	0.49	20.00
L.S.D, at 0.05 (0.07) and L.S.D, at 0.01 (0.10).													
Soil EC (dSm-1) in treated alluvial clayey soil													
4 g Ca/kg soil as RP	W.B.O		0.71	0.72	0.76	0.80	0.84	0.95	1.08	1.20	1.33	0.93	0
	CM		0.82	0.85	0.91	0,92	1.05	1.18	1.52	1.65	1.77	1.22	31.18
	FYM		0.83	0.88	0.96	1.00	1.13	1.35	1.67	1.98	1.98	1.31	40.86
	Bm		0.72	0.78	0.82	0.84	0.90	1.08	1.31	1.36	1.48	1.03	10.75
	Bp		0.72	0.74	0.76	0.80	0.87	0.99	1.17	1.29	1.40	0.97	4.30
	CM	Bm	0.91	0.95	1.06	1.10	1.27	1.55	1.94	2.17	2.38	1.48	59.14
		Bp	0.91	0.92	1.05	1.05	1.21	1.48	1.83	2.01	2.26	1.41	51.61
	FYM	Bm	0.96	1.02	1.15	1.22	1.42	1.76	2.23	2.55	2.75	1.67	79.57
		Bp	0.92	0.94	1.08	1.13	1.31	1.63	2.08	2.27	2.49	1.54	65.59
General Mean			0.81	0.85	0.92	0.96	1.07	1.28	1.58	1.75	1.89	1.23	32.26
L.S.D, at 0.05 (0.9) and L.S.D, at 0.01 (0.13).													

* Treatments**,** W.B.O. [Soil EC at without bio-organic addition (control)], RP (Rock Phosphate Fluorapatite), CM (compost), FYM (farmyard manure), Bm (Bacillus megaterium), Bp (Bacillus polymyx).

** RC: Rate of relative changes of the results , referring to the control W.B.O.

Noteworthy that, accumulation of TSS as EC (dSm-1) in soil, as long as their presence is at a moderate level, is a beneficial process, since most of those salts are plant nutrients, deriving from organic matter mineralization.

Effect of bio-organic additives and rock phosphate treatments on soluble calcium content in soils.

The tabulated data (Table 5) demonstrated that, all of the applied treatments significantly increased the soluble calcium contents progressively in both soils by advancing the experimental time. Incorporation of CM or FYM raised the soluble calcium content in the sandy soil by RC value of 88.89 and 57.78 % compared to the control (W.B.O.), respectively. On the other hand, introduction of the bio-fertilizers *Bm* or *Bp* to the same soil improved calcium release from the rock phosphate, and from organic fertilizers and soil as well, to reach RC 20.00 and 4.44%, respectively. The uppermost RC rates of soluble calcium in the sandy soil were induced by the combined additions of the bio- and organic supplements, CM + *Bm* and followed by CM + *Bp* to be 166.67 and 142.22 % comparison with the control, respectively. Likewise, dual application FYM and any biofertilizer stimulated calcium solubility, but at rates lower than the corresponding ones of CM. The increases above the control, expressed by the RC rates were 128.89 and 111.11 % for FYM + *Bm* and FYM +*Bp*, respectively. The mean RC value of all treatments of the sandy soil was 68.89 % above their control (W.B.O.).

The alluvial clayey soil featured a trend similar to that occurred for the sandy soil regarding the influence of the experimental treatments on the contents of soluble Ca. Addition of CM or FYM, as organic matter sources, and Bm and Bp, as biofertilizers, achieved increases of the soluble calcium contents in the clayey soil reaching RC rates of 98.26, 68.70, 26.09 and 9.57 % comparison with the control (W.B.O.), for CM, FYM, Bm and Bp, respectively (Table 5). Incorporation of the combined additives of the bio-organic sources significantly augmented the contents of soluble calcium in the alluvial clayey soil, when gave RC rates of 197.39, 161.74, 147.83 and 130.43% comparison with the control (W.B.O.), for CM + *Bm*, CM + *Bp*, FYM + *Bm* and FYM + *Bp*, respectively. The mean RC rate of the total treatments of the alluvial clayey soil was 80.87%, referring to the their control (W.B.O.).

1. Short chain fatty acids given off via the recovery of organic materials may form soluble simple organic calcium salts.
2. The phosphate solubilizing bacteria are known to be efficient biological agents on producing a number of organic acids that participate in mobilization of calcium in soil medium.

Collectively, the fine soil texture, pH value around neutrality, adequate water content and soil enrichment with organic matter and bio-fertilizers have powerfull impacts on the solubility of calcium in soil.

Accordingly, the proper characteristics of the clayey soil, in the present study, provided a suitable medium for calcium solubility, than the sandy soil. More and above, the preferential makeup of compost (CM), i.e. narrower C\N ratio, near-neutral pH value and lower EC content are behind its superiority upon the farmyard manure (FYM). Efficiency of the species *Bacillus megaterium* (Bm) on producing organic acids excelled that of *Bacillus polymyxa* (Bp). Combinations of the bio-organic fertilizers showed better results of Ca solubility than the single additions of each. Compost combinations surpassed the farmyard counterparts (Sanyal and Datta, 1991 and Sagoe *et al.,* 1998).

Effect of bio-organic additives on microbial activity in soils (soil respiration)

Changes in soil respiration (carbon dioxide evolution), as a measure of the overall microbial activity in soil, under the impact of bio-organic additives are presented in Table (6), data exhibited that, all the applied amendments significantly accelerated soil respiration with time in the sandy soil.

Incorporation of CM or FYM increased the rate of CO_2 evolution to attain RC by values were about 51.82 and 33.97 % compared to the control (W.B.O.), respectively. On the other hand, introduction of the bio-fertilizers Bm or *Bp* improved soil respiration in the same sandy soil by RC about 15.93 and 6.53%, respectively.

Table (5): Effect of bio-organic additives and rock phosphate treatments in sandy and clayey soils on soluble calcium content (meq 100 g^{-1} soil).

Treatments *			Incubation times (Days)									Mean	RC ** (%)
			0	15	30	45	60	75	90	105	120		
Soluble Ca (meq 100 g^{-1} soil) in treated sandy soil													
4 g Ca/kg soil as RP	W.B.O		0.18	0.20	0.25	0.31	0.34	0.47	0.63	0.77	0.89	0.45	-
	CM		0.29	0.40	0.44	0.47	0.96	0.86	1.20	1.44	1.55	0.85	88.89
	FYM		0.30	0.32	0.39	0.40	0.65	0.68	1.08	1.20	1.34	0.71	57.78
	Bm		0.19	0.25	0.32	0.31	0.43	0.57	0.84	0.94	1.02	0.54	20.00
	Bp		0.19	0.22	0.24	0.30	0.35	0.48	0.67	0.81	0.94	0.47	4.44
	CM	Bm	0.56	0.46	0.83	0.66	0.86	1.21	1.99	2.02	2.23	1.20	166.67
		Bp	0.50	0.56	0.76	0.58	0.78	1.11	1.60	1.91	2.02	1.09	142.22
	FYM	Bm	0.36	0.41	0.75	0.90	0.74	1.03	1.45	1.69	1.91	1.03	128.89
		Bp	0.41	0.39	0.65	0.52	0.98	0.98	1.35	1.54	1.75	0.95	111.11
General Mean			0.31	0.33	0.48	0.46	0.63	0.77	1.13	1.29	1.42	0.76	68.89
L.S.D at 0.05 (0.05), and L.S.D, at 0.01 (0.08)													
Soluble Ca (meq 100 g-1 soil) in treated alluvial clayey soil													
4 g Ca/kg soil as RP	W.B.O		0.46	0.51	0.61	0.73	0.87	1.20	1.60	1.97	2.37	1.15	-
	CM		0.80	0.98	1.24	1.35	1.75	2.45	3.43	4.11	4.40	2.28	98.26
	FYM		0.83	0.89	1.08	1.11	1.52	1.93	2.97	3.37	3.76	1.94	68.70
	Bm		0.51	0.69	0.80	0.85	1.05	1.58	2.30	2.46	2.84	1.45	26.09
	Bp		0.48	0.55	0.62	0.74	0.96	1.33	1.86	2.23	2.57	1.26	9.57
	CM	Bm	1.22	1.40	1.79	2.01	2.62	3.67	5.15	6.16	6.80	3.42	197.39
		Bp	1.10	1.17	1.58	1.74	2.30	3.29	4.69	5.29	5.97	3.01	161.74
	FYM	Bm	1.08	1.20	1.52	1.65	2.19	3.07	4.29	5.01	5.67	2.85	147.83
		Bp	1.08	1.11	1.54	1.51	2.01	2.87	3.95	4.51	5.30	2.65	130.43
General Mean			0.78	0.88	1.12	1.21	1.58	2.22	3.15	3.64	4.11	2.08	80.87
L.S.D at 0.05 (0.08), and L.S.D, at 0.01 (0.12)													

* Treatments**,** W.B.O. [Soil soluble Ca at without bio-organic addition (control)], RP (Rock Phosphate Fluorapatite), CM (compost), FYM (farmyard manure), Bm (Bacillus megaterium), Bp (Bacillus polymyx).
** RC: Rate of relative changes of the results , referring to the control W.B.O.

Table (6): Rates of CO_2 evolved (mg g^{-1} soil 24 h^{-1}) from the rock phosphate treated sandy and alluvial clayey soils, as affected by the bio-organic additives at different incubation periods.

Treatments *			Incubation times (Days) 0	15	30	45	60	75	90	105	120	Mean	RC ** (%)
CO_2 evolved (mg g^{-1} soil 24 h^{-1}) in treated sandy soil													
4 g Ca/kg soil as RP	W.B.O		3.39	3.56	3.90	4.29	4.68	5.33	6.29	7.40	8.08	5.21	0
	CM		4.29	5.01	5.33	5.66	8.23	8.32	10.44	11.69	12.22	7.91	51.82
	FYM		4.16	4.48	4.90	5.27	6.62	7.19	9.31	10.07	10.86	6.98	33.97
	Bm		3.55	3.91	4.45	4.58	5.50	6.38	8.15	8.65	9.20	6.04	15.93
	Bp		3.50	3.72	3.92	4.33	4.81	5.77	7.19	8.02	8.71	5.55	6.53
	CM	Bm	6.22	5.93	7.68	7.55	8.87	11.09	15.35	15.86	17.16	10.63	104.03
		Bp	5.80	6.26	7.13	6.65	7.90	9.95	13.01	14.73	15.60	9.67	85.60
	FYM	Bm	4.86	5.15	6.76	7.73	7.46	9.28	11.80	13.29	14.46	8.98	72.36
		Bp	4.82	4.82	6.09	5.73	8.03	8.53	10.91	11.98	13.23	8.24	58.16
General Mean			4.35	4.59	5.34	5.53	6.58	7.61	9.75	10.71	11.52	7.33	40.69
L.S.D at 0.05 (0.60), and L.S.D, at 0.01 (0.90)													
CO_2 evolved (mg g^{-1} soil 24 h^{-1}) in treated alluvial clayey soil													
4 g Ca/kg soil as RP	W.B.O		4.76	4.98	5.38	5.92	6.62	8.02	9.88	11.17	12.46	7.69	0
	CM		6.26	6.85	7.74	8.32	9.82	12.38	16.13	18.31	19.37	11.69	52.02
	FYM		6.07	6.36	7.03	7.37	8.85	10.55	14.29	15.69	17.01	10.36	34.72
	Bm		5.03	5.63	6.07	6.42	7.30	9.30	12.09	12.83	14.07	8.75	13.78
	Bp		4.90	5.18	5.49	6.05	6.98	8.52	10.76	12.07	13.21	8.13	5.72
	CM	Bm	8.38	9.03	10.51	11.65	13.89	17.75	22.86	26.03	28.13	16.47	114.17
		Bp	7.74	8.08	9.44	10.21	12.27	15.79	20.88	22.98	25.17	14.73	91.55
	FYM	Bm	7.30	7.89	8.86	9.74	11.59	14.72	19.01	21.51	23.61	13.80	79.45
		Bp	7.01	7.22	8.56	8.75	10.55	13.53	17.47	19.35	21.77	12.69	65.02
General Mean			6.16	6.55	7.37	7.94	9.33	11.70	15.14	16.85	18.37	11.05	43.69
L.S.D at 0.05 (1.02), and L.S.D, at 0.01 (1.61)													

* Control (CO_2 evolved at without any additions), RP (Rock Phosphate Fluorapatite), W.B.O. (without bio-organic addition), CM (compost), FYM (farmyard manure), Bm (Bacillus megaterium), Bp (Bacillus polymyx).
** RC: Rate of relative changes of the results , referring to the control W.B.O.

The highest RC values of CO_2 evolution in the sandy soil were obtained by submitting the dual additives of bio and organic amendments, i.e. CM + *Bm* were superior and descendingly followed by CM + *Bp,* as they gave RC rates 104.03 and

85.60% above their control (W.B.O.), respectively. Combinations of FYM with any bio-fertilizer also increased soil respiration, but at lower extents than the CM counterparts, as the gained values, in comparison with the control (W.B.O.), were of RC 72.36 and 58.16% for FYM + *Bm* and FYM + *Bp*, respectively. The mean RC rate of the total bio-organic treatments was 40.69% compared to their control (W.B.O.)

Resembling results of the sandy soil were detected for the alluvial clayey soil, concerning the pattern of CO_2 evolution, but at higher rates (Table 6). Addition of compost (CM) or farmyard manure (FYM), as organic fertilizers, and *Bacillus megaterium* (Bm) or *Bacillus polymyxa* (Bp), as bio-fertilizers, all increased the soil respiration, to reach RC rates of 52.02, 34.72, 13.78, and 5.72%, respectively, above their control (W.B.O.) treatment.

Application of the combined additives of bio-organic substrates, significantly encouraged the discharge of CO_2 in the alluvial clayey soil, inducing RC rates of 114.17, 91.55, 79.45 and 65.02% compared to the control (W.B.O.), with CM + *Bm*, CM + *Bp*, FYM + *Bm* and FYM + *Bp*, respectively. The mean RC rate of the total bio-organic treatments was 43.69% referring to their control treatment (W.B.O.).

Evolution of CO_2 is the major outcome of organic matter decomposition in soil, as it represents the respiration of the chemoorganotrophic microorganisms dominating in soils. Hence it reflects the biochemical activity in a soil. The heterotrophic microorganisms, including the phosphate dissolving bacteria, derive their carbon and energy from organic sources, and thus all participate in the breakdown of such materials and emission of CO_2. Consequently, the applied bio-organic fertilizers favour the availability of phosphate in soil, also via formation of carbonic acid from the produced CO_2, as well as other organic acids (Barber, 1995 and kiran and Phogat, 2008).

CONCLUSION

The results of this work reveals the importance of rock phosphate as a source of calcium in both sandy and alluvial clayey soils. The soullization of calcium from rock phosphate increased greatly when these soils treated by bio and organic amendments individually or in combination.

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تأثير الإضافات العضوية والحيوية على إذابة الكالسيوم من صخر الفوسفات تحت ظروف الأراضي الرملية والطينية

إبراهيم عبد الحميد حموده[1] ، محمد أحمد الحويطي[2] ، أميرة إسماعيل المسلاوي[2]

[1] معهد بحوث الأراضي والمياه والبيئة – مركز البحوث الزراعية – الجيزة.

[2] معهد بحوث الدراسات البيئية – جامعة السادات – مدينة السادات - المنوفية.

الملخص العربي

أُجري هذا البحث لدراسة تأثير الإضافات الحيوية العضوية (الكمبوست CM والسماد البلدي FYM) كأسمدة عضوية صناعية وطبيعية ، وبكتيريا باسيلوس ميغاتيريوم *Bacillus megaterium* (Bm) وباسيلوس بوليميكسا *Bacillus polymyxa* (Bp) كأسمدة بيولوجية في صورة لقاحات بكتيرية مُذيبة للفوسفات ، على إذابة الكالسيوم من صخر الفوسفات RP (فلورو أباتيت) المُضاف والمخلوط في كل من التربة الرسوبية الطينية والتربة الرملية ، وقد أُجريت تجربة أُصص في خلال 120 يوم خلال صيف 2014 في الصوبة المفتوحة بمعهد الدراسات والبحوث البيئية ، جامعة السادات ، محافظة المنوفية ، مصر .

تم تطبيق 9 معاملات من الإضافات السابق ذكرها على كل نوع من نوعي التربة المُستخدمة ، مُثلت كل معاملة في ثلاث مُكررات ، ووضع في كل إصيص 500 جرام من التربة من صخر الفوسفات كمصدر للكالسيوم مُنخفض الذوبان في الماء بمعدل 4 جرام من الكالسيوم الكلي / كجم تربة ، كما تم إضافة معاملات الأسمدة العضوية الصناعية CM والطبيعية FYM بمعدل 2٪ من الكربون العضوي . وخُلطت محتويات كل أصيص (تربة + صخر الفوسفات + سماد عضوي) خلطاً جيداً ومتجانساً ، ومع ترطيب التربة في بداية التجربة (الزمن صفر) للوصول بمحتواها الرطوبي عند السعة الحقلية FC تم إضافة اللقاحات البكتيرية المقررة لكل معاملة بمعدل 2 مل من معلق اللقاح (10^3 - 10^4 خليه/ مل) لكل أصيص (500 جم أرض) . ويمكن تلخيص أهم النتائج المُتحصل عليها من تلك الدراسة في ما يلي :-

1. انخفضت قيمة pH التربة بزيادة زمن التحضين حتى 60 يوماً.
2. إزداد كل من التركيز الكلي للأملاح الذائبة في المحلول الأراضي ، تركيز الكالسيوم الذائب في المحلول الأرضي ، معدل انطلاق ك أ$_2$ من التربة كمؤشر لزيادة النشاط الحيوي بها ، وكانت هذه الزيادة معنوية ومستمرة بزيادة زمن التحضين ، وكان معدل الزيادة في التربة الطينية أعلى من التربة الرملية . وكان أعلى معدل زيادة من مخلوط الأسمدة العضوية والحيوية عن أي منهما بمفرده .
3. تزايد تركيز الكالسيوم الذائب في المحلول الأرضي مع المعاملات فردية الإضافة العضوية أو الحيوية مع زمن التحضين ، وتبعا للترتيب التنازلي : CM > FYM , *Bm* >*Bp* . وكان الترتيب التنازلي للمعاملات المشتركة هو : CM + *Bm* > CM + *Bp* , FYM + *Bm* > FYM + *Bp* . وفيما يتعلق بالمقارنة الكلية فكان الترتيب هو : CM + *Bm* > CM + *Bp* > FYM + *Bm* > FYM + *Bp* > CM > FYM > *Bm* > *BP* .
4. وأظهر الإضافات الفردية لكل من الأسمدة الحيوية والعضوية كمعدل لمتوسطات الزيادات التالية لذوبان الكالسيوم بالمقارنة بالكنترول الخاص بالمعاملات W.B.O. (تربة + صخر فوسفات ، بدون أي إضافات عضوية أو حيوية) (CM) (88.89 و 98.26٪) و FYM (57.78 و 68.69٪) وBm (20.00 و 26.09٪) Bp (4.44 و 9.57٪)، هذا لكل من التربة الرملية والطينية على التوالي . وعند إضافة أي من CM أو FYM وخلطة مع أي نوع من الأسمدة الحيوية فإن هذا يؤدي إلى زيادة كبيرة في ذوبان الكالسيوم بالمقارنة مع الإضافات الفردية لأي من الأسمدة العضوية أو الحيوية . وقد بلغت أعلى معدلات الزيادات الناتجة في معدل ذوبان الكالسيوم من صخر الفوسفات المُضاف للتربة المُختبرة عن استخدام أي CM أو FYM مع كل من Bm والتي سجلت 166.67 و 128.89٪ و 197.39 و 147.83٪ بالمقارنة بالكنترول الخاص بالمعاملات W.B.O. (تربة + صخر فوسفات ، بدون أي إضافات عضوية أو حيوية) وذلك في كل من التربة الرملية والطينية ، على التوالي .